Mechanisms underlying the postexercise attenuation of skin blood flow and sweating

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Abstract

Reports indicate that postexercise heat loss is modulated by baroreceptor input; however, the mechanisms remain unknown. We examined the time-dependent involvement of adenosine receptors, noradrenergic transmitters, and nitric oxide (NO) in modulating baroreceptor-mediated changes in postexercise heat loss. Eight males performed two 15-min cycling bouts (85% VO_{2max}) each followed by a 45-min recovery in the heat (35°C). Lower body positive (LBPP), negative (LBNP), or no (Control) pressure was applied in three separate sessions during the final 30-min of each recovery. Four microdialysis fibres in the forearm skin were perfused with: *1*) lactated Ringer's (Ringer's); *2*) $4m_M$ Theophylline (inhibits adenosine receptors); *3*) $10m_M$ Bretylium (inhibits noradrenergic transmitter release); or *4*) $10m_{M\ L}$-NAME (inhibits NO synthase). We measured cutaneous vascular conductance (CVC; percentage of maximum) calculated as perfusion units divided by mean arterial pressure, and local sweat rate. Compared to Control, LBPP did not influence CVC at $_L$-NAME, Theophylline or Bretylium during either recovery (P>0.07); however, CVC at Ringer's was increased by ~5-8% throughout 30 min of LBPP during Recovery 1 (all P<0.02). In fact, CVC at Ringer's was similar to Theophylline and Bretylium during LBPP. Conversely, LBNP reduced CVC at all microdialysis sites by ~7-10% in the last 15 min of Recovery 2 (all P<0.05). Local sweat rate was similar at all treatment sites as a function of pressure condition (P>0.10). We show that baroreceptor input modulates postexercise CVC to some extent via adenosine receptors, noradrenergic vasoconstriction, and NO whereas no influence was observed for postexercise sweating.

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PART ONE: EMPIRICAL AND THEORETICAL CONSIDERATIONS

CHAPTER I

INTRODUCTION

1.1 Introduction

Humans, as homeotherms, are required to maintain the relatively constant core body temperature of 37 °C in order to ensure normal physiological function. A crucial component to temperature control in humans is the precise regulation of skin blood flow and sweating which are the body's autonomic responses mediating heat dissipation. It has been well established that during periods of increased heat load, such as passive heat stress and exercise, skin blood flow and sweating are increased in proportion to the change in core body and skin temperatures (Gagge & Gonzalez, 1996). However, there exist marked differences in thermoregulatory control during recovery from a bout of exercise (Kenny *et al.*, 2007; Kenny & Journeay, 2010; Kenny & Jay, 2013). This was first evidenced by a substantial and persistent elevation in core body temperature postexercise (Thoden *et al.*, 1994; Kenny *et al.*, 2000) and was subsequently attributed to a rapid reduction in both skin blood flow and sweating in the early stages of recovery (Kenny *et al.*, 1996; Kenny *et al.*, 2000). Specifically, studies have consistently reported skin blood flow and sweating to return to pre-exercise levels within the first 20 minutes postexercise causing core body (Kenny *et al.*, 1996; Wilkins *et al.*, 2004) and muscle temperatures (Kenny *et al.*, 2008a) to remain elevated for 60 to 90 minutes.

Several studies have been conducted to address this disruption in thermoregulatory control. For example, it has been clearly established that the core temperature at which the heat loss responses are activated (i.e., the onset threshold of cutaneous vasodilation and sweating) is elevated during recovery by ~0.4°C compared to the pre-exercise period (Jackson & Kenny, 2003; Kenny *et al.*, 2003a, b). In addition, this response has been shown to occur irrespective of any progressive increases in heat storage as evidenced during

repeated exercise bouts (Kenny *et al.*, 2008a). Therefore, the postexercise attenuation in heat loss responses, and thereby elevation in core body temperature, is likely due to a residual effect of the exercise bout mediated by changes in cardiovascular, metabolic, osmolality, and/or other endogenous factors (Fortney & Vroman, 1985; Horowitz, 1990; Kenny *et al.*, 1996; Kenny *et al.*, 2007).

On the other hand, it is also known that recovery from exercise is associated with pronounced cardiovascular adjustments. This has been clearly evidenced by a marked and prolonged decrease in mean arterial pressure for ~2 hours after exercise known as postexercise hypotension (Wilkins *et al.*, 2004; Halliwill *et al.*, 2013). It is believed that postexercise hypotension is the result of blood pooling in the skeletal muscle which causes a decrease in central venous pressure and subsequently a markedly reduced stroke volume (Halliwill, 2001; Halliwill *et al.*, 2013). The lower blood pressure activates pressure-sensing receptors (i.e., baroreceptors) which are unloaded during episodes of hypotension. More recently, empirical evidence has indicated that there exists a functional link between postexercise cardiovascular and thermoregulatory control such that baroreceptor unloading is associated with the suppression of skin blood flow and sweating (Kenny *et al.*, 2007; Kenny & Journeay, 2010; Kenny & Jay, 2013).

Kenny and colleagues (2000) were the first to establish a functional link between postexercise baroreceptor unloading (i.e., postexercise hypotension) and the attenuation of heat loss responses. Their findings illustrated that performing a head-down tilt manoeuvre (whereby the subjects were positioned 15° down from the horizontal to minimize venous pooling and reverse baroreceptor unloading) reversed the postexercise increase in the onset threshold for vasodilation. Further studies employed mechanical manipulations (i.e., lower

body negative and positive pressures) and consistently ascribed, at least in part, the postexercise increase in onset threshold for both vasodilation and sweating to nonthermal baroreceptor input (Jackson & Kenny, 2003; Journeay *et al.*, 2004a; Journeay *et al.*, 2004b; McInnis *et al.*, 2006).

