THE SEPARATE AND INTEGRATED INFLUENCE OF METABO- AND BAROREFLEX ACTIVTIY ON HEAT LOSS RESPONSES

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B.Sc., University of Ottawa, 2009

Thesis submitted to the
Faculty of Graduate and Postdoctoral Studies
in partial fulfillment of the requirements for the degree of
Master's of Science in Human Kinetics

Faculty of Health Sciences, School of Human Kinetics
University of Ottawa, Ottawa, Canada

2011

ACKNOWLEDGEMENTS

The development and completion of this thesis would not have been made possible without the guidance and support of the following individuals.

To Dr. Glen Kenny, thank you for giving me the opportunity to complete my Master's of Science degree under your supervision. Your ability to motivate, encourage, support and guide make you an excellent teacher, supervisor and mentor. Additionally your drive for excellence is transferred to your students, as your high expectations have helped me reach my potential as a student, researcher and maturing individual.

To my committee members Dr. Pascal Imbeault and Dr. Eric Doucet, thank you for your time, effort, and expertise in providing me with constructive feedback on my project.

To my fellow Human and Environmental Physiology Research Unit peers for their technical support and for creating an enjoyable working environment.

A special thanks to Aaron Lynn and Dan Gagnon, whose knowledge, advice and support played large parts in helping me complete my project.

A special thanks to the individuals who volunteered as subjects for my project, your time and commitment towards my thesis is greatly appreciated.

Finally, I would like to thank my mother, Lucy and my father, Frank. You have always been there to support me, helping me reach my toughest ambitions and goals.

ABSTRACT

Current knowledge indicates that nonthermal muscle metaboreflex activity plays a critical role in the modulation of skin vasodilation and sweating. However, the mechanisms of control have primarily been studied during isometric handgrip exercise in which muscle metaboreceptor activation is induced by a brief post-exercise ischemia of the upper limb. While the reflex increase in mean arterial pressure associated with this period of ischemia is consistent with the activation of muscle metaboreceptors, the change in baroreflex activity may in itself modulate the response. Thus, we sought to understand how these nonthermal stimuli interact in modulating the control of skin perfusion and sweating under conditions of elevated hyperthermia. Furthermore, we examined the mechanisms responsible for the maintenance of arterial blood pressure under varying levels of heat stress during isometric handgrip exercise.

Our study findings indicate that the parallel activation of muscle metaboreceptors and baroreceptors during post-exercise ischemia causes divergent influences on the control of skin blood flow and sweating; and these nonthermal stimuli are dependent on the level of hyperthermia. Moreover, we report that heat stress reduces the increase in arterial blood pressure during isometric handgrip exercise and this attenuation is attributed to a blunted increase in peripheral resistance, since cardiac output increased to similar levels for all heat stress conditions.

These results provide important insight and understanding into the role of muscle metabo- and baroreflex activity on the control of skin blood flow and sweating; along with further knowledge into the cardiovascular mechanisms responsible for the regulation of arterial blood pressure during hyperthermia.

TABLE OF CONTENTS

PART ONE:

EMPIRICAL AND THEORETICAL CONSIDERATIONS

CHAPTER 1

INTRODUCTION

1.0 Background

The capacity of the body to regulate core temperature around 37^{o}C is critical in overall human functioning. Humans can function independently of their environment to maintain a constant core temperature and through this ability, are classified as homeotherms. Human thermoregulation is the process by which the amount of heat being produced is equal to the amount of heat being lost and this is refereed to as heat balance. Physiological control of core body temperature is maintained at the hypothalamus. Thermoafferent input from peripheral hot and cold receptors in the skin and central thermal receptors within the hypothalamus provide continuous feedback to the hypothalamus regarding the thermal state of the individual (Brooks *et al.*, 2005). A deviation from the set-point or criterion hypothalamic temperature activates mechanisms which regulate heating and cooling of the body to maintain thermal balance.

There are a number of factors that influence the control of the heat loss responses of sweating and skin blood flow. It is well established that changes in skin and/or core temperature modulate the rate of heat loss during a thermal stimuli. Independent of thermal control, the actions of nonthermal factors such as central command, baroreflexes, mechanoreceptors and metaboreceptors have important consequences in the control of heat loss responses during and following exercise (Johnson, 1986; Shibasaki *et al.*, 2003a; Shibasaki *et al.*, 2006; Kenny & Journeay, 2010). Various experimental paradigms have been employed to study the separate and integrative influences of nonthermal factors on core body temperature regulation. These include the evaluation of

thermoefferent activity during orthostatic/postural stress (Jackson & Kenny, 2003; Kenny & Journeay, 2010), exercise-induced heat stress (Gagnon *et al.*, 2008), post exercise recovery period (Journeay *et al.*, 2004; Journeay *et al.*, 2005; Jay *et al.*, 2008) and isometric handgrip exercise (IHG) (Crandall *et al.*, 1998; Kondo *et al.*, 1999). As discussed below, these studies demonstrate that nonthermal factors act as either inhibitory or excitatory stimulus which displaces the set-point about which temperature is regulated.

In brief, central command involves sending neural signals down from the brain which recruits the skeletal muscle to contract and activate brainstem centers that control autonomic neural outflow directed to the cardiovascular system (Raven *et al.*, 1997). Baroreceptors are small stretch-sensitive receptors located in the carotid sinus and aortic arch (arterial baroreceptors) as well as in the atria, ventricles and pulmonary vessels (cardiopulmonary baroreceptors). The baroreceptor reflex is the body's primary reflex pathway for homeostatic control of blood pressure. Baroreceptor loading is associated with an increase in mean arterial pressure (MAP) which triggers a decrease in sympathetic activity and an increase parasympathetic activity. Through these mechanisms, heart rate and cutaneous vasoconstriction decrease in order to reduce blood pressure back to the resting set point. Conversely, baroreceptor unloading in response to a decrease in mean arterial pressure causes an increase in sympathetic nerve activity and a reduction in parasympathetic nerve activity, leading to skin blood flow vasoconstriction and an increase in heart rate, which returns the blood pressure back to normal. Finally, muscle mechanoreceptors and muscle metaboreceptors are neural afferents in the skeletal muscle which are sensitive to changes in muscle tension and a build up of metabolites (Rowell & O'Leary, 1990). Through efferent and afferent feedback, these nonthermal

mechanisms also stimulate the cardiorespiratory centers of the brainstem and therefore also influence the control of MAP.

Studies show that nonthermal baroreceptor activity has a significant influence on heat loss responses during passive heating and during and following exercise. Baroreceptor unloading has been shown to attenuate skin blood flow responses, albeit the level of influences is less with increasing hyperthermia (Rowell *et al.*, 1973; Solack *et al.*, 1985; Keller *et al.*, 2006). In contrast, the role of baroreceptors in the control of sweating is less conclusive. While some studies show that baroreceptor unloading (such as during a postural stress or the post exercise recovery period) enhances sweat rate (Mack *et al.*, 2001) others show no effect (Solack *et al.*, 1985; Mack *et al.*, 1995; Journeay *et al.*, 2004). In addition to the influence of baroreceptors, activation of central command and mechanoreceptors has also been shown to increase skin perfusion and sweating during and following exercise (Kondo *et al.*, 2010).

To date less is known about the mitigating influences of metaboreceptors. This is due primarily to the limitations associated with isolating the influences of metaboreceptors in exercise and/or post exercise scenarios. A number of studies have employed an isometric handgrip exercise model to evaluate these responses. In general these studies show that metaboreceptors enhance sweating and inhibit cutaneous vasodilation through a reduction in the active vasodilator system (Crandall *et al.*, 1998; Kondo *et al.*, 1999). In addition these responses are elevated at increasing intensities of IHG exercise. However a limitation of these studies is that the sweating response was similar to the blood pressure responses suggesting that baroreceptor loading status may be a confounding influence.

As noted above, various experimental protocols have been employed to study the roles of nonthermal factors on the control of heat loss responses. Of these protocols, the isometric handgrip exercise model has been used to study the role of metaboreceptors. In general, the IHG exercise model involves maintaining a constant isometric forearm contraction using a handgrip dynamometer for a specific length of time (typically 30-120 seconds) performed at percentage of maximal voluntary contraction (MVC) as pre-determined during a maximal hand-grip test. Five seconds prior to the end of exercise a blood pressure cuff, which has been placed on the upper arm prior to the exercise, is inflated to a systolic pressure exceeding 240 mm Hg for two minutes to occlude limb blood flow thereby trapping blood metabolites. After the occlusion period, the cuff is deflated permitting normal limb circulation.

An important benefit of the IHG exercise model is that it allows the study of metaboreflex activity in the absence of the confounding influences of central command and/or mechanoreceptors. During the period of muscle ischemia, it is thought that the accumulation of metabolites within the muscle triggers chemosensitive afferents (group III and IV afferents) and reflexively raises arterial blood pressure (Rowell & O'Leary, 1990). This maneuver maintains the stimulation of metaboreceptor muscle afferents and the reflex activation of muscle sympathetic nerve activity, while muscular relaxation eliminates both central command and the stimulation of mechanoreceptor muscle afferents (Victor *et al.*, 1988; Rowell & O'Leary, 1990; Saito *et al.*, 1990; Nishiyasu *et al.*, 1994; Vissing, 1997). Through this protocol it is possible to isolate and manipulate metaboreceptors to investigate the role of muscle metaboreflex on heat loss responses. Previous studies have shown that the activation of metaboreceptors through the IHG

model have increased sweating during IHG exercise and the ischemia phase when sweating has been initiated (Kondo *et al.,* 1999). Other studies have demonstrated that cutaneous vascular conductance is reduced following the handgrip exercise due to withdrawal of the active vasodilator system and this withdraw is more pronounced under higher levels of hyperthermia (Crandall *et al.,* 1998).

To date it remains unclear how changes in blood pressure induced by the handgrip exercise and the subsequent period ischemia might influence skin blood flow and sweat rate. This experimental model does not consider the possible confounding influences of baroreceptor activity. During the IHG exercise model and post exercise ischemia there is an increase in MAP which is thought to be a consequence of the build up of metabolites from the previously active muscle which triggers chemosensitive afferents. In addition to the influence of metaboreflex activity, it is plausible that the ischemia-induced increase in baroreceptor loading status may be involved in the modulation of heat loss responses. For example, some studies show that unloading the baroreceptors can result in a decrease in cutaneous vascular conductance and a further attenuation in sweating (Rowell *et al.*, 1973; Solack *et al.*, 1985). In contrast, studies have demonstrated that baroreceptor loading is associated with increases in skin blood flow and sweat rate (Jackson & Kenny, 2003; Journeay *et al.*, 2004).

Levels of hyperthermia have been shown to influence the relative contribution of nonthermal influences. Crandall *et al.* (1998) showed that the influence of muscle metaboreceptors on local sweat rate is dependant of the level of hyperthermia. Under normothermic conditions local sweating during exercise, throughout the post exercise ischemia and recovery remained unchanged from baseline values. In contrast, when

exercise was performed in a mild and moderately heat-stressed condition (an increase in core temperature of 0.55ºC and 0.75ºC above baseline resting respectively induced by whole-body warming), sweat rate increased during the exercise from an elevated pre-exercise level and remained elevated throughout the exercise bout, the post exercise ischemia and the recovery period. During the post exercise ischemia, heart rate returned to pre-exercise levels whereas mean arterial pressure remained elevated. It is possible however that the increase in sweat rate may be attributed to thermal factors associated with the progressive increase in core temperature (Nadel *et al.*, 1971), given that all three IHG exercise trials were performed consecutively in the same trial (i.e., resting under normothermic conditions followed by progressive heating).

With respect to the control of skin blood flow Kondo *et al.* (1999) showed that during passive hyperthermia muscle metaboreflex did not affect skin blood flow under mild hyperthermic conditions. In contrast Crandall *et al.* (1998) reported that metaboreflex stimulation under hyperthermia resulted in a significantly reduced level of cutaneous vascular conductance from baseline. They showed that skin blood flow was reduced during muscle ischemia under hyperthermic conditions, despite adrenergic vasoconstrictor blockade with bretylium suggesting that the attenuation of skin blood flow was due to altered cutaneous active vasodilation. Kondo *et al.* (2002) examined the effect of differences in core temperature, induced by passive heating, on skin blood flow during repeated bouts of IHG. They observed less of a reduction in cutaneous blood flow in the second bout (37.84 °C) relative to the moderately-heated conditions during the first bout (37.54°C). It was concluded that the reduction in skin blood flow may be due to varying degrees of active cutaneous vasodilation between the two levels of core

temperature, suggesting that nonthermal factors can be suppressed with increasing levels of hyperthermia. Consistent with previous reports, their findings indicate that a greater thermoregulatory drive leads to a lesser inhibition of blood flow during the ischemic handgrip stimulus manifested by a reduced active vasodilator response.

