THE INFLUENCE OF SEX ON THE OSMORECEPTOR MODULATION OF HEAT LOSS RESPONSES

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ABSTRACT

Females exhibit lower sudomotor response than males, which has been attributed to physiological differences between sexes. It is well accepted that non-thermal factors (i.e. baroreceptors and osmoreceptors) can influence thermoeffector responses. Even though there are sex-related differences in baroreceptor modulation of thermoeffector responses, it remains unknown if differences in smoreceptors modulation could explain the lower sudomotor response in females. Therefore, we examined if there are sex-related differences in osmoreceptor modulation of sweating and cutaneous vascular conductance (CVC). A group of nine males and nine females were passively heated while in an isosmotic and hyperosmotic state. The onset and thermosensitivity of sweating and CVC were calculated and compared between groups and conditions. We show that the delay in onset of sweating and CVC is similar between sexes. However, thermosensitivity of sweating was lower in females than males. We conclude that hyperosmolality does not modulate the decreased sudomotor activity in females.

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GLOSSARY

SKBF – skin blood flow

CVC – cutaneous vascular conductance

NTS – nucleus tractus solitaries

LBNP – lower body negative pressure

AVP – arginine vasopressin

BT – bretylium tosylate

SGO – sweat gland output

HASG – heat activated sweat glands

ISO – isosmotic

HYP – hyperosmotic

MAP – mean arterial pressure

$\overline{T}_b$ – mean body temperature

T_{es} – oesophageal temperature

$\overline{T}_{sk}$ – mean skin temperature

Hb – hemoglobin

Hct – hematocrit

PV – plasma volume

BSA – body surface area

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PART ONE:

THEORETICAL BACKGROUND

CHAPTER I

INTRODUCTION

1.3 Introduction

Extensive research has shown sex-related differences in sudomotor response with females exhibiting lower sweat rates than males (Burse, 1979; Kenney, 1985; Nunneley, 1978). These differences in sudomotor response were believed to be the result of different physical characteristics and fitness level between the sexes (Burse, 1979; Kenney, 1985; Nunneley, 1978). However, only until recently it was demonstrated that there are true physiological differences underlying the reduced sweat rate of females (Gagnon & Kenny, 2011). In general, it has been shown that sweating thermosensitivity is lower in females than males, with no changes in onset thresholds (Gagnon *et al.*, 2013; Gagnon & Kenny, 2012; Inoue *et al.*, 2005). Furthermore, no sex-related differences in cutaneous vasodilation have been identified (Gagnon et al., 2013; Inoue et al., 2005; Kolka *et al.*, 1987).

It is well accepted that non-thermal factors such as central command, baroreflexes, mechanoreceptors, metaboreceptors and osmoreceptors can influence the modulation of thermoeffector responses (Kenny & Journeay, 2010; Shibasaki *et al.*, 2003). It has been shown that baroreceptor unloading can increase the onset threshold of vascular vasodilation (Crandall *et al.*, 1996; Cui *et al.*, 2004; Lynn *et al.*, 2012; G. W. Mack *et al.*, 2001; G.W Mack *et al.*, 1995; Solack *et al.*, 1985; Wilson *et al.*, 2001) with mixed results on the sweating response (Cui et al., 2004; Dodt *et al.*, 1995; Lynn *et al.*, 2012; G. W. Mack *et al.*, 2001; G.W Mack *et al.*, 1995; Solack *et al.*, 1985; Vissing *et al.*, 1994; Wilson *et al.*, 2001). Studies focused on osmoreceptors and the effect of plasma hyperosmolality have consistently reported increased onset thresholds in sweating and cutaneous vasodilatation (Fortney *et al.*, 1984; Lynn et al., 2012; Shibasaki *et al.*,

2009; Takamata *et al.*, 1995a; Takamata *et al.*, 1997), with no changes in the thermosensitivity of either thermoeffector response (Lynn *et al*., 2012; Takamata *et al*., 1997). In addition, the combined effect of baroreceptor unloading and hyperosmolality has been shown to have an augmented inhibitory effect on cutaneous vasodilatation (Ito *et al.*, 2005; Lynn *et al*., 2012)

Given the known sex-related differences in non-thermal factors it is plausible to suggest that sex-related differences in the osmotically driven shifts in thermoeffector responses may also exist. However, since studies examining the effect of plasma hyperosmolality on heat loss responses have been done in a predominantly male population, it remains unknown if sex-related differences exist**.** To the best of our knowledge, no studies have conducted direct comparisons between males and females on the effects of hyperosmolality on thermoeffector responses (sweating and skin blood flow). Given the known influence of non-thermal factors on thermoregulatory activity, it is important to assess sex-related differences in thermoefferent modulation.

1.2 Rationale

Sex-related differences in the sudomotor thermoeffector response have been identified, with females exhibiting lower sweat rates than males. It is well known that non-thermal factors such as baroreceptors and osmoreceptors can influence the modulation of thermoeffector responses (sweating and skin blood flow). Given that sex-related differences in non-thermal modulation exist and the absence of information on the effects of hyperosmolality in females' thermoeffector responses, it is still unknown if changes in plasma osmolality could explain the known sex-related differences in sudomotor function.

1.3 Purpose

The present study will examine if plasma hyperosmolality in females will cause similar thermoeffector responses in sweating and skin blood flow (SkBF) as previously shown in males. Using whole-body passive heating to increase core temperature and promote increases in sweating and SkBF, we will assess any potential sex-related differences in onset threshold and thermosensitivity (slope) of both sweating and SkBF in young males and females.

1.4 Study Objectives

1. To compare the effects of plasma hyperosmolality between males and females on SkBF onset thresholds and thermosensitivity during whole-body passive heating.
2. To compare the effects of plasma hyperosmolality between males and females on sweating onset thresholds and thermosensitivity during whole-body passive heating.

1.5 Hypotheses

2. The effects of plasma hyperosmolality will result in a delayed onset threshold of both sweating and SkBF in males and females.
3. The overall delay in onset threshold of sweating and SkBF will be higher in females than males.

1.6 Relevance

Reduced sweating response observed in females can result in impaired heat loss, theoretically putting females at a higher risk of heat-stress related injuries. The majority of the

studies examining the effects of hyperosmolality on sweating and SkBF have been conducted predominantly on a male population. To the best of our knowledge no studies have performed a direct comparison between males and females in thermoefferent responses (sweating and SkBF). Therefore, the current study will further our understanding of sex-related differences in sudomotor response and the influence that hyperosmolality can have in both sweating and SkBF.

1.7 Delimitations and Limitations

The proposed study was conducted in young males and females between the ages of 18 and 35 years. As a result, the findings of this study do not apply to older adults or individuals with chronic conditions. Furthermore, female participants underwent testing during the follicular/low hormone phase of the menstrual cycle or the placebo/no pill week in oral contraceptive users.

CHAPTER II

REVIEW OF LITERATURE

2.1 Thermoregulation

Humans are considered homeotherms due to their ability to maintain internal temperature at a constant level (~37 °C) despite temperature changes in the environment. This is achieved through a continuous exchange of heat between the body and the environment, resulting in a balance between the amount of heat produced within the body, the heat obtained from the environment, and heat being lost to the environment (Parsons, 2003; Werner, 1981). To better understand this transfer of heat, it is important to recognize that metabolic heat is first produced at the cellular level. Given that the cells must maintain a normal temperature, heat is dissipated from the cell to the surrounding tissues (Parsons, 2003). This is followed by a transfer of heat from the core to the surface of the body where it can be easily dissipated through conduction, convection, radiation or evaporation (Parsons, 2003). This interplay is better illustrated with the heat balance equation (Parsons, 2003):

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

M = rate of metabolic heat production
W = rate of mechanical work
K = rate of conductive heat loss
C_{SK} = 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 respiration
E_{RES} = rate of evaporative heat loss from respiration
S = rate of body heat storage

(All variables expressed in W·m-2)

where metabolic heat production (M) provides the energy necessary to do work (W), and any residual energy is then released in the form of heat (M-W). To maintain a constant internal

temperature, residual energy must be dissipated through either dry heat exchange (K + C + R) or evaporative heat loss (E). Any imbalance between heat gained and lost will result in a change in heat storage (S). For instance, if heat being produced is greater than heat being lost, a positive net flux will result and heat will be stored in the body. As a consequence, internal temperature will rise (Parsons, 2003).

2.1.1 Models of Thermoregulation

Several models have been put forward to explain the process of human thermoregulation. While they all share the idea of a control center that receives and integrates sensory input to evoke an effector response (output) (Parsons, 2003; Werner, 1981), there are some fundamental differences. For instance, the Set-Point Theory describes thermoregulation as a system that consists of a control center which continuously compares core temperature against a "reference" temperature. Any deviations in core temperature will result in an "error signal" that will evoke an effector response to bring core temperature close to the "reference" point (Hammel, 1968; J. D. Hardy, 1961; Mekjavic & Eiken, 2006; Parsons, 2003). Contrary to the idea of a set-point temperature regulation, the Inter-Threshold/Null Zone Theory states that internal temperature continuously changes within a narrow range known as the "null" zone. Internal temperature is maintained within this range through changes in vasomotor tone (vasodilatation or vasoconstriction) (Mekjavic & Eiken, 2006). However, when external stressors cause drastic fluctuations in internal temperature that cannot be adjusted with a vasomotor response, other thermoeffector responses (sweating or shivering) are activated to enhance heat loss or heat production (Mekjavic & Eiken, 2006; Parsons, 2003).

2.1.2 Passive and Active Systems

The human thermoregulatory system consists of two main systems, passive and active. The passive system refers to the entity that is being controlled or in this case the human body. Opposite to that, the active system represents the factors that control the passive system, which include thermosensory receptors, central integrator and thermoeffector organs (Werner, 1981). Both systems modify each other through a negative feedback loop which first detects thermal information through thermosensitive receptors, some of which have been identified in the hypothalamus (Nakayama *et al.*, 1961), midbrain, medulla oblongata, spinal cord, skin (Parsons, 2003; Werner, 1981), blood vessels, and abdominal cavity (Hensel, 1981). This input is then relayed, via afferent neural pathways, to the controlling center where integration of afferent signals takes place. It is well accepted that thermoregulatory activity is primarily controlled by the preoptic/anterior hypothalamus (Charkoudian, 2003; Parsons, 2003) which is responsible for heat loss and vasodilatory responses (Hammel, 1968; Mack, 2004). The integration of afferent signals by the hypothalamus results in an effector response that travels down to an effector organ that acts on the passive system (Werner, 1981).

2.2 Mechanisms of Heat Dissipation

The human body relies on two main mechanisms, vasomotor tone and sudomotor response, to dissipate heat and thereby maintain internal temperature relatively constant. Vasomotor tone is the initial driver that helps regulate internal temperature through changes in SkBF. For instance, increases in SkBF enable the transport of warm blood from the core to the surface of the body (Charkoudian, 2010) where heat can then be transferred to the environment. This results in cooler blood being returned to the core thereby lowering internal temperature.

Even though vasomotor tone is able to maintain internal temperature constant, abrupt changes in heat production, as in the case of exercise, results in a heat load that cannot be readily dissipated through changes in SkBF alone. As a result, other effector responses such as sudomotor activity must be activated (Mekjavic & Eiken, 2006).

2.2.1 Skin Blood Flow

Cutaneous circulation plays a major role in thermoregulation given its ability to direct blood from the core to the surface of the body where heat can be dissipated in the form of dry heat (Mekjavic & Eiken, 2006). An increase in SkBF enhances the rate of heat loss by increasing convective transfer of heat from the core to the periphery (Charkoudian, 2003). In parallel, the evaporation of sweat cools the skin, which in turn cools the blood in the dilated skin vessels before it returns to the core (Charkoudian, 2003).

Skin blood flow is regulated by two main nerves of the sympathetic nervous system namely the sympathetic noradrenergic vasoconstrictor nerves and the sympathetic active vasodilator nerves, each responsible for vasoconstriction and vasodilation of the skin vasculature, respectively (Grant & Holling, 1938; Lewis & Pickering, 1931). The sympathetic noradrenergic vasoconstrictor system is well known for its role in maintaining "vasomotor tone" or sustained vasoconstriction at rest, which allows for a resting SkBF of around 250 mL/min (Brengelmann & Savage, 1997; Johnson & Proppe, 1996). However, this volume is subject to change based on environmental conditions. During cold exposure, sympathetic vasoconstrictor activity is increased through the release of norepinephrine, which binds to alpha (α) 1 and α2-receptors found in the cutaneous vasculature (Charkoudian, 2003, 2010). This evokes generalized vasoconstriction that reduces SkBF and promotes heat conservation. Conversely

during heat exposure, the sympathetic active vasodilator system, which does not contribute to vasomotor tone at rest, is activated resulting in a profound increase in SkBF (Johnson & Proppe, 1996; Rowell, 1983). It has been shown that during severe hyperthermia, SkBF can go up to 6 to 8 L/min, which is about 60% of total cardiac output (Johnson & Proppe, 1996). This active vasodilation is believed to result from the action of cholinergic nerves (Kellogg *et al.*, 1995), however, it is still not clear which substances (vasodilators) are involved (Charkoudian, 2010). Research on this area has suggested that nitric oxide (Shastry *et al.*, 1998), vasoactive intestinal peptide (Bennett *et al.*, 2003), substance P/NK-1 receptors (Wong & Minson, 2006), histamine/H1-receptor mediated (Wong *et al.*, 2004), and prostaglandins (McCord *et al.*, 2006) may play a role in this vasodilatory response.

2.2.2 Sweating

Sudomotor activity allows the body to dissipate large amounts of heat through the evaporation of water in the form of sweat, better known as evaporative heat loss. Regulation of sweating is believed to begin with efferent signals from the preoptic region of the hypothalamus. Signals then travel down via the tegmentum of the pons and medullary raphe regions to the intermediolateral cell column of the spinal cord where neurons emerge through the ventral horn and then synapse at the sympathetic ganglia. From the sympathetic ganglia, postganglionic non-myelinated C-fibers extend to the sweat gland (effector organ) (Uno, 1977). In the event of sudomotor activation, the sympathetic nerves that innervate the sweat glands release the neurotransmitter acetylcholine, which is quickly hydrolyzed in the synaptic cleft by the enzyme acetylcholinesterase (Randall & Kimura, 1955). It is the action of this enzyme that allows for the regulation of sweating during low sweat rate conditions (Randall & Kimura, 1955), however,

during profused sweating the action of acetylcholinesterase becomes diminished due to the increased concentration of the neurotransmitter in the synaptic cleft (Shibasaki & Crandall, 2001).

Humans possess apocrine and eccrine sweat glands, however, only the latter is involved in thermoregulatory processes (Sato *et al.*, 1989) and can be found throughout the surface of the body. Areas of the body that are heavily populated with eccrine sweat glands include the forehead (most), upper limbs, trunk and lower limbs (Sato & Dobson, 1970; Taylor & Machado-Moreira, 2013). These sweat glands are composed of a bulbous secretory coil that sits in the dermis and is connected to a duct that extends all the way through the epidermis up to the surface of the skin (Sato & Sato, 1983; Shibasaki *et al.*, 2006). The process of sweat production starts with the stimulation of muscarinic receptors on the surface of the sweat gland by acetylcholine. This is followed by an influx of calcium ions that activates the movement of sodium and chloride ions into the cell, thus creating an osmotic gradient that favors the movement of water from the plasma into the gland (Taylor & Machado-Moreira, 2013). This water based fluid is collected into the bulbous secretory coil of the gland where it is pushed upwards in a pulsatile manner. As this fluid travels through the duct, sodium and chlorine ions are pumped back into the plasma decreasing the sodium content of sweat, and thereby making it hypotonic to plasma (Sato, 1973). It is worth noting that the concentration of sodium in sweat is highly dependent on sweat rate, as higher sweat production results in the saturation of the pumps leaving more sodium ions in the sweat (Allan & Wilson, 1971).

2.2.3 Effector Response-Temperature Relationship

The control of sweating in humans was initially believed to depend upon changes in skin

temperature (Hammel, 1968), however, subsequent research showed that internal temperature also played a major role (McCook *et al.*, 1965; Nadel *et al.*, 1971b). As a result, mean body temperature (the weighted sum of internal and mean skin temperature) (Nadel *et al.*, 1971a; Nadel *et al.*, 1971b) is used to account for the influence of both core and skin temperatures. In addition, mean body temperature has been used to characterize the influence of thermal and non-thermal factors on the thermoeffector responses of SkBF and sweating. This characterization is based on the effector response-mean body temperature relationship, which consists of three main parts: onset threshold, thermosensitivity and plateau (Figure 1).

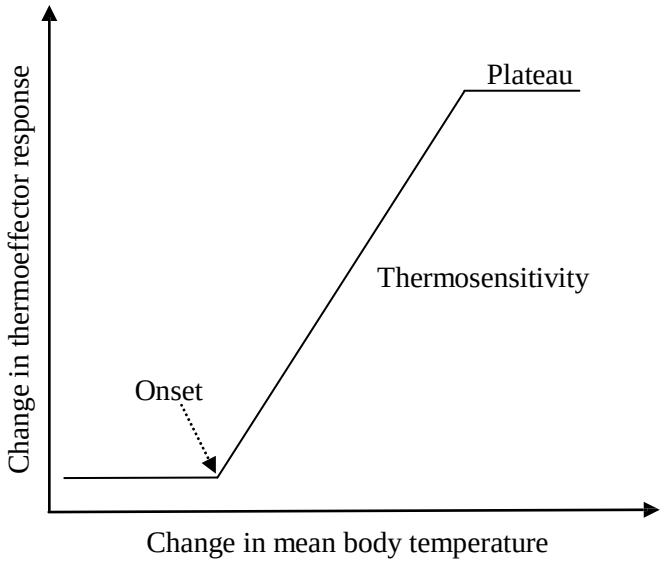

Figure 1. Schematic representation of a typical relationship between changes in thermoeffector response (SkBF or sweating) and changes in mean body temperature.