To date, few studies have been conducted to evaluate the mechanisms underlying the postexercise suppression in skin blood flow. Some evidence suggests that it is by the withdrawal of the active vasodilator system (Kenny *et al.*, 2003a). Using bretylium tosylate to block the release of vasoconstrictor transmitters (i.e., noradrenaline and neuropeptide Y) from sympathetic nerves, Kenny and colleagues (2003a) determined that the increased onset threshold for vasodilation postexercise was not related to enhanced vasoconstriction. However, the assessment of the onset threshold for vasodilation only occurred at ~40-50 minutes postexercise when skin blood flow had already returned to baseline levels. In fact, a recent study has uncovered a role for vasoconstriction in the early stages of recovery (i.e., ≤30 min postexercise) and a major role for adenosine receptors throughout 60 minutes of recovery (McGinn *et al.*, 2014). However, whether these modulators (i.e., vasoconstriction and/or adenosine receptors) represent the efferent branch of the baroreceptors remains to be determined.

Similar to skin blood flow, the underlying mechanisms responsible for the postexercise suppression of sweating remain to be elucidated. Sweating is known to be the primary avenue for heat loss during exercise and is activated predominantly by the release of acetylcholine by sympathetic cholinergic nerves which bind to muscarinic receptors on the sweat gland (Shibasaki & Crandall, 2010). Recent reports have identified an influence of nitric oxide on sweating during exercise in young adults (Welch *et al.*, 2009; Stapleton *et al.*,

2014); however, its role in modulating sweating during recovery has not been well examined. Further, while adrenergic receptors have been identified on the sweat glands and their influence on sweat rate is reported to be minimal (Buono *et al.*, 2010; Buono *et al.*, 2011; Martinez *et al.*, 2012), their involvement has not been assessed during high intensity exercise or in the subsequent recovery period. Reports have clearly established that sweating returns to baseline levels following an exercise bout (Journeay *et al.*, 2004a; Journeay *et al.*, 2004b; McInnis *et al.*, 2006) and that the onset threshold for sweating is elevated during recovery (Jackson & Kenny, 2003; Kenny *et al.*, 2003b). However, the mechanisms at the end-organ (i.e., sweat gland) underlying these observations have been largely ignored in the literature. Specifically, whether baroreceptor loading status modulates the level of sweating has been debated extensively (Jackson & Kenny, 2003; Kenny *et al.*, 2003a, b; Kenny *et al.*, 2007; Kenny & Journeay, 2010; Shibasaki & Crandall, 2010; Kenny & Jay, 2013). As such, the involvement of baroreceptors and the independent modulators (i.e., nitric oxide and adrenergic receptors) for the postexercise suppression in sweating must be uncovered.

1.2 Rationale and statement of the problem

Although the mechanisms of control for skin blood flow and sweating have been well established during passive heat stress and exercise, there is still little known about the mechanisms involved in the postexercise suppression of heat loss. We used intradermal microdialysis to infuse agents in order to characterize the involvement of nitric oxide, noradrenergic vasoconstriction, and adenosine receptors in modulating the time-dependent changes in local heat loss responses of skin blood flow and sweating following repeated exercise bouts. Furthermore, we employed three pressure conditions (i.e., no pressure

[control], lower body negative pressure, or lower body positive pressure) during the recovery periods to evaluate the mechanisms by which baroreceptors influence postexercise heat loss responses.

We examined the mechanisms underlying the control of the heat loss responses during two 15-min bouts of intermittent cycling (i.e., upright cycling) performed at a fixed percentage of maximal oxygen consumption (85%). This exercise intensity has been used previously when evaluating the postexercise suppression in heat loss (Kenny *et al.*, 2003a, b) and was used to ensure that the exercise bout was sufficiently challenging for all participants. Each bout was followed by 45 min of upright seated recovery.

1.3 Study objectives

The objective of the proposed study was to evaluate the mechanisms regulating the disturbance in thermoregulatory control during the postexercise period. Specifically, this study aimed to:

1) Evaluate whether nitric oxide synthase, noradrenergic vasoconstriction, and/or adenosine receptors are responsible for mediating the time-dependent reductions in skin blood flow and sweating following repeated bouts of moderate intensity exercise;
2) Evaluate the influence of baroreceptor loading status on the postexercise suppression in heat loss responses;
3) Determine whether the baroreceptor modulation of heat loss during recovery is carried out through nitric oxide, noradrenergic vasoconstriction, and/or adenosine receptors.

1.4 Hypothesis

We evaluated the hypothesis that the postexercise heat loss response of skin blood flow and sweating would be modulated by nonthermal baroreceptor input such that applying lower body positive pressure during recovery would attenuate the suppression of heat loss whereas applying lower body negative pressure would exacerbate the suppression of heat loss. Furthermore, it was hypothesized that baroreceptors would modulate the heat loss responses in part through nitric oxide such that heat loss during lower body positive would be attenuated when nitric oxide synthase is inhibited compared to a control site. In contrast, it was hypothesized that changes in baroreceptor loading status would not alter sweating or skin blood flow at either the bretylium or theophylline sites.

1.5 Relevance of the study

To date, there has been extensive research on the control of heat loss responses under passive heat stress and during exercise. However, there remains a significant gap in the understanding of how thermoregulatory control is impaired postexercise. Results obtained from this study are relevant in two bodies of knowledge. First, the study provides important advancements in the understanding of the mechanisms modulating skin blood flow and sweating during the postexercise period. Second, this research has practical applications in that we understand in greater depth the origins of and factors mediating heat-related injury. Specifically, we elucidated the mechanisms that are responsible for changing the body's ability to regulate core temperature following a period of exercise. As such, future studies may now use this information to develop potential preventative and therapeutic approaches to avoid heat-related injury.