Nonthermal Factor	**Influence**
Central Command	-Descending neural signals from motor centers in the brain -Recruit skeletal muscle to contract -Activate brainstem centers that control autonomic neural outflow directed to the cardiovascular system
Baroreceptors	-Small stretch sensitive receptors located in the carotid sinus, aortic arch (arterial) and atria, ventricles and pulmonary vessels (cardiopulmonary)
Metaboreceptors	-Neural afferents in skeletal muscle -Sensitive to build up of metabolites in the muscle
Mechanoreceptors	-Neural afferents in skeletal muscle -Sensitive to changes in muscle tension

Figure 1. Summary overview of nonthermal factors

1.1 Rationale

To date, little is known about the possible interactive influences of metaboreceptor and baroreceptor activity on the control of heat loss responses of sweating and skin blood flow. Further, it remains to be determined if the level of thermal input alters the pattern of response of nonthermal baro- and metabo- receptor activity as induced by passive heating. Therefore, the purpose of the present study was to investigate the influence of a change in baroreceptor loading status on metaboreceptor control of skin perfusion and sweating during post isometric handgrip exercise ischemia. In addition, this study examined the influence of increasing levels of hyperthermia (i.e., at normothermic

conditions and at a 0.6 and 1.4ºC increase in core temperature) on skin blood flow, sweating and cardiovascular responses during the isometric handgrip exercise paradigm.

1.2 Purpose

The following study was conducted to evaluate the separate and combined actions of the nonthermal influences of metaboreceptors and baroreceptors during the isometric handgrip exercise model, on the control of heat loss and cardiovascular responses during varying levels of heat stress. Through this examination, we are able to provide critical advancements in our understanding of the role of nonthermal muscle metaboreceptor and baroreceptor activity in the control of skin blood flow and sweating along with advancing our understanding the effects of elevated states of hyperthermia on the regulation of arterial blood pressure.

1.3 Objectives

1) To investigate, if baroreceptor loading status influences the metaboreflex mediated change in heat loss responses during post isometric handgrip exercise ischemia.

2) To evaluate if the influence of the baro- and metaboreflex mediated control of heat loss responses is influenced by the level of thermal stress.

3) To evaluate if heat stress attenuates the increase in arterial blood pressure observed during isometric handgrip exercise.

1.4 Hypothesis

We tested the hypothesis that baroreceptor unloading induced by application of lower body negative pressure (LBNP) would attenuate the metaboreflex mediated increase in sweating and exacerbate the reduction in skin blood flow. In contrast, we tested the hypothesis that baroreceptor loading induced by application of lower body positive pressure (LBPP) would increase the metaboreflex mediated increase in sweating and attenuate the decrease in skin blood flow. Further, we also tested the hypothesis that the magnitude of the response (i.e., increase during LBPP and decrease during LBNP) would be attenuated with increasing levels of hyperthermia. Finally we hypothesized that heat stress would attenuate the increase in mean arterial pressure during an isometric handgrip exercise.

1.5 Relevance

To date, recent work has shown that nonthermal factors can have a profound influence on the control of skin blood flow and sweating and therefore core temperature regulation. Advancing our understanding of the separate and competing influences of nonthermal factors on human thermoregulatory responses at different levels of hyperthermia is an important step in understanding potential factors which increase the risk and/or occurrence of heat-related injuries. By increasing our understanding of how nonthermal factors may modulate thermoregulatory and cardiovascular function during and following exercise, it will be possible to develop interventions (i.e., different recovery strategies, postural manipulation, cooling techniques, etc.) which can be used to mitigate or manage impairment in the capacity to dissipate heat.

1.6 Delimitations and Limitations

Subjects recruited for this study were healthy physically active males, aged between 18 and 45 years. Therefore, the results of this study cannot be applied to females, children, or older adults. Skin blood flow and sweating was only measured on the non-exercising (left) forearm. As such it is not possible to evaluate possible regional differences in the pattern of response. It is assumed that the level of activation of the metaboreceptors during the exercise and occlusion was the same during each thermal and pressure experimental session. It was not possible to measure the activation level of the metaboreceptors and therefore we assume that activation was the same during all experimental sessions as each participant exercised at the same relative handgrip intensity (60% maximal voluntary contraction).

CHAPTER 2

REVIEW OF LITERATURE

2.1 Human Thermoregulation

The human body maintains core body temperature at a constant internal temperature of 37°C while the skin temperature remains closer to the surrounding environment. Under conditions of thermal stress such as exercise or exposure to hot ambient conditions, the rate of whole-body heat loss increases to offset an increase in the rate of heat storage and therefore core temperature. The physiological control of core body temperature is directly mediated by the hypothalamus using feedback from both central and peripheral thermal sensors (Brooks *et al.,* 2005). Central and peripheral thermal stimuli are well known to modulate sweating activity at rest and during physical work. In response to elevations in core and/or skin temperature, skin vasodilation occurs and eccrine sweat glands are activated, thereby promoting heat loss. Activation of heat loss responses is primarily controlled from the preoptic and anterior regions of the hypothalamus where thermosensitive neurons are located. However, afferent stimuli originating from the skin, viscera, and spinal cord can influence this response.

Healthy humans regulate core temperature to maintain a near constant level (~37ºC) regardless of environmental conditions. To do this, a balance must be maintained between the heat produced within the body and the heat lost to the environment via a combination of dry heat exchange and evaporative heat loss. At rest in a thermoneutral environment (such as room temperature), resting metabolic heat production is balanced primarily by dry heat loss via conduction/convection and radiation. As ambient air temperature increases, the smaller temperature gradient between the skin surface and

ambient air reduces the capacity for dry heat exchange. When the environment is warmer than the skin, the body will gain heat via dry heat exchange, increasing the requirements for sweating and circulatory responses to achieve a given rate of heat dissipation.

Physical activity will also increase metabolic heat production, thereby creating a greater need for heat to be dissipated to the environment so that core temperature can be maintained at a safe level. The increase in core temperature at steady state is largely independent of the ambient air temperature (within the measured range of 5 to 36°C) and is proportional to the metabolic rate. With metabolic and/or environmental heat load, the increase in sweat rate and skin blood flow is proportional to the amount of evaporative heat loss required for heat balance. When changes in sweating and skin blood flow cannot facilitate a sufficiently high rate of heat loss (e.g. intense physical activity in hot/humid conditions and/or an impairment of normal thermoregulatory function), body core temperature continually rises. If left unchecked, this may lead to heat illness and eventually death.

2.2 Heat Balance

The ability of the body to regulate core temperature around 37°C is critical for normal biological functioning. The interaction of man with the thermal environment or body heat balance can be expressed by the following simple formula:

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

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

Under typical exercise or working conditions: metabolic heat production (M) increases due to the high intensity of physical activity; rate of evaporative heat loss from the skin is low due to the high partial pressure of water vapor in the ambient air and is further reduced by highly insulative and vapor impermeable clothing; rate of sensible heat exchange between the skin and the environment by conduction, convection and radiation (K + C + R) is often negative as the high environmental temperatures cause a net heat transfer towards the body if they are greater than that of the skin; respiratory heat exchange by conduction and evaporation (C_{RES} + E_{RES}) is relatively small. When coupled with an increased metabolic heat production from the physical demands of work as well as the potentially confounding effects of semi-permeable or impermeable clothing, fluid loss, fatigue, sleep deprivation or various pathological conditions, the body's physiological responses may not be sufficient to achieve thermal balance{Jay, 2010 #1818}. Consequently, an elevation in core temperature may occur, leading to a graded increase of heat stress and therefore an increased risk of heat injury.

2.3 Heat Loss Response

As outlined above the mechanisms of heat loss are critical in maintaining normal resting core body temperature during heat stress and these mechanisms include radiation, conduction, convection and evaporation. Radiation is the mechanism by which heat is transferred between the body and the environment via electromagnetic waves depending on the temperature gradient between the body and the environment. Conduction is the mechanism by which heat is transferred between two surfaces in contact with one another depending on the temperature gradient between the two surfaces. Convection is the

mechanism by which heat is transferred from a surface to a gas or liquid depending on the temperature gradient, rate of motion of the gas or liquid and its relative heat storage capacity. Finally evaporation is the mechanism by which heat is lost through the vaporization of water at the skin surface or through respiration.

Evaporation provides the major defense against overheating. The quantity of heat loss by vaporization of water (i.e., evaporation of sweat) depends on two factors: 1) the rate at which water is secreted by the sweat glands; and 2) the ability of the ambient environment to remove water vapor. If the air is dry and moving quickly, then heat loss by evaporation is limited only by the rate at which sweat can be secreted. Conversely, if the air is humid and still, then heat loss is restricted by the capacity of the surrounding air to remove water from the skins surface. With high humidity, the ambient vapor pressure approaches that of the moist skin, and evaporation greatly diminishes, despite an elevated sweat rate. Thus, water vapor pressure of the air is a major factor in determining evaporative heat loss.

2.3.1 Sweating

In the human body there are two types of sweat glands. These are apocrine and eccrine glands. Apocrine glands are found in the armpits and pubic regions whereas eccrine sweating glands are distributed all over the body (Parsons, 2003). The differences between eccrine and apocrine sweat are of physiologic importance. Eccrine sweat glands are not attached to hair follicles. They produce a watery secretion, are innervated by cholinergic nerves and are of major importance in thermoregulation. The ducts of

apocrine sweat glands are attached to hair follicles, are innervated by adrenergic nerves, start to function at puberty and these glands secrete a viscous fluid.

With the exception of the palms of the hands and soles of the feet, thermal sweat is produced by the approximately 2-4 million eccrine sweat glands located across almost the entire body surface. Sweat production is regulated by cholinergic sympathetic neurons. As such they differ markedly from other sympathetic end organs because they are cholinergic rather than adrenergic. Furthermore, these glands actively secrete sweat only when stimulated via nerve impulses. The volume of sweat secreted is proportional to the frequency of the efferent nerve impulses. This overall effect of the nervous system on sweat secretion is termed sudomotor activity. The body's ability to withstand high temperatures is directly related to the relative humidity of the air. Heat loss by vaporization of sweat can reach values as high as 900 calories/hour and air moving across a sweaty skin surface enhances evaporation during high humidity.

The rate of sweat secretion varies markedly depending on environmental temperature and degree of muscular work being performed. In a hot climate during a period of heavy physical work, a person can secrete over 1.5 L of sweat per hour. In a dry atmosphere, nearly all of this sweat can be evaporated. In general, an increase in sweating occurs through the combination of increasing the number of sweat glands that are activated and increasing the amount of sweat released per gland.

2.3.2 Skin Blood Flow

Skin blood flow is essential in the maintenance of normal body temperature during heat stress. The thermoregulatory apparatus can respond to a wide range of

temperatures to cause varying increases in the cutaneous circulation. It also serves to provide an avenue for heat loss even at rest with heat loss of 80-90 kcal/hour under thermoneutral conditions (Johnson & Proppe, 1996).

Under heat stress conditions, a change in the level of skin blood flow at a certain core temperature can be attributed to: 1) a change in the threshold for vasodilation (delayed or augmented response); 2) a change in the sensitivity (slope) of the blood flow increase, or 3) both factors (Charkoudian, 2003). Increases in skin temperature is a less sensitive driver of cutaneous blood flow than a similar increase in core temperature, which is representative of blood temperature sensed by the hypothalamic neurons (Wyss *et al.,* 1974). Skin blood flow can increase from 0.5 L/min to 6-8 L/min during severe hyperthermia (Johnson, 1986). Skin blood flow is under control of the sympathetic vasodilator system, which increases peripheral skin blood flow during heat stress. An increase in skin blood flow 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. During exercise or passive heating increases in skin blood flow and sweating are proportional to the increases in core body temperature. Consequently when a steady core temperature is reached there is a plateau in skin blood flow and sweating.

During dynamic exercise an increase in core temperature results in the activation of thermal control of heat loss responses. At the start of exercise there are competing demands for blood flow. The exercising muscle receives blood for metabolic requirements and this results in cutaneous vasoconstriction of the skin to meet this

demand, however the response is altered during prolonged exercise (Johnson, 1992). During prolonged exercise an initial rise in skin blood flow is followed by a plateau to meet the thermoregulatory demands of an increase in core temperature.

2.4 Nonthermal Influences of Heat Loss Responses

Numerous studies have examined the influence of the nonthermal factors on thermoefferent activity. Nonthermal reflexes such as central command, baroreflexes, muscle mechanoreceptors and metaboreceptors have been shown to modulate the pattern of heat loss responses during heat exposure (Johnson, 1986; Vissing *et al.*, 1991; Johnson, 1992; Kondo *et al.*, 1997; Kondo *et al.*, 1999; Shibasaki *et al.*, 2003a). Figure 2 shows the nonthermal factors which can influence heat loss responses and these responses are reviewed in the following section.