The onset threshold represents the mean body temperature at which an initial increase in thermoeffector response (sweating or SkBF) is observed (Charkoudian, 2003; Mekjavic & Eiken, 2006). The thermosensitivity corresponds to the slope of the line which reflects any subsequent increases in effector response due to continuous increments in temperature (Charkoudian, 2003).

Finally, the plateau is the flat portion of the relationship, which can reflect either a state of balance (heat dissipation and heat production are equal) or a state of maximal responsiveness (the effector response can no longer increase despite increases in temperature). In the latter state, a difference between maximal responsiveness and heat produced will reflect the amount of heat being stored in the body (Charkoudian, 2003). Changes in any of these components may lead to impaired or enhanced thermoregulatory ability (Gisolfi & Wenger, 1984) depending on the alteration observed. Potential changes in the effector response-mean body temperature relationship are further illustrated in Figure 2. Changes in onset thresholds or thermosensitivity can result from alterations in: 1) the thermoafferent signals coming from peripheral thermoreceptors, 2) the integration of afferent information at the control center, 3) the thermoefferent signals, 4) the response of thermoeffector organs, or 5) a combination of any of the four. As a result, examining changes in onset threshold and thermosensitivity can provide valuable insight into the mechanisms responsible for temperature regulation. Changes in onset threshold have been used as an indicator of central modulation of temperature regulation, while the thermosensitivity has been used to describe peripheral adaptations in effector responses (Nadel *et al*., 1971b; Nadel *et al.*, 1974). Furthermore, the plateau portion of the thermoeffector-mean body temperature relationship can also provide insight into the maximal capacity of the response. It has been shown that shifts in onset threshold can be attributed to factors such as hyperosmolality (Shibasaki *et al.*, 2009), circadian rhythm (Aoki *et al.*, 2001) and sex hormones (Stephenson & Kolka, 1993), while changes in thermosensitivity can result from hypovolemia (Fortney *et al.*, 1981).

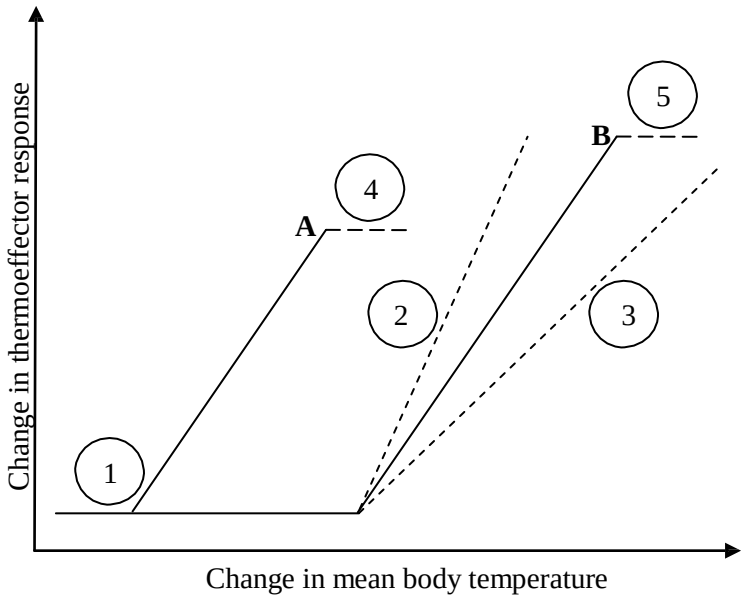

Figure 2. Schematic representation of observed alterations in the thermoeffector response-mean body temperature relationship. [1] The onset threshold of a thermoeffector response occurs at a lower core temperature in condition A compared to condition B. [2] The thermosensitivity (slope) is increased resulting in a greater increase in thermoeffector response (sweating or SkBF) per unit change in mean body temperature. In contrast, [3] a lower thermosensitivity leads to a smaller change in thermoeffector response for a given change in mean body temperature. [4] A lower plateau compared to [5] reflects a lower overall responsiveness, and therefore increased heat storage.

3.3 Hydration

Hypohydration, a state of body water deficit, can result from 1) inadequate fluid intake or 2) a mismatch between fluid loss and fluid intake (Sawka *et al*., 2001). During heat stress or exercise, body water content is quickly lost, mainly through sweating. This loss of fluid results in a redistribution of fluid among the body's compartments (Sawka, 1992). For instance, during mild fluid loss, most of the water comes from the extracellular compartments (interstitial fluid and plasma). However, when fluid loss is increased, there is a fluid shift that favors the

maintenance of blood volume and therefore, most of the water lost comes from the intracellular compartment (Costill *et al.*, 1976). Early studies conducted by Nose and Colleagues (1983) showed that the greatest proportion of water loss comes from the skin and the muscle, while water content in the brain and liver remains relatively stable (Nose *et al*., 1983). It is therefore important to examine the effects of hydration status on cardiovascular regulation, given the inherent fluid shifts that take place with fluid loss.

2.3.1 Cardiovascular Regulation during Hypohydration

Thermoregulatory mechanisms rely on the mobilization of water to dissipate heat. For instance, during sweating fluid is transferred from the extracellular compartments to the sweat gland generating sweat and dissipating large amounts of heat in the process (evaporative heat loss) (Sawka *et al*., 2001). However, this shift in fluids varies according to the degree of fluid loss. Fluid is initially and primarily drawn from the interstitial and plasma compartments to generate sweat. However, as sweat is being produced, the ion pumps in the sweat gland duct will pump sodium and chloride ions back into the extracellular compartments (Sato, 1973). As a result, the deficit in water, compounded by the increased concentration of ions relative to total plasma volume, will increase plasma osmolality. This increase in plasma osmolality (hyperosmolality) will then act on the intracellular compartment by driving water from the cells into the plasma (Nose *et al.*, 1988). This will in part help maintain blood volume and defend blood pressure (Sawka, 1992) in the face of profound fluid loss.

Further cardiovascular adjustments during hypohydration include reductions in cardiac output during exercise mainly driven by a consistent drop in stroke volume (Gonzalez-Alonso *et al.*, 1995, 1997; Nadel *et al.*, 1980; Sproles *et al.*, 1976). In some cases, the drop in cardiac

output has been reported to be as high as 13%, accompanied by a drop in mean arterial pressure (Gonzalez-Alonso *et al.,* 1997). These cardiovascular alterations have been attributed to compromised venous return due to: 1) decreased vascular volume, 2) increased fluid loss due to evaporative heat loss and 3) increased cutaneous blood volume for heat dissipation purposes (Nadel *et al*., 1980; Sawka, 1992). In addition to lower cardiac output, it has been noted that dehydrated individuals exhibit higher levels of hyperthermia when compared to euhydrated (normal body water content) and hyperhydrated (increased body water content) individuals (Montain & Coyle, 1992; Nadel *et al.*, 1980; Sawka *et al.*, 1985). This increased hyperthermia has been attributed to significant delays in the onset threshold of cutaneous vasodilation with no change in thermosensitivity (slope) (Nadel *et al*., 1980) and lower sweat rate in hypohydrated individuals (Greenleaf & Castle, 1971). Similarly, Sawka et al. (1985) found that progressive levels of hypohydration resulted in overall reduced sweat rates, which would imply an inability to dissipate heat and higher heat storage, thereby explaining the rise in internal temperature (Sawka *et al.*, 2001; Sawka *et al.*, 1985).

3.3.2 Cardiovascular Regulation during Passive Heat Stress

During whole-body passive heating, an increase in cardiac output is typically observed and in some cases it can increase above 10 L/min (Crandall *et al.*, 2008). Despite the increase in cardiac output, stroke volume has been shown to remain unchanged or at best mildly increased (Johnson & Proppe, 1996; Wilson *et al.*, 2007). Therefore, the main driver behind the increase in cardiac output has been suggested to be increases in heart rate during heating (Johnson & Proppe, 1996). Furthermore, decreased central venous pressure and preload have also been reported during whole body heating (Crandall *et al*., 2008; Wilson *et al*., 2007). However, stroke

volume is maintained irrespective of the reduction in preload thanks to increased ejection fraction (Crandall *et al*., 2008; Wilson *et al.*, 2009). In addition, mean arterial pressure has been shown to remain relatively stable (Cui *et al.*, 2004; Johnson & Proppe, 1996; Wilson *et al*., 2001) even though there is a dramatic increase in cutaneous perfusion at the expense of decreasing blood flow to the splanchnic and renal regions (Charkoudian, 2003; Crandall *et al*., 2008).

3.4 Non-thermal Factors of Thermoregulation

While thermal input is the main driver for changes in thermoeffector responses (sweating and SkBF), there are non-thermal factors that can also modulate theses responses. For instance, during exercise, a rise in internal core and skin temperature (thermal factors) results in the activation of sweating and SkBF, while at the same time other reflexes of non-thermal origin linked to exercise can also affect sweating and SkBF. Such non-thermal factors include central command, baroreflexes, mechanoreceptors, metaboreceptors and osmoreceptors (Gisolfi & Robinson, 1970; Kenny & Journeay, 2010; Shibasaki et al., 2003). Central to the present discussion are baroreceptors and osmoreceptors, which will be addressed next.

2.4.1. Influence of Baroreceptors on Heat Dissipation

Baroreceptors are stretch receptors that regulate blood pressure. These receptors are classified into two families: cardiopulmonary and arterial. Cardiopulmonary baroreceptors, also known as low pressure receptors, are located in the heart and the pulmonary vasculature. Arterial baroreceptors, on the other hand, are known as high pressure receptors and can be found in the carotid sinus and the aortic arch. Both groups of baroreceptors continuously monitor changes in pressure. More specifically, arterial baroreceptors are responsible for regulating arterial blood

pressure while cardiopulmonary baroreceptors monitor mainly venous and atrial pressure. In the event of a drop in arterial blood pressure, the firing rate of these stretch receptors decreases and is considered a state of baroreceptor unloading. Alternatively, when arterial blood pressure increases, the firing rate increases and is known as loading of baroreceptors. This afferent information (firing rate) is relayed to the nucleus tractus smorecept (NTS) in the medulla oblongata where it is integrated. It is important to note that each group of baroreceptors uses a different neural pathway to relay afferent signals to the NTS (Kenny & Journeay, 2010; Shibasaki *et al*., 2003). Once afferent input is integrated, the corresponding effector response results in the activation of the sympathetic and parasympathetic branches of the autonomic nervous system. In the event that baroreceptors are unloaded (drop in blood pressure), sympathetic stimulation will increase heart rate, stroke volume and cardiac output (Van Wynsberghe *et al.*, 1995), while at the same time inducing a state of cutaneous vasoconstriction (Charkoudian, 2003), thereby, increasing blood pressure.

It has been shown that baroreceptor unloading can modify thermoeffector responses by influencing SkBF modulation during passive heating (Crandall *et al*., 1996; Cui *et al*., 2004; Kenny *et al.*, 2010; Lynn *et al*., 2012; Solack *et al*., 1985; Wilson *et al.*, 2001) and during exercise (Mack *et al*., 2001; Mack *et al.*, 1995). Using lower body negative pressure (LBNP) to unload baroreceptors during cycle ergometer exercise (119±8 W), Mack et al. (2001) found that application of LBNP (-40 mmHg) resulted in the increase in onset threshold for cutaneous vascular conductance (CVC). Similar findings have also been reported when using LBNP during passive heating. Cui et al. (2004) examined changes in CVC during consecutive increments of LBNP (-3 mmHg to -40 mmHg) and noted that CVC significantly decreased from baseline at the two highest levels applied (-21 mmHg and -40 mmHg). Crandall et al. (1996) also used passive

heating while applying progressively increasing levels of LBNP (-5, -10 and -30 mmHg) and found that changes in CVC were significant at the highest level of LBNP. This suggests that CVC may only be affected at high levels of LBNP (Crandall *et al*., 1996).

Contrary to the known effect of baroreceptor unloading on SkBF, its effect on sweating remains controversial (Kenny & Journeay, 2010; Shibasaki *et al*., 2006). Several studies have reported changes in sweating as a result of baroreceptor unloading during exercise (Mack *et al.*, 2001; Mack *et al*., 1995). On the contrary, during passive heating some studies have reported changes in sweating (Dodt *et al.*, 1995; Solack *et al*., 1985), while others have not (Cui *et al*., 2004; Lynn *et al.*, 2012; Vissing *et al.*, 1994; Wilson *et al.*, 2001). Mack et al. (2001) showed an increase in the onset threshold of sweat rate during exercise and upon application of -40 mmHg of LBNP. Similarly, using LBNP and passive heating to induce sweating, it was found that the thermosensitivity was reduced by 28% from control (Solack *et al*., 1985). However, it was noted that after removing the LBNP, sweat rate did not recover (Solack *et al*., 1985) making the influence of baroreceptors inconclusive. Likewise, skin electrodermal activity (an index of sweating) was found to be reduced during both LBNP (-5 mmHg to -10 mmHg) and 30° head-up tilt in passively heated subjects (Dodt et al., 1995). In contrast to these results, no changes in sudomotor activity were reported after inducing an acute (~60 sec) and sustained (8-10 minutes) change in blood pressure with pharmacological agents (Wilson *et al*., 2001). More recently, Lynn et al. (2012) examined changes in both CVC and sweating response during whole-body passive heating with and without the application of LBNP (-29mmHg). They reported that the onset threshold for CVC increased with the application of LBNP. However, LBNP was shown to have no effect on the onset threshold of sweating and the thermosensitivity of either response was found to be unaffected by LBNP (Lynn *et al*., 2012).

Conflicting results on the role of baroreceptor loading status in sudomotor activity have been attributed to a number of confounding factors. First, the use of LBNP to induce unloading of baroreceptors can result in a concomitant decrease in skin temperature from enhanced convective cooling that can account for reductions in sweat rate (Shibasaki *et al*., 2003; Shibasaki *et al*., 2006). This was the case in the studies by Cui et al (2004) and Vissing et al. (1994), both of which observed slight decreases in sweating, that upon examination, were attributed to reduced skin temperatures and not baroreceptor unloading. Second, it has been suggested that different methods used to unload baroreceptors may result in the activation of different types of baroreceptors (cardiopulmonary vs. arterial) and thereby lead to conflicting findings (Shibasaki *et al*., 2006; Wilson *et al.*, 2001). For instance, application of LBNP and the use of head-up tilts can result in the unloading of cardiopulmonary baroreceptors, while pharmacological manipulation of blood pressure unloads mainly arterial baroreceptors. Last, given that not all studies have induced the same level of hyperthermia, it has been suggested that the difference in results may be due to inability of baroreceptors to modulate sweating at high levels of hyperthermia (Shibasaki *et al*., 2006).

2.4.2. Influence of Osmoreceptors on Heat Dissipation

Changes in plasma osmolality are detected by osmosensitive neurons known as osmoreceptors (Bourque *et al.*, 1994). These neurons have been identified in the central nervous system at the level of the forebrain lamina terminalis (Stocker *et al.*, 2008), as well as in the periphery of the body around the hepatic portal vein (Bourque *et al.*, 1994). Furthermore, these receptors are responsible for the release of arginine vasopressin (AVP), also known as antidiuretic hormone, which is involved in increased water reabsorption in the kidney (Bourque

et al., 1994). It has been documented that small changes in osmolality result in the release of AVP and activation of thirst sensation (Thompson *et al.*, 1987). However, this smoreceptors-driven response has been shown to be overridden by drinking. Thompson et al. (1987) demonstrated that drinking resulted in an immediate decrease in AVP release and thirst sensation even though plasma osmolality remained the same. This reflexive response has been identified as an oropharyngeal afferent nerve reflex (Thompson *et al*., 1987) that results from the withdrawal of inhibitory osmotic input (Takamata et al., 1995a).

In addition to the important role in fluid regulation (Bourque *et al.*, 1994), osmoreceptors have also been shown to influence thermoeffector responses (sweating and SkBF) (Shibasaki *et al*., 2003). Early studies by Fortney and colleagues (1981 & 1984) examined the effects of dehydration (hypovolemia and hyperosmolality) on heat loss responses. It was reported that a reduction of blood volume (hypovolemia) of 8.7% without changes in plasma osmolality resulted in a decrease in thermosensitivity (slope) of the sweat rate-esophageal temperature relationship (Fortney *et al*., 1981). However, when a state of plasma hyperosmolality was induced through exercise and infusion of hyperosmotic saline, thus maintaining normal blood volume, the onset threshold of sweating and cutaneous vasodilation was delayed without changes in the thermosensitivity (Fortney *et al*., 1984).

Studies focused on the independent effect of plasma hyperosmolality have consistently reported that heat loss responses (sweating and SkBF) are impaired through a delay in the onset threshold (Fortney et al., 1984; Lynn et al., 2012; Shibasaki et al., 2009; Takamata et al., 1995a; Takamata et al., 1997). For instance, Takamata and colleagues (1995) used isosmotic (0.9% NaCl) and hyperosmotic (3% NaCl) saline infusions along with passive heating through lower leg water immersion, to evaluate the onset threshold of sweating. They reported that the onset

was significantly delayed in the hyperosmotic (+0.5 °C esophageal temperature) compared to the isosmotic condition (Takamata et al., 1995a). Following on these initial results, Takamata et al. (1997) examined the effects of increasing levels of plasma osmolality (0.9%, 2% and 3% NaCl infusions) on both sweating and CVC. It was found from regression analyses that the esophageal temperature onset threshold for sweating and CVC increased by 0.034 °C and 0.044°C respectively, while no changes were noticed on the thermosensitivity of either thermoeffector response (Takamata *et al.*, 1997). A study by Shibasaki and colleagues (2009) attempted to elucidate the mechanism behind the osmotically induced impairment of SkBF. Using bretylium tosylate (BT) to block sympathetic vasoconstriction, they found that untreated and BT treated-sites exhibited delayed onset thresholds (0.88 °C vs. 0.86 °C) during hyperosmotic infusion (Shibasaki *et al.*, 2009). Therefore, it was concluded that the delayed onset could be the result of decreased activity of the sympathetic vasodilatory system (Shibasaki *et al.*, 2009). In addition, the authors also reported increased onset threshold in sweating while no changes in the thermosensitivity of either thermoefferent response (Shibasaki *et al*., 2009).