1.6 Delimitations and limitations

This study evaluated the postexercise regulation of heat loss responses in young males (18-35 years). Therefore, the findings may not be representative for females or older individuals. Specifically, we elected not to include females in the present study given the fundamental sex-related differences in heat loss responses (Gagnon & Kenny, 2012) and to baroreceptor activation (Kenny & Jay, 2007). In addition, the study excluded participants with chronic diseases that may alter thermoregulatory and/or cardiovascular control and therefore the results are limited to individuals who are healthy and somewhat physically active. Finally, an effect size of 1.55 and 1.43 for cutaneous blood flow and sweating respectively based on previous work (Journeay *et al.*, 2004a; McGinn *et al.*, 2014) resulted in a minimum calculated ($\beta = 0.95$, $\alpha = 0.05$) sample size of 6 participants. Therefore, we felt that 8 participants would be sufficient for the present study.

CHAPTER II

REVIEW OF THE LITERATURE

2.1 Basic human thermoregulation

Homeostasis within the human body can be profoundly disrupted by relatively minor changes in body temperature (Taylor, 2006) with temperature regulation within a narrow range being vital for survival. Various factors including environmental conditions and physical activity can pose a substantial threat to the maintenance of body temperature. During such challenges, afferent information is sent from the peripheral (in the skin and muscle) and central (in the brain) thermoreceptors to the body's thermoregulatory centre. This region is believed to reside in the pre-optic anterior hypothalamus of the brain, and can act to alter the rate of heat loss through activation of the thermoeffector responses (Boulant & Bignall, 1973; Hensel, 1981). The system operates in a negative feedback loop that is designed to balance the rate of heat gained with the rate of heat lost in order to achieve heat balance (Gagge & Gonzalez, 1996).

When the body is under heat stress, the hypothalamus sends a signal via sympathetic nerves to the cutaneous vasculature and to the eccrine sweat glands in order to induce vasodilation and sweating, respectively (Armstrong *et al.*, 1993). The action of vasodilation opens the vessels of the cutaneous circulation and thereby allows greater blood to flow to the skin surface. Consequently, the increase in skin blood flow allows for an increase in skin temperature, and thereby increases the gradient for dry heat exchange between the skin and the external environment. However, the primary avenue for heat loss in humans is through eccrine sweating (Shibasaki & Crandall, 2010). Upon stimulation from cholinergic nerves, the sweat gland expels sweat onto the skin's surface which is subsequently evaporated. The body's ability to initiate this increase in dry and evaporative heat exchange is crucial in order to maintain a constant temperature during heat stress (Figure 1). In contrast, the

hypothalamus will respond to cold stress by the activation of shivering (to increase metabolic heat production) and cutaneous vasoconstriction (to decrease blood flow to the skin). This leads to an increase in the heat gained and a concomitant decrease in the heat lost in order to defend a decrease in body temperature.

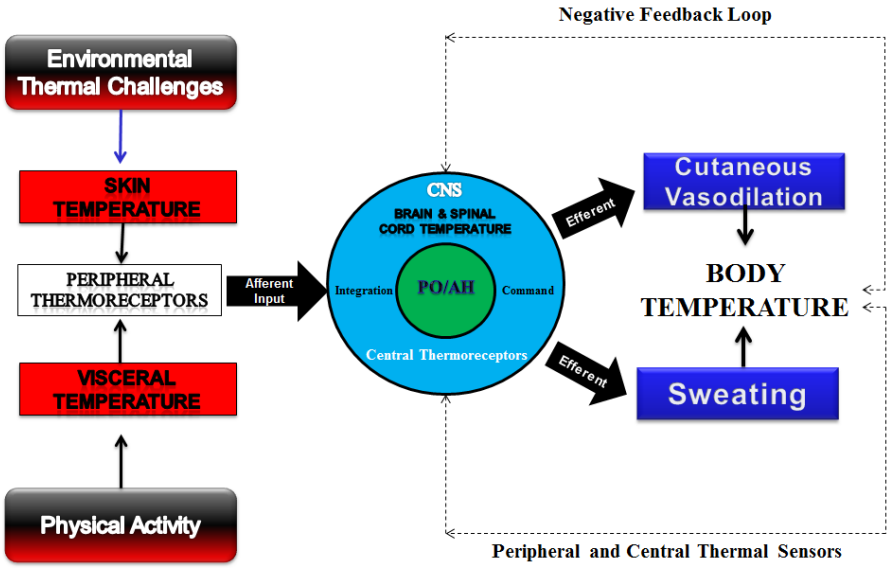

Figure 1. An illustration of the heat loss responses of increased skin blood flow (i.e., cutaneous vasodilation) and sweating in response to increasing skin and core temperature during heat stress. Adapated from (Nagashima *et al.*, 2000).

2.1.1 Heat Balance

Of the mechanisms for heat loss in humans, an equation has been derived that divides total heat loss into sensible and insensible heat transfer (Gagge & Gonzalez, 1996):

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

Where all terms have units of $W \cdot m^{-2}$ and,

S = rate of body heat storage
M = rate of metabolic heat production
W = rate of mechanical work
C = rate of convective heat loss from the skin
R = rate of radiative heat loss from the skin
E = rate of evaporative heat loss from the skin. (All values in $W \cdot m^{-2}$)

A change in the rate of body heat storage occurs when there is a mismatch between the rate of metabolic heat production (M ± W) and the rate of heat loss (±C ± R - E). Specifically, when metabolic heat production exceeds the total rate of heat loss, heat storage increases which will lead to increases in core body temperature. It is important to note from this equation that, depending on the environmental conditions, the rate of sensible heat exchange (±C ± R) can be either positive or negative. For instance, a hot environment whereby the ambient temperature is greater than skin temperature will result in a negative sensible heat loss (i.e., dry heat gain). Under such conditions, the total rate of heat loss is entirely determined by evaporative heat loss (i.e., sweating). This highlights the fact that the body can sense changes and integrate the appropriate effector responses to minimize changes in core body temperature irrespective of the environmental conditions and/or changes in the rate of metabolic heat production.