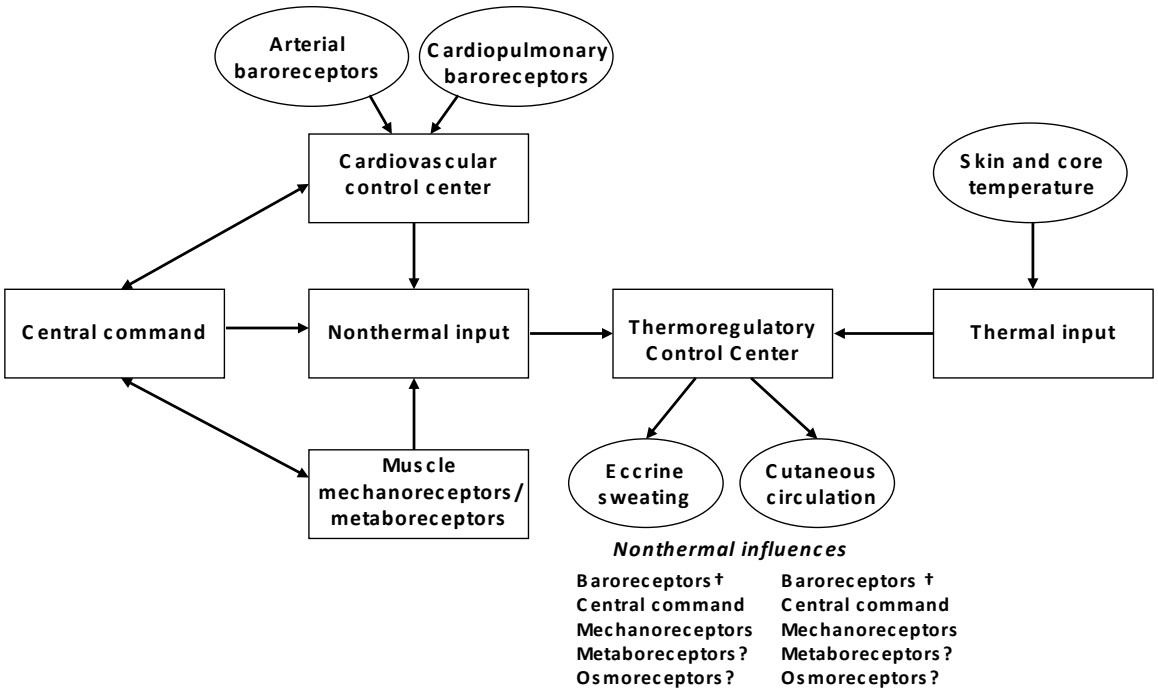

Figure 2. Schematic summary of nonthermal influences on heat loss responses. (Source: Kenny & Journeay, 2010)

2.4.1 Central Command

Central command signals arise from the higher brain centers and descend in close proximity to the cardiorespiratory centers in the medulla. Activation of central command (higher brain centers) generates effector activity, however the mechanism by which the pattern of the nerve activity remains unclear. Shibasaki *et al.* (2005) evaluated the effect of central command on skin blood flow by using a partial neuromuscular blockade technique through the use of cisatracurium. Cisatracurium is a non-depolarizing muscle relaxant which blocks action potential transmission at the myoneural junction by binding with cholinergic receptor sites. Their study showed that central command plays a role in the observed decrease in cutaneous vascular conductance during an isometric hand-grip exercise performed during heat stress.

Central command has been reported to be the primary mechanism that stimulates sympathetic outflow (vasoconstriction, vasodilation, and sudomotor activation) to the skin during intense handgrip exercise (Vissing *et al.*, 1991; Kondo *et al.*, 2002). Previous work by Shibasaki and colleagues (2003b) confirmed the influence of central command on local sweating using IHG exercise (2 minutes at 35% of MVC) with and without partial neuromuscular blockade (cisatracurium). The above mentioned experimental paradigm was employed in view of previous work demonstrating that: 1) isometric exercise evokes a variety of physiological responses, including increases in heart rate, arterial pressure, and skin and muscle sympathetic nerve activity (Secher, 1985; Victor *et al.*, 1989; Vissing *et al.*, 1991); 2) changes in sweat rate and skin blood flow during isometric handgrip exercise occur without changes in core and skin temperature (Crandall *et al.*, 1995); and, 3) neuromuscular blockade enhances central command during exercise

resulting in a greater increase in heart rate and blood pressure at a given work intensity (Leonard *et al.*, 1985). Trials were performed under normothermic, mild, and moderate hyperthermic conditions (0.5 and 1.0 ºC increase above baseline resting respectively). Neural blockade resulted in a significant reduction in maximal voluntary contraction and force during the submaximal isometric handgrip exercise. However, despite this decrease, a greater sweat rate was measured in both the normothermic and the mild hyperthermic conditions. In contrast, sweat rate was reduced in the moderate hyperthermic state relative to control. While these observations provide additional evidence to support the role of central command as a possible modulator of sweating during exercise, it also demonstrates that the relative influence of central command is diminished or non-existent with increases in the level of hyperthermia or thermal stress.

2.4.2 Metaboreceptors

Metaboreceptors are chemosensitive afferents that respond to metabolic products in muscle and can influence the control of circulation (Rowell & O'Leary, 1990). The metaboreceptors influences of heat loss responses in normothermic conditions was first studied by Vissing *et al.* (Vissing *et al.*, 1991; 1996) and Saito *et al.* (1990). In these studies two minutes of intense isometric hand-grip exercise (i.e., 30% of maximal voluntary contraction) was followed by post exercise muscle ischemia induced by forearm vascular occlusion performed upon the completion of exercise. Occlusion of the forearm blood flow has been shown to result in a sustained elevation of blood pressure above resting levels (Nishiyasu *et al.*, 1994) and this is an indicator that the muscle metaboreflex activity is elevated. The period of muscle ischemia is thought that the

accumulation of metabolites within the muscle triggers chemosensitive afferents (group III and IV afferents) and reflexively raises arterial blood pressure (Rowell & O'Leary, 1990). It is believed that the metabolites responsible for the stimulation of chemosensitive afferents include: lack of oxygen delivery (Sheriff *et al.*, 1987), lactate (Sinoway *et al.*, 1992b), hydrogen ion concentration (H+), pH (Victor *et al.*, 1988; Sinoway *et al.*, 1992a), arachidonic acid (Rotto & Kaufman, 1988), diprotonated phosphate (Sinoway *et al.*, 1994), and adenosine (Maclean *et al.*, 1997).

Muscle metaboreceptor afferents are isolated and activated during post- isometric handgrip forearm ischemia while muscle relaxation eliminates central command and stimulation of the muscle mechanoreceptor afferents (Kondo *et al.*, 1999). Saito *et al.* (1990) reported a sustained increase in mean arterial pressure during occlusion indicating that muscle metaboreflex activity was indeed elevated and this can suggest that thermoregulatory sweating is triggered during the activation of the muscle metaboreflex.

Studies show that the level of hyperthermia influences the pattern of response of nonthermal factors such as metaboreflex activity. Crandall *et al.* (1998) showed that in normothermic conditions, local sweat rate during exercise and during post exercise ischemia and recovery remained unchanged from baseline values. However, when the intense hand-grip exercise was performed in mild heat stressed conditions sweat rate increased during the exercise from an elevated pre-exercise level and remained elevated throughout the exercise bout, during the post exercise ischemia and subsequent recovery. However in this case, it is possible that the increase in sweat rate may be attributed to thermal factors associated with progressive increase in core temperatures (Nadel *et al.*, 1971). Kondo *et al.* (1999) observed a similar magnitude of increase in sweating response

during handgrip exercise performed under mild heat stress conditions (i.e., ambient air temperature of 35^{o}C). However, they showed that for a given level of hyperthermia the pattern of response was influenced by the duration (60 to 120 seconds) and intensity (30 to 45% of MVC) of exercise. A greater elevation in sweating was observed during the longer duration (120 seconds vs. 60 seconds) exercise bout performed at 30% of MVC. However, a similar response was observed for shorter exercise duration (i.e., 60 seconds) performed at a higher intensity of exercise (45% of MVC). For both conditions, a sustained elevation in sweating was observed during the post exercise ischemia period. Of note, however, the shorter duration exercise bout (i.e., 60 seconds) performed at the lower intensity exercise (i.e., 30% of MVC) did not result in a sustained reflex mediated elevation in MAP during the post exercise occlusion. Given that core and skin temperature remained unchanged during the trial and at similar levels for each of the experimental trials, their findings show that independent of the thermal influence, the metaboreflex mediated increase in sweating is influenced by the intensity and/or duration of the exercise.

As with the control of sweating, metaboreceptor activity has been shown to modulate skin blood flow response albeit the response is less conclusive. For example, Kondo *et al.* (1999) examined the effect of metaboreceptor stimulation on the cutaneous circulation during passive hyperthermia and concluded that the muscle metaboreflex did not affect skin blood flow under mild hyperthermic conditions. In contrast, Crandall *et al.* (1998) reported that metaboreflex stimulation under a hyperthermic condition resulted in a significantly reduced level of cutaneous vascular conductance from baseline. Specifically skin blood flow was reduced during muscle ischemia under an elevated state

of hyperthermia (0.55-0.75ºC above baseline resting). This reduction was shown to be the result of altered cutaneous active vasodilation rather than an increase in cutaneous vasoconstriction. Kondo *et al.* (2002) also showed that increasing levels of hyperthermia modified the pattern of response in skin blood flow during and following IHG exercise. They observed less of a reduction in cutaneous blood flow following a second bout performed at an elevated thermal state (37.84 °C) as compared to a first bout performed at a lower level of core temperature increase (37.54°C). Whole-body heating was performed to increase the level of hyperthermia between bouts and subsequently maintain core temperature during the IHG exercise at a pre-determined level of hyperthermia. They speculated that the attenuation in the skin blood flow observed at elevated levels of hyperthermia may be of the results of an attenuated active vasodilatory response as previously suggested by Crandall *et al.* (1995; Crandall *et al.*, 1998). This suggests that thermoregulatory drive on the active vasodilator system at greater core temperatures leads to a lesser inhibition of blood flow during the ischemic handgrip stimulus. Taken together, these studies show that muscle metaboreceptors tend to reduce active vasodilation. However, it remains unclear how changes in baroreceptor loading status associated with the reflex increase in blood pressure during the post exercise period of ischemia may affect this response.

2.4.3 Baroreceptors

There are two types of baroreceptors; arterial and cardiopulmonary baroreceptors. Baroreceptors as small stretch-sensitive receptors located in the carotid sinus and aortic arch (arterial baroreceptors) as well as in the atria, ventricles and pulmonary vessels

(cardiopulmonary baroreceptors). The baroreceptor reflex is the primary pathway for homeostatic control of blood pressure. The aortic arch receptors receives impulses through the vagus nerve and the impulses travel to the nucleus tractus solitarius (NTS) in the medulla while the carotid arch receptors act through the sinus nerve which send signals up to the glossopharyngeal nerve before being received by the nucleus tractus solitarius (Berne & Levy, 2001). These nerves regulate baroreceptor activity and blood pressure through neural control of heart rate and vascular resistance (Brooks *et al.*, 2005). Baroreceptor loading is associated with an increase in mean arterial pressure which triggers a decrease in sympathetic nerve activity and an increase in parasympathetic activity. This results in a decrease in heart rate and cutaneous vasoconstrictor tone bringing the blood pressure back to normal levels (Van Wynsberghe *et al.*, 1995). On the contrary, a decrease in mean arterial pressure unloads baroreceptors, which effectively increases sympathetic nerve activity and decreases parasympathetic activity. This elicits an increase in heart rate and cutaneous vasoconstriction returning the blood pressure back to normal (Van Wynsberghe *et al.*, 1995). Through this process the body can constantly maintain arterial blood pressure in response to stimuli which can affect blood pressure such as exercise or postural stress.

Increasing evidence for baroreceptor modulation of heat loss responses has been derived from studies examining the post-exercise period. Studies show that post-exercise sweating and skin blood flow responses are attenuated for a prolonged period and are paralleled by a sustained elevation in core temperature. The underlying mechanism for this perturbation of post-exercise thermoregulatory control has been linked to a nonthermal baroreflex mediated response associated with post-exercise hypotension. This

hypothesis has evolved from the initial observation that a greater level of post exercise hypotension, induced by exercise of increasing intensity, results in a relative increase in the local onset of sweating (Kenny *et al.*, 2003a) and skin vasodilation (Kenny *et al.*, 2003b). In subsequent work, Jackson and Kenny (2003) showed that the post-exercise increase in the onset threshold for sweating and skin blood flow is reversed with the application of +50 mmHg LBPP. Their findings demonstrate that LBPP, which is known to reverse post-exercise hypotension and load baroreceptors, is capable of reversing the post exercise suppression of vasomotor and sudomotor activity.

Additional evidence for the role of cardiopulmonary and/or arterial baroreceptors in the modulation of post-exercise heat loss responses is demonstrated by studies employing postural manipulation (McInnis *et al.*, 2006; Journeay *et al.*, 2007; Kenny *et al.*, 2008) to reverse the post-exercise hypotension. McInnis *et al.* (2006) showed that extended recovery from dynamic exercise in the 15º head-down tilt position attenuates the reduction in cutaneous vascular conductance and sweating when compared with the responses measured during recovery in the upright seated posture. Kenny *et al.* (2008) examined the effect of a supine recovery relative to the upright seated exercise posture. Consistent with the findings using LBPP and 15° head-down tilt, supine recovery was shown to attenuate the post-exercise reductions in mean arterial pressure, cutaneous vascular conductance, and sweat rate.