In addition to the independent effect of plasma hyperosmolality on sweating and SkBF, it has been suggested that the combination of baroreceptor unloading and plasma hyperosmolality can have an augmented inhibitory effect on thermoeffector responses (Ito *et al.*, 2005; Lynn *et al.*, 2012). Lynn et al. (2012) examined the independent and combined effect of baroreceptor unloading and plasma hyperosmolality during passive heat stress. Using an experimental design consisting of whole-body heating in combination with either an isosmotic (0.9% NaCl) or hyperosmotic (3% NaCl) state along with LBNP (-29 mmHg) or control sham pressure (0 mmHg), they assessed sweating and CVC in the forearm (Lynn *et al.*, 2012). It was reported that the onset threshold for CVC was delayed by the hyperosmotic infusion; however, the magnitude

of the delay was increased by the combination with LBNP. This suggested an additive effect of hyperosmolality and baroreceptor unloading (Lynn *et al*., 2012). Furthermore, the application of LBNP alone did not result in any changes in the onset threshold of sweating nor on the thermosensitivity of both CVC and sweating (Lynn *et al*., 2012).

3.5 Thermoregulation and Sex-related Differences

Sex-related differences in non-thermal modulation of heat loss responses have been identified in the literature. In particular, females have been shown to have a reduced ability to regulate blood pressure (Convertino, 1998). More specifically, it has been noted that females have lower tolerance to LBNP (Gotshall, 2000) and are more likely to experience orthostatic hypotension due to decreased muscle sympathetic nerve activity (Shoemaker *et al.*, 2001). Furthermore, females have also been shown to have lower baroreflex buffering of blood pressure than males (Christou *et al.*, 2005). Last, studies examining heat loss responses post-exercise have noted higher onset thresholds of sweating and CVC post-exercise and greater reductions in blood pressure post-exercise in females (Carter *et al.*, 2001; Kenny *et al.*, 2006). Given the known sex-related differences in non-thermal factors it is plausible to suggest that sex-related differences in the osmotically driven shifts in thermoeffector responses may also exist. However, studies examining the effects of plasma hyperosmolality on heat loss responses have been done in a predominantly male population, thus it remains unknown if sex-related differences exist**.** To the best of our knowledge, no studies have conducted direct comparisons between males and females on the effects of hyperosmolality on thermoeffector responses (sweating and SkBF). Given the known influence of non-thermal factors on thermoregulatory activity, it is important to assess sex-related differences in thermoefferent modulation.

2.5.1. Sex-related Differences on Heat Loss Responses

Sex-related differences have been long recognized with females exhibiting lower sudomotor response than males (Burse, 1979; Kenney, 1985; Nunneley, 1978). This was attributed to differences in physical characteristics between males and females, which included body mass, surface area to body mass ratio and fitness level (Burse, 1979; Kenney, 1985; Nunneley, 1978). This reduced sweating response in females has been observed during exercise (Bar-Or, 1998; Gagnon & Kenny, 2012; Morimoto *et al.*, 1967) as well as during passive heating (Bittel & Henane, 1975; Buono & Sjoholm, 1988; Fox *et al.*, 1969; Gagnon *et al.*, 2013; Inoue *et al.*, 2005). For instance, Bar-Or (1998) showed that females had a lower sweat rate while having a greater number of heat activated sweat glands. As a result, the reduced sweat rate was attributed to lower sweat output per gland (Bar-Or, 1998). Similar results have also been shown by Inoue et al. (2005) after 60 minutes of passive heating (lower leg immersion). They reported that female local sweat rate in the forehead, chest, back and forearm was consistently lower than in males, while having overall lower sweat gland outputs (Inoue *et al*., 2005). More recently, Gagnon et al. (2013) observed lower local sweat rates in females due to lower sweat gland output. It was further shown that females had lower sweating thermosensitivity than males, while having similar onset thresholds (Gagnon *et al.*, 2013).

Numerous studies have assessed sex-related differences in the sweating response; however, only a few studies have examined SkBF responses (Gagnon *et al.*, 2013; Inoue *et al.*, 2005; Kolka *et al*., 1987). Inoue et al. (2005) compared both sweating and cutaneous vasodilation in both males and females during passive heating. They found that while females exhibited lower sweat rate than males there were no differences in cutaneous blood flow between the two groups (Inoue *et al*., 2005). Moreover, no differences in onset threshold for both

sweating and SkBF between males and females were observed (Inoue *et al*., 2005). More recently, Gagnon et al. (2013) reported that both onset threshold and thermosensitivity of CVC were similar between males and females.

There remains considerable disagreement regarding the mechanism by which sex-related differences in thermoregulatory function occur (central vs. peripheral). While some research advocates for a central modulation demonstrated by altered onset thresholds (Fox *et al*., 1969), other studies sustain the idea of peripheral modifications (Gagnon *et al.*, 2013; Gibson & Shelley, 1948; Madeira *et al.*, 2010). The idea of a peripheral modulation is particularly evident in pharmacological studies that manipulate thermoeffector responses. Given that during pharmacological stimulation core temperature changes are eliminated, central command is not being activated and therefore peripheral modulation can be isolated (Madeira *et al*., 2010). It has been postulated that peripheral alterations can be the result of increased cholinergic sensitivity of sweat glands and/or altered glandular morphology (size) (Inoue *et al*., 2005). A recent study by Madeira and colleagues (2010) evaluated sex-related differences in sweat gland cholinergic sensitivity using iontophoretic administration of pilocarpine (sweat stimulant agent). They demonstrated that males exhibited greater cholinergic sensitivity than female counterparts, even after matching them on maximal aerobic capacity (Madeira *et al*., 2010). They concluded that sex-differences in cholinergic sensitivity were evident irrespective of differences in fitness (Madeira *et al.*, 2010). Similar results have also been reported by Gibson and Shelley (1948) where males showed higher sweating in response to pharmacological stimulation. Recently, Gagnon et al (2013) examined the sudomotor and vasomotor responses in males and females using incremental doses of acetylcholine, methylcholine, and sodium nitroprusside. It was noted that males and females did not show any differences in the activation of heat loss responses of

SkBF and sweating at the various concentrations employed; however, at the maximal dose of these pharmacological agents females exhibited lower maximal sweat rate (Gagnon et al., 2013). Within the same study, heat loss responses were also evaluated during whole-body passive heating (water perfused suit) and it was noted that females had a lower sweating thermosensitivity while no differences were observed in onset thresholds. In addition, no differences in cutaneous vasodilatation were observed. Taken together, it was concluded that sudomotor activity in females is likely the result of peripheral modulation rather than central.

Although research has been devoted to the understanding of sex-related differences in heat loss responses, the effect that hyperosmolality can have on these responses in males and females is yet to be determined. Furthermore, the consistently reported lower sweat rate in females has not been evaluated from the point of view of changes in plasma osmolality. Gagnon and Kenny (2012) used an exercise model of increasing requirements for heat loss to compare sweating and SkBF in both males and females. They found that while the onset threshold of sweating was similar between males and females, the thermosensitivity was lower in female participants (Gagnon & Kenny, 2012). This was parallel by overall lower sweating in females, which was attributed to lower sweat gland output (Gagnon & Kenny, 2012). Plasma osmolality was measured in a subset of participants at the end of each exercise bout with no differences in osmolality being detected; nonetheless, it is still unclear whether continuous fluctuations in plasma osmolality due to fluid loss from sweating could account for the differences observed in sweating response. Alternatively, sex-related changes in smoreceptors activation, and therefore sensitivity of the response could exist. This idea of smoreceptors sensitivity has been indirectly observed in studies looking at fluid regulation and changes in urine osmolality. It is well accepted that arginine vasopressin (AVP) stored in the posterior pituitary gland is released upon

stimulation from central osmoreceptors (Wenner & Stachenfeld, 2012), and its release has been shown to be very sensitive to changes in osmolality (Calzone *et al.*, 2001). Given that males have consistently been shown to have higher levels of AVP compared to females (Crofton *et al.*, 1986; Hancock *et al.*, 2010; Share *et al.*, 1988) it could be suggested that males exhibit increased sensitivity to changes in osmolality. In the study by Hancock and Colleagues (2010) a sample of males and females underwent a 24 hour dehydration (water deprivation) protocol while AVP concentrations were measured every 4 hours. It was noted that males had significantly higher levels of AVP throughout the initial 12 hours of water deprivation than females (Hancock *et al*., 2010). This increased concentration of AVP observed in males could indicate increased sensitivity to changes in osmolality, as AVP release can serve as an index of smoreceptors sensitivity (Hancock *et al.*, 2010). Similarly, Stachenfeld and Colleagues (2001) also concluded that males exhibit greater sensitivity to changes in osmolality, as indicated by a greater slope in the plasma AVP-plasma osmolality relationship than females (Stachenfeld *et al.*, 2001). This resulted in a greater release of AVP per increase in plasma osmolality (Stachenfeld *et al.*, 2001). Therefore, it would be appropriate to examine whether plasma osmolality could explain the sex-related differences in sudomotor response previously reported in the literature.

2.5.2. Female Hormones

Female sex hormones (estrogen and progesterone) have been shown to play a role in female thermoregulatory activity. For instance, female core temperature fluctuates between 0.3–0.5°C throughout the phases of the menstrual cycle (Charkoudian, 2003; Kaciuba-Uscilko & Grucza, 2001). Furthermore, resting core temperature and mean body temperature during the mid-lutheal (high hormone) phase are higher than the follicular phase (low hormone) (Inoue et

al., 2005; Kolka & Stephenson, 1997; Stephenson & Kolka, 1985). Studies examining the differences between phases of the menstrual cycle on heat loss responses have consistently found that both sweating and SkBF onset thresholds are shifted. For instance, Grucza et al. (1993) showed that the onset threshold for sweating in females was increased during the lutheal phase compared to the follicular phase during exercise (Grucza *et al.*, 1993). A more recent study by Inoue et al. (2005) further showed that overall local sweat rate was not affected by the phases of the menstrual cycle; however, the onset threshold for sweating and cutaneous vasodilation were higher during the mid-lutheal phase (Inoue *et al.*, 2005). Similarly, Kolka & Stephenson (1997) reported a higher onset threshold for cutaneous vasodilation with no changes in the thermosensitivity (slope) during the mid-lutheal phase. Similar to endogenous estrogen and progesterone exogenous sources of these hormones, as in the case of oral contraceptives, have been shown to have similar effects on thermoregulatory activity (Charkoudian & Johnson, 1997). Charkoudian et al. (1997) reported that high levels of exogenous estrogen and progesterone resulted in a higher onset threshold of cutaneous vasodilatation when compared to the low hormone phase of the menstrual cycle. Furthermore, Kenny and Colleagues (2008) demonstrated that heat loss responses post-exercise were not affected by the phase of menstrual cycle or oral contraceptive use (Kenny *et al.*, 2008). Therefore, when comparing thermoregulatory responses between males and females it is important to take into consideration the phases of the menstrual cycle and oral contraceptive use.

PART TWO:

METHODS AND RESULTS

Osmoreceptors do not exhibit a sex-dependent modulation of heat loss responses

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ABSTRACT

The present study examined if there are sex-related differences in the osmotically induced changes in both sweating and cutaneous vascular conductance (CVC). Nine young males and nine females were passively heated (water-perfused suit) to 1.5°C above baseline oesophageal temperature while in an isosmotic (0.9% NaCl saline infusion) (ISO) and hyperosmotic (3% NaCl saline infusion) (HYP) state. Forearm sweat rate (ventilated capsule), skin blood flow (laser-Doppler), oesophageal temperature and skin temperature were continuously recorded. Sweat gland output (SGO) on the forearm was calculated from the number of heat activated sweat glands (modified iodine-paper technique) at the end of heating. The onset threshold and thermosensitivity of sweating and CVC were determined using the linear portion of each response plotted against mean body temperature and analysed using segmented regression analysis. We show that the osmotically induced delay in the onset threshold of sweating and CVC is similar between males and females. Although the thermosensitivity of CVC was similar between sexes ($p=0.601$), the thermosensitivity of sweating was consistently lower in females compared to males ($p=0.018$). The lower thermosensitivity in sudomotor response of females was accompanied by a lower SGO ($p=0.003$), albeit similar sweat gland activation to males ($p=0.644$). We conclude that sex-related differences in thermoeffector activity are independent of osmoreceptor activation. Therefore, osmoreceptors do not exhibit sex-related differences in the modulation of CVC and sweating responses during heat stress.

ABBREVIATIONS

CVC, cutaneous vascular conductance; MAP, mean arterial pressure; SGO, sweat gland output, $\overline{T}_b$, mean body temperature; T_{es}, Oesophageal temperature; $\overline{T}_{sk}$, mean skin temperature.

INTRODUCTION

While thermoeffector responses (sweating and skin blood flow) are primarily modulated by changes in internal and skin temperature (i.e. thermal factors); these responses can also be influenced by factors of non-thermal origin (i.e. baroreceptors, mechanoreceptors, metaboreceptors, central command and osmoreceptors) (Shibasaki *et al.*, 2003; Kenny & Journeay, 2010). Furthermore, this non-thermal sensory receptor modulation of heat loss responses may differ between males and females. For instance, response to baroreceptor activation has been shown to differ between males and females such that females exhibit lower baroreflex buffering of blood pressure (Convertino, 1998; Christou *et al.*, 2005) and lower orthostatic tolerance (Shoemaker *et al.*, 2001). It has been further suggested that the non-thermal baroreceptor-mediated attenuation of thermoefferent responses may be more pronounced in females than males, as exemplified by higher onset thresholds of sweating and cutaneous vascular conductance in females post-exercise (Kenny *et al.*, 2007). Similarly, sex-related differences have been observed in both metabo- (Jarvis *et al.*, 2011) and mechano-reflexes (Ives *et al.*, 2013), with females exhibiting attenuated responses when compared to males. Even though extensive research has been devoted to the understanding of sex-related differences in non-thermal factors, it remains unclear whether osmoreceptors also exhibit a sex-specific response.

Given that prolonged exercise and/or exposure to heat can result in profound fluid loss through sweating (and thereby increases in osmolality) (Sawka *et al.*, 2001), examination of sex-related differences in the osmoreceptor modulation of thermoeffector responses remains an important area of consideration. Numerous studies have demonstrated that hyperosmolality impairs heat loss responses as evidenced by a delayed onset threshold of both sweating and skin

blood flow (Fortney *et al.*, 1984; Takamata *et al.*, 1995a; Takamata *et al.*, 1997; Shibasaki *et al.*, 2009; Lynn *et al.*, 2012), with no effect on the thermosensitivity of either response (Takamata *et al.*, 1997; Lynn *et al.*, 2012). However, it is still unknown whether this impairment varies between males and females. Recent studies on fluid regulation have noted that there are sex-differences in the osmotically driven release of vasopressin, with females having reduced vasopressin concentrations than males for a given level of plasma osmolality (Stachenfeld *et al.*, 2001). This suggests that for a given absolute value of osmolality there is a different response between males and females. Therefore, it is plausible to suggest that sex-related differences in the osmoreceptor modulation of heat loss responses may also exist.

Thus, the purpose of this study is to examine if there are sex-related differences in the osmotically induced changes in both sweating and cutaneous vascular conductance (CVC) during whole-body heat stress. We hypothesized that 1) an increase in osmolality will result in a delayed onset threshold of both sweating and CVC in males and females; and 2) the overall delay in onset threshold will be higher in females than in males. This will be accomplished using whole-body passive heating to increase core temperature and promote increases in sweating and skin blood flow.

METHODS

Ethical approval

The present study 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 participating in the study.

Participants

A total of nine young males (25 ± 5 years) and nine young females (22 ± 4 years) were recruited for this study. Participants were healthy, non-smokers and had no cardiovascular, renal, respiratory, or metabolic conditions. None were taking any prescription or over-the-counter medication, with the exception of oral contraceptives in some female participants (n=4). Female participants completed the experimental sessions during the follicular phase of the menstrual cycle or the placebo/non-pill week of oral contraceptive use. Participant characteristics are presented in table 1.

[Please insert table 1 about here]

Experimental protocol

Participants were asked to complete one preliminary session and two experimental sessions for this study.

Preliminary session

Participants completed a Physical Activity Readiness Questionnaire (Par-Q) and an American Heart Association/American College of Sports Medicine Health/Fitness Facility Pre-participation Screening Questionnaire to assess their eligibility to participate in the study. In order to determine levels of physical activity, participants completed the Baecke Sport Index Questionnaire and the Kohl Physical Activity Questionnaire (Met-hrs/week).

Experimental sessions

The present study consisted of two experimental sessions. Female participants completed

the experimental trials during the first 8-10 days after the start of their self-reported menses (follicular/low hormone phase). Females taking oral contraceptives completed the trials during the placebo/no-pill week. Hormone (17β-estradiol and progesterone) status was confirmed on the day of each trial.

Participants reported to the laboratory between 8:00 a.m. and 11:00 a.m. on each experimental session. They were asked to refrain from salty food, caffeinated beverages, alcohol and exercise 24 hours prior to the test day. In addition, participants were asked to hydrate well before the trial. Hydration status was verified upon arrival to the laboratory by providing a urine sample and measuring urine specific gravity.