2.2 Heat loss responses during heat stress

For humans, heat stress can result from 1) exposure to hot and/or humid ambient conditions, 2) an increase in metabolic heat production associated with exercise, or 3) their combination. In fact, it is believed that one of the greatest challenges to the thermoregulatory and cardiovascular systems is the dual effect of environmental heat stress during exercise (Crandall & Gonzalez-Alonso, 2010). With increasing core temperatures, physiological processes begin to fail which makes temperature regulation one essential component to being a healthy human. Afferent temperature information from the skin and core is sent to the thermoregulatory centre in the brain and is integrated into an efferent response in order to defend against large changes in body temperature (Taylor, 2006). In thermoregulatory research, skin and core temperature measurements are often used to calculate mean body

temperature, which is intended to reflect the integrated message received at the hypothalamus (Lenhardt & Sessler, 2006). Thus, control of the heat loss responses can be summarized into three main phases as a function of mean body temperature: the onset threshold, the thermosensitivity, and the plateau (Figure 2).

The mean body temperature at which skin blood flow and sweating (i.e., the heat loss responses) are activated is referred to as the onset threshold for vasodilation and sweating, respectively (Charkoudian, 2003). As observed in Figure 2a, the onset threshold is the point at which a marked and steady increase in heat loss is observed. Secondly, the thermosensitivity of the response is defined as the rate of increase in heat loss as a function of increasing mean body temperature (Figure 2b) (Charkoudian, 2003). Finally, the heat loss responses will enter the plateau phase when further increases in mean body temperature are not accompanied by increases in heat loss (Figure 2c). This tends to indicate the maximal capacity of the responses; however, a plateau phase will also be observed (in the absence of increasing mean body temperature) at heat balance (Gagge & Gonzalez, 1996).

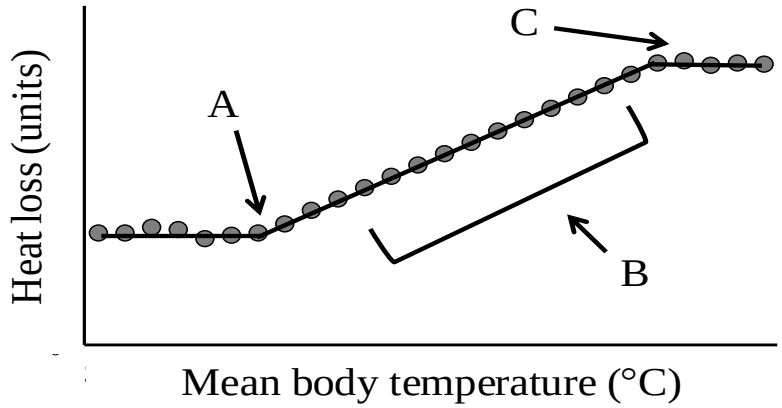

Figure 2. Mean body temperature increases initially before the heat loss responses are activated at a given onset threshold (A). Heat loss is subsequently increased proportionally to the increase in mean body temperature, the linear portion of which represents the thermosensitivity of the response (B). Once heat loss reaches maximal values, a plateau of the line is observed, whereby no further increase in heat loss occurs despite increasing mean body temperature (C). It should be noted that this response has been demonstrated using local, whole-limb, and whole-body measurements of heat loss responses. Adapted from (Gagnon & Kenny, 2012).

2.2.1 Skin blood flow

Although it is not considered the primary avenue for heat loss during passive heat stress or exercise, skin blood flow is an important component to thermoregulatory control (Charkoudian, 2003, 2010). For instance, small differences in skin blood flow over several hours can result in drastic fluctuations in mean body temperature (Charkoudian, 2003). In a thermoneutral environment, the skin is known to receive ~250 mL of blood, an equivalent of ~5-10% of the total volume of blood being circulated throughout the body each minute (i.e., cardiac output) (Rowell, 1974; Crandall *et al.*, 1996). However, in a state of heat stress and/or exercise, skin blood flow is increased substantially up to ~8 $L \cdot min^{-1}$ to allow for augmented convective heat transfer from the core to the periphery. It is well established that skin blood flow is regulated by two branches of the sympathetic nervous system: an active vasoconstrictor system and an active vasodilator system (Johnson & Proppe, 1996). The active vasoconstrictor system is generally responsible for 10-15% of the increase in skin blood flow during heat stress. Although for a number of years it was believed that the vasoconstrictor system was entirely mediated by the presynaptic release of noradrenaline (i.e., noradrenergic vasoconstriction; (Rowell, 1977)), it has recently been reported that neuropeptide Y and possibly ATP also have a crucial role in mediating cutaneous vasoconstriction (Stephens *et al.*, 2001; Stephens *et al.*, 2004).

In contrast, the active vasodilator system is responsible for 85-90% of the total increase in skin blood flow during heat stress. Although the search continues for the "unknown vasodilator" that is mentioned in numerous reviews (Charkoudian, 2003, 2010; Johnson & Kellogg, 2010; Johnson *et al.*, 2014), the involvement of a number of neurotransmitters and receptors has been identified. The most prominent vasodilator in the

literature is nitric oxide (Rowell, 1977; Kellogg *et al.*, 1998; Minson *et al.*, 2001; Charkoudian, 2003, 2010; Johnson & Kellogg, 2010; Fieger & Wong, 2012; Johnson *et al.*, 2014); however, others include prostaglandins (McCord *et al.*, 2006a), histamine receptors (Wong *et al.*, 2004), vasoactive intestinal peptide (Bennett *et al.*, 2003), substance P (Wong & Minson, 2006) and adenosine receptors (Fieger & Wong, 2012). In fact, nitric oxide has been ascribed to approximately 30-45% of the vasodilatory response during a passive heat stress (Kellogg *et al.*, 1998; Shastry *et al.*, 2000; McCord *et al.*, 2006a), and a pronounced role has also been identified during exercise (Gilligan *et al.*, 1994; Welch *et al.*, 2009; McGinn *et al.*, 2014).

