The Separate and Combined Contributions of Metabo- and Baroreceptors to Postexercise Heat Loss

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ABSTRACT

Acute (~2 min) baroreceptor unloading was reported to modulate metaboreflex control of postexercise cutaneous blood flow, but not sweating. We examined whether sustained changes in baroreceptor loading status during prolonged postexercise recovery can alter the metaboreceptors' influence on heat loss. Thirteen young males performed a 1-min isometric handgrip exercise (IHG) at 60% maximal voluntary contraction followed by 2-min of forearm ischemia (to activate metaboreceptors) before and 15, 30, 45 and 60-min after a 15-min intense treadmill running exercise (>90% maximal heart rate) in the heat (35°C). This procedure was repeated on three separate days with the application of lower-body positive (LBPP, +40 mmHg), negative (LBNP, -20 mmHg), or no pressure (Control) postexercise. Sweat rate (ventilated capsule; forearm, chest, upper back) and cutaneous vascular conductance (CVC; forearm, upper back) were measured. Relative to pre-IHG levels, sweating at all sites increased during IHG and remained elevated during ischemia at baseline and similarly at 30, 45, and 60-min postexercise (site average sweat rate increase during ischemia: Control, 0.13±0.02; LBPP, 0.12±0.02; LBNP, 0.15±0.02 $mg \cdot min^{-1} \cdot cm^{-2}$; all $P<0.01$), but not at 15-min (all $P>0.10$). LBPP and LBNP application did not modulate the pattern of sweating to IHG and ischemia (all $P>0.05$). At 15-min postexercise, forearm CVC was reduced from pre-IHG levels during both IHG and ischemia under LBNP only (ischemia: 3.9±0.8 $\%CVC_{max}$; $P<0.02$). Therefore, we show metaboreceptors modulate postexercise sweating in the mid-to-late stages (30-60 min) of recovery, independent of baroreceptor loading status and similarly between skin sites. In contrast, metaboreflex modulation of forearm but not upper back CVC occurs only in the early stages of recovery (15 min) and depends upon baroreceptor unloading.

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PART ONE:

THEORETICAL BACKGROUND

CHAPTER I
INTRODUCTION

1.1 Introduction

The heat loss responses of sweating and skin blood flow are critical to the control of core body temperature during exercise and/or environmentally-induced heat stress. However, following dynamic exercise, there is a disruption in postexercise thermoregulatory control characterized by a rapid reduction of the heat loss responses to near baseline levels within the early stages (~20 min) of recovery, despite sustained elevations of core body and muscle temperatures above baseline resting levels for up to 90 min (Kenny, Chen, Johnston, Thoden, & Giesbrecht, 1997; Kenny & Jay, 2007; Kenny et al., 2006; Thoden, Kenny, Reardon, Jette, & Livingstone, 1994; Wilkins, Minson, & Halliwill, 2004). This robust response was observed to remain intact with repeated exercise/rest cycles, irrespective of progressive increases in thermal drive as reflected by changes in body heat content and core temperature (Gagnon & Kenny, 2011; Kenny et al., 2009; Kenny & Gagnon, 2010). Therefore, this phenomenon has been ascribed to the actions of nonthermal factors (i.e., factors unrelated to body temperature). Specifically, several reports in the past 15 years have identified muscle pump/mechanoreceptors, metaboreceptors, and baroreceptors – which have been linked with significant cardiovascular adjustments and the accumulation exercise-induced metabolites associated with recovery – as having a profound impact on the control of postexercise sweating and skin blood flow (Kenny & Jay, 2013). As such, the influence of nonthermal factors on heat loss responses may have important consequences in the control of core body temperature.

Baroreceptors are thought to be primarily responsible for the disruption of postexercise thermoregulatory control (Kenny & Jay, 2013). Located in the vessel walls of the carotid sinus,

aortic arch, pulmonary vessels, as well as in the atria and ventricles, baroreceptors are specialized mechanoreceptors that sense changes in blood pressure and are referred to as “unloaded” during a state of hypotension. Following dynamic exercise, mean arterial pressure (MAP) often exhibits a pronounced and sustained reduction from pre-exercise levels (~5-10 mmHg) lasting ~2 hours, known as postexercise hypotension, which is the result of significant cardiovascular adjustments (Halliwill, Buck, Lacewell, & Romero, 2013). Specifically, postexercise hypotension has been attributed to a reduction in stroke volume secondary to a decrease in central venous pressure that is believed to be due to blood pooling in skeletal muscles, as well as a resetting of the arterial baroreflex (i.e., autonomic responses to changes in blood pressure) to lower operating pressures (Halliwill, 2001; Halliwill et al., 2013). The hypothesis that baroreceptors are the primary nonthermal modulator of postexercise heat loss has evolved from a body of empirical evidence associating the suppression of postexercise sweating and skin blood flow to baroreceptor unloading (Kenny & Journeay, 2010). This is supported by the observation that the reversal of postexercise hypotension via either application of positive pressure to the lower limbs (+45 mmHg), a 15º head-down tilt manoeuvre in supine position, or employing a passive or active recovery mode, all of which serve to increase venous return and subsequently stroke volume, resulted in a more rapid rate of core temperature decay secondary to an enhanced sweat rate and level of skin blood flow (Journeay, Reardon, Jean-Gilles, Martin, & Kenny, 2004; Journeay, Reardon, Martin, & Kenny, 2004; McInnis, Journeay, Jay, Leclair, & Kenny, 2006).

While baroreceptors are a significant mediator of the marked suppression of postexercise heat loss, recent evidence indicates that muscle metaboreceptors may have a more important role in modulating postexercise heat loss than previously thought. Metaboreceptors are known to elicit increases in sweating and reductions in skin blood flow when activated during passive heating at

rest at various levels of hyperthermia (e.g., core temperature increases ranging from 0.55 to 1.40ºC), as well as at 20 min following intense dynamic exercise (Binder, Lynn, Gagnon, Kondo, & Kenny, 2012; Crandall, Stephens, & Johnson, 1998; McGinn, Swift, Binder, Gagnon, & Kenny, 2014). The influence of metaboreceptor activation is typically studied using a 1-min isometric handgrip (IHG) exercise and 2 min of post-IHG ischemia (achieved by occlusion of the upper exercising arm). The period of ischemia is thought to trap the metabolites produced during IHG exercise, which then trigger group III and IV chemosensitive afferents (i.e., metaboreceptors) located in skeletal muscle (Rotto & Kaufman, 1988; Rowell & O'Leary, 1990). Metaboreceptors activation produces a concomitant reflex-increase in MAP, which indicates that the metaboreceptors have been activated – this is known as the metaboreflex (Shibasaki, Kondo, & Crandall, 2001).

Up until recently, the nonthermal factors and their influence on postexercise heat loss had been studied separately from one another during recovery. Given that high intensity exercise results in metabolite accumulation, elevations in core temperature, and pronounced cardiovascular adjustments (Halliwill et al., 2013; Hellsten, Maclean, Radegran, Saltin, & Bangsbo, 1998; Juel, Pilegaard, Nielsen, & Bangsbo, 2000; Kenny & Niedre, 2002), there may be interplay between metabo- and baroreceptors in modulating the heat loss responses during postexercise recovery. In fact, a recent study acutely (~2 min) applied lower-body negative pressure (a maneuver serving to unload the baroreceptors; LBNP) simultaneously with post-IHG ischemia to eliminate the reflex increase in MAP, thereby isolating the influence of the metaboreceptors from baroreceptors. This study determined that baroreceptor loading associated with post-IHG ischemia can modulate metaboreceptor control of postexercise cutaneous blood flow, but not that of sweating.

In addition to the existence of interactions between nonthermal sensory receptors in the modulation of postexercise heat loss, studies have clearly demonstrated that the effectiveness of nonthermal influences on heat loss can be altered as a function of the level of hyperthermia. That is, in the presence of a greater thermal drive, thermal input overrides nonthermal input (Binder et al., 2012; Gagnon, Jay, Reardon, Journeay, & Kenny, 2008; Kondo et al., 2002; McGinn, Paull, Meade, Fujii, & Kenny, 2014). Therefore, time-dependent changes in the relative contribution of nonthermal and thermal input to postexercise heat loss may exist during recovery from exercise as core temperature gradually reduces towards pre-exercise levels. For example, Gagnon et al. (2008) observed time-dependent changes in the control of postexercise heat loss responses in individuals rendered hyperthermic (~2.8°C increase in core temperature) such that the control of skin blood flow is governed by nonthermal influences by the early stages of recovery (e.g., >10min, core temperature elevation of 1.3°C from baseline) while nonthermal control over sweating is only observed at later stages (e.g., >50 min, core temperature elevation of 0.6°C from baseline).

It is currently unknown the extent to which metaboreceptors can modulate postexercise heat loss responses during a prolonged recovery and whether sustained changes in baroreceptor loading status can impinge on these responses differently throughout recovery. Given the fact that changes in the contribution of nonthermal factors to postexercise heat loss responses are time-dependent (i.e., related to thermal and/or cardiovascular changes), and that metaboreceptors are known to interact with baroreceptors, it is relevant to examine the role of metaboreceptors as a function of postexercise recovery time under different sustained baroreceptor loading statuses.

1.2 Rationale

Although it has been shown that metaboreceptors are capable of influencing postexercise heat sweating and skin blood flow, no study has yet assessed to what extent they can modulate

these responses during different sustained backgrounds of baroreceptor loading over a prolonged recovery period. Furthermore, while the sweating response to the metaboreflex has been shown to differ between different skin regions (e.g., non-glabrous such as the chest and forearm vs glabrous such as the palm) (Kondo et al., 1999), differences in the response at other regions [e.g., the forehead, known to exhibit emotional and gustatory sweating responses (McGregor, 1952)] are not known. To our knowledge, this is the first study that will examine the time-dependent contributions of metabo- and baroreceptors (both separate and combined) to postexercise sweating and skin blood flow, as well as characterize the regional differences in these responses at three different skin sites.

This project employed sustained mechanical manipulations [i.e., lower body positive pressure (LBPP) and LBNP application] to alter blood pressure with the aim of establishing longstanding differences in baroreceptor loading status during recovery from dynamic exercise in three experimental conditions. This design allowed for the evaluation of the transient influences of metaboreceptors (assessed using IHG exercise followed by post-IHG ischemia) on the control of skin blood flow and sweating at various consecutive time intervals over postexercise recovery during simultaneous sustained changes in baroreceptor loading status.

1.3 Objectives

The objective of the proposed study is to evaluate the changes in the control of local heat loss responses at different levels of baroreceptor loading during metaboreceptor activation throughout recovery from a single bout of high-intensity dynamic exercise. The specific objectives are to:

1) Evaluate the time-dependent influence of metaboreceptors on the control of sweating and skin blood flow during postexercise recovery;

2) Assess whether prolonged changes in baroreceptor loading status would alter the metaboreceptor influence on sweating and skin blood flow (i.e., the integrated influences);

3) Characterize the regional differences in sweating and skin blood flow to metaboreceptors activation.

1.4 Hypotheses

It was hypothesized that:

1) The sweating and cutaneous vascular responses to metaboreceptor activation would be blunted in the early stages (i.e., first 15 min) of recovery when the level of hyperthermia is expected to be elevated compared to later stages (i.e., >15 min);

2) Baroreceptor unloading or loading induced by the application of -20 mmHg LBNP or +40 mmHg LBPP respectively would influence the metaboreflex-induced changes in skin blood flow but not sweating;

3) There would be regional differences in the sweating and cutaneous vascular responses to the metaboreflex given previous studies have shown that different skin regions exhibit heterogeneity in the magnitude of the response [e.g., more elevated sweat rates are typically seen at the head, followed by the trunk, and then the limbs (Inoue, Shibasaki, Ueda, & Ishizashi, 1999; Kondo et al., 1998; Takano et al., 1996)].

1.5 Relevance of the Study

Taken together, nonthermal factors have a profound influence on the control of heat loss responses and therefore the regulation of core body temperature. By beginning to examine the separate and combined influences of nonthermal stimuli (e.g., metaboreceptors and baroreceptors), the current thesis will advance our knowledge of potential interplay that may exist between different nonthermal stimuli implicated in postexercise recovery, which have previously been shown to affect heat dissipation. Understanding how nonthermal factors impact the capacity to dissipate heat is necessary to expand on the current state of knowledge of potential underlying factors that increase the risk for heat-related injuries. The findings may have practical implications that could serve in the development of preventative measures (e.g., safety guidelines) and interventions (e.g., recovery strategies) to manage and/or mitigate impairments in heat dissipation and reduce the risk of heat stress-related illness and/or injury. Subsequently, these protocols can be implemented in workplaces, as well as in sport and civilian contexts that present a high risk for individuals to suffer a heat-related injury.

1.6 Delimitations and Limitations

This study examined the heat loss responses to nonthermal sensory receptor activation in young males only (i.e., age range of 18 to 23 years). Therefore, the results do not necessarily apply to older individuals and may not be representative of female responses. Furthermore, the study requires that participants be healthy and physically active (to be able complete the high intensity treadmill running protocol) and thus the conclusions from the current study are not directly applicable to individuals with chronic diseases that may further alter thermoregulatory and cardiovascular control.