Upon arrival to the laboratory participants changed into shorts and a sports bra (females) and provided a urine sample after which a nude weight was obtained. Participants then donned a liquid-perfusion garment (Allen-Vanguard Corp., Ottawa, ON, Canada) which covered the entire body except for the head, hands, feet, and right forearm. They then rested in supine posture for a baseline period of 30 minutes to ensure the stabilization of fluid compartments in the body before obtaining a baseline blood sample. During the baseline rest, a venous catheter was inserted in the right arm and connected to a secondary line in order to conduct a 90-minute infusion of either isosmotic 0.9% NaCl (**ISO**) or hyperosmotic 3% NaCl (**HYP**) saline to maintain or increase blood osmolality, respectively. The saline solutions were infused at a rate of 0.2 and 0.125 $ml \cdot min^{-1} \cdot kg^{-1}$ of body weight for 0.9% and 3.0% NaCl, respectively (Takamata *et al.*, 1997; Lynn *et al.*, 2012). Throughout the infusion period, blood pressure and heart rate were measured continuously, while thirst sensation ratings were obtained every 10 minutes. Baseline cardiac output measurements were obtained in duplicate during the last 10 minutes of the infusion period. Furthermore, participants were perfused with water at 34°C to stabilize skin

temperature. In addition, two sweat capsules and two laser-Doppler probes along with the heater modules (maintained at 35°C) were placed in the right forearm. At the end of the infusion a second blood draw was collected to confirm osmotic state in each session.

Upon completion of the saline infusion, the water being perfused through the suit was heated to 48.5°C and participants were covered with a thick blanket to minimize any heat lost to the environment. Whole-body passive heating continued until oesophageal temperature (**T_{es}**) had increased by 1.5°C from baseline (i.e. end of infusion). Throughout the heating protocol, blood pressure and heart rate were measured continuously. Thirst sensation ratings were recorded every 0.25°C increase in oesophageal temperature. Cardiac output and blood samples were obtained every 0.5°C increase (i.e. at 0.5°C, 1.0°C, and 1.5°C) in T_{es}. Upon completing the heating protocol (i.e. 1.5°C above baseline T_{es}), a sweat gland activation assessment was performed followed by a maximal skin blood flow measurement consisting of a 20 minute local heating period at 42°C and a subsequent 25 minute period at 44°C. Before leaving the laboratory, participants provided a second nude weight and a urine sample.

Measurements

Oesophageal temperature was measured using a pediatric thermocouple probe of 2 mm in diameter (Mon-aTherm Nasopharyngeal Temperature Probe, Mallinckrodt Medical, St-Louis, MO, USA). The probe was inserted through the participant's nostril and down the esophagus to the length of the probe (~40 cm) with the end of the probe sitting at approximately heart level for most individuals of average height. The probe was inserted while participants swallowed water through a straw. Skin temperature was measured at 10 points using T-type (copper/constantan) thermocouples integrated into heat flow sensors

(Concept Engineering Old Saybrook, CT, USA). The thermocouples were placed in the forehead, bicep, forearm, chest, abdomen, upper back, lower back, quadriceps, hamstrings, and front calf. All of these areas were previously shaved and thoroughly cleaned with alcohol. Each thermocouple was secured with a double sided adhesive ring and surgical tape (Blenderm, 3M, St. Paul, MN, USA). Mean skin temperature was calculated using a 10 point weighting of the regional proportions determined by Hardy and DuBois (1938). Temperature data was collected with an HP Agilent data acquisition module (model 3497A) at a rate of one sample every 15 seconds and simultaneously recorded on a personal computer with LabVIEW software (LabVIEW 7.0, National Instruments, TX, USA).

Blood pressure was measured using a Finometer (Finapress Medical Systems, Amsterdam, Netherlands). Mean arterial pressure (**MAP**) was estimated from the beat-to-beat recording of the left middle finger arterial pressure waveform with the volume-clamp method (Penaz, 1973). Prior to measurement recording, the Finometer was calibrated using the physiocal criteria followed by brachial artery pressure reconstruction (Gizdulich *et al.*, 1996; Gizdulich *et al.*, 1997). Heart rate was recorded continuously using a Polar coded transmitter and advantage interface (Polar Electro Oy, Finland). Cardiac output ($L \cdot min^{-1}$) was measured non-invasively using an Innocor™ inert gas re-breathing unit with breath-by-breath ergospirometry and an arterial oxygen saturation sensor (Innovisions, Odense, Denmark). This system has been previously validated against the direct Fick method and thermodilution (Peyton & Thompson, 2004). The following mixture of gases was employed: 5% nitrous oxide and 1% sulphur hexafluoride diluted with ambient air (Ayotte *et al.*, 1970). The participant was asked to breathe through a 3-way valve connected to a breathing filter (Pro-Tec Filters, PF30S, 30 mm ports, Odense, Denmark), an anti-static

rubber bag, and a gas analyzer. Breathing frequency was fixed at 20 breaths·min^{-1} with the assistance of an auditory metronome. Before each measurement, the rubber bag was filled with the mixture of gas with a volume previously determined for each participant, which enabled the participant to fully empty the bag with each inhalation. Stroke volume was calculated as cardiac output divided by heart rate.

Local sudomotor activity was measured on the right forearm in duplicate using 3.8 cm^2 ventilated plastic capsules. The right forearm was shaved and cleaned with alcohol prior to placing and securing the sweat capsules with double-sided adhesive rings, topical skin glue (Collodion HV, Mavidon Medical products, Lake Worth, USA), and surgical tape. Each capsule was ventilated with anhydrous compressed air at a rate of 1 L·min^{-1}. Water content of the effluent air was measured using dew point mirrors (RH systems, Albuquerque, NM, USA). Local sweat rate was calculated using the difference in water content between effluent and influent air and multiplied by the flow rate, and normalized for the skin surface area under the capsule (expressed in mg·min^{-1}·cm^{-2}). The number of heat activated sweat glands in the forearm was measured in triplicate in an area adjacent to the sweat capsules using the modified iodine-paper technique (Gagnon *et al.*, 2012) at the end of the passive heating protocol (i.e. at 1.5°C above baseline T_{es}). Sweat gland output was calculated by dividing the corresponding sweat rate by the number of activated sweat glands.

Local skin blood flow was assessed in duplicate using laser-Doppler velocimetry (PeriFlux System 5000, Perimed AB, Stockholm, Sweden) at the right forearm. Each laser-Doppler flow probe was placed adjacent to the sweat capsules and secured with double sided adhesive rings in an area of the forearm that was not highly vascularised as determined by visual inspection. The laser-Doppler flow probes remained in the same place

for the duration of the experimental session to ensure reliable measurements. Cutaneous vascular conductance was calculated as skin blood flow perfusion units divided by MAP and expressed as a percentage of maximum.

Plasma osmolality, serum osmolality, and plasma volume changes were determined using venous blood samples. An indwelling venous catheter was inserted in the antecubital vein of the right arm connected to a Luer-Lock extension (Microbore Extension, Clave™, Locking Spin Collar, Non-DEHP) and secured in place with a 6 x 7 cm film dressing (Tegaderm Film, 3M Health Care, St. Paul, MN, USA). Venous blood (approximately 10mL) was collected without stasis into K2 EDTA™, Serum™, and Lithium-Heparin™ vacutainers (BD Vacutainer, Franklin lakes, NJ, USA) for hematology, serum and plasma osmolality analysis, respectively. Blood samples in the K2 EDTA™ vacutainer were immediately analyzed for hemoglobin (**Hb**) concentration and hematocrit (**Hct**) ration. Blood samples in the Lithium-Heparin™ vacutainer were immediately centrifuged for 10 minutes and the aliquot was transferred to a plastic collection vile. Blood samples in the Serum™ vacutainer were allowed to sit for 20 minutes in order for the blood to fully coagulate before being centrifuged for 10 minutes and the aliquot separated. Both plasma and serum aliquots were immediately analyzed upon separation to determine osmolality using the freezing-point method (Osmometer, Advance Instruments). An additional sample of blood was collected without stasis into a SST^{TM} vacutainer from female participants in order to confirm that each experimental session took place during the follicular phase of the menstrual cycle or placebo/non-pill week of oral contraceptives. Samples were sent to an external laboratory (Gamma-Dynacare Medical Laboratories, Ottawa, ON, Canada) for the analysis of 17β-estradiol and progesterone.

Nude weight was recorded before and after each session using a calibrated scale

(Mettler Toledo, Model KCC, USA). Urine specific gravity was determined in duplicate using a handheld refractometer (TS400, Reichter Inc., Depew, NY, USA) before and after each experimental session. Thirst sensation was assessed using the Visual Analogue Thirst Sensation Scale (Marks *et al.*, 1988) which has been shown to provide a high correlation between thirst and plasma osmolality during hypertonic infusions (Takamata *et al.*, 1995b; Stachenfeld *et al.*, 1996). Participants were presented with a scale of 175 mm in length where the lower end was labeled as "*not thirsty at all*", at the length of 125 mm a horizontal line was labeled as "*extremely thirsty*" and a final line at 175 mm marked the end of the scale. Participants were instructed to draw a line at any point on the scale as they deem appropriate. Thirst sensation was recorded as the distance (in cm) from the lower end to the mark drawn by the participant. For the purpose of eliminating bias in the assessment of thirst sensation, this study followed a single-blind design. Although all participants were informed about the type of saline infusions used in the study, the type of saline being used in each experimental session was not disclosed.

Data Analysis

Throughout the experimental protocol, local sweat rate and skin blood flow were measured in duplicate and the averages of both sites were used for statistical analysis. Mean body temperature was calculated using the following equation (Wingo *et al.*, 2010):

$$\text{Mean Body Temperature } (\bar{T}_b) = 0.8 \times T_{es} + 0.2 \times \bar{T}_{sk} \quad [1]$$

Where T_{es} is oesophageal temperature and $\bar{T}_{sk}$ is mean skin temperature. This equation was selected because it accounts for the influence of both internal and skin temperature on heat loss responses previously identified in the literature. Changes in thermoeffector response (sweating and CVC) for each participant was plotted against changes in mean body temperature and the

onset threshold was defined as the mean body temperature at which sweating and CVC rapidly increased. Similarly, thermosensitivity was defined as the slope of the linear portion of the thermoeffector response-mean body temperature relationship. Onset threshold and thermosensitivity of both sweat rate and CVC were calculated using GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA) software and verified by visual inspection for each condition (isosmotic and hyperosmotic). Changes in plasma volume from baseline resting were calculated from changes in hemoglobin and hematocrit using the following equation (Dill & Costill, 1974):

$$\Delta PV\ (\%) = 100 \times (Hb_B/Hb_A) \times \{[1-(Hct_A/100)] / [1-(Hct_B/100]\} -100 \qquad [2]$$

where ΔPV is the percent change in plasma volume, Hb_B is the hemoglobin concentration at baseline, Hb_A is the hemoglobin concentration at a specific time point (i.e. end infusion, 0.5°C, 1.0°C, 1.5°C), Hct_A is the hematocrit at a specific time point (i.e. end infusion, 0.5°C, 1.0°C, 1.5°C), and Hct_B is the hematocrit at baseline. Finally, thirst sensation ratings were calculated from the Visual Analogue Scale by subtracting the distance from the start of the scale to the line drawn by the participant from the total length of the scale (175mm), this was divided by the total length of the scale and then multiplied by 100 to express it as a percentage.

Statistical Analysis

A two-by-two mixed ANOVA with the repeating factor of condition (levels: ISO and HYP) and the non-repeating factor of sex (levels: males and females) was used to determine the effect of condition and sex on the onset threshold and thermosensitivity of both sweating and CVC. A three-way mixed ANOVA with the repeating factors of condition (levels: ISO and HYP), time (levels: pre, end-infusion, at 0.5°C, at 1.0°C and at 1.5°C), and the non-repeating factor of sex (level: males and females) was used to analyze the following variables: osmolality,

plasma volume, MAP, cardiac output, heart rate, stroke volume and thirst sensation. Holm-Bonferroni post-hoc analysis of pre-planned comparisons was carried out whenever an ANOVA was rejected. The level of significance was set to an alpha level of $p \leq 0.05$. All statistical analysis was performed using SPSS 20.0 software package for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Male participants were taller ($p=0.010$) and heavier ($p<0.001$) than their female counterparts. There were no statistically significant differences in training status between males and females as estimated by the Baecke Sport Index Questionnaire ($p=0.821$) or Kohl Physical Activity Questionnaire Kohls (Met-hrs/week) ($p=0.891$) (Table 1). Female participants completed both experimental sessions during the follicular phase/no-pill week of oral contraceptives. 17β–estradiol levels during the ISO and HYP were 143 ± 74 and 156 ± 92 pmol/L ($p=0.604$), respectively. Similarly, progesterone levels for ISO and HYP conditions were 1.7 ± 0.5 and 1.8 ± 0.4 nmol/L ($p=0.393$), respectively. Both 17β–estradiol and progesterone levels were within the accepted reference range of 46-607 pmol/L and 0.6-4.7 nmol/L, respectively.

Hydration Status

No differences were observed in hydration status as determined by nude weight measurements between conditions in either males (ISO: 76.33 ± 6.56 kg; HYP: 76.52 ± 6.46 kg) or females (ISO: 60.19 ± 7.89 kg; HYP: 60.17 ± 8.53 kg) ($p=0.747$). Furthermore, urine specific gravity measurements were also similar between conditions ($p=0.203$) in both males (ISO: 1.013 ± 0.006; HYP: 1.017 ± 0.006) and females (ISO: 1.017 ± 0.004; HYP: 1.017 ± 0.006), as well as

between sexes (p=0.419).

Blood osmolality and changes in plasma volume

Baseline plasma osmolality measured before the start of saline infusion in both males and females were ISO: 290 ± 3 vs 287 ± 3 mosmol·kg of solvent^{-1} and HYP: 288 ± 2 vs 288 ± 2 mosmol·kg of solvent^{-1}. A main effect of condition (ISO vs HYP) in changes of plasma osmolality was observed ($p<0.001$), while no main effect of sex was measured ($p=0.388$). Infusion of hypertonic saline resulted in an increase in plasma osmolality from baseline levels (males: 299 ± 4 and females: 300 ± 4 mosmol·kg of solvent^{-1}) (all $p<0.001$), while infusion of isotonic saline resulted in a similar plasma osmolality at the end of infusion compared to baseline resting values (males: 291 ± 4 and females: 289 ± 4 mosmol·kg of solvent^{-1}) (all $p>0.06$) (Figure 1a).

Similarly, baseline serum osmolality for males and females were ISO: 289 ± 2 vs 287 ± 4 mosmol·kg of solvent^{-1} and HYP: 289 ± 3 vs 289 ± 3 mosmol·kg of solvent^{-1}. Similar to plasma, serum osmolality increased from baseline after infusion of hypertonic saline (males: 299 ± 3 and females: 300 ± 4 mosmol·kg of solvent^{-1}) (all $p<0.001$), while infusion of isotonic saline did not change osmolality levels (males: 290 ± 3 and females: 288 ± 3 mosmol·kg of solvent^{-1}) (all $p>0.07$). There was a main effect of condition (ISO vs HYP) in changes of serum osmolality ($p<0.001$), but no differences were observed between sexes ($p=0.277$).

[*Please insert Figure 1 about here*]

Changes in plasma volume at the end of infusion for males and females were ISO: +5.2 ± 3.4 % vs +5.2 ± 4.0 % and HYP: +7.6 ± 3.1 % vs +8.1 ± 3.3 %. These changes in plasma volume

were not different between males and females (p=0.668), however, there was a main effect of condition (i.e. ISO vs HYP) within groups ($p \leq 0.001$). Plasma volume decreased from end infusion levels with passive heat stress ($p \leq 0.001$), although the decrease was similar between sexes (p=0.153) and across conditions (p=0.394).

Thirst sensation

Infusion of hypertonic saline resulted in a significantly higher thirst sensation in comparison to isotonic saline ($p<0.001$). This increased thirst sensation during HYP was similar between males and females (p=0.392). Figure 1b shows the changes in thirst sensation over time.

Thermal responses

Heating time. The total time required to increase T_{es} by 1.5°C from baseline for males and females were (ISO: 60 ± 11 vs 56 ± 14 min; and HYP: 55 ± 8 vs 51 ± 9 min). Heating time was significantly lower during the hypertonic condition compared to isotonic (p=0.001), however, no differences between males and females were observed (p=0.433).

Skin and core temperatures. Table 2 summarizes baseline and total change in oesophageal temperature, mean skin temperature and mean body temperature for both males and females during the ISO and HYP conditions. There were no differences in baseline oesophageal temperature between conditions (p=0.951), however, there was a main effect of sex (p=0.009). Baseline mean skin temperature did not differ between conditions (p=0.099) or sexes (p=0.699). Furthermore, baseline mean body temperature was not different between conditions (p=0.607), but it was statistically different between males and females (p=0.026). Whole-body passive

heating resulted in a similar increase between males and females in oesophageal (p=0.505), mean skin (p=0.326) and mean body temperatures (p=0.933). Moreover, no differences were observed in the total change in oesophageal (p=0.543), mean skin (p=0.869) and mean body temperatures (p=0.745) between conditions (i.e. ISO and HYP).