PART TWO:

METHODS AND RESULTS OF THE THESIS

ARTICLE I:
Hyperthermia modifies muscle metaboreceptor and baroreceptor modulation of heat loss in humans

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Running head: Metabo- and baroreflex control of skin blood flow and sweating

Paper submitted to American Journal of Physiology (R-00463-2011R1)

ABSTRACT

The relative influence of muscle metabo- and baroreflex activity on heat loss responses during post-isometric handgrip (IHG) exercise ischemia remains unknown, particularly under heat stress. Therefore, we examined the separate and integrated influences of metabo- and baroreceptor mediated reflex activity on sweat rate and cutaneous vascular conductance (CVC) under increasing levels of hyperthermia. Twelve males performed 1-min of IHG exercise at 60% of maximal voluntary contraction followed by 2-min of ischemia with simultaneous application of either lower body positive (**LBPP**: +40 mmHg), negative (**LBNP**: -20 mmHg) or no pressure (**CON**) under no heat stress (**NHS**). On separate days, trials were repeated under heat stress conditions of 0.6ºC (**MHS**) and 1.4ºC (**HHS**) increase in esophageal temperature. For all conditions, mean arterial pressure was greater with **LBPP** and lower with **LBNP** relative to **CON** during ischemia (all $p \leq 0.05$). No differences in sweat rate were observed between pressure conditions regardless of the level of hyperthermia ($p > 0.05$). Under **MHS**, no differences in CVC were observed between pressure conditions. However, during **HHS**, **LBNP** significantly reduced CVC by 21 ± 4% ($p \leq 0.05$) and **LBPP** significantly elevated CVC by 14 ± 5% ($p \leq 0.05$) relative to **CON**. These results show that sweating during post-IHG exercise ischemia is activated by metaboreflex stimulation and not baroreflexes. In contrast, our results suggest that baroreflexes can influence the metaboreflex modulation of CVC, but only at greater levels of hyperthermia.

Keywords: Heat Stress, Thermoregulation, Isometric Handgrip Exercise, Post-exercise Ischemia

INTRODUCTION

Extensive studies have shown that mechanoreceptors and baroreceptors (cardiopulmonary and arterial) modulate the heat loss responses of cutaneous vasodilation and sweating during passive heating, exercise and post-exercise recovery, while central command can influence sweating and cutaneous vasodilation during exercise (12, 13, 23, 24, 31, 34). In contrast, there remains limited information regarding the influence of metaboreceptors on thermoeffector activity during and following exercise, largely due to the difficulties associated with isolating the muscle metaboreflex.

To date, much of our understanding of the influence of metaboreceptor activity on heat loss responses has been limited to findings obtained using an isometric handgrip (IHG) exercise model (18, 20, 30). After an IHG exercise bout at a given percentage of maximal voluntary contraction, a two minute ischemia period is induced by occluding all limb blood flow to the exercising arm, which is thought to trigger group III and IV chemosensitive afferents (26). The activation of the metaboreflex results in an enhanced sweating response (18) while attenuating cutaneous vascular conductance (3).

A potential caveat to these findings, however, is the fact that activation of the metaboreflex also results in a reflex increase in arterial blood pressure (26), thereby inducing a concomitant change in baroreflex activity. Previous research has established that changes in baroreflex activity alone, induced by postural (e.g. head-down tilt) or mechanical (lower body positive/negative pressure) challenges, can influence skin blood flow (14, 15, 21, 37) and sweating (11, 14, 16, 22). It is plausible therefore that the measured reduction of cutaneous vascular conductance and concomitant increases in

sweating during the post-IHG exercise ischemia period may not only be influenced by metaboreflex activity, but also by baroreflex activity or a combination of both reflexes.

To our knowledge, only one study has differentiated between metabo- and baroreflex activity on sweat rate during the post-IHG exercise ischemia period. Shibasaki et al., (30) determined that pharmacologically-induced changes in blood pressure (using sodium nitroprusside) did not modify the reflex elevations in sweating during the period of ischemia. Although they eliminated the possible influence of arterial baroreceptors on sweat rate during the period of ischemia, it remains unknown if cardiopulmonary baroreceptors could influence this response. Furthermore, skin blood flow was not measured during this study; as such there remains a critical gap in our understanding of the role of baroreflex activity on skin blood flow during post-IHG exercise ischemia. Finally, a number of studies suggest that the relative influence of nonthermal factors in modulating thermoefferent activity can change as a function of the level of hyperthermia (7, 16, 17). For example, Kondo et al., (17) demonstrated that the contribution of nonthermal activity (central command, metaboreceptors and mechanoreceptors) in modulating sweating during IHG exercise became reduced with moderate levels of heat stress (~0.3°C T_{es} increase). It is possible therefore that the metabo-reflex modulation of sweat rate during post-exercise ischemia may be attenuated under high levels of heat stress. However, no study has examined if an elevated level of hyperthermia (>0.8°C) during post-IHG exercise ischemia can influence metaboreflex modulation of sweating and cutaneous vascular conductance.

Therefore, the purpose of this study was two-fold; first we examined the interaction of metabo- and baroreflex activity on sweating and cutaneous vascular

conductance during post-IHG exercise ischemia, and second; we examined this relationship under increasing levels of heat stress. To isolate the influence of metabo- and baroreflex activity, the period of ischemia was performed while baroreflex activity was manipulated using lower body negative pressure (-20 mmHg), lower body positive pressure (+40 mmHg), or during a control (no pressure) condition. It was hypothesized that cardiopulmonary and arterial baroreceptor unloading would attenuate the metaboreflex mediated increase in sweating and exacerbate the reduction in cutaneous vascular conductance during heat stress. In contrast, it was hypothesized that cardiopulmonary and arterial baroreceptor loading would increase the metaboreflex mediated increase in sweating and attenuate the decrease in cutaneous vascular conductance during whole-body heating. Finally, we hypothesized that the influence of the metabo- and baroreflex activity on sweating and cutaneous vascular conductance would diminish as core temperature increased.

METHODS

Ethical approval

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

Participants

Twelve healthy (no history of respiratory, metabolic or cardiovascular disease, non-smoking and normotensive) and physically active (exercised 3-5 times per week for a minimum of 30 min) males volunteered for this study. Mean ± standard deviation characteristics of the participants were: age, 24 ± 7 yrs; height, 180 ± 6 cm; weight, 79 ± 10 kg.

Experimental Design

On the day prior to each experimental test session, subjects were instructed to abstain from caffeine, alcohol and strenuous physical activity for 24 hours prior to the trial. Participants were asked to arrive at the laboratory after eating a light meal. For each subject, trials were performed at the same time of day to avoid circadian variations in core temperature. All trials were separated by a minimum of 24 hours.

For all sessions, subjects were asked to wear socks and shorts. After entering an environmental chamber maintained at a temperature of 24°C and a relative humidity of 20%, monitoring instruments were attached. During the instrumentation phase, each subject performed two brief maximal voluntary contractions (MVC) with their right hand using a handgrip dynamometer. The highest value obtained was used to calculate the relative workload (60% MVC) to be performed during the experimental conditions. Following the instrumentation phase, subjects were inserted, up to the hips, in a pressure box. A custom made neoprene skirt ensured an air-tight seal at the level of the iliac crest, while the participants remained in an upright seated posture. The pressure box was custom designed to examine the application of positive and negative pressure to the lower body segments of the upright man. Baseline data were recorded for five minutes at rest. The subject then performed 60 seconds of IHG exercise at 60% MVC. A visual feedback system was used to ensure the subject maintained the desired force throughout the exercise period. Five seconds before the end of the exercise bout, a cuff around the upper arm performing the contraction was inflated to a super-systolic pressure (>240 mm Hg) for 120 seconds creating complete occlusion of forearm blood flow. During the period of ischemia, cardiopulmonary and arterial baroreceptors were either unaltered through no pressure (**CON**), loaded through the application of lower body positive pressure (**LBPP**, +40 mm Hg), or unloaded through the application of lower body negative pressure (**LBNP**, -20mm Hg). Following the period of ischemia, the cuff was deflated and post-exercise recovery data were collected for another 120 seconds. Trials were completed in random order and a 20-min recovery period between conditions was given to minimize muscular fatigue.

On separate days, the experimental protocol was repeated under different levels of thermal stress: (i) no heat stress (**NHS**), (ii) moderate heat stress (**MHS**), equal to an esophageal temperature (T_{es}) increase of 0.6°C, and (iii) high heat stress (**HHS**), equal to an T_{es} increase of 1.4°C. For the heat stress trials, subjects were dressed in a high density water-perfusion suit and 48°C water was perfused. The suit covered the entire body surface except for the face and left forearm where the laser-Doppler probe and sweat capsule were located. Upon reaching the desired core temperature increase, water bath temperature was reduced to 42°C to maintain a steady state body core temperature. Once a steady state temperature was achieved, baseline resting data were collected. During the heat stress conditions, room temperature was increased to 37°C after the instrumentation phase. The water-perfusion suit was not worn during the **NHS** condition.

Measurements

Esophageal temperature was monitored continuously using a pediatric esophageal temperature probe (Mon-a-therm®, Mallinckrodt Medical, St-Louis, USA) inserted through the nose to a depth of forty centimeters past the entrance of the nostril. Local skin temperature (T_{sk}) was measured at nine sites on the body (forehead, upper back, lower back, chest, abdomen, bicep, quadriceps, hamstring and back calf); to subsequently calculate mean skin temperature according to the proportions determined by Hardy and Dubois (10). Esophageal and skin temperature data were collected using a data acquisition module (HP Agilent model 3497A) at a sampling rate of 15 seconds and simultaneously displayed and recorded in spreadsheet format on a personal computer with LabVIEW software (Version 7.0, National Instruments, TX, USA).

Forearm sweat rate was measured continuously on the left mid-anterior forearm using a 3.8 cm^2 ventilated capsule attached to the skin with adhesive rings and topical Collodion HV skin glue (Mavidon Medical products, Lake Worth, FL, USA). Anhydrous compressed air was passed through the capsule over the skin surface at a rate of ~1.0 $L \cdot min^{-1}$. Water content of the effluent air was measured using a high precision dew point mirror (model 473, RH systems, Albuquerque, NM, USA). Sweat rate was calculated using the difference in water content between effluent and influent air, and the flow rate. This value was normalized for the skin surface area under the capsule and expressed in $mg \cdot min^{-1} \cdot cm^{-2}$.

Forearm skin blood flow (SkBF) was estimated using laser-Doppler velocimetry (PeriFlux System 5000, Perimed AB, Stockholm, Sweden) on the left mid-anterior forearm. Prior to the start of the experimental trial, the laser-Doppler flow probe (PR 401 Angled Probe, Perimed AB, Stockholm, Sweden) was affixed with an adhesive ring to the forearm in a site without superficial veins that demonstrated pulsatile activity. The probe was not moved until the end of the experimental trial. A maximum SkBF test was performed at the end of each experimental session by locally heating the SkBF site to 42°C for 20 min and then increasing local temperature to 44°C for an additional 25 min (2). Cutaneous vascular conductance was calculated as laser-Doppler velocimetry output in arbitrary perfusion units divided by mean arterial pressure and expressed as a percentage of maximum.

During the entire experimental trial, a Finometer (Finapres Medical Systems, Amsterdam, Netherlands) was used to measure mean arterial pressure from the beat-to-beat recording of the right middle finger arterial pressure waveform with the volume-

clamp method (25) and physiocal criteria (36). Prior to the start of each measurement period, brachial artery pressure reconstruction (8, 9) was calibrated with an upper arm return-to-flow systolic pressure detection (1). Furthermore, the right arm was supported at heart level, and a level calibration was performed.

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

Statistical Analysis

All dependent variables were averaged over the following time periods for each pressure condition: a 15 second pre-exercise period (baseline; Rest), the final 15 seconds of IHG exercise (end-exercise; EE), the final 15 seconds of post-exercise ischemia (occlusion; Occ), and the final 15 seconds of recovery from post-exercise ischemia (recovery; Rec). A two-way analysis of variance was performed to compare the dependent variables at each time point (Rest, EE, Occ, Rec) between the three different pressure conditions (**CON**, **LBNP**, **LBPP**). This analysis was repeated for each level of heat stress. The p value for significance was set at $\alpha \leq 0.05$. Post-hoc comparisons were carried out using paired samples T-tests. All analyses were performed using the statistical software package SPSS 18.0 for Windows (SPSS Inc. Chicago, IL, USA). All results are presented as means ± standard error, unless otherwise indicated.

RESULTS

Haemodynamic responses

Figure 1 shows mean arterial pressure and heart rate responses for each of the three pressure conditions at each level of hyperthermia. Baseline resting values were similar across all pressure conditions for each level of heat stress.