CHAPTER II
REVIEW OF LITERATURE

2.1 Thermoregulation & Heat Balance

Thermoregulation refers to ability to homeostatically regulate the body's internal temperature within a narrow range (around ~37°C), despite large fluctuations in internal stressors (e.g., metabolic heat production of the body tissues) and thermal gradients between the skin and the environment. The body maintains a relatively constant core temperature due to the management of a fine balance between the heat produced and the heat lost. Thus, constant exchanges of heat occur within the body between the different tissues types, as well as between the skin and the environment to maintain an equilibrium of heat content within the body. The rate of heat exchange is governed by the first law of thermodynamics as it relates to the conservation of energy: heat energy can neither be created nor destroyed; it can only change forms and be transferred from one place to another. Based on this law, all thermoregulatory processes in the body can be represented by this heat balance equation (Parsons, 2003):

$$S = M - (\pm W) \pm (R + C) - E$$

Where: S is the rate of body heat storage;
M is the rate of metabolic energy expenditure;
W is the rate of external work;
R is the rate of radiant heat exchange;
C is the rate of convective heat exchange and;
E is the rate of evaporative heat exchange.
All units in Watts

The left side of the equation is the rate of metabolic heat produced (M-W) by the body; the metabolic energy that can be used towards performing work is typically minimal, thus most of the metabolic energy produced is released in the form of heat. The right side of the equation represents the rate of heat exchange with the environment, which can be positive (heat loss) or negative (heat

gained), depending on the environmental conditions. Thus a mismatch between the rates of metabolic heat production and heat loss will result in a positive or negative rate of body heat storage, *S*. For example, when heat dissipation exceeds heat production [i.e., $M\text{-}W < E \pm (C+R+K)$], there is a negative rate of body heat storage ($S<0$) and over time core temperature will decrease accordingly. On the other hand, when the rate of metabolic heat produced combined with a dry heat gain in a hot environment [i.e., when air temperature is greater than skin temperature, $M\text{-}W \pm (C+R+K) >$ E] exceed the rate of heat lost, there is a positive rate of body heat storage ($S>0$) and core temperature will increase over time. Left unchecked, an ongoing rise in core temperature will eventually lead to hyperthermia, which can be potentially life-threatening (Armstrong et al., 2007).

2.1.1 Thermal Perception & Integration

Current understanding of thermoregulation is of an "interthreshold zone" – that is, a narrow range of core temperatures bound by the thresholds at which sweating and shivering responses are initiated – wherein core temperature is permitted to fluctuate (Mekjavic & Eiken, 2006). For example, during heat stress, core temperature is allowed to rise to a certain extent before a temperature threshold is reached and the appropriate effector responses are recruited. In order to initiate an effector response to counter core temperature fluctuations, the body must sense changes in temperature and integrate this information. Warm and cold thermoreceptors (i.e., receptors sensitive to changes in temperature) transmit thermal input to the central nervous system (Parsons, 2003). In normal ambient conditions (e.g., 25°C), thermoreceptors in the skin exhibit basal tonic activity and the firing rate of the cold and warm thermoreceptors increases as the skin temperature decreases or increases, respectively (Boulant, 1998). Thermoreceptors have also been found in the muscles, core, and throughout central nervous system in animal models, particularly in the

preoptic/anterior hypothalamic region of the brain where a concentrated cluster of thermoreceptors has been discovered (Hammel, Jackson, Stolwijk, Hardy, & Stromme, 1963; Nakayama, Eisenman, & Hardy, 1961). The integration of thermal input is thought to occur in this area, which is considered to be the body's thermoregulatory center since it is also coordinates the recruitment of thermoregulatory responses in attempt to mitigate and/or minimize the changes in core body temperature (Boulant, 2000). As an example, warm thermal input elicits the effector heat loss responses of cutaneous vasodilator and sudomotor activity (i.e., increases skin blood flow and sweating, respectively).

2.2 Heat Loss Responses

During physical activity and/or exposure to an environmental heat load, the ability of the body to regulate its core temperature is paramount. Humans can dissipate heat in two ways: increasing blood flow to the skin through cutaneous vasodilation (i.e., dry heat exchange), and more importantly by the evaporation of sweat from the skin.

2.2.1 Skin Blood Flow

Cutaneous vasculature is dually innervated by two branches of the autonomic sympathetic nervous system whose overall effects are termed "vasomotor activity" (Charkoudian, 2003; Johnson, Minson, & Kellogg, 2014; Kellogg, 2006). One branch includes the sympathetic noradrenergic nerves that release primarily norepinephrine (activating postsynaptic α-adrenergic receptors) and co-transmitters such as neuropeptide Y, both of which mediate cutaneous vasoconstriction (Stephens, Aoki, Kosiba, & Johnson, 2001; Stephens, Saad, Bennett, Kosiba, & Johnson, 2004). The second branch includes the cholinergic nerves which are responsible for cutaneous active vasodilation. Unlike the vasoconstrictor nerves, cholinergic nerves do not exhibit

tonic activity, are only found in nonglaborous skin, and are only active during hyperthermia. The exact mechanisms underlying active vasodilation are not well understood. The current understanding is that acetylcholine contributes to the early rise in skin blood flow and that the co-transmission of nitric oxide is needed for full expression of the response (e.g., the remainder ~30%) (Johnson et al., 2014; Kellogg, 2006; Wilkins, Holowatz, Wong, & Minson, 2003). To date, several other cotransmitters have been identified as potentially having a role in cutaneous active vasodilation: vasointestinal peptide, substance P, histamine, and prostaglandins (Johnson et al., 2014).

Changes in skin blood flow ultimately reflect the balance between the vasoconstrictor and active vasodilator systems, which is what gives a biphasic characteristic to the cutaneous vasodilatory response. The first phase of the response involves passive vasodilation due to the withdrawal of sympathetic vasoconstrictor tone in response to increases in skin temperature (Johnson & Kellogg, 2010). Further increases (the remaining ~85-95% of the response) are associated with increases in the level of hyperthermia (core body temperature) and involve the active vasodilator system (Kellogg et al., 1995).

At rest in normothermic environments, the vasoconstrictor system exhibits tonic activity that is responsible for a low total skin blood flow of ~250-500 mL/min (Pergola, Kellogg, Johnson, & Kosiba, 1994). Subtle changes in cutaneous vascular tonicity allow for the maintenance of a normal core body temperature, despite slight changes in daily activity levels or in thermoneutral ambient conditions without the need for sweating or shivering (Charkoudian, 2003). This is made possible by the fact that small changes in skin blood flow over the entire body surface can result in large changes in heat dissipation (e.g., core temperature would increase by 0.5˚C over the course of 24 h if total skin blood flow were to decrease by 2%) (Johnson & Kellogg, 2010).

During heat stress, a given increase in core temperature will initiate increases in skin blood flow. Thereafter, skin blood flow rises linearly with core temperature. Once the heat loss response of skin blood flow reaches maximal values, a flattening of the response is observed, whereby no further increase in skin blood flow occurs despite increasing body core temperature. While increases in skin blood flow are primarily stimulated by elevations in core temperature, skin temperature can also influence the response (Wyss, Brengelmann, Johnson, Rowell, & Niederberger, 1974). Ultimately, increasing skin blood flow allows for the convective transfer of heat from within the core to the skin surface where it can then be exchanged with the environment. This is made possible by the fact that increases in skin blood flow induce concomitant changes in skin temperature, and thus the thermal gradient between the skin and the environment, which will then affect the rate of dry heat exchange (Hardy, 1961). Additionally, a greater skin temperature assists in the evaporation of sweat and minimizes heat gain from the environment when air temperature exceeds that of the skin (Charkoudian, 2003).

2.2.2 Sweating

Evaporative heat loss is by far the body's primary avenue for heat dissipation (Kenny & Journeay, 2010), representing 25% of heat loss at rest and >80% during exercise (Gisolfi & Wenger, 1984). It becomes the only means by which humans can dissipate heat when ambient air temperature exceeds skin temperature (~≥35°C) since heat can be gained from this environment.

Evaporation of sweat requires energy to make the phase change from liquid to gas. This energy comes from the heat at the skin surface; thus as the sweat evaporates, it eliminates heat from the skin, effectively cooling the body. However, high ambient humidity will reduce the capacity to evaporate sweat (i.e., air is saturated with water). Thus with increasing environmental temperatures and humidity, evaporating sweat becomes increasingly limited. While sweat

production would continue, the rate of evaporation would be reduced thus sweat would be wasted by dripping off the skin without actually cooling it. Therefore, if strenuous work is combined with an environmental heat load (e.g., hot, humid conditions), the result is a more rapid rise in core temperature.

Thermal sweat is produced by approximately 2-4 million eccrine sweat glands located throughout the body's non-glabrous skin regions (Shibasaki, Kondo, & Crandall, 2003). Much like in the control of cutaneous active vasodilation, core and skin temperatures are the dominant input for activating sweating such that a given increase in core temperature will trigger sudomotor efferent nerve impulses (originating from the preoptic hypothalamus) to induce sweating (Kondo, Nishiyasu, Inoue, & Koga, 2010). Sudomotor neurons (i.e., sweat gland innervation) are sympathetic cholinergic fibres that release acetylcholine, which is the primary neurotransmitter that triggers sweating upon binding to muscarinic receptors located on the sweat gland (Shibasaki & Crandall, 2010). Increases in sweat rate are achieved by increasing the number of activated sweat glands and/or by increasing the amount of sweat secreted per gland.

The rate of sweat production varies as a function of the environmental heat load and the rate of metabolic heat production during exercise. This can be referred to as the evaporative requirement to achieve heat balance, which defines the level of whole-body sweating required to achieve a state of thermal balance (Gagnon, Jay, & Kenny, 2013). Thus an individual performing work in a hot environment can produce up to 2 L/hour of sweat (Sawka & Noakes, 2007). Further, an environment in which there is dry, fast moving ambient air facilitates sweat evaporation whereas humid, stagnant air hampers the ability to evaporate sweat, resulting in an increase in the amount of sweat dripping (i.e., a decrease in sweating efficiency).

2.3 Recovery from Exercise

Depending on the environmental conditions, core temperature typically becomes elevated during dynamic exercise. However, recovery from exercise results in a persistent elevation in core and muscle temperatures from baseline levels for up to 90 min (Kenny & Jay, 2007; Kenny et al., 2006; Thoden et al., 1994; Wilkins et al., 2004). Despite this excess residual heat load following exercise (Kenny et al., 2009), there is a rapid reduction in heat loss to near baseline levels at both the local (i.e., sweating and skin blood flow) and whole-body levels within the first ~20 min of recovery (Journeay, Reardon, Jean-Gilles, et al., 2004; Kenny et al., 1997; Kenny et al., 2009; Thoden et al., 1994). Importantly, this marked suppression of postexercise heat loss is observed to remain intact with successive exercise/recovery cycles, despite progressive increases in body heat storage during intermittent exercise bouts (Gagnon & Kenny, 2011; Kenny et al., 2009; Kenny & Gagnon, 2010).

Another key observation associated with the postexercise period is that of pronounced cardiovascular adjustments (Kenny, Jay, & Journeay, 2007) – specifically a period of reduced blood pressure (i.e., postexercise hypotension) lasting up to 2 hours (Halliwill, 2001). Postexercise hypotension has been attributed to a sustained increase in vascular conductance in muscles that is believed to be dependent upon the activation of H_1 and H_2 histamine receptors in previously active muscle tissue (Halliwill et al., 2013). In support of this view are the findings that the blockade of histamine receptors attenuated postexercise muscle vasodilation and hypotension by ~80 % (Lockwood, Wilkins, & Halliwill, 2005; McCord, Beasley, & Halliwill, 2006). Other hypotheses stipulate that the mechanism underlying postexercise hypotension implicate a resetting of the arterial baroreflex to lower operating pressures as well as reduced signal transduction of from sympathetic adrenergic outflow to vasoconstriction (Halliwill et al., 2013).

Given that both phenomena (i.e., postexercise hypotension and suppression of postexercise heat loss) are observed simultaneously, it is believed that there is a functional relationship between postexercise thermoregulatory and cardiovascular control.

2.4 Nonthermal influence of thermoregulation

While thermal factors (i.e., core and skin temperatures) are primarily responsible for modulating thermoeffector activity, other factors of nonthermal origin (i.e., unrelated to body tissue temperatures) are also known to modulate the heat loss responses. These nonthermal factors include central command, muscle mechanoreceptors and metaboreceptors, osmoreceptors, and baroreceptors (Kenny & Journeay, 2010). The influence of these factors on the control of skin blood flow and sweating can be assessed through the observation of changes in thermoeffector activity that occur in the absence of changes in body tissue temperatures.

It was previously reported that nonthermal baroreceptor input associated with postexercise hypotension is a primary mediator of the postexercise suppression in heat loss (Gagnon et al., 2008; Kenny & Journeay, 2010). However, a recent study suggests that while metaboreceptors may play a greater role than previously thought in modulating postexercise sweating and skin blood flow. The following sections will provide an overview of the nonthermal influences on heat loss.

2.4.1 Central command

Central command, consisting of signals that arise from higher brain centers, is defined as the parallel activation of autonomic and motor centers that simultaneously increase sympathetic and motor neuron activity (Charkoudian & Wallin, 2014). Studies have attempted to isolate the influence of central command on skin blood flow and sweating using an IHG protocol. During IHG, central command is the primary mechanism stimulating sympathetic outflow to the skin (i.e., vasomotor and sudomotor activity). This was demonstrated by Vissing and Hjortso (1996) who showed that IHG elicited large increases in skin sympathetic nerve activity (indicator of central command activity) in the presence of partial neuromuscular blockade. Neuromuscular blockade blunts muscular contraction such that a given stimulus will elicit a substantially lower force. Thus a greater level of input from the higher brain centers (i.e., central command) is required to evoke the desired external force while the influence of muscle metaboreceptors and mechanoreceptors is minimized.

In hyperthermic conditions, IHG reduces cutaneous vascular conductance (CVC, calculated as skin blood cell flux divided by the prevailing MAP) via withdrawal of active cutaneous vasodilation and also potentially via a non-neuronal mechanism (G. R. McCord & Minson, 2005; Shibasaki, Rasmussen, Secher, & Crandall, 2009) while sweat rate increases in parallel (Binder et al., 2012; Crandall, Musick, Hatch, Kellogg, & Johnson, 1995). Central command's specific effects on modulating sweating and CVC during IHG have been separately evaluated. Shibasaki, Secher, Selmer, Kondo, and Crandall (2003) showed that central command is capable of increasing sweating during both normothermia (only when the sweating response was sensitized via inhibition of acetylcholinesterase) and hyperthermia (0.5 and 1.0 $^{\circ}$C increase in core temperature above baseline levels) when subjects performed IHG under partial neuromuscular

blockade. However, it is noteworthy that the contribution of central command to increases in sweating was diminished under moderate hyperthermia (1.0 ˚C increase in core temperature) when sweating was already elevated. Using the neuromuscular blockade technique, Shibasaki, Secher, Johnson, and Crandall (2005) later determined that central command was also responsible for mediating the observed reduction in CVC with IHG (during hyperthermia only) since CVC returned to baseline levels during post-IHG ischemia when central command influences are absent.