2.2.2 Sweating

The activation of sweating is the most effective mechanism for dissipating heat in humans. As metabolic heat production increases with exercise and/or ambient temperature becomes greater than skin temperature, evaporation (i.e., sweating) is the crucial and/or only avenue by which heat is lost (Wendt *et al.*, 2007). Under such conditions, sweating represents >80% of total body heat loss compared to only 25% under resting conditions (Cain & McLellan, 1998; Gavin, 2003). Accordingly, a recent study determined that >90% of whole-body evaporative heat loss is determined by the requirement for heat loss, which incorporates both dry heat exchange and metabolic heat production (Gagnon *et al.*, 2013).

The human sweat glands are usually classified into two subtypes based on the origin of their activation. The apocrine sweat glands are found sparsely throughout the body, are sensitive to adrenaline, and subsequently are involved in “emotional” sweating (i.e., stress, fear, and/or pain) (Saga, 2002). In contrast, the eccrine sweat glands are much more

prominent in humans, and represent thermoregulatory sweating (Shibasaki *et al.*, 2006; Shibasaki & Crandall, 2010). For the purposes of this thesis, any further mention of sweat glands will refer to eccrine sweat glands. It is estimated that humans have anywhere from 2 to 4 million eccrine sweat glands, and they can be found in the forehead, upper limbs, trunk, and lower limbs in order from greatest to lowest density (Sato & Dobson, 1970; Kondo *et al.*, 1998). As mentioned previously, the rate of sweating is primarily determined by the requirement for heat loss, however, humans have the capacity to achieve whole body sweat rate as high as 2 $L \cdot hr^{-1}$ (Sawka *et al.*, 2007). The control of sweating originates in the sympathetic nervous system which releases acetylcholine peripherally from synaptic terminals to bind to the muscarinic receptors located on the sweat glands (Shibasaki & Crandall, 2010). Furthermore, recent evidence has also found a role for nitric oxide in modulating the sweating response during methacholine administration (Lee & Mack, 2006), and during exercise (Welch *et al.*, 2009; Stapleton *et al.*, 2014).

2.3 Postexercise responses

Despite the well regulated mechanisms of body temperature control during passive heat stress and exercise, there exist substantial differences in thermoregulation during recovery from exercise (Kenny *et al.*, 2007; Kenny & Journeay, 2010; Kenny & Jay, 2013). Exercise results in a marked increase in temperature of central core and muscle tissue which is paralleled by concomitant increases in heat loss responses of sweating and skin blood flow and marked elevations in mean arterial pressure and heart rate. The magnitude of the response for each of these variables is strongly dependent upon the intensity of exercise. However, upon the cessation of exercise, the heat loss responses are reduced to baseline

levels as quickly as 20 minutes into recovery which subsequently results in a persistent elevation in core body temperature for greater than 60 to 90 minutes postexercise (Wilkins *et al.*, 2004; Kenny *et al.*, 2007; Kenny *et al.*, 2008a; Kenny & Journeay, 2010). In addition, mean arterial pressure is simultaneously reduced compared to baseline levels, a phenomenon known as postexercise hypotension, whereas heart rate is elevated in an attempt to sustain cardiac output (Halliwill, 2001; Halliwill *et al.*, 2013). In summary, the postexercise period is associated with pronounced thermoregulatory and cardiovascular impairments as evidenced by a persistent elevation in core body temperature and simultaneous decrease in mean arterial pressure.

2.3.1 Postexercise skin blood flow

Numerous reports have shown that postexercise skin blood flow regulation is clearly altered compared to its control during exercise or passive heat stress (Kenny *et al.*, 2000; Kenny *et al.*, 2003a; Wilkins *et al.*, 2004; Kenny *et al.*, 2007). Specifically, skin blood flow has been demonstrated to decrease rapidly following exercise despite a persistent elevation in core body temperature (Wilkins *et al.*, 2004). This decrease in skin blood flow following exercise has been attributed to a central modulation defined by an elevated onset threshold for vasodilation measured during recovery that is intensity-dependent (Jackson & Kenny, 2003; Kenny *et al.*, 2003a); however, the mechanisms at the level of the end-organ (i.e., vascular smooth muscle) have yet to be fully elucidated.

2.3.1.1 Nitric oxide

It is well established that nitric oxide is an important component of cutaneous active vasodilation during whole-body heat stress such that inhibiting its production reduces skin blood flow by ~30-45% (Kellogg *et al.*, 1998; Shastry *et al.*, 2000; Minson *et al.*, 2001; McCord *et al.*, 2006a). Given that it has been previously postulated that attenuated active vasodilation is responsible for the postexercise suppression in skin blood flow (Kenny *et al.*, 2003a), it seems plausible that nitric oxide would be involved. This has recently been addressed in a study by McGinn and colleagues (2014). Specifically, the study found that nitric oxide only had a role in the very early stages (i.e., ≤10 minutes) of recovery. However, it remains unclear how the contribution of nitric oxide would differ with progressive increases in the level of hyperthermia as would be observed following repeated exercise bouts.

2.3.1.2 Noradrenergic vasoconstriction

As mentioned above, it is currently believed that the postexercise suppression in skin blood flow is the result of attenuated active vasodilation (Kenny *et al.*, 2003a). The authors used iontophoresis of bretylium tosylate during the assessment of the onset threshold for active vasodilation pre- and postexercise. Their findings indicated that the onset threshold for vasodilation did not differ between the control site and the sites receiving bretylium tosylate, which reflected that any changes in skin blood flow regulation was not a result of reflex vasoconstrictor activity. Furthermore, it was found that the postexercise onset threshold for vasodilation was elevated by ~0.2 – 0.5 °C and that this response was strongly dependent upon the exercise intensity such that the onset threshold for vasodilation was

higher when exercise was performed at increasing intensity (Kenny *et al.*, 2003a). However, the assessment of the onset threshold for vasodilation was only performed ~40-50 minutes postexercise when skin blood flow had returned to baseline levels. In fact, a recent study determined using microdialysis of bretylium tosylate that noradrenergic vasoconstriction exhibits a time-dependent role in modulating skin blood flow during the first 30 minutes of recovery (McGinn *et al.*, 2014). Therefore, it seems that the suppression of skin blood flow postexercise is the result of increased vasoconstrictor activity and attenuated active vasodilation simultaneously.