Under no heat stress, isometric handgrip exercise resulted in a similar increase in mean arterial pressure relative to baseline for all pressure conditions (Table 1). During post-exercise ischemia, mean arterial pressure remained elevated above baseline resting for all pressure conditions ($p \leq 0.05$). However, relative to **CON**, a greater elevation was observed during **LBPP** ($p \leq 0.001$), whereas mean arterial pressure was lower during **LBNP** ($p \leq 0.001$). During the recovery period from post-exercise ischemia, mean arterial pressure returned to baseline resting levels for all pressure conditions ($p > 0.05$) and no differences between conditions were observed ($p > 0.05$). A similar pattern of response was observed in both the moderate and high heat stress conditions, with the exception that under high heat stress, the application of **LBNP** returned mean arterial pressure to baseline resting levels ($p = 0.376$) (Table 1, Figure 1).

Under no heat stress, isometric handgrip exercise increased heart rate significantly above baseline resting to similar levels between pressure conditions (Table 1, Figure 1). During post-exercise ischemia, heart rate returned to baseline resting during **CON** ($p = 0.701$) whereas it decreased to levels below baseline resting during **LBPP** ($p = 0.001$). In contrast, during **LBNP**, heart rate remained significantly elevated above baseline resting values ($p = 0.047$). As such, heart rate was significantly lower during **LBPP**

(p=0.002) and significantly greater during **LBNP** (p=0.006) compared to **CON**. Following post-exercise ischemia, heart rate returned to baseline resting values for all pressure conditions (p>0.05). A similar pattern of response was observed under both moderate and high heat stress conditions (Table 2, Figure 1).

Thermal responses

Baseline esophageal (**NHS**: 36.88 ± 0.06°C, **MHS**: 36.88 ± 0.66°C, **HHS**: 36.88 ± 0.44°C) and mean skin (**NHS**: 32.27 ± 0.10°C, **MHS**: 32.67 ± 0.26°C, **HHS**: 33.42 ± 0.20°C) temperatures were not significantly different between heat stress conditions. Within the no heat stress condition, esophageal and mean skin temperatures remained unchanged from baseline levels throughout the experimental protocol and were not affected by the application of lower body pressure (p>0.05). As expected, whole-body heating increased esophageal and mean skin temperatures during moderate (T_{es}: 37.54 ± 0.04°C, T_{sk}: 35.86 ± 0.11°C) and high (T_{es}: 38.28 ± 0.04°C, T_{sk}: 36.84 ± 0.08°C) heat stress conditions. Under both moderate and high heat stress conditions, esophageal temperature did not change from its elevated level throughout the experimental protocol (p>0.05). Furthermore, mean skin temperature did not change during isometric handgrip exercise for all pressure conditions (p>0.05). However, the application of **LBNP** (**MHS**: 35.06 ± 0.11°C, **HHS**: 35.96 ± 0.11°C) and **LBPP** (**MHS**: 35.07 ± 0.15°C, **HHS**: 36.16 ± 0.09°C) resulted in a significant decrease in mean skin temperature ($p \leq 0.05$). Although mean skin temperature returned toward baseline values during the recovery periods of the moderate and high heat stress conditions, they remained lower than baseline rest ($p \leq 0.05$)

following the application of **LBNP** (**MHS**: 35.18 ± 0.10°C, **HHS**: 36.19 ± 0.07°C) and **LBPP** (**MHS**: 35.17 ± 0.13°C, **HHS**: 36.30 ± 0.07°C).

Sweat rate and cutaneous vascular conductance

Figure 2 depicts sweat rate and cutaneous vascular conductance for all pressure conditions at each level of heat stress. Baseline resting responses for each of the three levels of heat stress were similar across all pressure conditions.

No differences across time ($p=0.087$) and between pressure conditions ($p=0.286$) were observed in sweat rate under no heat stress. Under moderate heat stress, sweat rate for all pressure conditions was significantly elevated ($p\leq0.05$) above baseline resting during isometric handgrip exercise and the subsequent period of post-exercise ischemia. The magnitude of increase was similar for all pressure conditions ($p>0.05$). During recovery, sweat rate returned to resting levels ($p>0.05$). A similar pattern of response was observed under high heat stress.

No differences across time ($p=0.420$) and between pressure conditions ($p=0.088$) were observed in cutaneous vascular conductance during no heat stress. During moderate heat stress, a significant reduction in cutaneous vascular conductance below baseline resting was observed during isometric handgrip exercise ($p\leq0.05$) and the magnitude of reduction was similar between pressure conditions ($p>0.05$). Cutaneous vascular conductance remained significantly reduced below baseline resting during post-exercise ischemia for **CON** and **LBNP** ($p\leq0.05$), whereas it returned to baseline resting levels for **LBPP** ($p=0.334$). However, cutaneous vascular conductance did not differ between pressure conditions ($p>0.05$). During the recovery period, cutaneous vascular

conductance gradually returned towards baseline values, but remained reduced during the **LBNP** condition ($p=0.015$), while it returned to resting levels for **CON** and **LBPP** ($p>0.05$).

During high heat stress, a reduction in cutaneous vascular conductance below baseline resting values was observed during isometric handgrip exercise for all pressure conditions ($p\leq0.05$) and the magnitude of reduction was similar between pressure conditions ($p>0.05$). During post-exercise ischemia, cutaneous vascular conductance remained attenuated for **CON** and **LBNP** ($p\leq0.05$), albeit the magnitude of reduction was greater in **LBNP** compared to **CON** ($p=0.039$). In contrast, cutaneous vascular conductance increased to levels significantly above baseline resting levels during **LBPP** ($p=0.034$), being significantly different than **CON** ($p=0.011$). Cutaneous vascular conductance gradually returned towards baseline values following the application of lower body pressure, but remained significantly reduced for the **LBNP** condition ($p=0.007$), whereas it returned to baseline resting for **LBPP** and **CON** ($p>0.05$).

DISCUSSION

This is the first study to examine the role of cardiopulmonary and arterial baroreflex activity on the metaboreflex modulation of heat loss responses under a steady state condition of increasing levels of heat stress. A key finding of this study is that, contrary to our hypothesis, baroreflex activity did not influence sweat rate during the activation of the metaboreflex under both moderate and high heat stress. In contrast, we show that baroreflex activity can modulate the metaboreflex-induced reductions in cutaneous vascular conductance, albeit this was only evidenced during high heat stress. These results suggest that changes in cutaneous vascular conductance during post-IHG exercise ischemia are modulated by both metaboreceptors and baroreceptors, while changes in sweat rate are solely mediated by the activation of muscle metaboreceptors. Furthermore, we show that metabo- and baroreflex activity can influence heat loss responses at elevated levels of heat stress.

As expected from previous studies (3, 30), sweat rate and cutaneous vascular conductance did not change throughout the experimental protocol without heat stress, confirming that isometric handgrip exercise and post-exercise ischemia does not influence heat loss responses under normothermic conditions. Previous studies show that under moderate levels of heat stress (<0.8°C), sweat rate increases above baseline rest during isometric handgrip exercise (4, 19) and remains elevated during the period of post-exercise ischemia (3, 18, 30). In contrast, cutaneous vascular conductance decreases during isometric handgrip exercise and remains reduced during the subsequent period of ischemia (3, 4). Only when the occlusion is removed do both sweat rate and cutaneous vascular conductance return to baseline values. During the isometric handgrip exercise

period, it is believed that the increase in sweat rate and reduction of cutaneous vascular conductance is attributed to the activation of metaboreceptors, mechanoreceptors and central command (18, 32, 33, 35), whereas metaboreceptors are solely responsible for the elevated levels of sweat rate and reduction in cutaneous vascular conductance during the ischemia period (3, 18, 30). However, the accumulation of muscle metabolites during the period of ischemia triggers chemosensitive afferents which induce a reflex rise in mean arterial pressure (26). As such, post-exercise ischemia not only activates the metaboreflex, but also the baroreflex through changes in mean arterial pressure. Prior to the current study, it was unclear to what extent baroreflex activity modulated the previously observed alterations in sweat rate and cutaneous vascular conductance during post-exercise ischemia, and if such interactions would be evidenced under high heat stress conditions (>0.8°C).

Shibasaki et al., (30) examined the influence of arterial baroreflex activity on sweat rate during the activation of the metaboreflex, by having subjects perform the isometric handgrip exercise paradigm under moderate levels of heat stress (increase in sublingual temperature of ~0.5°C). During the post-exercise ischemia period, arterial baroreflex activity was removed through a bolus injection of sodium nitroprusside (5). Despite eliminating the reflex increase in mean arterial pressure during the ischemia period, sweat rate nonetheless remained elevated, suggesting that arterial baroreflex activity did not influence the metaboreflex induced increases in sweating during the ischemia period. Our results support the findings of Shibasaki et al., (30) and extend them by showing that altering the influence of both arterial and cardiopulmonary baroreceptors, through the application of lower body negative/positive pressure (6, 27-

29), does not alter the metaboreflex modulation of sweat rate. As such, a role for baroreflex activity in modulating the elevations in sweat rate during the ischemia period can be ruled out.

Previous studies have suggested that the contribution of nonthermal factors in modulating heat loss responses is attenuated with progressive increases in hyperthermia (7, 16, 17). Specifically, Kondo et al., (17) demonstrated that increases in sweat rate during isometric handgrip exercise became significantly attenuated with moderate increases in core temperature (~0.3°C). These findings suggest that the contribution of central command, baroreceptors, mechanoreceptors, and metaboreceptors, all of which are activated during isometric handgrip exercise, is reduced as core temperature increases. Similarly, in the current study, increases in sweat rate during the isometric handgrip exercise period of the control condition at high levels of heat stress (+0.04 $mg \cdot min^{-1} \cdot cm^{2}$) were relatively less than those observed at moderate levels of heat stress (+0.09 $mg \cdot min^{-1} \cdot cm^{2}$), which supports the observations of Kondo et al., (17) and extends them to high levels of thermal stress (~1.4°C). In contrast, however, elevations in sweat rate during the post-exercise ischemia period did not differ between the moderate (+0.04 $mg \cdot min^{-1} \cdot cm^{2}$) and high (+0.03 $mg \cdot min^{-1} \cdot cm^{2}$) heat stress conditions. These results suggest that the metaboreflex maintains the ability to modulate sweat rate as core temperature increases, but only when its influence is isolated from other nonthermal factors. Future studies are warranted to examine the functional importance of these diverging responses.

The current results also demonstrate that the metaboreflex attenuation of cutaneous vascular conductance is evidenced to a greater extent when the competing influence of baroreflex activity is removed (i.e., application of **LBNP**) during high levels of heat stress (>0.8°C increase in core temperature). Additionally, when the cardiopulmonary and arterial baroreflexes were loaded to a greater extent with the application of lower body positive pressure, an increase in cutaneous vascular conductance was observed during the ischemia period, indicating that baroreflex activity can override the attenuation of cutaneous vascular conductance observed during the activation of muscle metaboreceptors. This is in contrast to moderate heat stress, during which the application of lower body negative and positive pressure during post-exercise ischemia did not alter the cutaneous vascular conductance response, although it is clear that lower body negative and positive pressure created a diverging cutaneous vascular conductance response (see Fig. 2). These results suggest that the modulation of cutaneous vascular conductance by the metaboreflex may be of greater importance than previously evidenced (3) at low to moderate levels of heat stress (<0.8°C increase in core temperature).

Limitations

In the present study, a short duration but high intensity isometric handgrip exercise model was employed. To our knowledge, previous studies have typically used a longer duration (2-3 minutes) and lower intensity (30-45%) isometric handgrip exercise paradigm (3, 4, 18, 19, 30). Therefore, it is unknown if the same pattern of response would be observed at lower intensities and longer durations. During moderate and high

heat stress, we observed a reduction in mean skin temperature during the period of ischemia and recovery with the application of both lower body negative and positive pressure. As such, the reduction in cutaneous vascular conductance observed during the application of **LBNP** may be attributed to air movement across the lower legs in the sealed pressure box. However, we observed an increase in cutaneous vascular conductance with similar reductions in mean skin temperature during the application of **LBPP**. Therefore, we do not believe that reductions in mean skin temperature influenced the cutaneous vascular conductance response.

CONCLUSION

We show that the concurrent application of lower body negative or positive pressure does not alter the sustained elevations in sweat rate typically associated with post-exercise ischemia both under moderate and high heat stress conditions. These results confirm a muscle metaboreceptor modulation of sweat rate which is not influenced by cardiopulmonary and arterial baroreflex activity. In contrast, both lower body negative and positive pressure altered the cutaneous vascular conductance response associated with post-exercise ischemia, but only under high heat stress conditions. As such, our results demonstrate that cardiopulmonary and arterial baroreflex activity influences the metaboreflex modulation of cutaneous vascular conductance, but not sweat rate during heat stress.

ACKNOWLEDGEMENTS

The authors wish to thank all the participants who volunteered for the present study.

GRANTS

This study was supported by the Natural Sciences and Engineering Research Council (RGPIN-298159-2009, held by Dr. Kenny) and Leaders Opportunity Fund from the Canada Foundation for Innovation (22529, held by Dr. Kenny). Dr. Kenny is supported by a University of Ottawa Research Chair in Environmental Physiology. Mr. Konrad Binder (MSc) was supported by a Natural Sciences and Engineering Research Council Graduate Fellowship.