Central command has also been reported to alter heat loss responses during postexercise recovery. Journeay, Reardon, Martin, et al. (2004) sought to delineate the relative influences of certain nonthermal factors on postexercise heat loss responses by using three different recovery modes: 1) active loadless pedaling, which involves central command, baroreceptor loading, mechanoreceptor/ muscle pump activation, 2) passive assisted pedaling, which involves baroreceptor loading, and mechanoreceptor/muscle pump activation, and 3) inactive recovery, which involves only baroreceptor unloading. It was found that central command, along with mechanoreceptor stimulation, could modulate postexercise sweating in males; however, the attenuated reduction in postexercise CVC was attributed primarily to enhanced venous return and baroreceptor loading during passive and active recovery modes.

2.4.2 Metaboreceptors

Muscle metaboreceptors consist of groups III and IV chemosensitive afferents that respond to the accumulation of metabolites arising from exercising muscle (Nishiyasu et al., 1994; Rotto & Kaufman, 1988; Rowell & O'Leary, 1990; Victor, Bertocci, Pryor, & Nunnally, 1988). The response to metaboreceptor afferent input is a reflex activation of sympathetic pathways and an increase in blood pressure to maintain adequate perfusion of metabolically active muscles (i.e., pressor reflex). In the past two decades, a growing body of empirical evidence suggests that lactic

acid and ATP (metabolites released from exercising muscle that act through acid-sensing ion channels and purinergic 2 receptors, respectively – receptors that have been found on group III and IV muscle afferent endings) play a large role in the generation of the exercise pressor reflex (J. L. McCord & Kaufman, 2010).

Classically, metaboreceptor activation has been studied using the IHG and post-IHG ischemia model, wherein central command, baroreceptors, metaboreceptors and mechanoreceptors/skeletal muscle pump are implicated during IHG while the post-IHG ischemia period eliminates the involvement of central command and mechanoreceptors (Kenny & Journeay, 2010). Performing IHG exercise is known to increase heart rate, cardiac output (CO), MAP, and muscle and skin sympathetic nerve activity (Binder, Gagnon, Lynn, Kondo, & Kenny, 2013; Hisdal, Toska, Flatebo, Waaler, & Walloe, 2004; Mark, Victor, Nerhed, & Wallin, 1985; Vissing, Scherrer, & Victor, 1991). Post-IHG ischemia has been shown to maintain MAP and muscle sympathetic nerve activity increased above baseline levels while heart rate and skin sympathetic nerve activity return to baseline levels (Binder et al., 2012; Rowell & O'Leary, 1990; Victor, Pryor, Secher, & Mitchell, 1989; Vissing et al., 1991). However, whether the metaboreflex induces increased CO and/or total peripheral resistance (TPR) has been subject to debate with some studies reporting increases in CO (Binder et al., 2013; Lind, Taylor, Humphreys, Kennelly, & Donald, 1964; Shepherd, Blomqvist, Lind, Mitchell, & Saltin, 1981) whereas others report an increase in TPR with no change to CO (Chirinos et al., 2010; Hisdal et al., 2004; Toska, 2010).

Among the first studies to evaluate metaboreceptor stimulation of sweating were Saito, Naito, and Mano (1990) who employed 2 min of IHG at 30% of maximal voluntary contraction (MVC) followed by 2 min of post-IHG ischemia under normothermic conditions. They observed that the IHG-induced increase in MAP was maintained during the period of ischemia, which is

consistent with the known reflex increase in MAP above baseline levels that occurs with the metaboreflex (Rowell & O'Leary, 1990), and this was paralleled by a rapid return to pre-IHG baseline of electrodermal function (an index of sudomotor activity). Crandall et al. (1998) extended on this observation, being the first to showcase the metaboreflex modulation of sweating during mild and moderate hyperthermia (i.e., 0.55ºC and 0.75ºC increase in core temperature above baseline levels, respectively). Sweat rate increased during 3 min of IHG (30% of MVC) and since it remained elevated throughout post-IHG ischemia and decreased once the occlusion was released, this was attributed to metaboreceptor activation. Kondo et al. (1999) later demonstrated that with a sufficient intensity of the stimulus (e.g., 60 s of IHG at 45% of MVC or a longer IHG at a lower intensity), the same pattern of response also occured during a mild heat load (i.e., exposure to 35°C ambient temperature).

As for the cutaneous vascular response to metaboreceptor activation, Kondo et al. (1999) also reported that under the same mild heat load described previously, the metaboreflex did not affect skin blood flow. In contrast, Crandall et al. (1998) showed that in mild hyperthermia (a 0.55°C elevation in sublingual temperature) metaboreceptor activation resulted in a reduction in CVC from pre-IHG resting levels. Furthermore, since a similar reduction in CVC was found at skin sites with and without a vasoconstrictor blockade (iontophoresis of bretylium tosylate), the authors argued that this was mediated by withdrawal of activate vasodilation. However, it has been argued that the reduction in CVC during IHG (and by extension, perhaps post-IHG ischemia) is not likely due to withdrawal of active vasodilation but rather to local (i.e., non-neural) mechanisms that serve an autoregulatory protective function associated with the elevations in perfusion pressure secondary to the increased MAP that accompanies IHG (and also post-IHG ischemia) (G. R. McCord & Minson, 2005). This is supported by the observation that the IHG-induced reduction

in CVC only occurred at more elevated levels of CVC (at least ~40-50% CVC_{max}) that were achieved before IHG in both normothermic (via local heating) and hyperthermic individuals. However, a more recent study showed that in normothermia, regardless of the level of CVC achieved prior to IHG, CVC did not change during IHG, which suggests that non-neural mechanisms alone are insufficient to induce reductions in CVC during IHG in normothermia (Shibasaki, Rasmussen, et al., 2009). Furthermore, the authors clearly show that in hyperthermia, CVC was reduced in both an arm that had received a complete neural blockade and an intact arm, with more substantial reductions observed at the unblocked arm's site. Taken together, these observations suggest that both neural and non-neural components mediate the IHG- (and perhaps metaboreflex-) induced reduction in CVC during hyperthermia.

Recent work demonstrated that metaboreceptors are capable of modulating heat loss responses at 20 min into recovery from dynamic exercise. McGinn, Swift, et al. (2014) are the first to show that following 15 min of high intensity treadmill running, post-IHG ischemia induced a reduction in CVC (~6%) while sweat rate increased (~0.11 $mg \cdot min^{-1} \cdot cm^{-2}$) in parallel. A surprising result was that 2 min of ischemia alone (i.e., without a prior period of IHG) resulted in a similar magnitude of reduction in CVC as that observed during the post-IHG ischemia condition while in parallel sweat rate was increased relative to the control condition (i.e., when no IHG exercise or post-IHG ischemia was performed). These results suggest that the level of remaining metabolites in circulation from the high intensity exercise were sufficient enough to activate the metaboreflex during postexercise recovery.

2.4.3 Baroreceptors

Baroreceptors are stretch-sensitive receptors responding to changes in the tension of blood vessel walls. Two groups of baroreceptors exist: the arterial baroreceptors, located in the aortic

arch and carotid sinus, are responsible for monitoring changes in central blood pressure and the brain's blood supply and, respectively; the low-pressure baroreceptors (known as cardiopulmonary receptors), located in large systemic veins and in the right atrium, are responsible for monitoring blood volume levels. Afferent signals arising from arterial baroreceptors ascend the vagus and the glossopharyngeal nerves to the nucleus tractus solitarius in the medulla oblongata where the firing rate is interpreted. A greater degree of stretch (i.e., baroreceptor "loading", associated with an increase in MAP) increases the firing rate of baroreceptor action potentials. This results in an increase in parasympathetic activity, which reduces heart rate and myocardium contractility, and also an inhibition of sympathetic activity, which reduces vasomotor tone to reduce TPR (Charkoudian & Wallin, 2014). Alternatively, less of a stretch (i.e., baroreceptor "unloading", associated with a decrease in MAP) reduces the firing rate, which causes an increase in sympathetic tone to produce the opposite responses.

The baroreflex has been shown to have a significant influence on thermoeffector activity during passive heating, exercise, and postexercise recovery (Kenny & Journeay, 2010). The baroreflex control of heat loss responses has been studied by employing techniques that alter hemodynamics via postural (i.e., head-down and head-up tilt) or mechanical [i.e., the application of lower-body positive or negative pressure (LBPP or LBNP, respectively)] manipulations. Specifically, LBNP and head-up tilt manoeuver induce baroreceptor unloading whereas LBPP and head-down tilt induce baroreceptor loading. Another method to alter blood pressure is through the use of pharmacological agents that eliminate potentially confounding influences such as convective cooling associated with lower-body pressure application (Shibasaki et al., 2001).

The baroreflex control of skin blood flow in normothermia has been evidenced using various levels of LBNP (-10 to -50 mmHg) to reduce cardiac filling. This resulted in reductions in

forearm skin blood flow in a graded fashion according to the level of LBNP both at rest and during mild cycle ergometer exercise (Mack, Nose, & Nadel, 1988; Tripathi & Nadel, 1986). The underlying mechanism for the reductions in skin blood flow is believed to be a baroreflex-mediated vasoconstriction in skeletal muscle and skin to redirect blood to the central cavity and increase blood pressure (Tripathi, Shi, Wenger, & Nadel, 1984). With regards to hyperthermia, when Kellogg, Johnson, and Kosiba (1990) engaged the active vasodilator system using whole-body heating, they observed a decrease in forearm CVC during application of LBNP at a skin site treated with a vasoconstrictor blockade and attributed this to a baroreflex-mediated withdrawal of active vasodilation. Taken together, it appears the baroreflex exerts control over skin blood flow via the active vasodilator pathway during hyperthermia (Kellogg et al., 1990) and the vasoconstrictor pathway during nomorthermia (Tripathi & Nadel, 1986).

The involvement of baroreceptors in the control of sweating is controversial (Kenny & Jay, 2013; Kenny & Journeay, 2010; Shibasaki, Kondo, et al., 2003). Mack, Nishiyasu, and Shi (1995) reported an attenuated sweat rate during moderate exercise in a normothermic environment with the simultaneous application of LBNP (-40 mmHg), and this response was reversed upon LBNP removal. In contrast, Solack, Brengelmann, and Freund (1985) found that the baroreflex was not involved in modulating sweating when LBNP was applied during whole-body heating in supine position. The disparity in findings remains evident in studies examining the influence of baroreceptors on sweating during the postexercise period. For example, Gagnon et al. (2008) as well as Journeay, Reardon, Jean-Gilles, et al. (2004) showed that the reversal baroreceptor unloading associated with postexercise hypotension (using active/passive recovery modes or LBPP application, respectively) could enhance both the level of sweating and CVC during recovery from dynamic exercise, which resulted in a more rapid rate of core temperature decay.

On the other hand McGinn, Paull, et al. (2014) showed that baroreceptors do not influence postexercise sweating. The contradictory findings may be explained by the possibility that different techniques used to manipulate MAP may engage a specific group of baroreceptors which may have different effects from one another. However, it is not currently possible to distinguish which groups are affected at any given time.

Several studies in the past decade have attributed the perturbation in postexercise thermoregulatory control to a baroreflex-mediated response associated with postexercise hypotension (Kenny & Jay, 2013; Kenny et al., 2007; Kenny & Journeay, 2010). For example, when LBPP (+50 mmHg) was applied during recovery from exercise to reverse postexercise hypotension, it eliminated the postexercise increase in the onset thresholds typically observed for sweating and skin blood flow (Jackson & Kenny, 2003). Furthermore, other studies that reversed postexercise hypotension using postural manipulations showed that a 15^{o} head-down tilt position (McInnis et al., 2006) and supine recovery (Kenny et al., 2008) mitigated the postexercise suppression of CVC and sweating and resulted in a faster esophageal temperature decay compared to recovery in the upright seated posture. Similar findings were observed with the use of +45 mmHg of LBPP or different postexercise recovery modes (Journeay, Reardon, Jean-Gilles, et al., 2004; Journeay, Reardon, Martin, et al., 2004). Taken together, there is ample evidence to suggest that baroreceptors play a major role in the suppression of postexercise heat loss and that reversing baroreceptor unloading can serve to attenuate this suppression.

2.4.4 The relative contribution of nonthermal factors to postexercise heat loss responses

It is important to consider that metaboreceptor stimulation results in a reflex increase in MAP and thus a concomitant change in baroreflex activity. Therefore, it is challenging to isolate the influence of metaboreceptors alone (i.e., in the absence of changes in baroreceptor loading

status). Shibasaki et al. (2001) eliminated this potential confounding factor by systemically infusing sodium nitroprusside (SNP) during post-IHG ischemia only to return MAP to baseline levels. Similar elevations in sweat rate during post-IHG exercise ischemia were observed independent of MAP manipulations and it was concluded that the sweating response associated with metaboreflex activation is not influenced by changes baroreflex activity.

Binder et al. (2012) went a step further by examining the separate and combined influences of both the metaboreflex and baroreflex on the heat loss responses during increasing levels of hyperthermia induced by whole-body heating with a water-perfused suit. While a metaboreceptor-induced reduction in CVC was observed during moderate and high levels of hyperthermia (i.e., core temperature that was +0.6 and +1.4 °C from baseline, respectively), it was found that baroreceptors (manipulated with acute LBPP and LBNP during simultaneous post-IHG ischemia) could only mediate this response at high levels of hyperthermia. On the other hand, the metaboreflex modulation of sweating was uninfluenced by changes in baroreflex activity brought on by LBNP or LBPP application. McGinn, Swift, et al. (2014) later evaluated the interactions between the baro- and metaboreflexes on the heat loss responses following high intensity dynamic exercise by employing acute -40 mmHg LBNP application during post-IHG ischemia. Similarly as with Binder et al, they found that baroreceptor loading associated with post-IHG ischemia could mediate the metaboreflex modulation of postexercise CVC but not sweating. Although both studies support the assessment that baroreceptors may not modulate sweating, these studies present acute manipulations of baroreceptor loading status. However, postexercise hypotension is long lasting; therefore it remains to be determined whether baroreceptors are capable of modulating the metaboreflex influence of sweating and CVC during more a more prolonged change in baroreceptor loading status.