[*Please insert table 2 about here*]

Sweating. Table 3 outlines the mean body temperature absolute onset threshold, delta change in onset threshold and thermosensitivity of local sudomotor response in both males and females. The absolute mean body temperature at which the onset of forearm local sweat rate occurred was higher in HYP compared to ISO (p<0.001). Furthermore, a main effect of sex was observed in the absolute onset of sweating (p=0.004), with females exhibiting a higher absolute onset. The change in mean body temperature from baseline to the onset of sweating was significantly higher during HYP (p<0.001), however, there were no differences in the relative change in mean body temperature between males and females (p=0.522) (Table 3). Finally, the thermosensitivity of sweating as represented by the slope of the sweating-mean body temperature relationship was similar between conditions (ISO vs. HYP) (p=0.186). However, the thermosensitivity was consistently lower in females than in males (p=0.018). The number of heat activated sweat glands per cm^2 during the isotonic condition did not differ between sexes (p=0.644) or condition (p=0.678) (Table 4). Contrary to these results, total sweat gland output was consistently lower in females than males (p=0.003) irrespective of condition (Table 4). The sweating response of two representative participants during ISO and HYP conditions is shown in Figure 2.

[*Please insert Table 3, Table 4 and Figure 2 about here*]

Cutaneous vascular conductance (CVC). Table 3 shows the mean body temperature absolute onset threshold, delta change in onset threshold and thermosensitivity of CVC in both males and females. Absolute mean body temperature onset threshold of CVC was significantly higher during HYP when compared to ISO ($p=0.001$). In addition, a main effect of sex was observed in the absolute threshold of CVC ($p=0.003$) with females having a higher absolute threshold. The change in mean body temperature from baseline to the onset of CVC was significantly higher during HYP ($p<0.001$), however, no differences were observed between males and females ($p=0.275$). Finally, no differences the thermosensitivity of CVC were observed between conditions (ISO vs. HYP, $p=0.621$) or sexes (males vs. females, $p=0.601$). The CVC response of two representative participants during ISO and HYP conditions is shown in Figure 3.

[*Please insert Figure 3 about here*]

Cardiovascular responses

Table 5 outlines baseline values of cardiac output, heart rate, stroke volume and MAP for both males and females during ISO and HYP conditions. Baseline heart rate and stroke volume did not differ across conditions ($p=0.107$ and $p=0.132$) or sexes ($p=0.173$ and $p=0.059$). Cardiac output at baseline was consistently lower in females during both ISO ($p=0.036$) and HYP ($p=0.027$) conditions. However, no main effect of condition was observed ($p=0.735$). During whole-body heating, heart rate and cardiac output significantly increased (all $p<0.001$), while stroke volume significantly decreased ($p<0.001$). No main effect of condition was observed for the changes in any of the responses with increases in oesophageal temperature (all $p>0.1$).

Changes in heart rate, cardiac output and stroke volume as a function of increases in oesophageal temperature are shown in Figure 4. No differences in MAP were observed between males and females ($p=0.630$); however, MAP significantly decreased ($p<0.001$) during passive heating, and to the same extent in both ISO and HYP conditions ($p=0.085$).

[*Please insert Table 5 and Figure 4 about here*]

DISCUSSION

The purpose of the present study was to examine if there are sex-related differences in the osmotically induced changes in both sweating and CVC. The main finding is that the overall delay in onset threshold of sweating and CVC was similar between males and females. We hypothesized that an increase in plasma osmolality would result in a delayed onset threshold of sweating and CVC in both sexes. Congruent with this hypothesis, our results showed a delayed onset threshold in both thermoefferent responses in males and females. However, our results did not support our second hypothesis which stated that the overall delay in onset threshold would be greater in females than males. On the contrary, there were no differences in the overall change in mean body temperature required to elicit an increase in sweating and CVC between sexes.

Sweating

The present study showed a higher onset threshold of sweating during the hyperosmotic condition in both males and females. This delay in onset threshold of local sweating after inducing a hyperosmotic state supports previous findings by others (Fortney *et al.*, 1984; Takamata *et al.*, 1997; Lynn *et al.*, 2012) and further expands on these studies by showing, for the first time, the effects of hyperosmolality in young females. Furthermore, our results showed

that while hyperosmolality resulted in a delayed onset threshold of sweating, there were no differences in the overall delay between males and females. To the best of our knowledge no studies to date have conducted a direct comparison between sexes on the effects of hyperosmolality on the local sudomotor response. Nonetheless, similar to our results in the isosmotic condition, studies examining sex-related differences in heat loss responses have reported no differences in the onset threshold of sweating between males and females during passive heat stress (Inoue *et al.*, 2005; Gagnon *et al.*, 2013). The present study also showed that sweating thermosensitivity was consistently lower in females irrespective of osmotic state (isosmotic vs. hypertonic). While a lower sweating thermosensitivity in females has been previously reported during passive heat stress (Gagnon *et al.*, 2013) and exercise (Gagnon & Kenny, 2012b), our results further expand on previous observations on females by showing that the reduced sweating thermosensitivity is also evident in a hyperosmotic state. More importantly, our findings suggest that the extent to which sweating thermosensitivity is reduced in females is not affected by hyperosmolality, as the slope of the response did not differ between osmotic conditions. Until recently, the idea that hyperosmolality does not affect sweating thermosensitivity had only been shown in males (Takamata *et al.*, 1997; Lynn *et al.*, 2012), however, our results demonstrate that previous observations in males also extend to females.

Measurements of heat activated sweat glands in the forearm at the end of heating showed no differences between males and females. Therefore, the reduced sweating response observed in females is mainly driven by lower sweat gland output. In keeping with our results, previous studies have reported no differences in heat activated glands in the forearm while having lower sweat gland outputs in females during both passive heating (Inoue *et al.*, 2005; Gagnon *et al.*, 2013) and exercise (Gagnon & Kenny, 2012b). However, no studies to date examining the

effects of hyperosmolality on heat loss responses have assessed sweat gland activation in either males or females. Therefore, our study is the first to show that after controlling for osmotic state, there were no sex-related differences in sweat gland activation and sweat gland output between conditions (isotonic vs. hypertonic). This suggests that hyperosmolality does not affect either one of these variables. These observations expand the current literature on the effects of hyperosmolality on sudomotor response in males, and provide novel information on the female response. It should be noted that higher sweat gland activation in females has been reported in the chest and upper back during exercise (Gagnon & Kenny, 2012b), which suggests regional variations in sweat gland activation. This regional variation cannot be assessed from the present study given that local sweating and sweat gland activation was only measured in the forearm. Future research should evaluate the potential influence of hyperosmolality on regional variation in sudomotor response in both males and females.

Cutaneous Vascular Conductance

The present study showed that hyperosmolality results in a delayed onset threshold of CVC, which supports findings by previous studies (Fortney *et al.*, 1984; Takamata *et al.*, 1997; Shibasaki *et al.*, 2009; Lynn *et al.*, 2012). Our study also showed that the onset threshold of CVC is not different between males and females in either condition (iosmotic vs. hyperosmotic). Even though no previous studies have compared the effects of hyperosmolality on the onset threshold of CVC between males and females, some recent studies examining sex-related differences in CVC during passive heating reported no differences in onset thresholds between sexes (Inoue *et al.*, 2005; Gagnon *et al.*, 2013). While this matches our findings during the isosmotic condition, the present study is the first to report such changes during a hyperosmotic state. These

observations suggest that the overall delay in the onset threshold induced by hyperosmolality occurs to the same extent in both males and females. Furthermore, our results showed no differences in the thermosensitivity of CVC between males and females, which is in keeping with recent studies using passive heat stress (Inoue *et al.*, 2005; Gagnon *et al.*, 2013). Finally, the thermosensitivity of CVC did not differ with osmotic conditions, which is similar to previous results on males during passive heating (Takamata *et al.*, 1997; Lynn *et al.*, 2012). However, this is the first study to show such results in females.

Limitations

The present study was designed to examine osmoreceptor modulation only; as such, baroreceptor activity was not assessed. This is an important limitation given that baroreceptor unloading alone can result in a delayed onset threshold of CVC (Crandall *et al.*, 1996; Cui *et al.*, 2004; Lynn *et al.*, 2012) and when combined with hyperosmolality it can have an additive effect (Lynn *et al.*, 2012). Nonetheless, studies examining the effects of baroreceptor unloading on CVC, using consecutive increments of lower body negative pressure, have shown that the onset threshold of CVC was only affected at higher levels of lower body negative pressure, usually greater than -21 mmHg (Crandall *et al.*, 1996; Cui *et al.*, 2004). Taking this into consideration, along with the fact that the present study was conducted in the supine posture and no differences in MAP were observed between groups and conditions, it is unlikely that baroreceptor unloading may have confounded our results. However, if baroreceptor unloading played a role in our results, it would have been to the same extent in both sexes given that no differences in MAP were observed between groups. Furthermore, it should be noted that while there is still significant disagreement on the effects of baroreceptor unloading on sudomotor response

(Shibasaki *et al.*, 2006), a recent study by Lynn et al. (2012) demonstrated that unloading the baroreceptors had no effect in local sweating during passive heating in an isosmotic and hyperosmotic condition. Therefore, we are confident that our sweating responses are not attributed to baroreceptor loading status. Given that our experimental design does not allow us to detect the influence, if any, of baroreceptors further research should examine sex-related differences and the combined effect of baroreceptors and osmoreceptors in local heat loss responses.

Female participants were asked to complete both experimental sessions during the follicular/low hormone phase of the menstrual cycle, therefore, our results cannot be extended to other phases. Further research is needed to evaluate the effects of different phases of the menstrual cycle on the hyperosmotic inhibition of thermoeffector responses (sweating and CVC). Finally, our sample consisted of young males and females, therefore, our results are limited to this specific population, and cannot be extrapolated to older adults or populations with chronic conditions such as diabetes or obesity. The sex-related differences in osmoreceptors modulation of local heat loss responses on these vulnerable populations remain to be elucidated.

Perspective

Our results demonstrate that 1) there are no sex-related differences in the onset threshold of sweating, 2) the overall delay in the onset threshold of sweating was similar between sexes, and 3) females exhibited consistently lower sweating thermosensitivity than males irrespective of plasma osmolality. Taken together, these findings suggest that previously reported differences in sudomotor activity between males and females are not modulated by changes in blood osmolality via osmoreceptors. To the best of our knowledge, the study by Gagnon and Kenny (2012) has

been the only to report plasma osmolality when examining sex-related differences in thermoeffector activity (sweating and skin blood flow) during incremental exercise. However, plasma osmolality was only measured in a subgroup of participants during a separate experimental session and heat loss responses were not reported in this sub-group. Therefore, the relationship between plasma osmolality and thermoefferent responses could not be established. Nonetheless, the lack of differences in plasma osmolality in this sub-group of males and females led the authors to speculate that osmoreceptors were most likely not involved in the reduced sudomotor response observed in females. In fact, the present study provides, for the first time, direct evidence that reduced sweat rate in females cannot be attributed to hyperosmolality, and therefore, these sex-differences in sudomotor response are likely mediated by an alternative mechanism. As previously suggested by Inoue et al. (2005) these differences may therefore be at the end-organ level (i.e. sweat gland) as either differences in the morphology or cholinergic sensitivity of the sweat gland. However, the recent study by Gagnon et al. (2013) discarded the latter possibility by showing no differences in the response to cholinergic agonists between males and females. As concluded by Gagnon et al. (2013) sex-related differences in sweating may therefore be in the morphology of the gland (i.e. size of the sweat gland) and future research should examine this possibility.

Irrespective of osmotic state, females exhibit reduced sweating thermosensitivity and lower sweat gland output. Given that sweating (evaporative heat loss) is the main mechanism to dissipate heat from the body and that females have an impaired ability to dissipate heat (Gagnon & Kenny, 2011, 2012b; Gagnon *et al.*, 2013), they are at an increased risk of suffering from heat stress related illnesses. Although the present study demonstrates that young females have a similar response to hyperosmolality as young males, it should be noted that there are age-related

changes that could render older adults more susceptible to heat stress. More specifically, older adults have reduced stimulation of thirst sensation and sensitivity to thirst (Kenney & Chiu, 2001), therefore, this could lead to dehydration and increased osmolality. Further research is warranted on the effects of hyperosmolality in local heat loss responses in both older males and females.

Significance

The present study provides novel information on the effects of hyperosmolality on thermoefferent responses in females. This work showed that while the onset threshold of both sweating and CVC increased in the hyperosmotic condition, this delay was similar between males and females. Furthermore, females also exhibited a reduced sweating thermosensitivity and sweat gland output irrespective of condition. Although the lack of differences between males and females on the delay in onset thresholds may point towards a similar response to a given level of osmolality, the net effect on the ability to dissipate heat may be very different. It is well accepted that changes to the onset threshold or the thermosensitivity of thermoeffector responses can profoundly affect heat dissipation, and therefore, heat storage. For instance, a delay in onset threshold and reduction in thermosensitivity will compromise the ability to achieve heat balance, resulting in greater heat storage (Gagnon & Kenny, 2012a). This is particularly true for females since, as shown by the present study, they display a delay in onset threshold compounded by an already reduced sweating thermosensitivity. As a result, during a state of dehydration (high plasma osmolality) females are at a higher risk for heat-related illness because, compared to men, they will store more heat before achieving heat balance. Therefore, maintenance of proper hydration is of major importance in the female population given that they already have an

impaired sudomotor response.

CONCLUSION

In summary, the present study examined if there are sex-related differences in the osmotically induced changes in both sweating and CVC. Our results suggest that the osmotically induced delay in the onset threshold of sweating and CVC is similar between males and females. Whereas the thermosensitivity of CVC was similar between sexes, the thermosensitivity of sweating was consistently lower in females compared to males. The lower thermosensitivity in sudomotor response of females was accompanied by a lower sweat gland output, albeit similar sweat gland activation to males. As a result, the sex-related differences in thermoeffector activity are independent of osmoreceptor activation. We conclude that osmoreceptors do not exhibit a sex-related dependent modulation of sweating and CVC responses during heat stress, and therefore, the lower sudomotor activity of females cannot be attributed to osmoreceptor modulation.

REFERENCES

Ayotte B, Seymour J & McIlroy MB. (1970). A new method for measurement of cardiac output with nitrous oxide. *J Appl Physiol* **28,** 863-866.

Christou DD, Jones PP, Jordan J, Diedrich A, Robertson D & Seals DR. (2005). Women have lower tonic autonomic support of arterial blood pressure and less effective baroreflex buffering than men. *Circulation* **111,** 494-498.

Convertino VA. (1998). Gender differences in autonomic functions associated with blood pressure regulation. *Am J Physiol* **275,** R1909-1920.

Crandall CG, Johnson JM, Kosiba WA & Kellogg DL, Jr. (1996). Baroreceptor control of the cutaneous active vasodilator system. *J Appl Physiol* **81,** 2192-2198.

Cui J, Wilson TE & Crandall CG. (2004). Orthostatic challenge does not alter skin sympathetic nerve activity in heat-stressed humans. *Auton Neurosci* **116,** 54-61.

Dill DB & Costill DL. (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol* **37,** 247-248.

Fortney SM, Wenger CB, Bove JR & Nadel ER. (1984). Effect of hyperosmolality on control of blood flow and sweating. *J Appl Physiol* **57,** 1688-1695.

Gagnon D, Crandall CG & Kenny GP. (2013). Sex differences in postsynaptic sweating and cutaneous vasodilation. *J Appl Physiol* **114,** 394-401.

Gagnon D, Ganio MS, Lucas RA, Pearson J, Crandall CG & Kenny GP. (2012). Modified iodine-paper technique for the standardized determination of sweat gland activation. *J Appl Physiol* **112,** 1419-1425.

Gagnon D & Kenny GP. (2011). Sex modulates whole-body sudomotor thermosensitivity during exercise. *J Physiol* **589,** 6205-6217.

Gagnon D & Kenny GP. (2012a). Does sex have an independent effect on thermoeffector responses during exercise in the heat? *J Physiol* **590,** 5963-5973.

Gagnon D & Kenny GP. (2012b). Sex differences in thermoeffector responses during exercise at fixed requirements for heat loss. *J Appl Physiol* **113,** 746-757.

Gizdulich P, Imholz BP, van den Meiracker AH, Parati G & Wesseling KH. (1996). Finapres tracking of systolic pressure and baroreflex sensitivity improved by waveform filtering. *J Hypertens* **14,** 243-250.

Gizdulich P, Prentza A & Wesseling KH. (1997). Models of brachial to finger pulse wave distortion and pressure decrement. *Cardiovasc Res* **33,** 698-705.

Hardy J & DuBois E. (1938). The technique of measuring radiation and convection. *J Nutr* **15,** 461-475.

Inoue Y, Tanaka Y, Omori K, Kuwahara T, Ogura Y & Ueda H. (2005). Sex- and menstrual cycle-related differences in sweating and cutaneous blood flow in response to passive heat exposure. *Eur J Appl Physiol* **94,** 323-332.

Ives SJ, McDaniel J, Witman MA & Richardson RS. (2013). Passive limb movement: evidence of mechanoreflex sex specificity. *Am J Physiol Heart Circ Physiol* **304,** H154-161.

Jarvis SS, VanGundy TB, Galbreath MM, Shibata S, Okazaki K, Reelick MF, Levine BD & Fu Q. (2011). Sex differences in the modulation of vasomotor sympathetic outflow during static handgrip exercise in healthy young humans. *Am J Physiol Regul Integr Comp Physiol* **301,** R193-200.

Kenney WL & Chiu P. (2001). Influence of age on thirst and fluid intake. *Med Sci Sports Exerc* **33,** 1524-1532.

Kenny GP, Jay O & Journeay WS. (2007). Disturbance of thermal homeostasis following dynamic exercise. *Appl Physiol Nutr Metab* **32,** 818-831.

Kenny GP & Journeay WS. (2010). Human thermoregulation: separating thermal and nonthermal effects on heat loss. *Front Biosci* **15,** 259-290.

Lynn AG, Gagnon D, Binder K, Boushel RC & Kenny GP. (2012). Divergent roles of plasma osmolality and the baroreflex on sweating and skin blood flow. *Am J Physiol Regul Integr Comp Physiol* **302,** R634-642.