2.3.1.3 Adenosine receptors

The adenosine metabolite has been shown to be an important modulator of blood flow in a variety of tissues (Tabrizchi & Bedi, 2001). The effect of adenosine has been found to influence both vessel dilation (Wilson *et al.*, 1990; Hansen & Schnermann, 2003) and constriction (Biaggioni *et al.*, 1989; Hansen & Schnermann, 2003), depending on the tissue type and species studied. While these findings seem contradictory in nature, they have been ascribed to the presence of multiple adenosine receptor subtypes. Specifically in human skin vessels, A_1 and A_2 adenosine receptor subtypes have been identified (Stojanov & Proctor, 1989; Tabrizchi & Bedi, 2001). Recent work has characterized a role for adenosine receptors in the cutaneous vasoconstrictor response to whole-body cooling (Swift *et al.*, 2014) and the vasodilator response to local heating (Fieger & Wong, 2010); however, these findings were not paralleled during whole-body heat stress when adenosine receptors were inhibited alone (Fieger & Wong, 2012). Consistent with the latter observations, adenosine receptors have been reported to have no influence on skin blood flow during exercise (McGinn *et al.*, 2014).

However, the study by McGinn and colleagues (2014) uncovered a major role for adenosine receptors in modulating postexercise skin blood flow such that its suppression was attenuated by ~40-60% throughout 60 minutes of recovery during theophylline administration.

2.3.2 Postexercise sweating

Similar to the postexercise skin blood flow response, rapid reductions in sweating are also observed despite the persistent elevation in core temperature (Kenny *et al.*, 2007; Kenny & Journeay, 2010; Kenny & Jay, 2013). This has been consistently observed with local and whole-body measurements of sweating. Furthermore, this response was found to be irrespective of the heat load accumulated during exercise such that successive exercise bouts produced the same reduction in whole-body evaporative heat loss during recovery (Kenny *et al.*, 2008a).

The mechanism underlying this response has also been assessed with respect to the postexercise onset threshold for sweating. Specifically, it has been identified that the postexercise onset threshold for sweating is elevated by ~0.3 °C compared to pre-exercise levels (Jackson & Kenny, 2003) and that the magnitude of increase in the onset threshold for sweating also exhibits an exercise intensity-dependent response (Kenny *et al.*, 2003b). Taken together, these findings indicated that the increased onset threshold for sweating postexercise was related to nonthermal baroreceptor input associated with postexercise hypotension. However, the precise modulators involved in the suppression of sweating postexercise remain to be elucidated.

Recent evidence has uncovered a role for nitric oxide in modulating sweat rate during exercise of short duration (~15-30 min) and moderate intensity (~50-60% of maximal oxygen consumption) (Welch *et al.*, 2009; Stapleton *et al.*, 2014). Findings from these studies indicated that inhibition of nitric oxide synthase causes impairments in local sweating during exercise in young adults; however, it remains unclear whether nitric oxide could be involved in modulating postexercise sweating. In addition, studies have identified adrenergic receptors proximal to the eccrine sweat glands in humans (Uno & Hokfelt, 1975; Uno, 1977). While studies infusing adrenergic agonists have found that the contribution of adrenergic receptors to sweating is minimal at best (Buono *et al.*, 2010; Buono *et al.*, 2011; Martinez *et al.*, 2012), their role in modulating sweating during recovery has not been assessed. Considering the level of catecholamines is rapidly changing during and following exercise, it seems plausible that adrenergic receptors may be involved.

2.3.3 Postexercise cardiovascular regulation

The postexercise period is also associated with pronounced cardiovascular adjustments. This is commonly evidenced by postexercise hypotension which is characterized by a marked and prolonged decrease in mean arterial pressure during recovery for ~2 hours (Halliwill, 2001; Halliwill *et al.*, 2013). This phenomenon has generally been attributed to a persistent increase in systemic vascular conductance (i.e., skeletal muscle vasodilation) that is not completely offset by increases in cardiac output (Halliwill, 2001; Halliwill *et al.*, 2013). Several studies have been conducted to determine the source of this systemic vasodilation and how postexercise sympatholysis (i.e., a decrease in sympathetic activity) could be a factor (Halliwill *et al.*, 1996). Halliwill and colleagues (2000) first ruled

out the potent vasodilator nitric oxide, by systemically inhibiting its production. Following this original work, follow-up studies were conducted to eliminate changes in alpha-adrenergic vascular responsiveness (Halliwill *et al.*, 2003), prostaglandin-dependent peripheral vasodilation (Lockwood *et al.*, 2005a), excess postexercise oxygen consumption (Williams *et al.*, 2005), and oxidative stress (Romero *et al.*, 2015) as potential contributors. However, it was found that systemic inhibition of H_1 (Lockwood *et al.*, 2005b) and H_2 (McCord *et al.*, 2006b) histamine receptors reduced the level postexercise hypotension. In fact, the postexercise hypotensive response was blunted by ~80% when H_1 and H_2 receptors were blocked simultaneously (McCord & Halliwill, 2006).

Along with postexercise hypotension there also exists is a substantial decrease in the volume of blood in the central cavity (Halliwill, 2001). According to the Frank-Starling principle, a decrease in central blood volume causes a reduction in venous return, and this is experienced as a substantial decrease in stroke volume during the postexercise period (Kenny & Gagnon, 2009). Consequently, heart rate is elevated for an extended period during recovery in the attempt to maintain a sufficient level of cardiac output. These responses have been shown to be dependent upon the duration and intensity of exercise such that a greater intensity of exercise results in a more pronounced decrease in mean arterial pressure and stroke volume as well as a concomitant increase in postexercise heart rate (Kenny & Niedre, 2002; Jackson & Kenny, 2003). Furthermore, it is currently believed that postexercise hypotension and the associated cardiovascular adjustments alter central thermoregulatory drive, resulting in altered thermoregulatory control following exercise (Kenny *et al.*, 2007; Kenny & Journeay, 2010; Kenny & Jay, 2013).