It is important to consider that other nonthermal factors such as muscle mechanoreceptors and osmoreceptors can modulate sweating and skin blood flow and thus may influence these responses during postexercise recovery in this thesis project. In fact, mechanoreceptors, which are activated by a change in skeletal muscle length, have already been shown to influence postexercise sweating and skin blood flow (Carter, Wilson, Watenpaugh, Smith, & Crandall, 2002; Gagnon et al., 2008; Journeay, Reardon, Martin, et al., 2004). In addition, osmoreceptors (specialized neurons in the hypothalamus that detect changes in plasma osmolality) are thought to have a profound influence on heat loss responses, in particular during conditions of plasma hyperosmolality (i.e., a high concentration of blood plasma solutes) (Kenny et al., 2007). Indeed, it is well documented that plasma hyperosmolality increases the temperature threshold for the onset of sweating and cutaneous vasodilation (Barrera-Ramirez, McGinn, Carter, Franco-Lopez, & Kenny, 2014; Fortney, Wenger, Bove, & Nadel, 1984; Lynn, Gagnon, Binder, Boushel, & Kenny, 2012; Shibasaki, Aoki, Morimoto, Johnson, & Takamata, 2009; Takamata, Nagashima, Nose, & Morimoto, 1997). Hyperosmolality is typically associated with dehydration, which can be brought on by excessive sweating as can be observed during prolonged exercise without fluid replacement. It is likely that the protocol employed in this thesis project will bring about some extent of dehydration and thus the involvement of osmoreceptors in the nonthermal modulation of heat loss responses during postexercice recovery should not be discounted.

Lastly, the level of hyperthermia is known to modify the effectiveness of nonthermal modulation of heat responses. For example, nonthermal factors wer more effective at modulating the heat loss responses during moderate increases in core temperature (~0.3°C) while thermal factors overrode the nonthermal influence at greater elevations in core temperature (~1.4°C) (Binder et al., 2012; Kondo et al., 2002). Importantly for this thesis project, it is expected that core

temperature will be substantially elevated (by ~1.0 °C above baseline levels) during the early stages of recovery while it will be only moderately elevated during the later stages of recovery, and therefore the relative contribution of thermal and nonthermal factors to heat loss may change throughout recovery. In fact, Gagnon et al. (2008) observed a shift in contributions during a 60-min recovery period such that nonthermal influences on sweating were not evident until 50 min. Their results further revealed that these contributions were divergent for the heat loss responses such that nonthermal control of CVC was observed earlier in recovery (~10 min).

PART TWO:

METHODS AND RESULTS

Muscle Metaboreceptors Modulate Postexercise Sweating, but not Cutaneous Blood Flow, Independent of Baroreceptor Loading Status

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Running Head: Metabo- and baroreflex modulation of postexercise heat loss

ABSTRACT

We examined whether sustained changes in baroreceptor loading status during prolonged postexercise recovery can alter the metaboreceptors' influence on heat loss. Thirteen young males performed a 1-min isometric handgrip exercise (IHG) at 60% maximal voluntary contraction followed by 2-min of forearm ischemia (to activate metaboreceptors) before and 15, 30, 45 and 60-min after a 15-min intense treadmill running exercise (>90% maximal heart rate) in the heat (35°C). This was repeated on three separate days with continuous lower-body positive (LBPP, +40 mmHg), negative (LBNP, -20 mmHg), or no pressure (Control) from 13- to 65-min postexercise. Sweat rate (ventilated capsule; forearm, chest, upper back) and cutaneous vascular conductance (CVC; forearm, upper back) were measured. Relative to pre-IHG levels, sweating at all sites increased during IHG and remained elevated during ischemia at baseline and similarly at 30, 45, and 60-min postexercise (site average sweat rate increase during ischemia: Control, 0.13±0.02; LBPP, 0.12±0.02; LBNP, 0.15±0.02 $mg \cdot min^{-1} \cdot cm^{-2}$; all $P<0.01$), but not at 15-min (all $P>0.10$). LBPP and LBNP did not modulate the pattern of sweating to IHG and ischemia (all $P>0.05$). At 15-min postexercise, forearm CVC was reduced from pre-IHG levels during both IHG and ischemia under LBNP only (ischemia: 3.9±0.8 $\%CVC_{max}$; $P<0.02$). Therefore, we show metaboreceptors increase postexercise sweating in the mid-to-late stages of recovery (30-60 min), independent of baroreceptor loading status and similarly between skin sites. In contrast, metaboreflex modulation of forearm but not upper back CVC occurs only in the early stages of recovery (15-min) and is dependent upon baroreceptor unloading.

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Key words: thermoregulation, postexercise, metaboreflex, baroreflex, heat loss responses

INTRODUCTION

It is well-documented that recovery from exercise is associated with a disturbance in thermal homeostasis. This response is characterized by a marked and rapid suppression of heat loss responses (i.e., sweating and cutaneous blood flow) within ~20 min following exercise cessation despite persistent elevations in core and muscle temperatures for up to 90 min postexercise (19, 20, 22, 34, 37). The body's compromised ability to dissipate heat postexercise has been attributed to the actions of nonthermal factors (21). In fact, numerous studies in the past decade have demonstrated that nonthermal factors (e.g., baroreceptors, muscle mechano-/metaboreceptors) have a pivotal role in the modulation of postexercise heat loss and therefore core temperature regulation (8, 15, 17, 18, 22, 28, 29). A number of these studies have alluded to the hypothesis that baroreceptor activity associated with postexercise hypotension, characterized by a marked reduction in blood pressure from pre-exercise levels lasting ~2 h (13), is primarily responsible for mediating the attenuation of postexercise heat loss. However, recent evidence indicates that there may be interplay between nonthermal baroreceptors and metaboreceptors in their modulation of postexercise heat loss (28).

McGinn and colleagues (28) were the first to demonstrate that briefly stimulating the metaboreceptors during postexercise recovery can evoke a transient increase in sweating and reduction in cutaneous vascular conductance (CVC). The authors used a classic model to study the metaboreflex whereby isometric handgrip (IHG) exercise was followed by forearm ischemia to trap the metabolites, resulting in a concomitant increase in mean arterial pressure (MAP) (indicating that the metaboreceptors have been activated) (35). Moreover, an acute application of lower body negative pressure (LBNP) was employed during forearm ischemia to reverse the acute reflex increase in MAP such that the effect of the metaboreflex was assessed in the absence of

changes in baroreceptor loading status. It was determined that a forearm ischemia-induced loading of the baroreceptors was itself a key modulator of the cutaneous vascular response, but not the sweating response to the metaboreflex (28). However, the metaboreceptor influence was only assessed at 20-min following high intensity treadmill running in order to coincide with the approximate point of nadir of postexercise hypotension (19, 22, 23) and the point at which sweating and cutaneous blood flow have returned to near baseline levels (21). Postexercise hypotension, and therefore baroreceptor unloading, has a well-documented role in modulating postexercise heat loss (8, 17, 29). In particular, this is supported by the observation that reversing postexercise hypotension with lower body positive pressure (LBPP) application leads to a greater rate of core temperature decay due to an enhanced rate of heat dissipation associated with elevated sweating and cutaneous blood flow responses (17). However, it is unknown how the influence of metaboreceptors on heat loss would be altered when superimposed over a sustained change in baroreceptor loading status during prolonged recovery.

Importantly, Gagnon and colleagues (8) reported that the contributions of thermal and nonthermal factors to the control of postexercise heat loss are time-dependent such that a mechano- and baroreceptor modulation of sweating was only observed in the later stages (≥50 min) of a 60-min recovery period. In contrast, nonthermal modulation of CVC was evidenced earlier in recovery (>10 min). It remains to be determined how the contribution of metaboreceptors would be altered over the course of an extended recovery during which time there is a gradual return of core temperature to baseline resting levels. Thus, the objectives of this study were: 1) to examine the time-dependent influence of metaboreceptors on postexercise sweating and CVC; and, 2) to assess whether prolonged changes in postexercise baroreceptor loading status would alter these responses. We hypothesized 1) that metaboreceptor modulation of sweating and CVC would be

attenuated in the early stages of recovery; and, 2) that the metaboreflex-induced changes in sweating, but not CVC, would occur independently of baroreceptor loading status. A secondary objective was to characterize the regional variation of the heat loss responses given that an increasing number of reports have demonstrated regional heterogeneity in the nonthermal influence of the heat loss responses (25, 37).

METHODS

Ethical approval. The current experimental protocol was approved by the University of Ottawa Health Sciences and Science Research Ethics Board and is in accordance with the Declaration of Helsinki. Prior their participation in the study, written informed consent was obtained on a voluntary basis from all participants.

Participants. Thirteen healthy (non-smoking and normotensive with no history of respiratory, metabolic, or cardiovascular disease) and physically active (exercise 3-6 times per week for ≥30 min) males volunteered for the study. Their age, height, body mass, body surface area, and maximal oxygen consumption were as follows (mean ± SD): 20 ± 2 years, 177 ± 6 cm, 75 ± 8 kg, 1.92 ± 0.10 m^2, 52.0 ± 6.1 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$ respectively.

Experimental design. Subjects volunteered for a screening visit as well as three experimental sessions that were conducted on different days and separated by a minimum of 48 h. The screening visit consisted of informing participants of the study protocol and familiarizing them with the equipment and procedures prior to obtaining written consent. The day before each experimental session, participants were instructed to drink water throughout the day and 500 mL at night before sleeping to ensure euhydration. Participants were also asked to abstain from alcohol, caffeine, and medications including antipyretics, as well as to refrain from heavy or prolonged exercise for at least 24 h before each session. Each participant performed three experimental sessions in a randomly assigned order at the same time of day to avoid circadian variations in core body temperature.

On the day of the experimental sessions, participants reported to the laboratory after eating a light meal and drinking 500 mL of water no less than 2 h before their arrival. Upon arrival, participants provided a urine sample and voided the remainder of their bladder before weighing themselves nude using a digital weight scale platform (Model CBU150X, Mettler Toledo, Scherzenbach, Switzerland) with a weighing terminal (Model IND560, Mettler Toledo). Thereafter participants' body height was measured using a stadiometer.

Participants performed two brief (<3 s) maximal voluntary contractions with the right hand using a handgrip dynamometer. The higher of the two maximal voluntary contractions was used to determine the relative workload for the isometric handgrip (IHG) exercise (60% of maximal voluntary contraction, average workload: 30 ± 2 kg). Thereafter, participants were instrumented with sweat capsules (~20 min) in a thermoneutral room outside an environmental chamber (Can-Trol Environmental Systems, Markham, ON, Canada) such that sweating was avoided for proper adhesion of the capsules. The remainder of the instrumentation period took place inside the environmental chamber regulated at 35°C and 20% relative humidity with participants seated upright (~60 min).

After a 10-min baseline resting period, participants performed the metaboreceptor activation protocol. This consisted of 1 min of IHG exercise at 60% maximal voluntary contraction (as verified by visual feedback) immediately followed by a 2-min occlusion of blood flow to the arm (forearm ischemia) after which participants recovered for 2 min. For the occlusion, a pressure cuff was inflated to suprasystolic levels (>240 mmHg) and was rapidly deflated for the recovery period. Participants were then required to perform 15 min of treadmill running at >90% of maximal heart rate (average heart rate across participants: 182 ± 1 bpm). After treadmill exercise, participants were immediately transferred (~5 min) to an upright seated pressure box sealed at the

iliac crests with custom-made neoprene shorts. Thereafter participants remained seated for an additional 8 min to allow for a brief undisturbed natural recovery. This ensured that the thermal and cardiovascular responses returned to similar levels in each condition prior to pressure application. The metaboreceptor activation protocol was then repeated at 15, 30, 45 and 60 min of recovery (i.e., R15, R30, R45, and R60, respectively). On separate days, either LBPP (+40 mmHg), LBNP (-20 mmHg), or no pressure (0 mmHg with circulating air to standardize this effect across conditions, Control) was applied continuously starting at 13 min of recovery and lasting for the duration of the 65-min recovery period. Given that previous studies showed marked changes in baroreceptor loading status within 2 min of pressure application (3, 28), we selected the 13-min time point of recovery to initiate changes in baroreceptor loading status (i.e., application of LBNP, LBPP or no pressure) to ensure that the acute responses to altered baroreflex activity passed before the first assessment of metaboreceptor activation at 15 min of recovery (i.e., R15). Finally, at the end of the experimental session, peak cutaneous blood flow was determined by local heating at the measurement sites to 42°C for ~10 min followed by an additional ~20 min at a temperature of 44°C until a plateau was observed for at least 2-3 min.

Measurements. Esophageal temperature was measured continuously using a general purpose thermocouple temperature probe (Mallinckrodt Medical Inc., St-Louis, MO, USA) ~2 mm in diameter that was inserted ~40 cm past the nostril and into the esophagus while the participants sipped 100-300 mL of water through a straw. Skin temperatures were measured at six skin sites using thermocouples (Concept Engineering, Old Saybrook, CT, USA) attached with surgical tape. Mean skin temperature was calculated using 6 skin temperatures weighted to the regional proportions as determined by Hardy & Dubois (14): upper back 21%, chest 21%, quadriceps 9.5%, hamstrings 9.5%, front calf 20%, and biceps 19%. All temperature data were collected with an HP

Agilent data acquisition module (Model 34970A; Agilent Technologies Canada Inc., Mississauga, ON, Canada) at 15 s intervals, and simultaneously displayed with LabVIEW software (Version 7.0, National Instruments, Austin, TX, USA) and recorded in spreadsheet format on a desktop computer.