DISCLOSURES

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

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Table 1. Mean (±SE) baseline values for three thermal conditions; no heat stress (**NHS**), moderate heat stress (**MHS**), high heat stress (**HHS**) and pressure conditions; no pressure (**CON**), lower body positive pressure (**LBPP**), or lower body negative pressure (**LBNP**).

Thermal Condition	Pressure Condition	MAP (mmHg)	HR (beats/min)	CVC (% of max)	SR ($mg \cdot min^{-1} \cdot cm^{2}$)
	CON	90(3)	66(3)	13(1)	0.06(0.01)
NHS	**LBPP**	89(2)	65(3)	13(1)	0.07(0.01)
	LBNP	89(3)	66(3)	13(1)	0.06(0.01)
	CON	87(2)	100(4)	40(3)	0.86(0.07)
MHS	**LBPP**	86(2)	100(4)	39(2)	0.89(0.07)
	LBNP	87(3)	101(4)	39(2)	0.89(0.10)
	CON	86(2)	121(4)	49(5)	1.32(0.07)
HHS	**LBPP**	86(2)	118(4)	50(4)	1.30(0.08)
	LBNP	87(2)	121(4)	50(4)	1.31(0.09)

Table 2. Mean (±SE) relative changes from baseline for three pressure conditions; no pressure (**CON**), lower body positive pressure (**LBPP**), and lower body negative pressure (**LBNP**) during an isometric handgrip exercise protocol.

	Pressure Condition	IHG Exercise	Post-exercise ischemia	Recovery
No Heat Stress				
MAP (mmHg)	**CON**	+35(3)*	+16(1)*	+1(0)
	LBPP	+33(3)*	+26(2)*	+1(1)
	LBNP	+35(3)*	+10(2)*	0(1)
HR (beats/min)	**CON**	+33(4)*	-1(2)	-2(1)
	LBPP	+36(4)*	-8(2)*	-1(1)
	LBNP	+37(4)*	+6(3)*	-2(2)
CVC (% of max)	**CON**	0(0)	0(0)	0(0)
	LBPP	0(0)	-1(1)	-1(0)
	LBNP	0(0)	-1(1)	0(1)
SR ($mg \cdot min^{-1} \cdot cm^2$)	**CON**	0.00(0.00)	0.00(0.00)	0.00(0.00)
	LBPP	0.00(0.00)	0.00(0.00)	0.00(0.00)
	LBNP	0.00(0.00)	0.00(0.00)	0.00(0.00)
Moderate Heat Stress				
MAP (mmHg)	**CON**	+28(2)*	+14(1)*	+1(1)
	LBPP	+29(2)*	+22(2)*	0(1)
	LBNP	+28(3)*	+4(2)*	+1(1)
HR (beats/min)	**CON**	+25(3)*	-2(3)	-1(2)
	LBPP	+27(2)*	-17(2)*	-1(1)
	LBNP	+24(3)*	+11(1)*	-3(2)
CVC (% of max)	**CON**	-6(1)*	-4(1)*	-1(1)
	LBPP	-5(1)*	+1(1)	-2(1)
	LBNP	-5(1)*	-9(1)*	-4(2)*
SR ($mg \cdot min^{-1} \cdot cm^2$)	**CON**	+0.09(0.02)*	+0.04(0.01)*	+0.01(0.02)
	LBPP	+0.07(0.01)*	+0.05(0.02)*	-0.01(0.01)
	LBNP	+0.08(0.02)*	+0.03(0.01)*	-0.03(0.02)
High Heat Stress				
MAP (mmHg)	**CON**	+18(1)*	+10(1)*	0(1)
	LBPP	+19(2)*	+20(1)*	-1(1)
	LBNP	+17(1)*	-2(2)	-2(2)
HR (beats/min)	**CON**	+22(3)*	+2(2)	+1(2)
	LBPP	+26(3)*	-14(2)*	-1(1)
	LBNP	+23(3)*	+15(2)*	-3(3)
CVC (% of max)	**CON**	-5(1)*	-2(0)*	0(1)
	LBPP	-5(1)*	+7(3)*	-1(1)
	LBNP	-4(1)*	-10(2)*	-5(2) *
SR ($mg \cdot min^{-1} \cdot cm^2$)	**CON**	+0.04(0.01)*	+0.03(0.00)*	-0.01(0.01)
	LBPP	+0.05(0.01)*	+0.03(0.01)*	+0.01(0.01)
	LBNP	+0.05(0.02)*	+0.02(0.01)*	0.00(0.00)

MAP, mean arterial pressure; HR, heart rate; CVC, cutaneous vascular conductance; SR, sweat rate. *denotes significantly different from baseline rest, $p \leq 0.05$.

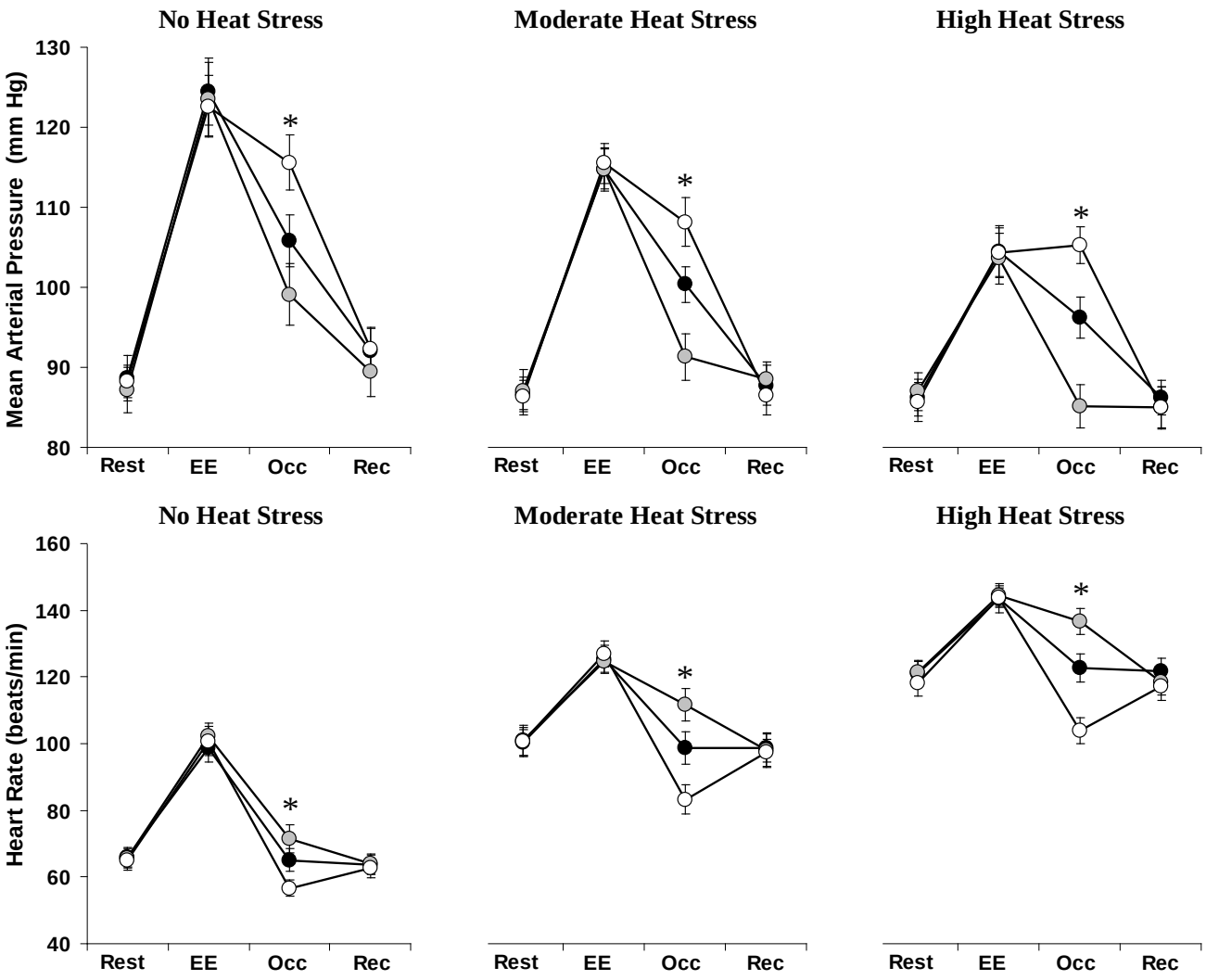

Figure 1. Mean (±SE) arterial pressure and heart rate at Rest, End-Exercise (EE), Occlusion (Occ) and Recovery (Rec) during control (**CON** ●), the application of **LBNP** (●) and **LBPP** (○) without heat stress (**NHS**, left panel), during moderate (**MHS**, centre panel) and high (**HHS**, right panel) heat stress. ***** denotes **LBPP** and **LBNP** are significantly different from **CON** ($p \leq 0.05$).

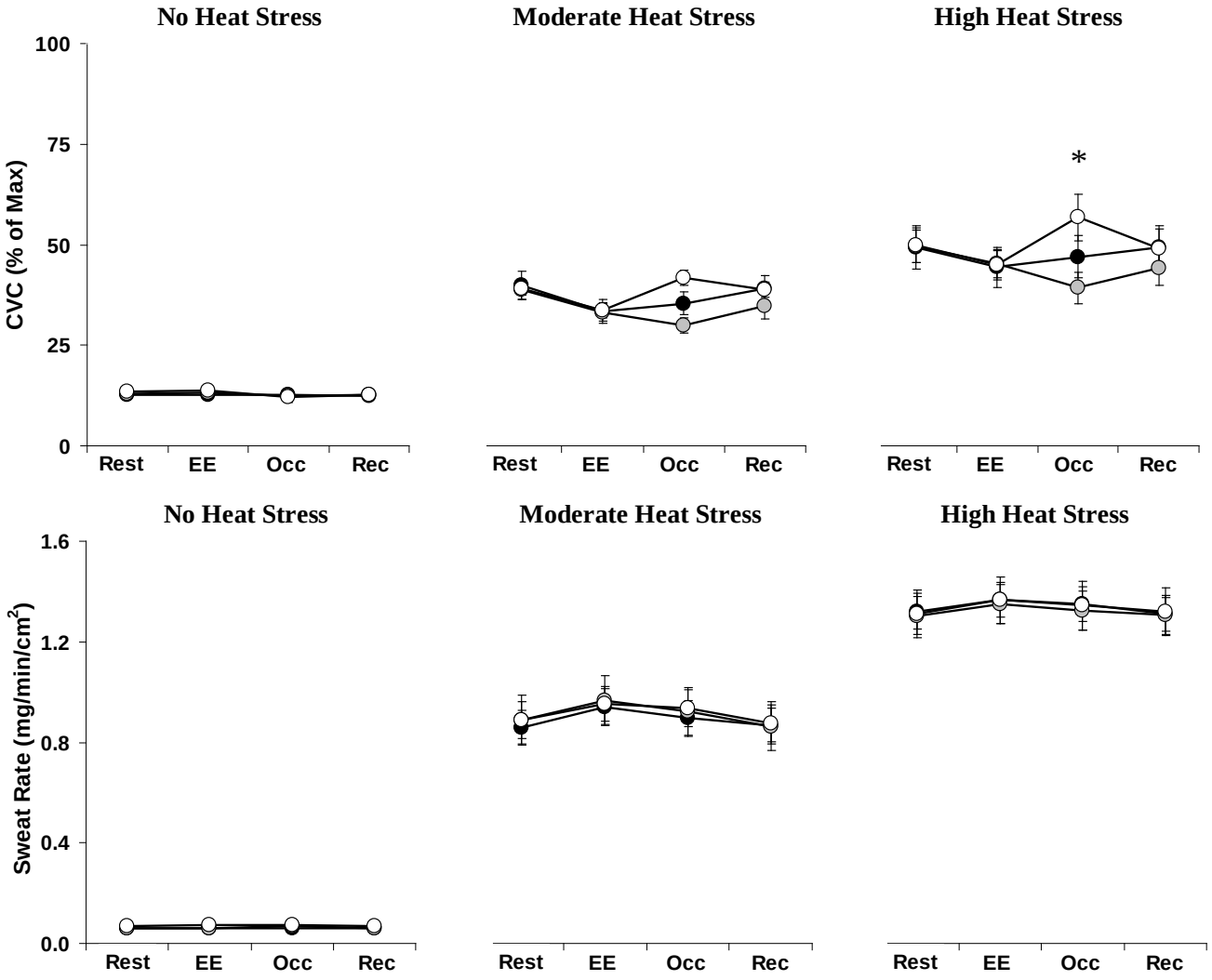

Figure 2. Mean (±SE) cutaneous vascular conductance and sweat rate at Rest, End-Exercise (EE), Occlusion (Occ) and Recovery (Rec) during control (**CON** ●), the application of **LBNP** (●) and **LBPP** (○) without heat stress (**NHS**, left panel), during moderate (**MHS**, centre panel) and high (**HHS**, right panel) heat stress. ***** denotes **LBPP** and **LBNP** are significantly different from **CON** ($p \leq 0.05$).