Marks LE, Stevens JC, Bartoshuk LM, Gent JF, Rifkin B & Stone VK. (1988). Magnitude-matching: the measurement of taste and smell. *Chem Senses* **13,** 63-87.

Penaz J. (1973). photoelectric measurement of blood pressure, volume and flow inthe finger. *Digest 10th int Conf Med Biol Engng* **104**.

Peyton PJ & Thompson B. (2004). Agreement of an inert gas rebreathing device with thermodilution and the direct oxygen Fick method in measurement of pulmonary blood flow. *J Clin Monit Comput* **18,** 373-378.

Sawka MN, Montain SJ & Latzka WA. (2001). Hydration effects on thermoregulation and performance in the heat. *Comp Biochem Physiol A Mol Integr Physiol* **128,** 679-690.

Shibasaki M, Aoki K, Morimoto K, Johnson JM & Takamata A. (2009). Plasma hyperosmolality elevates the internal temperature threshold for active thermoregulatory vasodilation during heat stress in humans. *Am J Physiol Regul Integr Comp Physiol* **297,** R1706-1712.

Shibasaki M, Kondo N & Crandall CG. (2003). Non-thermoregulatory modulation of sweating in humans. *Exerc Sport Sci Rev* **31,** 34-39.

Shibasaki M, Wilson TE & Crandall CG. (2006). Neural control and mechanisms of eccrine sweating during heat stress and exercise. *J Appl Physiol* **100,** 1692-1701.

Shoemaker JK, Hogeman CS, Khan M, Kimmerly DS & Sinoway LI. (2001). Gender affects sympathetic and hemodynamic response to postural stress. *Am J Physiol Heart Circ Physiol* **281,** H2028-2035.

Stachenfeld NS, Mack GW, Takamata A, DiPietro L & Nadel ER. (1996). Thirst and fluid regulatory responses to hypertonicity in older adults. *Am J Physiol* **271,** R757-765.

Stachenfeld NS, Splenser AE, Calzone WL, Taylor MP & Keefe DL. (2001). Sex differences in osmotic regulation of AVP and renal sodium handling. *J Appl Physiol* **91,** 1893-1901.

Takamata A, Mack GW, Gillen CM, Jozsi AC & Nadel ER. (1995a). Osmoregulatory modulation of thermal sweating in humans: reflex effects of drinking. *Am J Physiol* **268,** R414-422.

Takamata A, Mack GW, Stachenfeld NS & Nadel ER. (1995b). Body temperature modification of osmotically induced vasopressin secretion and thirst in humans. *Am J Physiol* **269,** R874-880.

Takamata A, Nagashima K, Nose H & Morimoto T. (1997). Osmoregulatory inhibition of thermally induced cutaneous vasodilation in passively heated humans. *Am J Physiol* **273,** R197-204.

Wingo JE, Low DA, Keller DM, Brothers RM, Shibasaki M & Crandall CG. (2010). Skin blood flow and local temperature independently modify sweat rate during passive heat stress in humans. *J Appl Physiol* **109,** 1301-1306.

COMPETING INTERESTS

The authors have no conflict of interest to disclose.

AUTHOR CONTRIBUTIONS

J.B-R and G.P.K. contributed to the conception and design of the experiments. J.B-R., R.M., M.R.C., H.F-L. and G.P.K. all contributed to the collection, analysis and interpretation of data, as well as, to the preparation of the manuscript. All authors approved the final version of the manuscript. All experiments took place at the University of Ottawa.

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FIGURE LEGENDS

Figure 1. Plasma osmolality (panel A) and thirst sensation ratings (panel B) for males (□) and females (○) during isosmotic (ISO) and hyperosmotic (HYP) conditions throughout the experimental protocol (i.e. baseline, end of infusion and throughout passive heating). Values represent mean ± standard error. (*) Denotes Male-HYP is significantly different from Male-ISO and (†) denotes Female-HYP is significantly different from Female-ISO ($p \leq 0.05$).

Figure 2. Forearm sweating response as a function of increases in mean body temperature during whole-body passive heating in isotonic (open symbols) and hypertonic (closed symbols) conditions for two representative subjects (panel A: male; panel B: female). Arrows indicate the onset threshold for sweating.

Figure 3. Forearm cutaneous vascular conductance (CVC) response as a function of increases in mean body temperature during whole-body passive heating in isotonic (open symbols) and hypertonic (closed symbols) conditions for two representative subjects (panel A: male; panel B: female). Arrows indicate the onset threshold for CVC.

Figure 4. Mean cardiac output (panel A), heart rate (panel B), stroke volume (panel C), and mean arterial pressure (MAP) (Panel D) for both males (□) and females (○) during isosmotic (ISO) and hyperosmotic (HYP) conditions at baseline and throughout whole body passive heating. Values represent mean ± standard error. (‡) Denotes Male-ISO is significantly different from Female-ISO and (§) denotes Male-HYP is significantly different from Female-HYP ($p \leq 0.05$).

Table 1. Participant characteristics.

Sex	Age (yrs)	Body Mass (kg)	Height (cm)	Baecke Index	Kohl (Met-hr/wk)
Males	25 ± 5	76.16 ± 6.51*	175 ± 8*	2.99 ± 0.56	25.71 ± 10.0
Females	22 ± 4	60.44 ± 8.38	166 ± 5	2.92 ± 0.76	24.87 ± 15.2

*Significantly different from females ($p \leq 0.05$). Values represent mean ± standard deviation.

Table 2. Baseline and total change in oesophageal temperature (°C), mean skin temperature (°C), and mean body temperature (°C) for both males and females during the isosmotic and hyperosmotic conditions.

Condition	Sex	T_{es}		$\overline{T}_{sk}$		$\overline{T}_{b}$	
		Baseline	ΔT_{es}	Baseline	$\Delta \overline{T}_{sk}$	Baseline	$\Delta \overline{T}_{b}$
ISO	Male	36.68 ± 0.19*	1.62 ± 0.13	33.80 ± 0.42	3.92 ±0.50	36.10 ± 0.17*	2.08 ± 0.17
	Female	36.93 ± 0.38	1.56 ± 0.21	33.85 ± 0.57	4.13 ± 0.65	36.31 ± 0.38	2.08 ± 0.15
HYP	Male	36.63 ± 0.19*	1.64 ± 0.12	33.94 ± 0.51	3.85 ± 0.60	36.09 ± 0.19*	2.09 ± 0.20
	Female	36.97 ± 0.26	1.59 ± 0.26	34.07 ± 0.59	4.14 ± 0.76	36.39 ± 0.26	2.10 ± 0.21

T_{es}, oesophageal temperature, ΔT_{es}, change in oesophageal temperature, $\overline{T}_{sk}$, mean skin temperature, $\Delta \overline{T}_{sk}$, change in mean skin temperature, $\overline{T}_{b}$, mean body temperature, $\Delta \overline{T}_{b}$, change in mean body temperature, ISO, isotonic condition, HYP, hypertonic condition. *Significantly different from females ($p \leq 0.05$). Values represent mean ± standard deviation.

Table 3. Mean body temperature onset threshold and thermosensitivity of sweating and CVC in both males and females during isosmotic and hyperosmotic conditions.

Condition	Sex	Sweating			CVC		
		Threshold	$\Delta\overline{T}_b$	Slope	Threshold	$\Delta\overline{T}_b$	Slope
		°C	°C	$mg \cdot min^{-1} \cdot cm^{-2}/°C$	°C	°C	$\%CVC_{max}/°C$
ISO	Male	37.12 ± 0.24*†	1.02 ± 0.30†	1.53 ± 0.70*	36.89 ± 0.32*†	0.78 ± 0.27†	83.26 ± 33.07
	Female	37.39 ± 0.26†	1.08 ± 0.24†	0.92 ± 0.26	37.25 ± 0.28†	0.93 ± 0.23†	97.13 ± 29.78
HYP	Male	37.38 ± 0.30*	1.30 ± 0.33	1.31 ± 0.57*	37.12 ± 0.29*	1.05 ± 0.29	86.64 ± 25.44
	Female	37.80 ± 0.18	1.41 ± 0.26	0.83 ± 0.34	37.58 ± 0.20	1.19 ± 0.34	85.21 ± 29.70

CVC, cutaneous vascular conductance, $\Delta\overline{T}_b$, increase in mean body temperature, ISO, isosmotic condition, HYP, hyperosmotic condition. *Significantly different from females and † significantly different from HYP ($p \leq 0.05$). Values represent mean ± standard deviation.

Table 4. Total number of heat activated sweat glands and sweat gland output per gland in both males and females during isosmotic and hyperosmotic conditions.

Condition	Sex	HASG (number per cm^2)	SGO (μg/gland)
ISO	Male	95 ± 32	10.97 ± 4.23*
	Female	107 ± 28	6.11 ± 2.08
HYP	Male	100 ± 30	9.04 ± 2.88*
	Female	99 ± 18	5.42 ± 1.29

HASG, Heat activated sweat glands, SGO, Sweat gland output, ISO, isosmotic condition, HYP, hyperosmotic condition.

*Significantly different from females ($p \leq 0.05$). Values represent mean ± SD.

Table 5. Baseline values for cardiac output, heart rate, stroke volume and MAP during isosmotic and hyperosmotic conditions.

Condition	Sex	$\dot{Q}$ (L/min)	HR (bpm)	SV (mL)	MAP (mmHg)
ISO	Male	7.2 ± 0.8*	60 ± 8	120.8 ± 11.2	85 ± 3
	Female	6.3 ± 0.9	68 ± 14	97.7 ± 32.1	85 ± 6
HYP	Male	7.1 ± 0.7*	62 ± 9	116.0 ± 10.0	85 ± 3
	Female	6.3 ± 0.9	69 ± 14	95.3 ± 29.2	86 ± 6

$\dot{Q}$, cardiac ouput, HR, heart rate, SV, stroke volume, MAP, mean arterial pressure, ISO, isosmotic condition, HYP, hyperosmotic condition. *Significantly different from females and † significantly different from HYP ($p \leq 0.05$). Values represent mean ± standard deviation.

FIGURES

Figure 1.

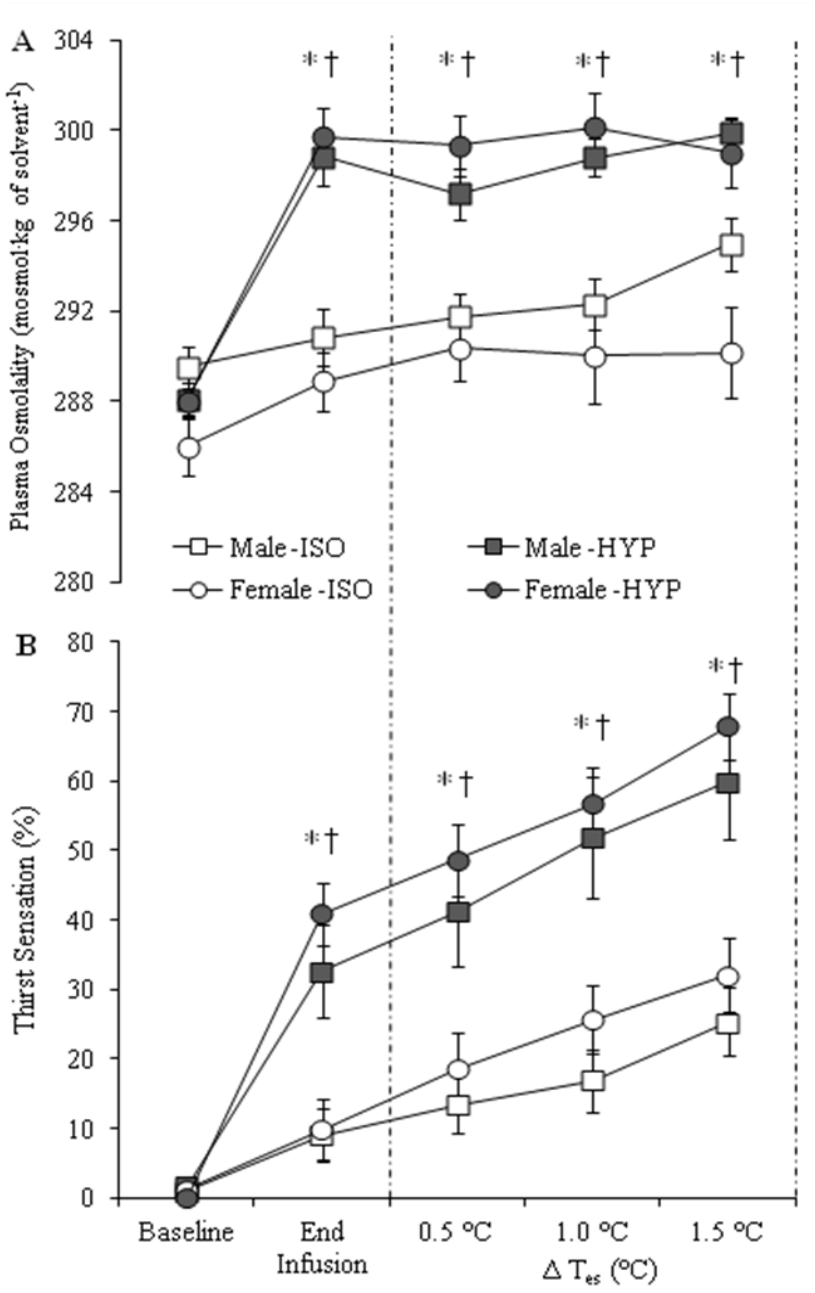


Figure 2.

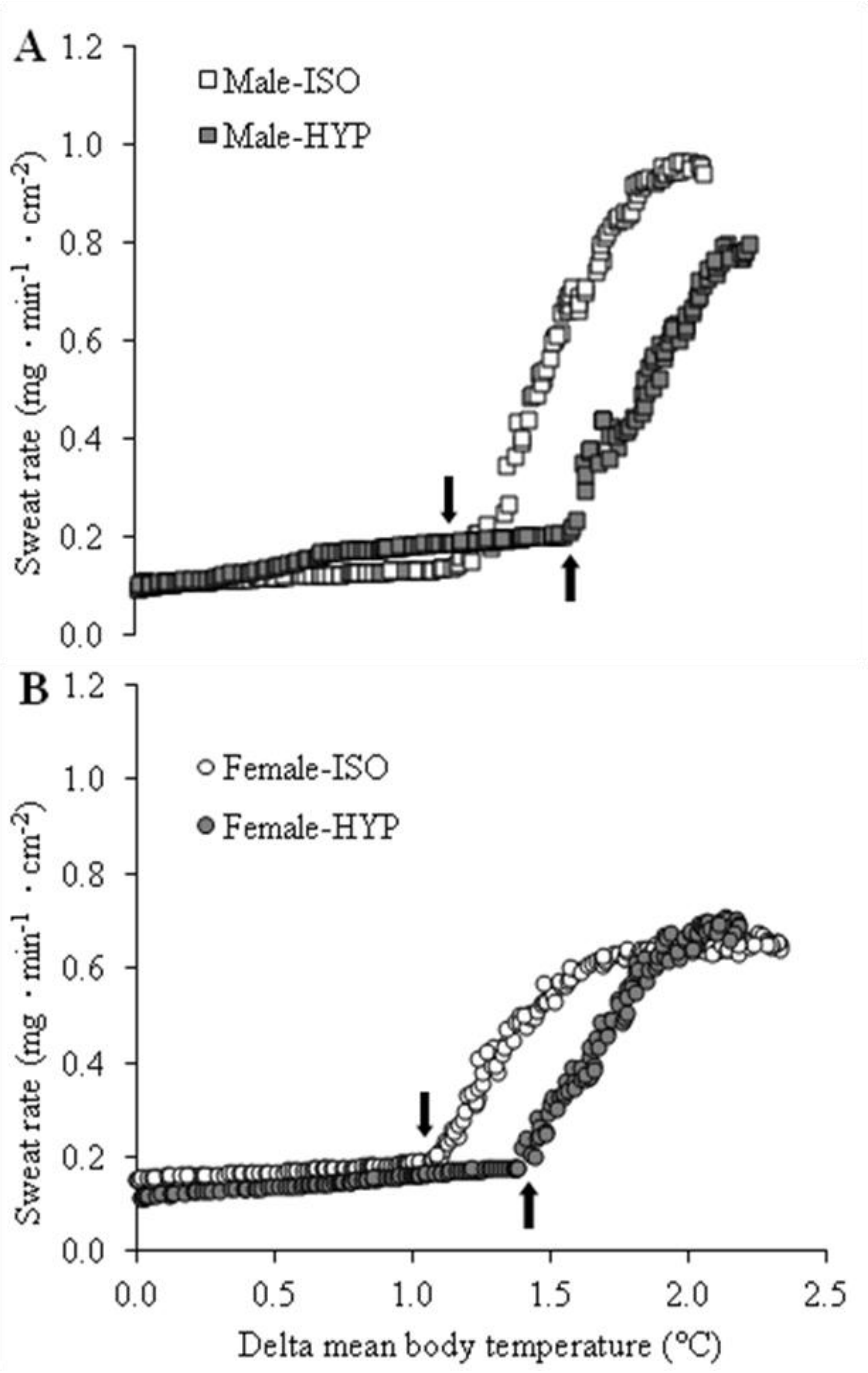

A
Male-ISO
Male-HYP
Sweat rate (mg · min-1 · cm-2)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
B
Female-ISO
Female-HYP
Sweat rate (mg · min-1 · cm-2)
0.0
0.5
1.0
1.5
2.0
2.5
Delta mean body temperature (°C)

Figure 3.