Sweat rate was measured at three skin sites (chest, forearm, and upper back) with a ventilated plastic capsule that was fixated at each site using adhesive rings, topical skin glue (Collodion HV, Mavidon Medical Products, Lake Worth, FL), and surgical tape. Anhydrous nitrogen gas was passed through each capsule over the skin surface at a rate of 1 $L \cdot min^{-1}$. Water content from the effluent air was measured using capacitance hygrometry (Model HMT333, Vaisala, Helsinki, Finland). Long vinyl tubes connected the gas tank (positioned in the chamber) to the sweat capsule and the sweat capsule to the hygrometer so that the gas temperature was equilibrated to room temperature (~35˚C) before reaching the sweat capsule. Local sweat rate was calculated every 5 s as the difference in water content between the effluent and influent air, multiplied by the flow rate, and then normalized for the skin surface area under the capsule ($mg \cdot min^{-1} \cdot cm^{-2}$).

Cutaneous blood flow was measured using laser-Doppler flowmetry (PeriFlux System 5000, Perimed AB, Stockholm, Sweden) at the forearm and upper back. Each laser-Doppler flow probe with a 7-laser array (Model 413, Perimed) was housed in a local heating element (PF5020 Temperature Unit, Perimed AB, Stockolm, Sweden) and affixed using a double-sided adhesive ring to an area of the skin that appeared minimally vascularized. Cutaneous vascular conductance (CVC) was subsequently calculated as the ratio of skin red blood cell flux (measured in perfusion units) to MAP, and then expressed as a percentage of peak CVC (as evaluated during the maximal cutaneous blood flow protocol; $\%CVC_{max}$).

Using a Finometer (Finapres Medical Systems, Amsterdam, The Netherlands), MAP was estimated from the beat-to-beat recording of the left middle finger arterial pressure waveform using the volume-clamp method (31). Physiocal criteria (36) were used to calibrate the finger arterial size to the point where the finger cuff air pressure equals the finger arterial blood pressure. The brachial artery pressure reconstruction (10, 11) was calibrated with an upper arm return-to-flow systolic pressure detection (4) with the left arm supported at heart level. Subsequent to each Finometer calibration period (i.e., at baseline resting and within 5 min postexercise), blood pressure was verified by manual auscultation using a validated mercury column sphygmomanometer (Baumanometer Standby Model, WA Baum Co, Copiague, NY, USA). A final blood pressure measurement at the end of the maximal cutaneous blood flow protocol was taken via manual auscultation and calculated as MAP (diastolic blood pressure plus one-third of pulse pressure).

Heart rate was recorded continuously using a Polar-coded wearlink transmitter and stored every 15 s with a Polar RS400 interface and Polar Trainer 5 software (RS400, Polar Electro, Kempele, Finland). A handheld total solids refractometer (Model TS400, Reichter, Inc., Depew, NY, USA) was used to assess urine-specific gravity from the urine samples obtained at the start and end of each experimental session.

Data analysis. For the three pressure conditions, absolute local sweat rate, CVC, MAP, and heart rate were averaged over the four stages of each of the five metaboreceptor activation protocols performed prior to and following treadmill exercise. These were as follows: the 15 s of pre-IHG resting, the final 15 s of IHG exercise, the final 15 s of forearm ischemia, and the final 15 s of recovery from forearm ischemia. These averages were also calculated for the change in sweat rate (Δsweat rate) and in MAP (ΔMAP) relative to the corresponding pre-IHG resting of each

metaboreceptor protocol. Esophageal and mean skin temperatures were averaged over a 1-min period preceding each IHG exercise. All values are presented as means ± SE. Due to technical difficulties, local sweat rate as well as esophageal and mean skin temperatures are presented for 12 participants and forearm CVC is presented for 11 participants.

Statistical analysis. Local sweat rate, CVC, MAP and heart rate obtained during the five metaboreceptor protocols were each analyzed using a three-way repeated-measures analysis of variance (3R-ANOVA) with the factors of pressure condition (three levels: LBPP, LBNP and Control), time (five levels: baseline resting along with R15, R30, R45 and R60), and metaboreceptor protocol stage (four levels: Baseline, IHG, forearm ischemia, and Recovery). In addition, a 3R-ANOVA was performed with the factors of skin site (three levels: chest, forearm, and upper back), pressure condition (three levels), and time (five levels) to compare the absolute level of sweating between skin sites prior to each metaboreceptor protocol. For the purpose of comparing the sweating response to the metaboreflex between skin sites, a 3R-ANOVA with the repeated factors of skin site (3 levels), time (5 levels) and metaboreceptor protocol stage (IHG and forearm ischemia) was performed for Δsweat rate obtained in the Control condition. Esophageal and mean skin temperatures were analyzed using a 2R-ANOVA with the factors of time (five levels) and pressure condition (three levels). A 2R-ANOVA with the factors of time (two levels: pre- and post-trial) and pressure condition (three levels) was performed for urine specific gravity. To verify that a similar intensity was achieved in the experimental sessions for IHG exercise and treadmill running exercise, a 1R-ANOVA with the factor of pressure condition (three levels) was performed for maximal voluntary contraction and for end treadmill running exercise heart rate, respectively. A 1R-ANOVA was also performed for CVC, absolute local sweat rate, MAP, and heart rate measured at 10 min postexercise to verify that similar thermoeffector activity levels were

achieved between conditions prior to pressure treatment. Pre-session body mass was assessed using a 1R-ANOVA with the factor of pressure condition (three levels). When a significant interaction or main effect was observed, *post-hoc* comparisons were carried out using paired samples *t* tests. For all analyses, differences were considered significant when $P \leq 0.05$. All statistical analyses were performed using the statistical software package SPSS 22.0 for Windows (SPSS Inc. Chicago, IL). Finally, it should be noted that the three-way ANOVA used in our study has a relatively increased potential for type II error. As our aim was to simultaneously assess the effects of pressure condition, recovery time, and metaboreceptor protocol stage, this method was the most appropriate to achieve the set objectives.

RESULTS

Hydration status and Exercise intensity

Hydration status, as assessed by pre-nude body mass, was similar between conditions (Control, 74.98 ± 2.27; LBPP, 74.91 ± 2.19; and LBNP, 74.66 ± 2.29 kg, $P = 0.44$). Urine specific gravity was increased from the start (Control, 1.009 ± 0.002; LBPP, 1.011 ± 0.002; LBNP, 1.008 ± 0.002) to the end (Control, 1.016 ± 0.001; LBPP, 1.017 ± 0.001; LBNP, 1.019 ± 0.002) of the experimental sessions ($P < 0.01$). The maximal voluntary contraction used to determine IHG exercise intensity (Control, 50 ± 2; LBPP, 51 ± 3; LBNP, 51 ± 3 kg, $P = 0.51$) as well as end-treadmill running exercise heart rate (Control, 184 ± 2; LBPP, 182 ± 1; LBNP, 181 ± 2 bpm, $P = 0.08$) did not differ between pressure conditions.

Temperature responses

Esophageal temperature. An interaction of pressure condition and time was detected for esophageal temperature ($P < 0.01$, Table 1). Specifically, esophageal temperature was similar between conditions from baseline until 15 min postexercise inclusively (all $P \geq 0.14$). During LBNP, esophageal temperature was greater compared to Control at 30, 45, and 60 min postexercise ($P \leq 0.05$) whereas it decreased more rapidly with LBPP compared to Control from 45 min postexercise until the end of the session (all $P \leq 0.01$).

Mean skin temperature. An interaction of pressure condition and time was observed for mean skin temperature ($P < 0.01$, Table 1). During LBPP application, mean skin temperature was similar to Control throughout the experimental session (all $P \geq 0.25$). In contrast, while mean skin temperature did not differ between LBNP and Control up to 30 min postexercise (all $P \geq 0.24$), a

reduction in mean skin temperature was noted during LBNP relative to Control by 0.39 ± 0.13 and 0.43 ± 0.14°C at 45 and 60 min postexercise respectively (both $P \leq 0.01$).

Cardiovascular responses

Heart rate. Heart rate exhibited an interaction of pressure condition and time ($P < 0.01$, Fig. 1A) such that while it was similar between conditions during baseline resting (all $P \geq 0.08$), heart rate gradually decreased during LBPP by 13 ± 3 bpm more than Control by 60 min of recovery ($P < 0.01$, Table 2). Conversely, the application of LBNP resulted in a heart rate greater than Control by 9 ± 3 bpm by 60 min postexercise ($P < 0.01$). In addition, heart rate increased during each IHG exercise relative to pre-IHG levels in all conditions (all $P \leq 0.01$). Although heart rate decreased below pre-IHG levels with forearm ischemia during baseline metaboreceptor protocol in all conditions ($P \leq 0.04$), we did not observe this response in the four metaboreceptor protocols performed in the postexercise recovery period under LBNP.

MAP. An interaction of pressure condition and time was detected for MAP ($P < 0.01$, Fig. 1B). Specifically, MAP was similar between conditions during baseline resting (all $P \geq 0.11$). However, LBPP application resulted in an elevation in MAP relative to both Control and LBNP by ~11 ± 3 mmHg throughout postexercise recovery (all $P \leq 0.03$), whereas Control and LBNP did not differ from one another, except during IHG exercise at R15 and R60 ($P < 0.05$). Within each condition, there were no time-dependent changes in pre-IHG resting values as R30, R45 and R60 were similar to R15 (all $P \geq 0.12$; Table 2). Additionally, in all conditions MAP increased during IHG exercise and remained elevated during forearm ischemia relative to pre-IHG levels in all five metaboreceptor protocols (all $P \leq 0.01$). Lastly, ΔMAP during each metaboreceptor protocol is presented in Table 2. Noteworthy, ΔMAP during forearm ischemia was attenuated

during R15 compared to baseline resting and remained similarly attenuated at R30, R45 and R60 in all pressure conditions (all $P \leq 0.05$; interaction of stage and time $P < 0.01$).

Heat loss responses

Sweating – Effect of pressure condition. The pressure condition did not influence the level of sweating throughout the session ($P = 0.23$), or the pattern of the sweating response during any metaboreceptor protocol (no interaction of pressure and stage $P = 0.15$).

Sweating – Effect of metaboreceptor activation. At all sites, sweat rate was increased during IHG exercise and remained elevated with forearm ischemia in all conditions during the baseline metaboreceptor protocol (Δsweat rate presented as average of the three skin sites during ischemia: Control, +0.12 ± 0.02; LBPP, +0.14 ± 0.03; LBNP, +0.16 ± 0.03 $mg \cdot min^{-1} \cdot cm^{-2}$; all $P \leq 0.01$; Fig. 2). During R15, while sweat rate was increased from resting levels during IHG exercise for LBPP and LBNP ($P < 0.05$), it was not different during forearm ischemia in any condition ($P \geq 0.12$). Conversely, at R30, R45 and R60, sweat rate was increased during IHG exercise from resting levels and remained elevated with forearm ischemia (all $P < 0.01$). Importantly, the magnitude of this response (i.e., Δsweat rate) was similar to that observed at baseline resting (all $P \geq 0.14$).

Sweating – Skin sites. An interaction of time and skin site ($P = 0.001$) was detected for absolute local sweat rate. Specifically, at pre-IHG resting of each metaboreceptor protocol, forearm sweating was lower compared to the chest and upper back ($P \leq 0.05$), while the latter two sites exhibited similar levels of sweating ($P \geq 0.05$; Fig. 3A, B, C). Additionally, Δsweat rate during forearm ischemia did not differ between skin sites at any time (all $P > 0.05$; Table 4).

CVC – Effect of pressure condition. No differences in forearm or upper back CVC were noted between conditions during baseline resting (all $P > 0.05$). However, effects of pressure were observed postexercise such that LBNP application was associated with reduced forearm and upper back CVC relative to LBPP and Control at 30-60 min postexercise (all $P < 0.05$, Table 2). On the other hand, CVC during LBPP application was similar to Control (all $P \geq 0.16$).

CVC – Effect of metaboreceptor activation. During the baseline resting metaboreceptor protocol, forearm CVC was increased from pre-IHG levels during IHG exercise for LBPP and LBNP only ($P \leq 0.05$), whereas CVC did not differ from pre-IHG levels with forearm ischemia in any condition (all $P \geq 0.12$; Fig. 4A). Upper back CVC was increased during IHG exercise from pre-IHG levels during the baseline metaboreceptor protocol in all conditions ($P \leq 0.01$; Fig. 4B). In the subsequent period of ischemia, upper back CVC returned to pre-IHG levels (Control, $P > 0.05$) or remained slightly elevated (LBPP and LBNP, both $P < 0.01$). During R15 and under Control conditions, forearm CVC was reduced from pre-IHG levels during IHG exercise ($P < 0.02$), but not forearm ischemia ($P = 0.10$). However, LBNP application resulted in reductions in forearm CVC from resting levels during both IHG exercise and the subsequent period of ischemia ($P < 0.02$). No effect of IHG exercise was observed on upper back CVC in any condition at R15 ($P \geq 0.36$). However the application of LBNP resulted in a reduction in upper back CVC during forearm ischemia at R15 ($P = 0.02$). For the remainder of recovery, forearm ischemia did not affect forearm and upper back CVC in any condition (all $P \geq 0.07$).

DISCUSSION

We show that metaboreceptors can influence postexercise sweating by maintaining sweat rates during forearm ischemia at elevated levels induced by the isometric handgrip exercise in the mid-to-late stages (i.e., 30 to 60 min) of recovery. The metaboreflex-mediated increase in sweating occurred despite a progressive decay in the level of hyperthermia over the course of recovery. Further, this pattern of response was homogenous across each site (forearm, chest, and upper back) and was similar for all pressure conditions and therefore independent of the baroreceptor loading status. In contrast, we show no effect of metaboreceptors on CVC at any point in recovery for either the forearm or upper back. However, we show that baroreceptor loading status does modulate this response such that forearm CVC was reduced with LBNP application in the early stages (i.e., 15 min) of recovery only.