ARTICLE II:

Heat stress attenuates the increases in arterial blood pressure during isometric handgrip exercise

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Key words: Cardiovascular; Hyperthermia; Thermoregulation; Mean Arterial Pressure; Vascular Resistance

Paper submitted to European Journal of Applied Physiology (EJAP-D-11-00903)

ABSTRACT

The mechanisms by which heat stress impairs the control of blood pressure are not thoroughly understood. The purpose of this study was to examine blood pressure responses during a sympathoexcitatory stimulus performed at increasing levels of heat stress. Twelve male subjects performed 1-min of isometric handgrip (IHG) exercise at 60% of maximal voluntary contraction under no heat stress (NHS). On separate days, trials were repeated under heat stress conditions of 0.6ºC (moderate heat stress, MHS) and 1.4ºC (high heat stress, HHS) increases in esophageal temperature. For all conditions, IHG exercise significantly elevated mean arterial pressure (MAP) above baseline, albeit there was less of an increase in MAP during MHS (115±2 mmHg) and HHS (105±3 mmHg) relative to NHS (124±4 mmHg). Furthermore, the attenuated increase in MAP was greater during HHS relative to MHS ($p<0.05$). In contrast, cardiac output increased to similar extents (NHS: 7.4±0.7, MHS: 7.9±0.3, HHS: 8.6±0.5 L/min) during IHG exercise for all temperature conditions ($p>0.05$). IHG exercise elevated total peripheral resistance above baseline rest for NHS (18±2 mmHg/L/min), while a lower non-sginificant increase in total peripheral resistance was observed during MHS (15±1 mmHg/L/min, $p=0.395$). During HHS, however, the increase in peripheral resistance (13±1 mmHg/L/min) was significantly blunted compared to both NHS ($p=0.015$) and MHS ($p=0.041$) during IHG exercise. Our findings show that heat stress attenuates the increase in arterial blood pressure during a sympathoexcitatory stimulus and this attenuation is vascular resistant dependent, since cardiac output increased to similar levels for all temperature conditions.

INTRODUCTION

It is well known that the human body's ability to regulate arterial blood pressure is compromised during combined heat stress and orthostatic challenge. In theory, the regulation of blood pressure results from the interaction between cardiac output and peripheral vascular resistance (Rowell *et al.*, 1969). As such, a compromised blood pressure response during combined heat and orthostatic stress could result from either an inadequate cardiac output and/or vascular resistance response.

During heat stress in the absence of orthostatic stress, decreases in peripheral vascular resistance due to skin vasodilation are adequately offset by increases in cardiac output such that mean arterial pressure (MAP) is well maintained (Rowell *et al.*, 1969). However, during the superimposition of an orthostatic challenge, decreases in cardiac output and a lack of increase in vascular resistance combine to create substantial reductions in MAP (Johnson & Proppe, 1996; Crandall, 2000). Since both cardiac output and vascular resistance respond to the orthostatic challenge, it is difficult to isolate which mechanism is predominantly responsible for the altered regulation of blood pressure.

In contrast to orthostatic challenges which cause decreases in arterial blood pressure (e.g. lower body negative pressure, head-up tilt, etc.), sympathoexcitatory stimuli typically cause elevations in arterial pressure (e.g. mental stress, cold pressor test, etc.). Recently, Cui et al. (2010) used the cold pressor test (CPT) to isolate the contribution of the vascular response to the regulation of blood pressure during heat stress. The CPT causes increases in arterial blood pressure primarily through elevations in vascular resistance, with little to no effect on cardiac output, stroke volume and heart rate (Vojacek *et al.*, 1982). Cui et al. (2010) observed that heat stress minimizes the

increase in arterial blood pressure in response to the CPT due to an attenuated increase in vascular resistance. Although their results provide evidence that an altered vascular response provides a significant contribution to altered blood pressure regulation during heat stress, it remains to be determined how this response is manifested when cardiac output is implicated. One sympathoexcitatory stimulus which can elicit increases in arterial blood pressure through both increases in vascular resistance and cardiac output is isometric handgrip (IHG) exercise (Lind, 1983).

Similarly to the CPT, IHG exercise performed without heat stress has been shown to evoke increases in mean arterial pressure (MAP), muscle sympathetic nerve activity and vascular resistance (Lind, 1983). However, it also stimulates increases in heart rate, and therefore cardiac output (Nishiyasu *et al.*, 1994). Therefore, IHG exercise provides a simple sympathoexcitatory stimulus to examine the relationship between cardiac output and vascular resistance in the regulation of arterial blood pressure during heat stress. To our knowledge, however, no study has specifically examined the cardiovascular responses to IHG exercise while heat stressed.

The present study examined the cardiovascular responses during IHG exercise performed at 60% maximal voluntary contraction (MVC) during increasing levels of heat stress (i.e., normothermia and at 0.6°C and 1.4°C increase in esophageal temperature above resting levels). We hypothesized that increases in MAP typically caused by IHG exercise at 60% MVC would be attenuated as a function of the level of heat stress. Furthermore, we hypothesized that this attenuated increase in MAP would be the result of a reduced increase in peripheral resistance, as opposed to a reduced increase in cardiac output.

METHODS

The current data were collected as part of a larger study examining the interaction between metabo- and baroreflex activity on sweating and skin blood flow during post isometric handgrip exercise ischemia. In contrast, the present study examines the cardiovascular responses during isometric handgrip exercise during increasing levels of heat stress.

Ethical approval

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

Participants

Twelve healthy (no history of respiratory, metabolic or cardiovascular disease, non-smoking and normotensive) and physically active (exercised 3-5 times per week for a minimum of 30 min) males volunteered for this study. Mean (± standard deviation) characteristics of the subjects were: age, 24 ± 7 yrs; height, 180 ± 6 cm; weight, 79 ± 10 kg.

Experimental Design

On the day prior to each experimental test session, subjects were instructed to abstain from caffeine, alcohol and strenuous physical activity for 24 hours. Subjects were asked to arrive at the laboratory after eating a light meal. For each subject, trials were

performed at the same time of day to avoid circadian variations in core temperature and all trials were separated by a minimum of 24 hours.

During the no heat stress (**NHS**) experimental trial, subjects were asked to dress in shorts and sit in an environmental chamber which was maintained at a temperature of 24°C and a relative humidity of 20% while monitoring instruments were attached. During this period, subjects were asked to perform two brief maximal voluntary contractions with their dominant hand using a handgrip dynamometer. The maximal value obtained was used to calculate the relative workload (60% MVC) to be performed during the experimental trials. After all instruments were attached, subjects were asked to sit in an upright seated posture and baseline data were then recorded for five minutes at rest. Following the baseline rest period, subjects were then asked to perform 60 seconds of IHG exercise at 60% MVC. To ensure the desired force was obtained, a visual feedback system was used throughout the exercise period.

On two different days, subjects repeated the experimental protocol under different levels of thermal stress: moderate heat stress (**MHS**), equal to an esophageal temperature (T_{es}) increase of 0.6°C, and high heat stress (**HHS**), equal to a T_{es} increase of 1.4°C. To increase and maintain esophageal temperature, room temperature was increased to 37°C after the instrumentation perio, subjects were dressed in a high density water-perfusion suit (Delta Temax Inc., Pembroke, ON, CAN), which was perfused with 48°C water. The perfusion suit covered the entire body surface except for the face and left forearm where the laser-Doppler flow probe was located. Upon reaching the desired core temperature, the water bath temperature was reduced to 43°C to maintain a steady state body core

temperature. Once a steady state temperature was achieved and maintained for at least 10 min, baseline resting data was then collected for a 5 minute period.

Measurements

Esophageal temperature was monitored continuously using a pediatric esophageal temperature probe (Mon-a-therm®, Mallinckrodt Medical, St-Louis, USA) inserted through the nose to a depth of forty centimeters past the entrance of the nostril. Skin temperature was measured at nine sites on the body: head 7%, upper back 9.5%, chest 9.5%, lower back 9.5%, abdomen 9.5%, bicep 10%, quadriceps 19%, hamstring 10%, front calf 16% to subsequently calculate mean skin temperature according to the method of Hardy and Dubois (1938). Esophageal temperature and skin temperature data were collected using a data acquisition module (HP Agilent model 3497A) at a sampling rate of 15 seconds and simultaneously displayed and recorded in spreadsheet format on a personal computer with LabVIEW software (Version 7.0, National Instruments, TX, USA).

Forearm skin blood flow (SkBF) was estimated using laser-Doppler velocimetry (PeriFlux System 5000, Perimed AB, Stockholm, Sweden) on the left mid-anterior forearm. Prior to the start of the experimental trial, the laser-Doppler flow probe (PR 401 Angled Probe, Perimed AB, Stockholm, Sweden) was affixed with an adhesive ring to the forearm in a site without superficial veins that demonstrated pulsatile activity. The probe was not moved until the end of the experimental trial. A maximum SkBF test was performed at the end of the experimental session by locally heating the SkBF site to 42°C for 20 min and then increasing local temperature to 44°C for an additional 25 min

(Charkoudian, 2003). Cutaneous vascular conductance (CVC) was subsequently calculated as laser-Doppler velocimetry output in arbitrary perfusion units divided by MAP and expressed as a percentage of maximum.

During the entire experimental trial, a Finometer (Finapres Medical Systems, Amsterdam, Netherlands) was used to measure MAP from the beat-to-beat recording of the right middle finger arterial pressure waveform with the volume-clamp method (Penaz, 1973) and physiocal criteria (Wesseling *et al.*, 1995). Prior to the start of each measurement period, brachial artery pressure reconstruction (Gizdulich *et al.*, 1996; Gizdulich *et al.*, 1997) was calibrated with an upper arm return-to-flow systolic pressure detection (Bos *et al.*, 1996). Furthermore, the right arm was supported at heart level, and a level calibration was performed. The Finometer finger arterial pressure waveform was also used to obtain an estimation of cardiac output using the modelflow method (Wesseling KH, 1993). Total peripheral resistance was subsequently calculated as the quotient of MAP and cardiac output.

Heart rate was monitored using a Polar coded transmitter, recorded continuously and stored with a Polar Advantage interface and Polar Precision Performance software (Polar Electro, Oy, Finland). Stroke volume was calculated as the quotient of cardiac output and heart rate.

Statistical Analysis

All dependent variables were averaged over the following time periods: a 15 second pre-exercise period (baseline; **Rest**), the final 15 seconds of IHG exercise (end-

exercise; **EE**), and the final 15 seconds of recovery (recovery; **Rec**) for each heat stress condition. A two-way analysis of variance was performed to compare the dependent variables at each time point (**Rest**, **EE**, **Rec**) between the three different heat stress conditions (**NHS**, **MHS**, and **HHS**). The p value for significance was set at ≤ 0.05. Post-hoc comparisons were carried out using paired samples T-test. All analyses were performed using the statistical software package SPSS 18.0 for Windows (SPSS Inc. Chicago, IL, USA). All results are presented as means ± standard error, unless otherwise indicated.

RESULTS

IHG exercise during no heat stress

Under NHS, IHG exercise significantly increased ($p \leq 0.05$) MAP, cardiac output, total peripheral resistance and heart rate above baseline resting, whereas stroke volume was significantly reduced ($p \leq 0.05$) . Furthermore, IHG exercise did not change CVC during NHS ($p > 0.05$).

Passive Heat Stress

Prior to performing IHG exercise, whole-body heating significantly elevated esophageal temperature ($p \leq 0.05$) above NHS for MHS (0.6 ± 0.04°C). Furthermore, under HHS, esophageal temperature (1.4 ± 0.06°C) was significantly greater than both NHS and MHS ($p \leq 0.05$). Within each heat stress condition esophageal temperature remained unchanged from resting levels during the entire experimental protocol ($p > 0.05$). Whole-body heating also elevated ($p \leq 0.05$) mean skin temperature above no heating during MHS (3.19±0.1°C), while mean skin temperature was further elevated ($p \leq 0.05$) above NHS and MHS during HHS (3.42 ± 0.08°C).

Prior to performing IHG exercise, whole-body heating increased cardiac output and heart rate above NHS for both MHS and HHS ($p \leq 0.05$). Furthermore, during HHS, heart rate was further increased above MHS ($p \leq 0.05$), whereas there were no differences in cardiac output ($p > 0.05$). Additionally, heat stress decreased total peripheral resistance and stroke volume below NHS ($p \leq 0.05$) to a similar extent for both MHS and HHS conditions. Consequently, there were no significant differences in MAP between all

conditions ($p>0.05$). Finally, under MHS and HHS CVC was significantly elevated above NHS and a further increase was observed in CVC from MHS to HHS (all $p\leq0.05$).

IHG exercise during moderate and high heat stress

IHG exercise significantly elevated MAP, cardiac output and heart rate during both moderate and HHS conditions ($p\leq0.05$). However, the magnitude of increase in MAP during IHG exercise was reduced for both MHS and HHS compared to NHS ($p\leq0.05$), with the greatest attenuation occurring during HHS ($p\leq0.05$) (Figure 1). In contrast, the relative increase in cardiac output and heart rate during IHG did not differ between the three heat stress conditions ($p>0.05$). During MHS, IHG exercise significantly elevated total peripheral resistance ($p\leq0.05$), albeit the magnitude of increase was less than in the NHS condition (Figure 1). During HHS, total peripheral resistance did not change from baseline rest during IHG exercise ($p=0.404$). Furthermore, stroke volume was reduced during IHG exercise during MHS, albeit this reduction was not significant ($p=0.178$) (Figure 2). Under HHS, stroke volume did not change from baseline rest during IHG exercise ($p>0.05$). Finally, during MHS and HHS, IHG exercise significantly reduced CVC below baseline resting ($p\leq0.05$) (Figure 2).