2.4 Baroreceptors

It is well established that factors of nonthermal origin can have a profound influence on the heat loss responses during recovery from exercise (Kenny *et al.*, 2007; Kenny & Journeay, 2010; Kenny & Jay, 2013). These nonthermal factors include mechanoreceptors, osmoreceptors, and metaboreceptors, but the baroreceptors have been the most prominent factor studied. The baroreceptor reflex is the body's primary reflex pathway for the control of blood pressure and can be divided into two main groups: arterial (or high pressure-sensing) baroreceptors and cardiopulmonary (or low pressure-sensing) baroreceptors. Baroreceptor loading is associated with increases in mean arterial pressure which triggers a decrease in sympathetic activity and subsequent increase in parasympathetic activity. Through these mechanisms, heart rate decreases along with peripheral resistance (i.e., vasodilation) in order to restore blood pressure to baseline levels. Conversely, baroreceptor unloading occurs during a decrease in mean arterial pressure leading to increases in sympathetic and decreases in parasympathetic nerve activity resulting in increases in heart rate and total peripheral resistance (i.e., vasoconstriction). Importantly, baroreceptors are unloaded during periods of postexercise hypotension and have been implicated in the modulation of both skin blood flow and sweating during passive heating and exercise as well as during the postexercise period.

2.4.1 Baroreceptor modulation of skin blood flow

Prior studies have identified that baroreceptor unloading with lower body negative pressure can decrease skin blood flow during passive heating (Kellogg *et al.*, 1990; Crandall *et al.*, 1996) and exercise (Mack *et al.*, 1995b; Mack *et al.*, 2001b). Whether baroreceptor unloading modulates skin blood flow via withdrawal of cutaneous active vasodilation or

increases in cutaneous vasoconstriction seems to depend on the thermal status of the individual. Specifically, baroreceptor unloading under normothermic conditions will enhance vasoconstrictor activity (Tripathi & Nadel, 1986) whereas baroreceptor unloading during heat stress will tend to withdraw active vasodilator input (Kellogg *et al.*, 1990).

It was demonstrated by Jackson and Kenny (2003) that application of lower body positive pressure (i.e., increasing baroreceptor loading) during recovery from exercise resulted in a substantial increase in skin blood flow. Specifically, the lower body positive pressure eliminated the increased onset threshold for active vasodilation during recovery and thereby allowed for increased blood flow to the skin. Further, it was reported that the increased onset threshold for vasodilation was entirely related to withdrawal of active vasodilation as iontophoresis of bretylium tosylate did not alter the results compared to a control site. In contrast, application of lower body negative pressure during recovery from exercise when the baroreceptors are already unloaded (i.e., postexercise hypotension) has not been observed to influence skin blood flow (Journeay *et al.*, 2004a). Therefore, in taking these findings together it has been postulated that postexercise skin blood flow is modulated, at least in part, by nonthermal baroreceptor input due to altered active vasodilation.

Empirical evidence suggests that the nonthermal baroreceptor modulation of skin blood flow is dependent upon the level of hyperthermia (Gagnon *et al.*, 2008; Binder *et al.*, 2012). Specifically, Gagnon and colleagues (2008) determined that reversing baroreceptor unloading did not influence the suppression of skin blood flow in the early stages of recovery (i.e., when core temperature remained ≥1.2°C above resting levels). However, with a progressive decay in core temperature to <1°C above resting levels, the attenuation of baroreceptor unloading via passive recovery (i.e., subjects remained seated while their legs

were passively driven through the full range of pedaling motion) was shown to reverse the attenuation of skin blood flow. It remains unclear the mechanisms by which baroreceptor unloading is involved in suppressing skin blood flow in recovery, and also whether this modulation differs as a function of repeated exercise bouts.

2.4.2 Baroreceptor modulation of sweating

The influence of nonthermal baroreceptor input is less established for the control of sweating (Kenny *et al.*, 2007; Kenny & Journeay, 2010; Kenny & Jay, 2013). However, several studies of the postexercise sweating response have attributed the suppression of sweating to baroreceptor unloading (Jackson & Kenny, 2003; Kenny *et al.*, 2003b; Journeay *et al.*, 2004a; McInnis *et al.*, 2006). This is evidenced by the attenuated reduction in sweating during the application of lower body positive pressure (Jackson & Kenny, 2003; Journeay *et al.*, 2004a) and head-down tilt (McInnis *et al.*, 2006) during the postexercise period. Taken together, it is believed that the postexercise suppression of sweating is largely mediated by the increased onset threshold; however, the influence of baroreceptor unloading can substantially alter the response.

Similar to skin blood flow, the nonthermal baroreceptor control of sweating has been shown to be mediated by the level of hyperthermia (Gagnon *et al.*, 2008; Binder *et al.*, 2012). Following exercise-induced heat stress to a core temperature of 39.5°C, Gagnon and colleagues (2008) reported no effect of reversed baroreceptor unloading on local sweat rate until core temperature was ≤0.6°C above baseline levels. In line with these findings, a recent study also reported that baroreceptor unloading during severe passive heat stress (i.e., core temperature ~2ºC above baseline levels) does not limit the increase in sweat rate (Schlader *et*

al., 2015). However, the modulators acting as the efferent arm of the baroreceptors controlling sweat rate have yet to be determined. Further, it remains unclear whether the baroreceptor modulation of sweat rate would differ following repeated bouts of exercise considering the level of hyperthermia and the magnitude of hypotension would both be increased.