Sweating

A recent study by McGinn and colleagues (28) was the first to demonstrate that metaboreceptors may play a pivotal role in modulating the postexercise suppression of sweating. However, metaboreceptors were only assessed at 20 min postexercise. Our study extends on these findings such that the metaboreflex induced a similar sustained increase in sweat rate from 30 to 60 min of recovery. This level of increase in sweating (~0.14 $mg \cdot min^{-1} \cdot cm^{-2}$) was similar to that measured during the baseline resting period across all three skin sites, indicating that the influence of metaboreceptors on sweating remains intact in a moderate exercise-induced state of hyperthermia (i.e., core temperature elevation of ~0.7°C). Noteworthy, McGinn et al. (28) recently reported that forearm ischemia in the absence of a preceding IHG exercise bout in the postexercise period maintained an elevated sweat rate (i.e., sweat rate did not increase further) rather than rapidly

decreasing as was reported in a separate condition whereby no IHG exercise or forearm ischemia were employed. In this case, it was argued that metaboreceptors still modulated sweating through a transient delay in the suppression of postexercise sweating towards baseline levels. Likewise, we did not detect a metaboreflex-induced increase in sweating during forearm ischemia at 15 min postexercise in our study; however, it appears that metaboreceptor activation prevented a further decrease in sweat rate (Figure 3). Interestingly, mechano- and baroreceptors have been shown to modulate postexercise sweating at lower levels of hyperthermia [i.e., core temperature increases of ~0.4°C (17) and ~0.6°C (8)] whereas we show metaboreceptors to modulate postexercise sweating under a high level of hyperthermia (i.e., core temperature increase of ~1ºC at 15 min of recovery). Taken together, these findings indicate that metaboreceptors play an important role in modulating sweating even under elevated levels of exercise-induced hyperthermia.

The postexercise suppression of sweating has long been attributed to baroreceptor unloading (21). This relationship was best evidenced in the study by Journeay et al. (17) who demonstrated that prolonged application of LBPP substantially increased heat loss via sweating and cutaneous blood flow and thereby accelerated the decrease in core temperature relative to LBNP and no pressure application. However, a recent study by McGinn et al. (27) reported that prolonged application of LBPP, LBNP or no pressure had no impact on postexercise sweat rate, which was ascribed to the greater levels of hyperthermia recorded relative to Journeay et al. (17). Our findings are consistent with McGinn et al. (27) such that postexercise sweat rate before each IHG exercise (i.e., pre-IHG baseline at 15, 30, 45, and 60 min of recovery, see Figure 3) was not impacted by the sustained application of LBPP or LBNP as with the no pressure condition. However, a baroreceptor-mediated component to postexercise sweating cannot be entirely discounted in our study given that similar levels of sweat rate were observed despite core

temperature being lower (by as much as 0.3°C) during LBPP relative to LBNP. On the other hand, a challenge that is inherent to metaboreceptor activation is the reflex increase in MAP which may itself influence the sweating response. Three studies have shown that eliminating the metaboreflex-induced changes in MAP does not alter the sweating response to metaboreceptor activation both during passive heating (3, 32) and in the postexercise period (28). In parallel, we showed that the metaboreflex-induced sweating response was not modulated by differences in baroreceptor loading status achieved by prolonged LBPP and LBNP application postexercise.

Previous reports have suggested that the magnitude of increase in MAP (30) and sweating (24) during forearm ischemia can infer the level of circulating metabolites, and therefore the magnitude of metaboreceptor activation. Along these lines, we show comparable metaboreflex-induced increases in sweating during baseline resting as well as at 30-, 45-, and 60- min postexercise. However, we also report that the change in MAP (ΔMAP) associated with forearm ischemia was persistently attenuated during postexercise recovery by ~8 mmHg compared to that at baseline resting despite the same IHG exercise intensity (i.e., 60% of maximal voluntary contraction). Moreover, this response occurred in all three pressure conditions, irrespective of pronounced differences in baroreceptor loading status. These conflicting observations of a similar sweating response despite differences in the ΔMAP may indicate a reduced magnitude of metaboreceptor activation combined with an increased sweating sensitivity. Alternatively, the blunted ΔMAP may indicate an attenuated increase in cardiac output for a similar magnitude of metaboreceptor activation, as was observed during moderate and high levels of passive heat stress (2). Further studies are required to elucidate these possibilities.

Cutaneous Vascular Response

While we showed that upper back CVC was unchanged during the activation of metaboreceptors throughout recovery, a reduction in forearm CVC during forearm ischemia was observed in the early stages of recovery (i.e., 15 min) during LBNP application only. The mechanism(s) underlying these disparate responses cannot presently be elucidated. However, the response at the forearm cannot be fully attributed to metaboreceptors given that a metaboreflex-mediated decrease in CVC did not occur during the Control condition. This indicates that the greater level of baroreceptor unloading associated with LBNP application (as evidenced by a higher heart rate compared to Control) was involved in reducing CVC during forearm ischemia. Our data are consistent with the findings by McGinn and colleagues (28) whereby the reduction in CVC during forearm ischemia was primarily mediated by changes in baroreceptor loading status induced with LBNP at 20 min postexercise (i.e., the approximate point of nadir for postexercise hypotension) (19, 23). In the present study, we evaluated the influence of metaboreceptors before and after this period (i.e., at 15 and ≥30 min postexercise) and under different conditions of baroreceptor loading status (i.e., with the continuous application of LBPP, LBNP or no pressure) for the duration of the 65-min recovery. Thus, any contribution of metaboreceptors to postexercise CVC may have been masked by time-dependent changes in hemodynamic responses at these stages of recovery. Therefore, in parallel to the time-dependent shift in the relative contribution of thermal and nonthermal control of postexercise cutaneous blood flow (and sweating) (8), a time-dependent shift may exist between the different nonthermal factors in terms of their relative control of CVC that may be closely related to postexercise cardiovascular adjustments.

It is well-established that changes in baroreflex activity alone can modulate CVC during postexercise recovery (8, 17, 27, 29). Accordingly, in the present study baroreceptor unloading

associated with LBNP application resulted in greater reductions in both forearm and upper back CVC towards baseline resting levels compared to Control and LBPP conditions. On the other hand, the application of LBPP did not affect CVC, which contrasts previous work that mechanically altered baroreceptor loading status via the application of lower body pressure (17, 27). The disparity may be explained in part by the distinct nature of the experimental paradigm employed in the present study wherein repeated bouts of IHG exercise and forearm ischemia to activate the metaboreflex were superimposed on the sustained application of pressure to the lower limbs. In contrast to previous studies (17, 27), we also did not observe postexercise hypotension prior to the application of pressure (i.e., at 13 min postexercise). However, this is consistent with the recent study by McGinn et al. (28) who employed a similar experimental paradigm consisting of an identical exercise protocol (i.e., treadmill running at 90% of maximum heart rate for 15 min) followed by a comparable short recovery period preceding a metaboreceptor activation maneuver. It is plausible that the differences between studies may be attributed to differences in exercise mode, level of exercise-induced hyperthermia, training status, or other factors (12). Nonetheless, the lack of any effect of metaboreceptor activation on CVC beyond the early stages of recovery may suggest that the attenuation of postexercise CVC is primarily mediated by baroreceptor input whereas metaboreceptors seem to have less of an important role in modulating postexercise CVC.

Limitations

Our findings may not be generalizable to other populations. For example, females have been shown to exhibit altered baroreflex activity (5, 6, 20) as well as an attenuated metaboreflex-induced increase in mean arterial pressure and muscle sympathetic nerve activity relative to their male counterparts (16). It has also been shown that fitness can modulate the responses to the

metaboreflex (1). Given that we included only young males who were sufficiently fit to complete the high intensity running protocol, it is unknown if a similar pattern of response would be observed in females, sedentary individuals, and/or older adults who are known to have an impaired capacity to dissipate heat (7, 9, 26, 33). Further studies are required to determine whether the influence of metaboreceptor activation on postexercise heat loss responses would differ in different population groups.

Perspectives and Significance

The suppression of postexercise heat loss has been primarily ascribed to baroreceptor control (21). Our findings strongly suggest that metaboreceptors can also impact postexercise heat loss, even at higher levels of hyperthermia (i.e., core temperature elevation of ~1ºC). An important question that remains is whether the metaboreflex can induce meaningful changes in whole-body heat exchange during the postexercise period. Importantly, our results lend evidence to suggest that metaboreceptor activation may well increase whole-body sweating such that we observed similar regional sweating responses during forearm ischemia at the forearm, chest, and upper back (Figure 2). However, future studies are warranted to assess this hypothesis of a metaboreflex-induced increase of whole-body heat loss, especially under conditions of elevated heat stress when the requirements of heat dissipation are greatest.

In summary, we show that metaboreceptors influence postexercise sweating by maintaining sweat rate at elevated levels as induced during IHG exercise from 30 to 60 min of recovery without any regional differences at the forearm, chest, and upper back despite the progressive decay of postexercise core temperature. Our findings also indicate that metaboreceptors can influence sweat rate (albeit without a detectable increase in sweat rate) as

early as 15 min of recovery. In addition, we show that the effect of metaboreceptors on sweating is unaltered by sustained changes in baroreceptor loading status. On the other hand, we demonstrated a minimal role for metaboreceptors in modulating CVC following the early stages of recovery such that CVC appears to be mediated primarily by baroreceptor loading status.

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Disclosures

None to declare.

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Table 1. Baseline values (i.e., pre-IHG exercise) for core, mean skin and body temperatures during pre- and postdynamic exercise periods.

	Baseline resting	End-dynamic exercise	Postexercise			
			15 min	30 min	45 min	60 min
T_{eso}, °C						
Control	36.67 ± 0.08	38.50 ± 0.12	37.53 ± 0.12	37.20 ± 0.08	37.16 ± 0.08	37.16 ± 0.07
LBPP	36.65 ± 0.09	38.48 ± 0.14	37.59 ± 0.13	37.10 ± 0.10	37.00 ± 0.08*	36.99 ± 0.07*
LBNP	36.65 ± 0.08	38.43 ± 0.13	37.51 ± 0.13†	37.32 ± 0.09*†	37.29 ± 0.08*†	37.27 ± 0.08†
T_{sk}, °C						
Control	34.72 ± 0.10	35.35 ± 0.10	36.22 ± 0.12	35.75 ± 0.11	35.62 ± 0.09	35.56 ± 0.10
LBPP	34.77 ± 0.09	35.39 ± 0.12	36.35 ± 0.13	35.74 ± 0.09	35.67 ± 0.07	35.68 ± 0.07
LBNP	34.81 ± 0.10	35.49 ± 0.11	36.31 ± 0.10	35.65 ± 0.14	35.23 ± 0.12*†	35.08 ± 0.12*†

Values presented as means ± SE (n = 12). T_{eso}, esophageal temperature; T_{sk}, mean skin temperature. Experimental conditions: LBPP, lower-body positive pressure; LBNP, lower-body negative pressure. Significantly different from *Control and †LBPP ($P < 0.05$).

Table 2. Baseline values (i.e., pre-IHG exercise) for hemodynamic and CVC responses during pre- and postdynamic exercise periods.

		Baseline resting	Postexercise			
			15 min	30 min	45 min	60 min
MAP, mmHg	Control	87 ± 2	87 ± 2	87 ± 2	89 ± 2†	90 ± 2†
	LBPP	90 ± 1	88 ± 2	88 ± 2	98 ± 2	100 ± 2
	LBNP	88 ± 1	86 ± 1	86 ± 1	86 ± 2†	89 ± 2†
HR, beats·min^{-1}	Control	74 ± 3	115 ± 3†	103 ± 2†	101 ± 3†	100 ± 3†
	LBPP	72 ± 3	105 ± 2	94 ± 2	89 ± 2	87 ± 2
	LBNP	72 ± 2	122 ± 3*†	114 ± 3*†	108 ± 3*†	109 ± 3*†
$CVC_{forearm}$, %max	Control	20 ± 3	38 ± 3	33 ± 3	25 ± 2	20 ± 2
	LBPP	18 ± 3	41 ± 2	33 ± 3	22 ± 3	18 ± 2
	LBNP	17 ± 3	41 ± 2	26 ± 2*†	17 ± 1*†	14 ± 1*
CVC_{back}, %max	Control	14 ± 2	31 ± 3	23 ± 3	17 ± 2	13 ± 2
	LBPP	11 ± 1	36 ± 4	25 ± 3	16 ± 2	13 ± 1
	LBNP	14 ± 1	32 ± 3	18 ± 2†	12 ± 1*†	10 ± 1†

Values presented as means ± SE (n = 13 for MAP, HR, and $CVC_{upper\ back}$; n=11 for $CVC_{forearm}$). MAP, mean arterial pressure; HR, heart rate; CVC, cutaneous vascular conductance. Experimental conditions: LBPP, lower-body positive pressure; LBNP, lower-body negative pressure. Significantly different from *Control and †LBPP ($P < 0.05$).

Table 3: Baseline (BL) values for MAP (mmHg) and the relative change from BL at end isometric handgrip exercise (IHG) and end of forearm ischemia (FI) during the pre- and postdynamic exercise periods.

	Baseline resting			*15 min postexercise*			*30 min postexercise*			*45 min postexercise*			*60 min postexercise*		
	BL	IHG	FI	BL	IHG	FI	BL	IHG	FI	BL	IHG	FI	BL	IHG	FI
Control	87 ± 2	27 ± 2	16 ± 2	87 ± 2	20 ± 2	8 ± 2	88 ± 2	22 ± 2	9 ± 2	89 ± 2	20 ± 2	8 ± 1	90 ± 2	23 ± 2	8 ± 1
LBPP	90 ± 1	27 ± 2	16 ± 2	88 ± 2	20 ± 3	11 ± 2	96 ± 2*	22 ± 3	9 ± 2	98 ± 2*	23 ± 3	8 ± 2	100 ± 2*	22 ± 3	8 ± 2
LBNP	88 ± 1	28 ± 2	17 ± 2	86 ± 1	16 ± 2*	8 ± 2	86 ± 2†	20 ± 2	10 ± 1	86 ± 2†	15 ± 2†	5 ± 2	89 ± 2†	17 ± 2*	7 ± 1

Values presented as means ± SE (n = 13). MAP, mean arterial pressure. Experimental conditions: LBPP, lower-body positive pressure; LBNP, lower-body negative pressure. Significantly different from *Control or †LBPP ($P < 0.05$).