DISCUSSION

This is the first study to examine the influence of whole-body heating on cardiovascular responses during IHG exercise performed at 60% MVC. The main finding of this study is that whole-body heating attenuates the increase in MAP during IHG proportionately to the level of thermal stress. Furthermore, we show that the attenuated increase in MAP is the result of a reduced increase in peripheral resistance rather than a reduced cardiac output response. These findings support our hypothesis and suggest that an altered peripheral vascular response is the main component of compromised blood pressure regulation during heat stress.

In the absence of heat stress, IHG exercise increased MAP, cardiac output and total peripheral resistance. Consistent with previous studies (Lind, 1983), the increase in cardiac output was solely the result of an increase in heart rate, since reductions in stroke volume were observed. In addition, we observed an increase in peripheral resistance, indicating peripheral vasoconstriction (Lind, 1983). As expected, we did not observe any reductions in CVC during exercise (Crandall *et al.*, 1995), such that forearm skin does not contribute to peripheral vasoconstriction without heat stress. As such, both elevations in cardiac output (through heart rate) and peripheral resistance contribute to the elevations in MAP observed during IHG exercise in the absence of heat stress.

During whole-body heating, active cutaneous vasodilation serves to increase skin blood flow in an attempt to dissipate heat for the regulation of body temperature. To accommodate the increase in skin blood flow and subsequent decrease in peripheral vascular resistance, cardiac output increases mainly through elevations in heart rate with little or no change in stroke volume (Johnson & Proppe, 1996). The net effect is

maintenance of MAP near resting values (Rowell, 1974). In the current study, the increase in MAP during IHG exercise was significantly attenuated during both the moderate and high heat stress conditions. This reduced increase in arterial blood pressure was solely the result of an attenuated increase in peripheral resistance, since elevations in cardiac output from baseline rest were similar between conditions. Interestingly, the graded attenuation of MAP increase during IHG exercise was paralleled by a gradual inability of the peripheral vasculature to respond to the IHG stimulus. Since the majority of circulating blood flow during heat stress is directed towards the skin (Rowell *et al.*, 1969), the lack of increase in peripheral vascular resistance is likely attributed to an inability of the skin to vasoconstrict during heat stress (Johnson & Proppe, 1996). Although CVC decreased during IHG for both the moderate and high heat stress conditions, these reductions were not sufficient to elevate peripheral vascular resistance in order to increase MAP to levels observed without heat stress (Crandall *et al.*, 2010). In fact, peripheral vascular resistance did not change from pre-exercise values during the high heat stress condition, suggesting that the ability of the skin to vasoconstrict may become absent at high levels of heat stress.

Previous work has shown that the responsiveness of the skin vasculature to a vasoconstriction stimulus is greatly reduced during whole body heating (Wilson *et al.*, 2002; Shibasaki *et al.*, 2007; Shibasaki *et al.*, 2008), suggesting that vasoconstriction may be attenuated by the active vasodilator system during heat stress. However, during a whole-body cold stress test, reductions in skin perfusion, is controlled by the sympathetic adrenergic system (Johnson & Proppe, 1996; Kellogg, 2006), whereas during heat stress, the active vasodilator system mediates 90-95% of the increase in skin blood flow

(Johnson & Proppe, 1996; Kellogg, 2006; Shibasaki *et al.*, 2008). Recent research has shown that the responsiveness of the sympathetic adrenergic system can be modulated by factors associated with the cutaneous active vasodilator system (Shibasaki *et al.*, 2007). Notably, Shibasaki *et al.* (2008), demonstrated that nitric oxide is capable of attenuating the cutaneous vasoconstrictor response to norepinephrine (the key neurotransmitter to control vasoconstriction). As such, the inability of the skin to sufficiently vasoconstrict during increasing levels of whole-body heating resulted in a blunted increase in peripheral resistance in the current study, thereby attenuating the increase in arterial pressure during a sympathoexcitatory stimulus. In addition to the present work, a recent study by Cui *et al.* (2010) reported that whole-body heating (~0.7°C) attenuated the increase in arterial blood pressure during the CPT. They concluded that the attenuation of arterial blood pressure during heat stress was due to a reduction in vascular resistance, since the CPT has little effect on heart rate and therefore cardiac output. In contrast, we examined which component, cardiac output and/or peripheral vascular response is responsible for the reduced increase in MAP during a sympathoexcitatory stimulus under increasing levels of heat stress. From the findings of the present study, we show that the reduced increase in arterial blood pressure from a sympathoexcitatory stimulus during heat stress is primarily vascular dependent, since cardiac output increased to similar levels during IHG exercise under both heat stress conditions. These observations provide a greater insight into the mechanisms responsible for the control of arterial blood pressure during heat stress by highlighting the importance of vascular resistance in maintaining blood pressure during a sympathoexcitatory stimulus.

Limitations

In the present study, one concern is whether the IHG stimulus was equivalent between all temperature conditions. To help overcome this possible limitation, participants were instructed to maintain the desired force through the aid of visual feedback on the handgrip dynamometer. By using visual feedback, participants maintained the desired force throughout the exercise period. Another possible limitation to the present study is the use of the finometer modelflow technique to estimate cardiac output during heat stress. Previous work suggests that the finometer flow technique has been shown to underestimate cardiac output. However, it is important to note that the assessment cardiac output using the modelflow method by Shibasaki et al. (2011) is specific to the conditions employed in their study, namely passive heating and subsequent lower body negative pressure in the supine posture. In contrast we report cardiac output as the relative change from baseline during IHG exercise for each temperature condition while sitting in the upright posture.

CONCLUSION

In conclusion, the present data demonstrates that heat stress attenuates the increase in arterial blood pressure in response to a sympathoexcitatory stimulus (i.e., IHG exercise) and this attenuation is vascular dependent, since cardiac output increased to similar levels for all temperature conditions during exercise. Furthermore, higher levels of heat stress can further reduce the observed increase in mean arterial blood pressure during IHG exercise and this is attributed to a blunted increase in peripheral resistance when heat stressed.

ACKNOWLEDGEMENTS

The authors wish to thank all the participants who volunteered for the present study. This study was supported by the Natural Sciences and Engineering Research Council (RGPIN-298159-2004 and RGPIN-298159-2009, held by Dr. Kenny) and Leaders Opportunity Fund from the Canada Foundation for Innovation (22529, held by Dr. Kenny). Dr. Kenny is supported by a University of Ottawa Research Chair in Environmental Physiology. Mr. Konrad Binder (MSc) was supported by a Natural Sciences and Engineering Research Council Graduate Fellowship.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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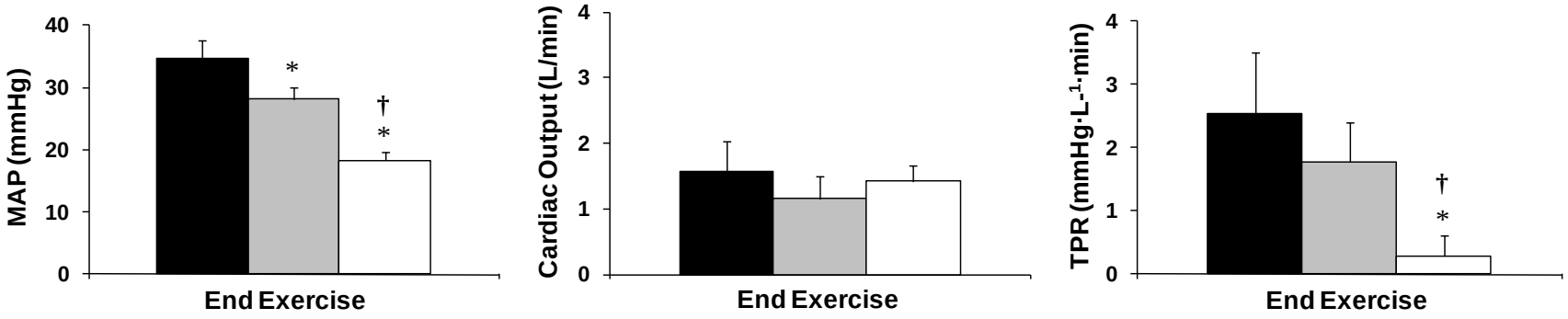

Fig. 1 Mean (±SE) relative changes from baseline in mean arterial pressure (MAP, left panel), cardiac output (middle panel) and total peripheral resistance (TPR, right panel) at End-Exercise (EE) during no heat stress (NHS, ■), moderate heat stress (MHS, ■) and high heat stress (HHS, □). * denotes significantly different from NHS ($p \leq 0.05$). † denotes significantly different from MHS ($p \leq 0.05$)

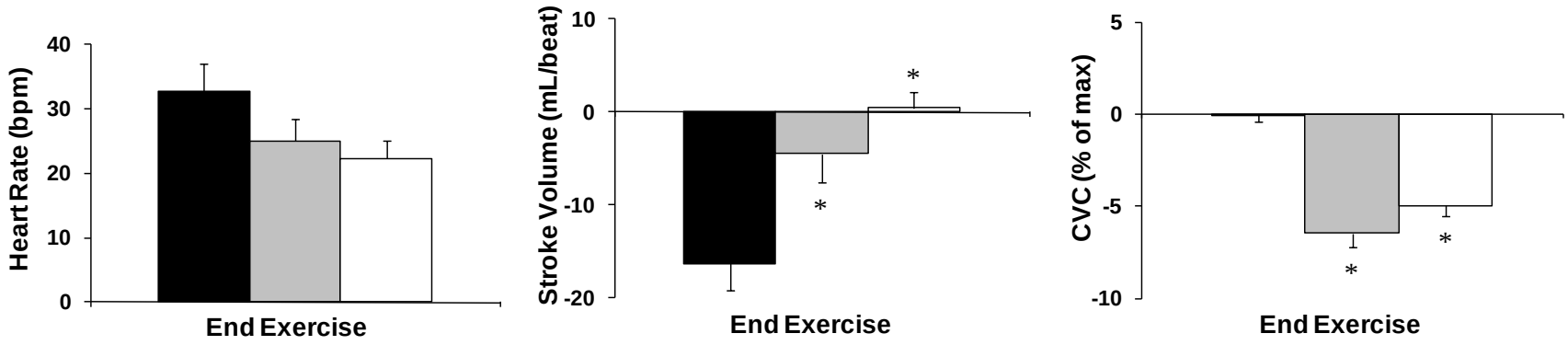

Fig. 2 Mean (±SE) relative changes from baseline in heart rate (left panel), stroke volume (middle panel), and cutaneous vascular conductance (CVC, right panel) at End-Exercise (EE) during no heat stress (NHS, ■), moderate heat stress (MHS, ■) and high heat stress (HHS, □). * denotes significantly different from NHS ($p \leq 0.05$)

PART THREE:

GENERAL CONCLUSIONS OF THE THESIS

The primary purpose of the first article was to examine the separate and integrated influence of metabo- and baroreceptor mediated activity on heat loss responses of sweating and skin blood flow under increasing levels of heat stress. In order to examine these responses we employed an isometric handgrip exercise followed by post-exercise ischemia during simultaneous lower body positive and negative pressure. It is evident from our findings that concurrent application of lower body negative or positive pressure does not alter the sustained elevations in sweat rate, typically associated with post-exercise ischemia both under moderate and high heat stress conditions. These results confirm a muscle metaboreceptor modulation of sweat rate which was not influenced by baroreceptor loading status. In contrast to the control of sweating, both lower body negative and positive pressure altered the cutaneous vascular conductance response associated with post-exercise ischemia, but this was only observed under high heat stress conditions. As such, our results demonstrate that baroreceptor loading status influences the metaboreflex modulation of cutaneous vascular conductance, but not sweat rate during heat stress.

The objective of the second article was to examine the cardiovascular responses during isometric handgrip exercise, performed at increasing levels of heat stress (i.e., normothermia and at 0.6°C and 1.4°C increase in esophageal temperature above resting levels). The novel observation from this study is that heat stress attenuates the increase in arterial blood pressure in response to isometric handgrip exercise and that this attenuation was vascular dependent, since cardiac output increased to similar levels for all

temperature conditions during IHG exercise. Furthermore, our findings show that higher levels of heat stress can further reduce the observed increase in mean arterial blood pressure during isometric handgrip exercise and this is attributed to a blunted increase in peripheral resistance when heat stressed.

The findings from the above studies provide important insight and understanding into the role of nonthermal muscle metaboreceptor and baroreceptor activity on the control of skin blood flow and sweating. Moreover, the findings provide a further understanding of the cardiovascular mechanisms responsible for the regulation of arterial blood pressure during hyperthermia.

PART FOUR:

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