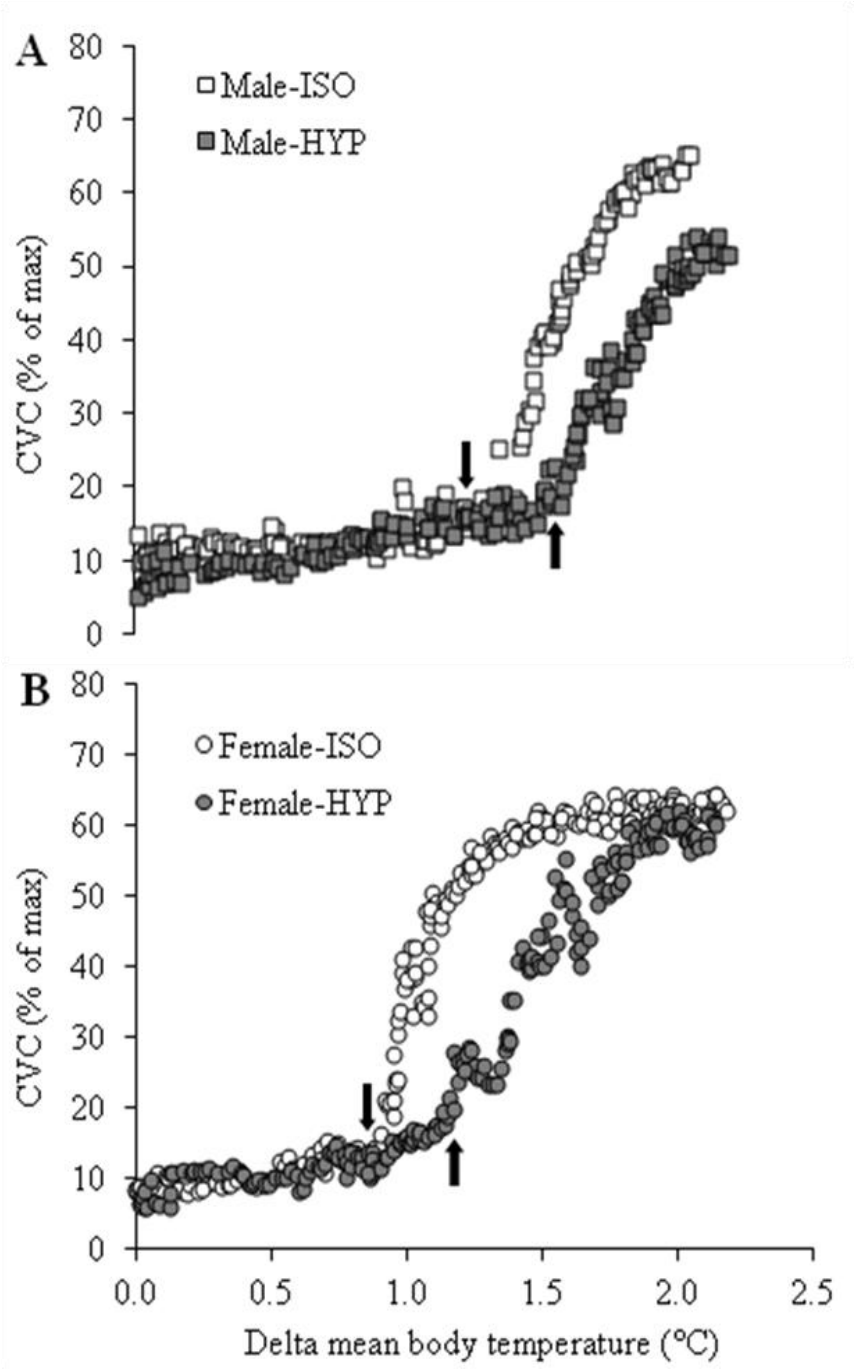
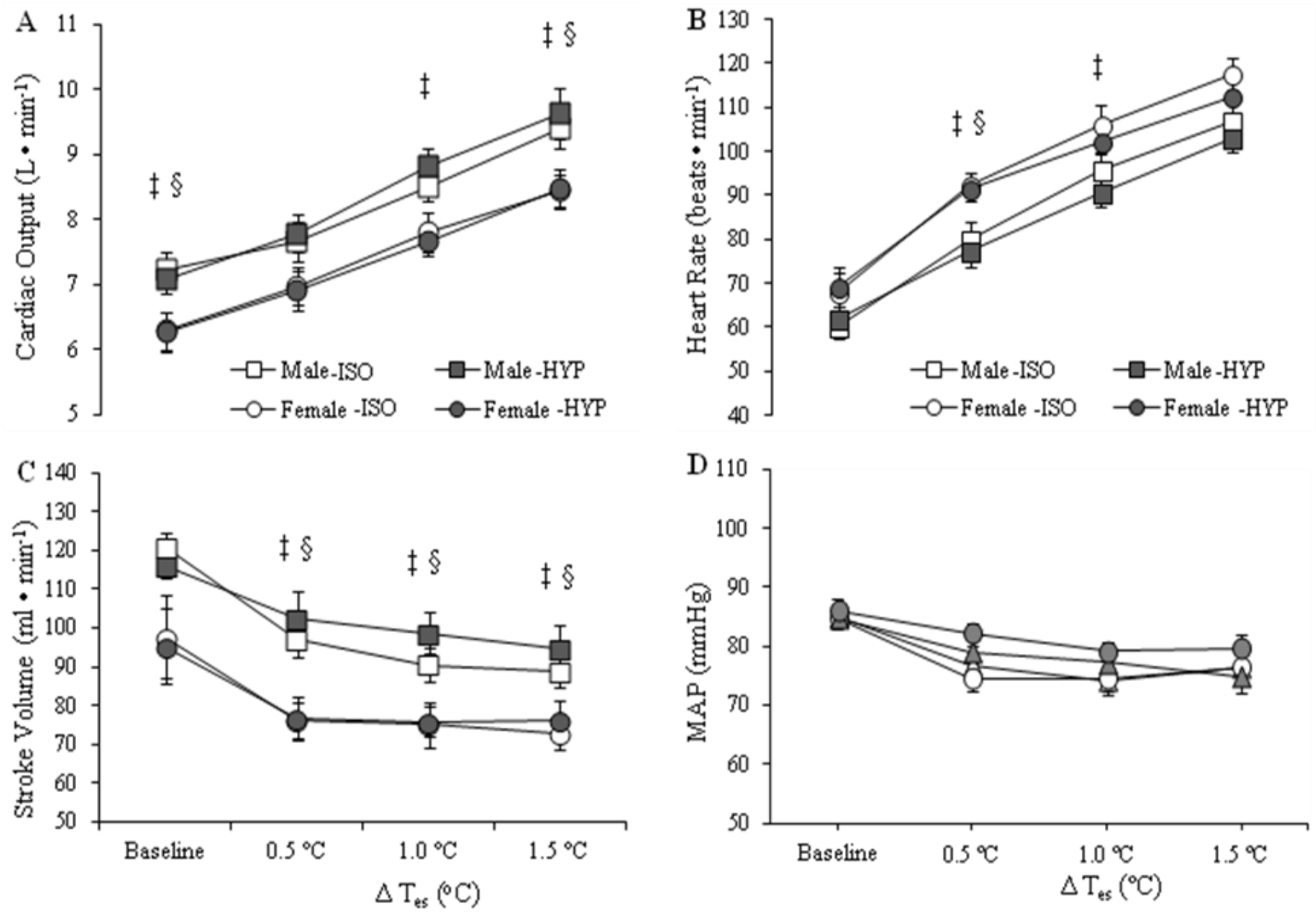

A
Male-ISO
Male-HYP
CVC (% of max)
0
10
20
30
40
50
60
70
80
B
Female-ISO
Female-HYP
CVC (% of max)
0
10
20
30
40
50
60
70
80
0.0
0.5
1.0
1.5
2.0
2.5
Delta mean body temperature (°C)

Figure 4.


A
Cardiac Output (L • min-1)
11
10
9
8
7
6
5
‡ §
‡
‡ §
Male -ISO
Male -HYP
Female -ISO
Female -HYP
B
Heart Rate (beats • min-1)
130
120
110
100
90
80
70
60
50
40
‡ §
‡
Male -ISO
Male -HYP
Female -ISO
Female -HYP
C
Stroke Volume (ml • min-1)
140
130
120
110
100
90
80
70
60
50
‡ §
‡ §
‡ §
Baseline
0.5 ℃
1.0 ℃
1.5 ℃
Δ Tes (°C)
D
MAP (mmHg)
110
100
90
80
70
60
50
Baseline
0.5 ℃
1.0 ℃
1.5 ℃
Δ Tes (℃)

PART THREE:

GENERAL CONCLUSION OF THE THESIS

PART THREE:

GENERAL CONCLUSION OF THE THESIS

The present thesis aimed to determine if there are sex-related differences in the osmotic attenuation of sweating and cutaneous vascular conductance. This was accomplished by evaluating the influence of central osmoreceptors on the mean body temperature onset threshold and thermosensitivity of both sweating and cutaneous vascular conductance in a sample of young males and females. In order to do this, blood osmolality was maintained or increased from baseline levels using saline infusions of isosmotic and hyperosmotic NaCl, respectively, while at the same time inducing passive heat stress.

The main finding from this thesis shows that the osmotically induced delay in onset threshold of sweating and cutaneous vascular conductance is similar between males and females. Furthermore, we show that females had consistently lower sweating thermosensitivity and sweat gland output. Additionally, no sex-related differences were observed in the cutaneous vascular conductance response.

It can be concluded from this thesis that the sex-related differences in sudomotor response are not modulated by osmolality via osmoreceptors. Thus, our results further support the idea that central modulation, via osmoreceptors, does not seem to influence local thermoeffector responses. Future research should focus on peripheral factors that may account for the reduced sudomotor response in females, such is the case of potential differences in sweat gland morphology.

PART FOUR:

REFERENCES

REFERENCES

Allan, J. R., & Wilson, C. G. (1971). Influence of acclimatization on sweat sodium concentration. *J Appl Physiol, 30*(5), 708-712.

Aoki, K., Stephens, D. P., & Johnson, J. M. (2001). Diurnal variation in cutaneous vasodilator and vasoconstrictor systems during heat stress. *Am J Physiol Regul Integr Comp Physiol, 281*(2), R591-595.

Ayotte, B., Seymour, J., & McIlroy, M. B. (1970). A new method for measurement of cardiac output with nitrous oxide. *J Appl Physiol, 28*(6), 863-866.

Bar-Or, O. (1998). Effects of age and gender on sweating pattern during exercise. *Int J Sports Med, 19 Suppl 2*, S106-107.

Bennett, L. A., Johnson, J. M., Stephens, D. P., Saad, A. R., & Kellogg, D. L., Jr. (2003). Evidence for a role for vasoactive intestinal peptide in active vasodilatation in the cutaneous vasculature of humans. *J Physiol, 552*(Pt 1), 223-232.

Bittel, J., & Henane, R. (1975). Comparison of thermal exchanges in men and women under neutral and hot conditions. *J Physiol, 250*(3), 475-489.

Bourque, C. W., Oliet, S. H., & Richard, D. (1994). Osmoreceptors, osmoreception, and osmoregulation. *Front Neuroendocrinol, 15*(3), 231-274.

Brengelmann, G. L., & Savage, M. V. (1997). Temperature regulation in the neutral zone. *Ann N Y Acad Sci, 813*, 39-50.

Buono, M. J., & Sjoholm, N. T. (1988). Effect of physical training on peripheral sweat production. *J Appl Physiol, 65*(2), 811-814.

Burse, R. L. (1979). Sex differences in human thermoregulatory response to heat and cold stress. *Hum Factors, 21*(6), 687-699.

Calzone, W. L., Silva, C., Keefe, D. L., & Stachenfeld, N. S. (2001). Progesterone does not alter osmotic regulation of avp. *Am J Physiol Regul Integr Comp Physiol, 281*(6), R2011-2020.

Carter, R., 3rd, Watenpaugh, D. E., & Smith, M. L. (2001). Gender differences in cardiovascular regulation during recovery from exercise. *J Appl Physiol, 91*(4), 1902-1907.

Charkoudian, N. (2003). Skin blood flow in adult human thermoregulation: How it works, when it does not, and why. *Mayo Clin Proc, 78*(5), 603-612.

Charkoudian, N. (2010). Mechanisms and modifiers of reflex induced cutaneous vasodilation and vasoconstriction in humans. *J Appl Physiol, 109*(4), 1221-1228.

Charkoudian, N., & Johnson, J. M. (1997). Modification of active cutaneous vasodilation by oral contraceptive hormones. *J Appl Physiol, 83*(6), 2012-2018.

Christou, D. D., Jones, P. P., Jordan, J., Diedrich, A., Robertson, D., & Seals, D. R. (2005). Women have lower tonic autonomic support of arterial blood pressure and less effective baroreflex buffering than men. *Circulation, 111*(4), 494-498.

Convertino, V. A. (1998). Gender differences in autonomic functions associated with blood pressure regulation. *Am J Physiol, 275*(6 Pt 2), R1909-1920.

Costill, D. L., Cote, R., & Fink, W. (1976). Muscle water and electrolytes following varied levels of dehydration in man. *J Appl Physiol, 40*(1), 6-11.

Crandall, C. G., Johnson, J. M., Kosiba, W. A., & Kellogg, D. L., Jr. (1996). Baroreceptor control of the cutaneous active vasodilator system. *J Appl Physiol, 81*(5), 2192-2198.

Crandall, C. G., Wilson, T. E., Marving, J., Vogelsang, T. W., Kjaer, A., Hesse, B., et al. (2008). Effects of passive heating on central blood volume and ventricular dimensions in humans. *J Physiol, 586*(1), 293-301.

Crofton, J. T., Dustan, H., Share, L., & Brooks, D. P. (1986). Vasopressin secretion in normotensive black and white men and women on normal and low sodium diets. *J Endocrinol, 108*(2), 191-199.

Cui, J., Wilson, T. E., & Crandall, C. G. (2004). Orthostatic challenge does not alter skin sympathetic nerve activity in heat-stressed humans. *Auton Neurosci, 116*(1-2), 54-61.

Dill, D. B., & Costill, D. L. (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol, 37*(2), 247-248.

Dodt, C., Gunnarsson, T., Elam, M., Karlsson, T., & Wallin, B. G. (1995). Central blood volume influences sympathetic sudomotor nerve traffic in warm humans. *Acta Physiol Scand, 155*(1), 41-51.

Fortney, S. M., Nadel, E. R., Wenger, C. B., & Bove, J. R. (1981). Effect of blood volume on sweating rate and body fluids in exercising humans. *J Appl Physiol, 51*(6), 1594-1600.

Fortney, S. M., Wenger, C. B., Bove, J. R., & Nadel, E. R. (1984). Effect of hyperosmolality on control of blood flow and sweating. *J Appl Physiol, 57*(6), 1688-1695.

Fox, R. H., Lofstedt, B. E., Woodward, P. M., Eriksson, E., & Werkstrom, B. (1969). Comparison of thermoregulatory function in men and women. *J Appl Physiol, 26*(4), 444-453.

Gagnon, D., Crandall, C. G., & Kenny, G. P. (2013). Sex differences in postsynaptic sweating and cutaneous vasodilation. *J Appl Physiol, 114*(3), 394-401.

Gagnon, D., Ganio, M. S., Lucas, R. A., Pearson, J., Crandall, C. G., & Kenny, G. P. (2012). Modified iodine-paper technique for the standardized determination of sweat gland activation. *J Appl Physiol, 112*(8), 1419-1425.

Gagnon, D., & Kenny, G. P. (2011). Sex modulates whole-body sudomotor thermosensitivity during exercise. *J Physiol, 589*(Pt 24), 6205-6217.

Gagnon, D., & Kenny, G. P. (2012). Sex differences in thermoeffector responses during exercise at fixed requirements for heat loss. *J Appl Physiol, 113*(5), 746-757.

Gibson, T. E., & Shelley, W. B. (1948). Sexual and racial differences in the response of sweat glands to acetylcholine and pilocarpine. *J Invest Dermatol, 11*(2), 137-142.

Gisolfi, C., & Robinson, S. (1970). Central and peripheral stimuli regulating sweating during intermittent work in men. *J Appl Physiol, 29*(6), 761-768.

Gisolfi, C. V., & Wenger, C. B. (1984). Temperature regulation during exercise: Old concepts, new ideas. *Exerc Sport Sci Rev, 12*, 339-372.

Gonzalez-Alonso, J., Mora-Rodriguez, R., Below, P. R., & Coyle, E. F. (1995). Dehydration reduces cardiac output and increases systemic and cutaneous vascular resistance during exercise. *J Appl Physiol, 79*(5), 1487-1496.

Gonzalez-Alonso, J., Mora-Rodriguez, R., Below, P. R., & Coyle, E. F. (1997). Dehydration markedly impairs cardiovascular function in hyperthermic endurance athletes during exercise. *J Appl Physiol, 82*(4), 1229-1236.

Gotshall, R. W. (2000). Gender differences in tolerance to lower body negative pressure. *Aviat Space Environ Med, 71*(11), 1104-1110.

Grant, R., & Holling, H. (1938). Further observations on the vascular responses of the human limb to body warming: Evidence for sympathetic vasodilator nerves in the normal subject. *Clin Sci, 3*, 273-285.

Greenleaf, J. E., & Castle, B. L. (1971). Exercise temperature regulation in man during hypohydration and hyperhydration. *J Appl Physiol, 30*(6), 847-853.

Grucza, R., Pekkarinen, H., Titov, E. K., Kononoff, A., & Hanninen, O. (1993). Influence of the menstrual cycle and oral contraceptives on thermoregulatory responses to exercise in young women. *Eur J Appl Physiol Occup Physiol, 67*(3), 279-285.

Hammel, H. T. (1968). Regulation of internal body temperature. *Annu Rev Physiol, 30*, 641-710.