Table 4: The change in sweat rate ($mg \cdot min^{-1} \cdot cm^{-2}$) relative to pre-IHG resting levels at end isometric handgrip exercise (IHG) and end of forearm ischemia (FI) during the pre- and postdynamic exercise periods under Control conditions.

	Baseline resting		*15 min postexercise*		*30 min postexercise*		*45 min postexercise*		*60 min postexercise*	
	IHG	FI	IHG	FI	IHG	FI	IHG	FI	IHG	FI
Chest	0.21 ± 0.05†	0.12 ± 0.03	-0.01 ± 0.03	-0.04 ± 0.05	0.14 ± 0.05†*	0.13 ± 0.05	0.16 ± 0.04†*	0.09 ± 0.02	0.14 ± 0.04	0.09 ± 0.03
Forearm	0.20 ± 0.04†	0.08 ± 0.02	0.07 ± 0.03	0.01 ± 0.04	0.21 ± 0.04	0.17 ± 0.04	0.24 ± 0.04	0.15 ± 0.03	0.20 ± 0.03	0.10 ± 0.02
Upper back	0.31 ± 0.06	0.16 ± 0.03	0.03 ± 0.02	-0.06 ± 0.05	0.22 ± 0.04	0.15 ± 0.04	0.25 ± 0.03	0.14 ± 0.03	0.21 ± 0.04	0.13 ± 0.03

Values presented as means ± SE (n = 12). Experimental conditions: LBPP, lower-body positive pressure; LBNP, lower-body negative pressure. Significantly different from *Forearm and †Upper back ($P < 0.05$).

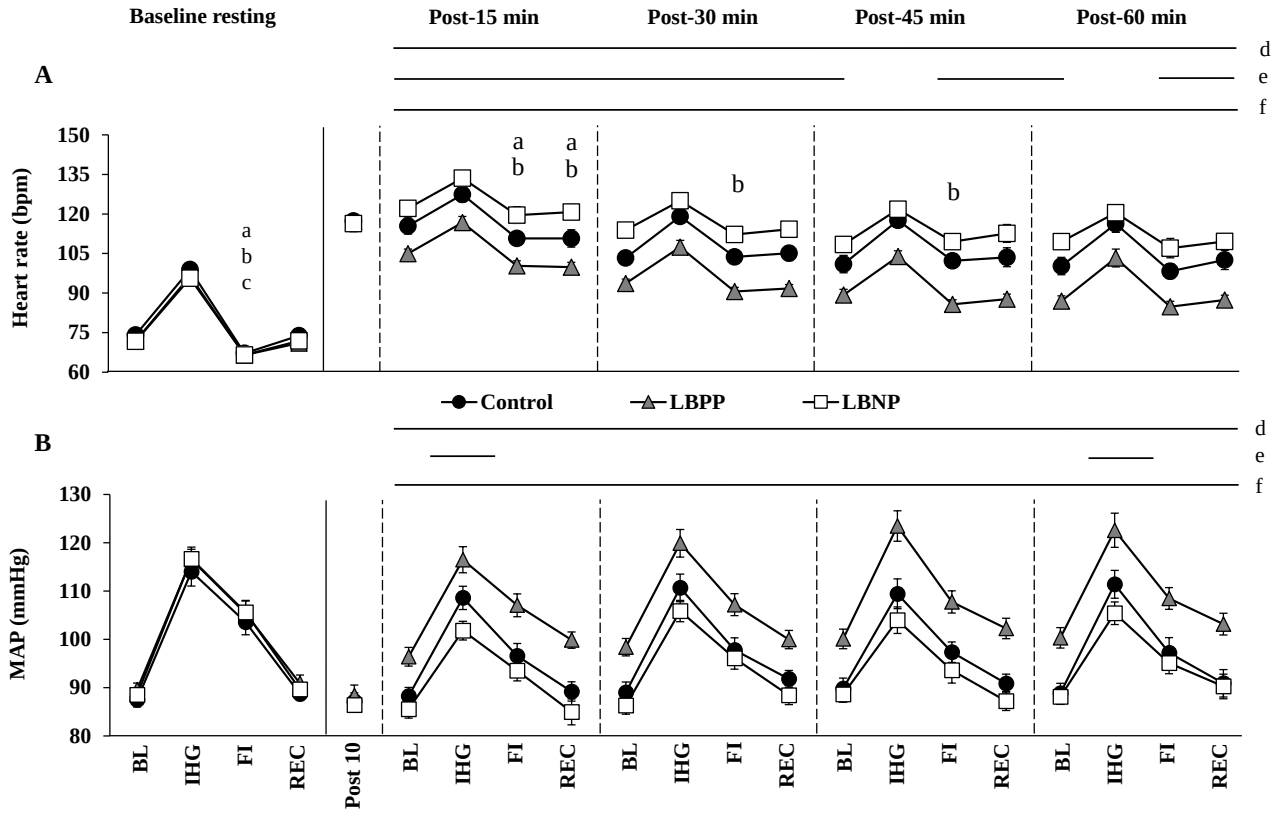

Figure 1: Cardiovascular responses at baseline (BL), end isometric handgrip exercise (IHG), end of ischemia (FI), and recovery from FI (REC) during Control, lower-body positive pressure (LBPP), and lower-body negative pressure (LBNP). Measurements were performed during a predynamic exercise period (Baseline resting) and throughout 65 min postdynamic exercise. Values expressed as means ± SE (n = 13). Mean arterial pressure (MAP, Panel A) was significantly elevated from BL during IHG and FI in all conditions. Heart rate (Panel B) was significantly elevated from BL during IHG in all conditions. Significantly different from BL within [a]Control, [b]LBPP, and [c]LBNP ($P < 0.05$). Control significantly different from [d]LBPP and [e]LBNP ($P < 0.05$). [f]LBNP significantly different from LBPP ($P < 0.05$).

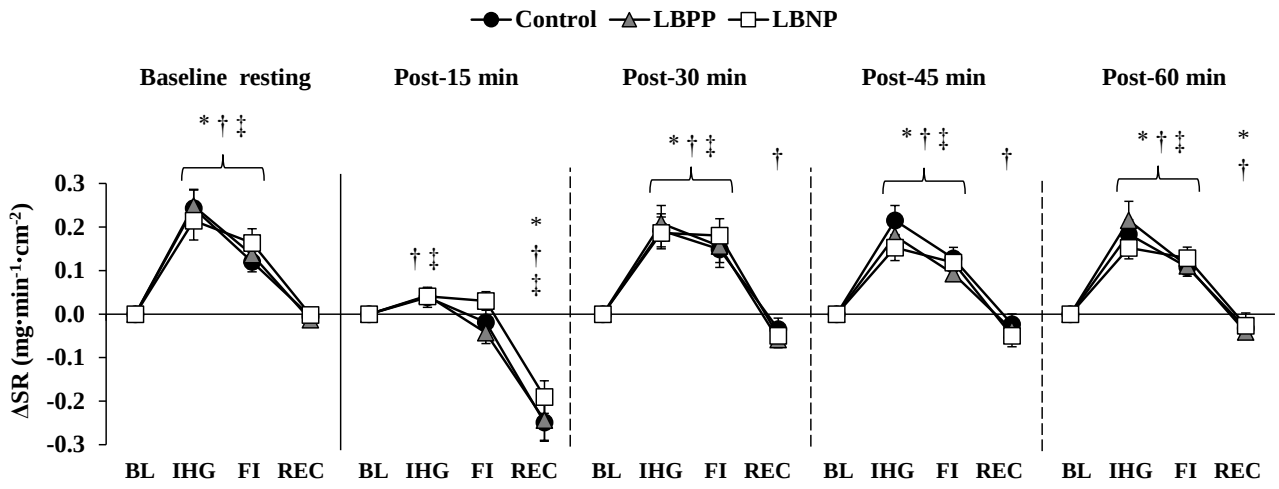

Figure 2: Relative changes in sweat rate (average of chest, forearm, and upper back; ΔSR) in comparison to baseline (BL) at end isometric handgrip exercise (IHG), end of ischemia (FI), and recovery from FI (REC) during Control, lower-body positive pressure (LBPP), and lower-body negative pressure (LBNP). Measurements were performed during a predynamic exercise period (Baseline resting) and throughout 65 min postdynamic exercise. Values expressed as means ± SE (n = 12). Significantly different from BL within *Control, †LBPP, and ‡LBNP ($P < 0.05$).

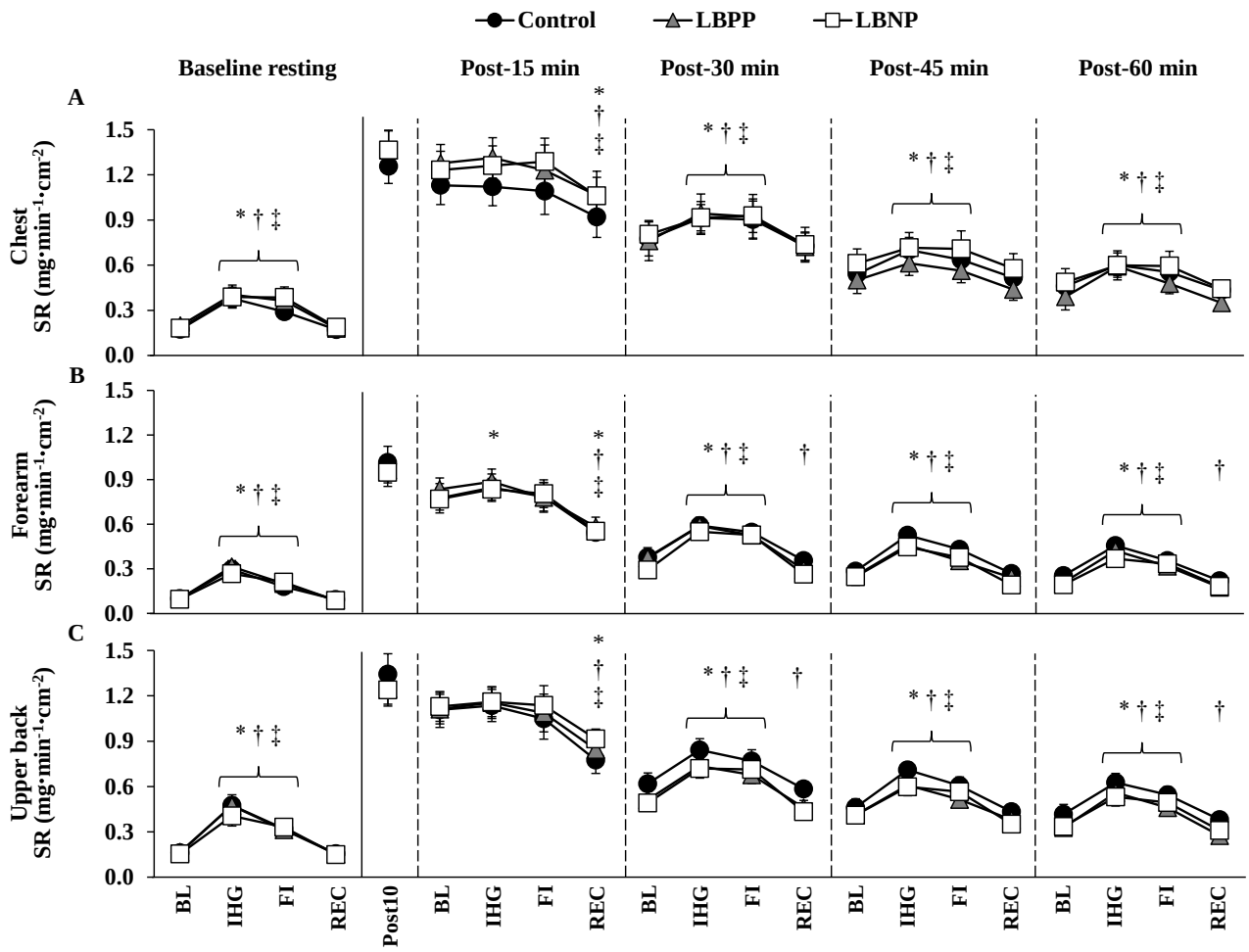

Figure 3: Absolute sweat rate (SR) at the chest (panel A), forearm (panel B), and upper back (panel C) measured at baseline (BL), end isometric handgrip exercise (IHG), end of ischemia (FI), and recovery from FI (REC) during Control, lower-body positive pressure (LBPP), and lower-body negative pressure (LBNP). Measurements were performed during a predynamic exercise period (Baseline resting) and throughout 65 min postdynamic exercise. Values expressed as means ± SE (n = 12). Significantly different from BL within *Control, †LBPP, and ‡LBNP ($P < 0.05$).

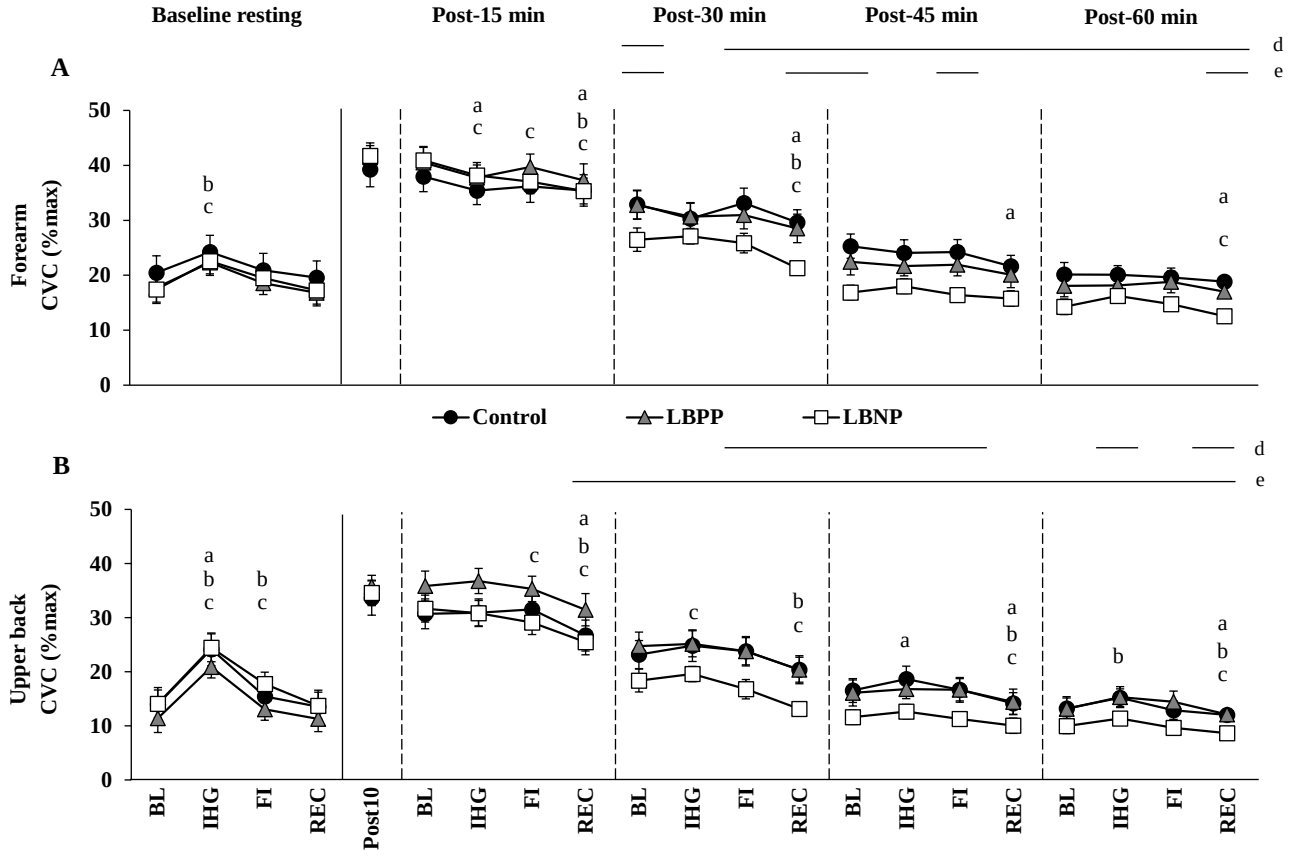

Figure 4: Cutaneous vascular conductance (CVC) of the forearm (panel A) and upper back (panel B) measured at baseline (BL), end isometric handgrip exercise (IHG), end of ischemia (FI), and recovery from FI (REC) during Control, lower-body positive pressure (LBPP), and lower-body negative pressure (LBNP). Measurements were performed during a predynamic exercise period (Baseline resting) and throughout 65 min postdynamic exercise. Values expressed as means ± SE (n = 11). LBPP was similar to Control throughout the trial ($P > 0.05$). Significantly different from BL within [a]Control, [b]LBPP, and [c]LBNP ($P < 0.05$). LBNP significantly different from [d]Control, and [e]LBPP ($P<0.05$).

PART THREE:

GENERAL CONCLUSIONS OF THE THESIS

GENERAL CONCLUSIONS

The primary purpose of the current thesis was to examine the combined influences of two nonthermal factors, metaboreceptors and baroreceptors, on sweating and skin blood flow during prolonged recovery from dynamic exercise. This was accomplished by employing a classic model used in the study of metaboreceptors (i.e., isometric handgrip exercise followed by a period of forearm ischemia) at several time intervals throughout a period of recovery from dynamic exercise, superimposed with the sustained application of lower-body positive and negative pressure (to maintain a continuous change in baroreceptor loading status). The most important finding of this study is that transiently stimulating the metaboreceptors following dynamic exercise maintained sweat rate at elevated levels as induced during IHG exercise in the mid-to-late stages of the recovery period (i.e., 30 to 60 min). Furthermore, metaboreceptors were found to be capable of influencing sweating as early as 15 min postexercise, albeit without a detectable increase in sweat rate during the preceding IHG exercise, in the presence of an elevated level of hyperthermia. Importantly, this response was unaffected by prolonged changes in baroreceptor loading status, thereby establishing that there is no interplay between baro- and metaboreceptors in the modulation of postexercise sweating. In contrast to the control of sweating, skin blood flow was influenced by metaboreceptors only in the early stages of recovery (i.e., 15 min) and only under baroreceptor unloading. Thus, the study results reveal divergent influences of nonthermal factors on the control of sweating and of skin blood flow as a function of recovery time.

In addition, this study presents important new findings in regards to the regional responses to the metaboreflex postexercise. The pattern of the sweating response to metaboreceptor activation (i.e., relative changes in sweat rate) was determined to be homogenous between the chest, forearm, and upper back regions; albeit the absolute level of sweating was lowest at the

forearm. Moreover, only skin blood flow evaluated at the forearm and not the upper back responded to metaboreceptor activation. Therefore, the current study shows that the nonthermal control of postexercise heat loss, which was distinct for sweating compared to skin blood flow, differs as a function of body region.

It is important to note that the sample in the present student consisted of young, physically fit males and therefore our results are not generalizable to other population groups. For example, sedentary individuals, females, and older adults display an impaired capacity to dissipate heat compared to young, physically fit males (Gagnon, Crandall, & Kenny, 2013; Gagnon & Kenny, 2012; Larose, Boulay, et al., 2013; Stapleton, Fujii, et al., 2014). In addition, sex has been shown to modulate nonthermal sensory receptor activity and control of sympathetic output (Christou, Jones, et al., 2005; Convertino, 1998; Kenny & Jay, 2011; Jarvis, VanGundy, et al., 2011), while physical fitness has been shown to modulate the sweat response induced by metaboreceptor activation (Amano, Ichinose, et al., 2011). Therefore one can speculate that these population groups would display an attenuated sweat response to metaboreceptor activation postexercise. Further studies are required to explore nonthermal modulation of heat loss in different population groups and the thermoregulatory implications for these individuals.

Ultimately, this study provides important insight into the integrated central modulation (via metaboreceptors and baroreceptors) of postexercise heat loss responses. Previous reports have attempted to delineate the relative contributions of different nonthermal factors (e.g., baroreceptors, mechanoreceptors/muscle pump, and central command) to the control of postexercise sweating and skin blood flow (Gagnon et al., 2008; Journeay, Reardon, Martin, et al., 2004; Journeay, Reardon, McInnis, & Kenny, 2005). However, future work should be directed at further examining the interplay between different nonthermal stimuli in the control of postexercise

heat loss responses given that there are often several factors simultaneously implicated during recovery from dynamic exercise. Additionally, a recent study examined the peripheral pathways through which baroreceptors mediate the postexercise suppression of skin blood flow and sweating (McGinn, Paull, et al., 2014). However, the local mechanisms underpinning nonthermal modulation of sweating and skin blood flow remain largely unknown. Therefore, future research should be conducted to delineate the local mechanisms through which nonthermal stimuli (e.g., including metaboreceptor activation) mediate their effects on skin blood flow and sweating in order to advance our understanding of postexercise thermoregulatory control.

In conclusion, the findings from the current study could serve in the development of interventions (e.g., recovery strategies). Such strategies could include recovery in the supine position, passive recovery (i.e., inactive movement of lower limbs to increase venous return), the use of compression socks, and adequate fluid replenishment to mitigate reductions blood volume (i.e., baroreceptor unloading) that occurs though profuse sweating. These interventions could then be used to mitigate and/or manage the postexercise suppression in heat dissipation that may place individuals at a greater risk for heat-related injuries and/or illness, particularly in vulnerable populations such as older adults and those with chronic health conditions (e.g., diabetes mellitus). Subsequently, these strategies can be implemented as part of protocols and safety procedures in workplaces, and sport and civilian contexts that present a high risk for individuals to suffer a heat-related injury.

PART FOUR:

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PART FIVE:

APPENDICES

APPENDIX A – Ethical Approval Certificate

File Number: H01-14-01 **Date (mm/dd/yyyy):** 03/05/2014

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

Ethics Approval Notice

Health Sciences and Science REB

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

First Name	Last Name	Affiliation	Role
Glen	Kenny	Health Sciences / Human Kinetics	Principal Investigator
Naoto	Fujii	Health Sciences / Physiotherapy	Co-investigator
Ryan	McGinn	Health Sciences / Human Kinetics	Co-investigator
Rob	Meade	Health Sciences / Human Kinetics	Co-investigator
Gabrielle	Paull	Health Sciences / Human Kinetics	Co-investigator
Martin	Poirier	Health Sciences / Human Kinetics	Co-investigator

File Number: H01-14-01

Type of Project: Professor

Title: Human thermoregulation: separating thermal and nonthermal effects on the body's capacity to dissipate heat

Approval Date (mm/dd/yyyy)	Expiry Date (mm/dd/yyyy)	Approval Type
02/28/2014	02/27/2015	Ia

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

Special Conditions / Comments:
N/A

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File Number: H01-14-01 **Date (mm/dd/yyyy):** 03/05/2014

Université d'Ottawa University of Ottawa

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

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

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

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

If you have any questions, please do not hesitate to contact the Ethics Office at extension 5387 or by e-mail at: ethics@uOttawa.ca.

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

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APPENDIX B – Ethical Renewal Certificate

File Number: h01-14-01 **Date (mm/dd/yyyy):** 02/11/2015

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

Ethics Approval Notice

Health Sciences and Science REB

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

First Name	Last Name	Affiliation	Role
Glen	Kenny	Health Sciences / Human Kinetics	Principal Investigator
Naoto	Fujii	Health Sciences / Physiotherapy	Co-investigator
Ryan	McGinn	Health Sciences / Human Kinetics	Co-investigator
Robert	Meade	Health Sciences / Human Kinetics	Co-investigator
Gabrielle	Paull	Health Sciences / Human Kinetics	Co-investigator
Martin	Poirier	Health Sciences / Human Kinetics	Co-investigator
Brian	Friesen	Health Sciences / Human Kinetics	Research Assistant
Baies	Mohammed Haqani	Health Sciences / Human Kinetics	Research Assistant

File Number: h01-14-01

Type of Project: Professor

Title: Human thermoregulation: separating thermal and nonthermal effects on the body's capacity to dissipate heat

Renewal Date (mm/dd/yyyy)	Expiry Date (mm/dd/yyyy)	Approval Type
02/28/2015	02/27/2016	Ia

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

Special Conditions / Comments:
N/A

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File Number: h01-14-01 **Date (mm/dd/yyyy):** 02/11/2015

Université d'Ottawa University of Ottawa

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

This is to confirm that the University of Ottawa Research Ethics Board identified above, which operates in accordance with the Tri-Council Policy Statement (2010) and other applicable laws and regulations in Ontario, has examined and approved the ethics application for the above named research project. Ethics approval is valid for the period indicated above and subject to the conditions listed in the section entitled "Special Conditions / Comments".

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

Please submit an annual report to the Ethics Office four weeks before the above-referenced expiry date to request a renewal of this ethics approval. To close the file, a final report must be submitted. These documents can be found at: http://www.research.uottawa.ca/ethics/forms.html.

If you have any questions, please do not hesitate to contact the Ethics Office at extension 5387 or by e-mail at: ethics@uOttawa.ca.

Mélanie Rioux
Ethics Coordinator
For Catherine Paquet, Director of the Office of Research Ethics and Integrity

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APPENDIX C – Experimental Timeline

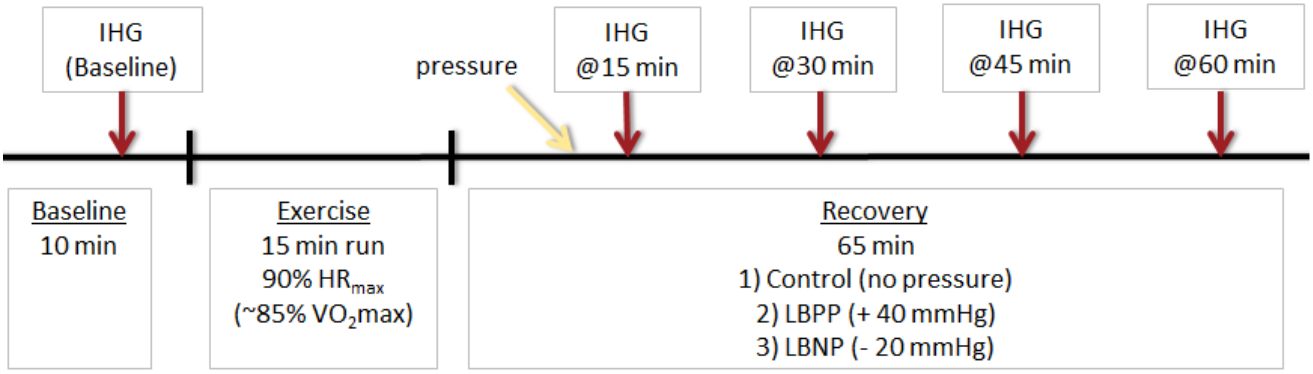

Figure 1. The timeline of the experimental protocol that will be employed in the current thesis proposal. HR, heart rate; IHG, isometric hand-grip exercise; LBNP, lower-body negative pressure; LBPP, lower-body positive pressure.

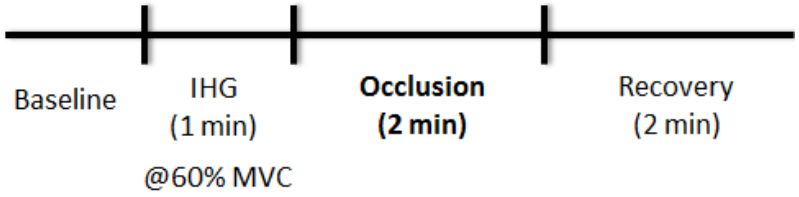

Figure 1. The metaboreceptor activation protocol timeline employed before dynamic exercise and at 15, 30, 45 and 60 min after dynamic exercise. IHG, isometric hand-grip exercise; MVC, maximal voluntary contraction