Hancock, M. L., 2nd, Bichet, D. G., Eckert, G. J., Bankir, L., Wagner, M. A., & Pratt, J. H. (2010). Race, sex, and the regulation of urine osmolality: Observations made during water deprivation. *Am J Physiol Regul Integr Comp Physiol, 299*(3), R977-980.

Hardy, J., & DuBois, E. (1938). The technique of measuring radiation and convection. *J Nutr, 15*, 461-475.

Hardy, J. D. (1961). Physiology of temperature regulation. *Physiol Rev, 41*, 521-606.

Hensel, H. (1981). *Thermoreception and temperature regulation*. London: Academic Press.

Inoue, Y., Tanaka, Y., Omori, K., Kuwahara, T., Ogura, Y., & Ueda, H. (2005). Sex- and menstrual cycle-

related differences in sweating and cutaneous blood flow in response to passive heat exposure. *Eur J Appl Physiol, 94*(3), 323-332.

Ito, T., Itoh, T., Hayano, T., Yamauchi, K., & Takamata, A. (2005). Plasma hyperosmolality augments peripheral vascular response to baroreceptor unloading during heat stress. *Am J Physiol Regul Integr Comp Physiol, 289*(2), R432-R440.

Johnson, J., & Proppe, D. (1996). *Cardiovascular adjustments to heat stress.* New York: Oxford University Press.

Kaciuba-Uscilko, H., & Grucza, R. (2001). Gender differences in thermoregulation. *Curr Opin Clin Nutr Metab Care, 4*(6), 533-536.

Kellogg, D. L., Jr., Pergola, P. E., Piest, K. L., Kosiba, W. A., Crandall, C. G., Grossmann, M., et al. (1995). Cutaneous active vasodilation in humans is mediated by cholinergic nerve cotransmission. *Circ Res, 77*(6), 1222-1228.

Kenney, W. L. (1985). A review of comparative responses of men and women to heat stress. *Environ Res, 37*(1), 1-11.

Kenney, W. L., & Chiu, P. (2001). Influence of age on thirst and fluid intake. *Med Sci Sports Exerc, 33*(9), 1524-1532.

Kenny, G. P., Gagnon, D., Shiff, D., Armstrong, R., Journeay, W. S., & Kilby, D. (2010). Influence of

nonthermal baroreceptor modulation of heat loss responses during uncompensable heat stress. *Eur J Appl Physiol, 108*(3), 541-548.

Kenny, G. P., & Journeay, W. S. (2010). Human thermoregulation: Separating thermal and nonthermal effects on heat loss. *Front Biosci, 15*, 259-290.

Kenny, G. P., Leclair, E., Sigal, R. J., Journeay, W. S., Kilby, D., Nettlefold, L., et al. (2008). Menstrual cycle and oral contraceptive use do not modify postexercise heat loss responses. *J Appl Physiol, 105*(4), 1156-1165.

Kenny, G. P., Murrin, J. E., Journeay, W. S., & Reardon, F. D. (2006). Differences in the postexercise threshold for cutaneous active vasodilation between men and women. *Am J Physiol Regul Integr Comp Physiol, 290*(1), R172-179.

Kolka, M. A., & Stephenson, L. A. (1997). Effect of luteal phase elevation in core temperature on forearm blood flow during exercise. *J Appl Physiol, 82*(4), 1079-1083.

Kolka, M. A., Stephenson, L. A., Rock, P. B., & Gonzalez, R. R. (1987). Local sweating and cutaneous blood flow during exercise in hypobaric environments. *J Appl Physiol, 62*(6), 2224-2229.

Lewis, T., & Pickering, G. (1931). Vasodilation in the limbs in response to warming the body with evidence for sympathetic vasodilator nerves in man. *Heart, 16*, 33-51.

Lynn, A. G., Gagnon, D., Binder, K., Boushel, R. C., & Kenny, G. P. (2012). Divergent roles of plasma

osmolality and the baroreflex on sweating and skin blood flow. *Am J Physiol Regul Integr Comp Physiol, 302*(5), R634-642.

Mack, G. W. (2004). Hypothalamic control of body temperature: Insights from the past. *J Appl Physiol, 97*(5), 1593-1594.

Mack, G. W., Cordero, D., & Peters, J. (2001). Baroreceptor modulation of active cutaneous vasodilation during dynamic exercise in humans. *J Appl Physiol, 90*(4), 1464-1473.

Mack, G. W., Nishiyasu, T., & Shi, X. (1995). Baroreceptor modulation of cutaneous vasodilator and sudomotor responses to thermal stress in humans. *J Physiol, 483 (Pt 2)*, 537-547.

Madeira, L. G., da Fonseca, M. A., Fonseca, I. A., de Oliveira, K. P., Passos, R. L., Machado-Moreira, C. A., et al. (2010). Sex-related differences in sweat gland cholinergic sensitivity exist irrespective of differences in aerobic capacity. *Eur J Appl Physiol, 109*(1), 93-100.

Marks, L. E., Stevens, J. C., Bartoshuk, L. M., Gent, J. F., Rifkin, B., & Stone, V. K. (1988). Magnitude-matching: The measurement of taste and smell. *Chem Senses, 13*(1), 63-87.

McCook, R. D., Wurster, R. D., & Randall, W. C. (1965). Sudomotor and vasomotor responses to changing environmental temperature. *J Appl Physiol, 20*(3), 371-378.

McCord, G. R., Cracowski, J. L., & Minson, C. T. (2006). Prostanoids contribute to cutaneous active vasodilation in humans. *Am J Physiol Regul Integr Comp Physiol, 291*(3), R596-602.

Mekjavic, I. B., & Eiken, O. (2006). Contribution of thermal and nonthermal factors to the regulation of body temperature in humans. *J Appl Physiol, 100*(6), 2065-2072.

Montain, S. J., & Coyle, E. F. (1992). Influence of graded dehydration on hyperthermia and cardiovascular drift during exercise. *J Appl Physiol, 73*(4), 1340-1350.

Morimoto, T., Slabochova, Z., Naman, R. K., & Sargent, F., 2nd. (1967). Sex differences in physiological reactions to thermal stress. *J Appl Physiol, 22*(3), 526-532.

Nadel, E. R., Bullard, R. W., & Stolwijk, J. A. (1971a). Importance of skin temperature in the regulation of sweating. *J Appl Physiol, 31*(1), 80-87.

Nadel, E. R., Fortney, S. M., & Wenger, C. B. (1980). Effect of hydration state of circulatory and thermal regulations. *J Appl Physiol, 49*(4), 715-721.

Nadel, E. R., Mitchell, J. W., Saltin, B., & Stolwijk, J. A. (1971b). Peripheral modifications to the central drive for sweating. *J Appl Physiol, 31*(6), 828-833.

Nadel, E. R., Pandolf, K. B., Roberts, M. F., & Stolwijk, J. A. (1974). Mechanisms of thermal acclimation to exercise and heat. *J Appl Physiol, 37*(4), 515-520.

Nakayama, T., Eisenman, J. S., & Hardy, J. D. (1961). Single unit activity of anterior hypothalamus during local heating. *Science, 134*(3478), 560-561.

Nose, H., Mack, G. W., Shi, X. R., & Nadel, E. R. (1988). Shift in body fluid compartments after dehydration in humans. *J Appl Physiol, 65*(1), 318-324.

Nose, H., Morimoto, T., & Ogura, K. (1983). Distribution of water losses among fluid compartments of tissues under thermal dehydration in the rat. *Jpn J Physiol, 33*(6), 1019-1029.

Nunneley, S. A. (1978). Physiological responses of women to thermal stress: A review. *Med Sci Sports, 10*(4), 250-255.

Parsons, K. (2003). *Human thermal environments*. london: Taylor & Francis.

Penaz, J. (1973). Photoelectric measurement of blood pressure, volume and flow inthe finger. *Digest 10th int Conf Med Biol Engng, 104*.

Peyton, P. J., & Thompson, B. (2004). Agreement of an inert gas rebreathing device with thermodilution and the direct oxygen fick method in measurement of pulmonary blood flow. *J Clin Monit Comput, 18*(5-6), 373-378.

Randall, W. C., & Kimura, K. K. (1955). The pharmacology of sweating. *Pharmacol Rev, 7*(3), 365-397.

Rowell, L. (1983). *Cardiovascular adjustments to thermal stress.* (Vol. III). Bethesda: Am. Physiol. Soc.

Sato, K. (1973). Sweat induction from an isolated eccrine sweat gland. *Am J Physiol, 225*(5), 1147-1152.

Sato, K., & Dobson, R. L. (1970). Regional and individual variations in the function of the human eccrine sweat gland. *J Invest Dermatol, 54*(6), 443-449.

Sato, K., Kang, W. H., Saga, K., & Sato, K. T. (1989). Biology of sweat glands and their disorders. I. Normal sweat gland function. *J Am Acad Dermatol, 20*(4), 537-563.

Sato, K., & Sato, F. (1983). Individual variations in structure and function of human eccrine sweat gland. *Am J Physiol, 245*(2), R203-208.

Sawka, M. N. (1992). Physiological consequences of hypohydration: Exercise performance and thermoregulation. *Med Sci Sports Exerc, 24*(6), 657-670.

Sawka, M. N., Montain, S. J., & Latzka, W. A. (2001). Hydration effects on thermoregulation and performance in the heat. *Comp Biochem Physiol A Mol Integr Physiol, 128*(4), 679-690.

Sawka, M. N., Young, A. J., Francesconi, R. P., Muza, S. R., & Pandolf, K. B. (1985). Thermoregulatory and blood responses during exercise at graded hypohydration levels. *J Appl Physiol, 59*(5), 1394-1401.

Share, L., Crofton, J. T., & Ouchi, Y. (1988). Vasopressin: Sexual dimorphism in secretion, cardiovascular actions and hypertension. *Am J Med Sci, 295*(4), 314-319.

Shastry, S., Dietz, N. M., Halliwill, J. R., Reed, A. S., & Joyner, M. J. (1998). Effects of nitric oxide synthase

inhibition on cutaneous vasodilation during body heating in humans. *J Appl Physiol, 85*(3), 830-834.

Shibasaki, M., Aoki, K., Morimoto, K., Johnson, J. M., & Takamata, A. (2009). Plasma hyperosmolality elevates the internal temperature threshold for active thermoregulatory vasodilation during heat stress in humans. *Am J Physiol Regul Integr Comp Physiol, 297*(6), R1706-1712.

Shibasaki, M., & Crandall, C. G. (2001). Effect of local acetylcholinesterase inhibition on sweat rate in humans. *J Appl Physiol, 90*(3), 757-762.

Shibasaki, M., Kondo, N., & Crandall, C. G. (2003). Non-thermoregulatory modulation of sweating in humans. *Exerc Sport Sci Rev, 31*(1), 34-39.

Shibasaki, M., Wilson, T. E., & Crandall, C. G. (2006). Neural control and mechanisms of eccrine sweating during heat stress and exercise. *J Appl Physiol, 100*(5), 1692-1701.

Shoemaker, J. K., Hogeman, C. S., Khan, M., Kimmerly, D. S., & Sinoway, L. I. (2001). Gender affects sympathetic and hemodynamic response to postural stress. *Am J Physiol Heart Circ Physiol, 281*(5), H2028-2035.

Solack, S. D., Brengelmann, G. L., & Freund, P. R. (1985). Sweat rate vs. Forearm blood flow during lower body negative pressure. *J Appl Physiol, 58*(5), 1546-1552.

Sproles, C. B., Smith, D. P., Byrd, R. J., & Allen, E. (1976). Circulatory responses to submaximal exercise

after dehydration and rehydration. *J Sports Med Phys Fitness, 16*(2), 98-105.

Stachenfeld, N. S., Mack, G. W., Takamata, A., DiPietro, L., & Nadel, E. R. (1996). Thirst and fluid regulatory responses to hypertonicity in older adults. *Am J Physiol, 271*(3 Pt 2), R757-765.

Stachenfeld, N. S., Splenser, A. E., Calzone, W. L., Taylor, M. P., & Keefe, D. L. (2001). Sex differences in osmotic regulation of avp and renal sodium handling. *J Appl Physiol, 91*(4), 1893-1901.

Stephenson, L. A., & Kolka, M. A. (1985). Menstrual cycle phase and time of day alter reference signal controlling arm blood flow and sweating. *Am J Physiol, 249*(2 Pt 2), R186-191.

Stephenson, L. A., & Kolka, M. A. (1993). Thermoregulation in women. *Exerc Sport Sci Rev, 21*, 231-262.

Stocker, S. D., Osborn, J. L., & Carmichael, S. P. (2008). Forebrain osmotic regulation of the sympathetic nervous system. *Clin Exp Pharmacol Physiol, 35*(5-6), 695-700.

Takamata, A., Mack, G. W., Gillen, C. M., Jozsi, A. C., & Nadel, E. R. (1995a). Osmoregulatory modulation of thermal sweating in humans: Reflex effects of drinking. *Am J Physiol, 268*(2 Pt 2), R414-422.

Takamata, A., Mack, G. W., Stachenfeld, N. S., & Nadel, E. R. (1995b). Body temperature modification of osmotically induced vasopressin secretion and thirst in humans. *Am J Physiol, 269*(4 Pt 2), R874-880.

Takamata, A., Nagashima, K., Nose, H., & Morimoto, T. (1997). Osmoregulatory inhibition of thermally induced cutaneous vasodilation in passively heated humans. *Am J Physiol, 273*(1 Pt 2), R197-204.

Taylor, N. A., & Machado-Moreira, C. A. (2013). Regional variations in transepidermal water loss, eccrine sweat gland density, sweat secretion rates and electrolyte composition in resting and exercising humans. *Extrem Physiol Med, 2*(1), 4.

Thompson, C. J., Burd, J. M., & Baylis, P. H. (1987). Acute suppression of plasma vasopressin and thirst after drinking in hypernatremic humans. *Am J Physiol, 252*(6 Pt 2), R1138-1142.

Uno, H. (1977). Sympathetic innervation of the sweat glands and piloarrector muscles of macaques and human beings. *J Invest Dermatol, 69*(1), 112-120.

Van Wynsberghe, D., Noback, C. R., & Carola, R. (1995). *Human anatomy & physiology*. New York: McGraw-Hill.

Vissing, S. F., Scherrer, U., & Victor, R. G. (1994). Increase of sympathetic discharge to skeletal muscle but not to skin during mild lower body negative pressure in humans. *J Physiol, 481 (Pt 1)*, 233-241.

Wenner, M. M., & Stachenfeld, N. S. (2012). Blood pressure and water regulation: Understanding sex hormone effects within and between men and women. *J Physiol, 590*(Pt 23), 5949-5961.

Werner, J. (1981). Control aspects of human temperature regulation. *Automatica, 17*, 351-362.

Wilson, T. E., Brothers, R. M., Tollund, C., Dawson, E. A., Nissen, P., Yoshiga, C. C., et al. (2009). Effect of thermal stress on frank-starling relations in humans. *J Physiol, 587*(Pt 13), 3383-3392.

Wilson, T. E., Cui, J., & Crandall, C. G. (2001). Absence of arterial baroreflex modulation of skin sympathetic activity and sweat rate during whole-body heating in humans. *J Physiol, 536*(Pt 2), 615-623.

Wilson, T. E., Tollund, C., Yoshiga, C. C., Dawson, E. A., Nissen, P., Secher, N. H., et al. (2007). Effects of heat and cold stress on central vascular pressure relationships during orthostasis in humans. *J Physiol, 585*(Pt 1), 279-285.

Wong, B. J., & Minson, C. T. (2006). Neurokinin-1 receptor desensitization attenuates cutaneous active vasodilatation in humans. *J Physiol, 577*(Pt 3), 1043-1051.

Wong, B. J., Wilkins, B. W., & Minson, C. T. (2004). H1 but not h2 histamine receptor activation contributes to the rise in skin blood flow during whole body heating in humans. *J Physiol, 560*(Pt 3), 941-948.

PART FIVE:

APPENDIX

5.1 Health Science and Sciences REB Ethics Approval Certificate

File Number: H03-10-03 **Date (mm/dd/yyyy):** 07/11/2012

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
Matthew	Coutsos	Health Sciences / Human Kinetics	Co-investigator
Daniel	Gagnon	Health Sciences / Human Kinetics	Co-investigator
Jill	Stapleton	Health Sciences / Human Kinetics	Co-investigator
Heather	Wright	Health Sciences / Human Kinetics	Co-investigator
Juliana	Barrera Ramirez	Health Sciences / Human Kinetics	Student Researcher

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
07/22/2012	07/21/2013	Ia

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

Special Conditions / Comments:
N/A

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http://www.research.uottawa.ca/ethics/index.html
http://www.recherche.uottawa.ca/deontologie/index.html

File Number: H03-10-03 **Date (mm/dd/yyyy)**: 07/11/2012

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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5.2 Supplemental Table

Participant characteristics

Sex	Age (yrs)	Body Mass (kg)	Height (cm)	BSA (m^2)	Body Fat (%)	$\dot{V}O_{2max}$ ($L \cdot min^{-1}$)	$\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)
Males	25 ± 5	76.16 ± 6.51*	175 ± 8*	1.92 ± 0.13*	18.48 ± 5.43*	3.46 ± 6.92*	43.6 ± 7.0*
Females	22 ± 4	60.44 ± 8.38	166 ± 5	1.66 ± 0.11	23.85 ± 3.59	2.30 ± 5.39	37.0 ± 4.9

BSA, body surface area; $\dot{V}O_{2max}$, maximal oxygen consumption. *Significantly different from females ($p \leq 0.05$). Values represent mean ± standard deviation. $\dot{V}O_{2max}$ and BSA values represent the mean of 9 males and 7 females.