PART TWO:

METHODS AND RESULTS OF THE THESIS

ARTICLE:

Mechanisms underlying the postexercise baroreceptor-mediated suppression of heat loss

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Mechanisms underlying the postexercise baroreceptor-mediated suppression of heat loss

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ABSTRACT

Reports indicate that postexercise heat loss is modulated by baroreceptor input; however, the mechanisms remain unknown. We examined the time-dependent involvement of adenosine receptors, noradrenergic transmitters, and nitric oxide (NO) in modulating baroreceptor-mediated changes in postexercise heat loss. Eight males performed two 15-min cycling bouts (85% VO_{2max}) each followed by a 45-min recovery in the heat (35°C). Lower body positive (LBPP), negative (LBNP), or no (Control) pressure were applied in three separate sessions during the final 30-min of each recovery. Four microdialysis fibres in the forearm skin were perfused with: *1*) lactated Ringer's (Ringer's); *2*) $4m_M$ Theophylline (inhibits adenosine receptors); *3*) $10m_M$ Bretylium (inhibits noradrenergic transmitter release); or *4*) $10m_M$ $_L$-NAME (inhibits NO synthase). We measured cutaneous vascular conductance (CVC; percentage of maximum) calculated as perfusion units divided by mean arterial pressure, and local sweat rate. Compared to Control, LBPP did not influence CVC at $_L$-NAME, Theophylline or Bretylium during either recovery (P>0.07); however, CVC at Ringer's was increased by ~5-8% throughout 30 min of LBPP during Recovery 1 (all P<0.02). In fact, CVC at Ringer's was similar to Theophylline and Bretylium during LBPP. Conversely, LBNP reduced CVC at all microdialysis sites by ~7-10% in the last 15 min of Recovery 2 (all P<0.05). Local sweat rate was similar at all treatment sites as a function of pressure condition (P>0.10). We show that baroreceptor input modulates postexercise CVC to some extent via adenosine receptors, noradrenergic vasoconstriction, and NO whereas no influence was observed for postexercise sweating.

ABBREVIATIONS

ANOVA, analysis of variance; CVC, cutaneous vascular conductance; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; $_{L}$-NAME, N^{G}-nitro-$_{L}$-arginine methyl ester; NOS, nitric oxide synthase; nNOS, neuronal nitric oxide synthase; LBPP, lower body positive pressure; LBNP, lower body negative pressure

INTRODUCTION

A growing body of empirical evidence indicates that postexercise thermoregulatory control is altered in humans. The cessation of exercise is associated with a suppression of heat loss responses (i.e., cutaneous blood flow and sweating) such that only ~50% of the heat gained during exercise is lost during the first hour of recovery (Kenny *et al.*, 2008b). It is believed that nonthermal factors of central origin are involved in this response (Kenny & Journeay, 2010) including a baroreceptor-mediated suppression of heat loss. Specifically, previous work has indicated that baroreceptor loading status has important implications in the control of cutaneous blood flow during passive heating at rest (Kellogg *et al.*, 1990; Crandall *et al.*, 1996), during exercise (Johnson & Park, 1981; Mack *et al.*, 1995a; Mack *et al.*, 2001a), and in the postexercise period (Kenny *et al.*, 2003a; Journeay *et al.*, 2004a; Gagnon *et al.*, 2008). Similarly, a baroreceptor-mediated modulation of sweating has been reported (Mack *et al.*, 1995a; Kenny *et al.*, 2003b; Journeay *et al.*, 2004a); however, it has not been consistently observed across all studies (Mack *et al.*, 2001a; Wilson *et al.*, 2001, 2005). In addition, some findings indicate that a greater magnitude of hyperthermia reduces the baroreceptor-mediated modulation of cutaneous blood flow and sweating following exercise (Gagnon *et al.*, 2008). However, there remains no information regarding the mechanisms by which the baroreceptors modulate postexercise heat loss at the level of the end-organ (i.e., skin vessels and/or sweat gland).

Heat stress is associated with substantial increases in cutaneous blood flow mediated initially by the removal of tonic vasoconstrictor activity followed by the onset of cutaneous active vasodilation (Grant & Holling, 1938; Johnson & Proppe, 1996). The mechanism(s) by which cutaneous blood flow is suppressed following exercise remains to be fully elucidated;

however, a recent study has implicated the time-dependent involvement of adenosine receptors and noradrenergic vasoconstriction (McGinn *et al.*, 2014). In particular, inhibition of adenosine receptors reportedly sustained cutaneous blood flow above a control skin site for the entire recovery (60 min) whereas inhibiting noradrenergic vasoconstriction was found to attenuate the suppression in cutaneous blood flow during the first 30 min postexercise. However, it was unclear whether these observations were the resultant effector response mediated by central baroreceptor input. Conversely, inhibition of nitric oxide production did not substantially alter cutaneous blood flow, albeit a role could not be discounted (McGinn *et al.*, 2014). In fact, given that nitric oxide remains one of the most prominent active vasodilator substances (Kellogg *et al.*, 1998; McCord *et al.*, 2006a; Stanhewicz *et al.*, 2012; Wong, 2013) and that changes in shear stress (indicated by changes in blood flow and/or pressure) can modulate nitric oxide production (Kuchan & Frangos, 1994; Vequaud & Freslon, 1996), it is plausible to suggest that changes in baroreceptor loading status during the postexercise period may impair nitric oxide production, and thereby cutaneous blood flow. On the other hand, sweating is the major avenue for heat loss during exercise in the heat, and is primarily mediated by cholinergic nerves (Shibasaki & Crandall, 2010; Machado-Moreira *et al.*, 2012). In addition, nitric oxide has been shown to mediate sweating during exercise-induced heat stress (Welch *et al.*, 2009; Stapleton *et al.*, 2014). A study by Stapleton *et al*. (2014) employed intermittent exercise (at ~52% of maximal oxygen consumption) and found that the role of nitric oxide in sweating was observed to a similar extent with each subsequent exercise bout. In contrast, a role for nitric oxide during each recovery period was not evident. As with cutaneous blood flow, it is plausible to suggest that baroreceptor unloading associated with postexercise hypotension (i.e., a decrease in shear

stress) may inhibit nitric oxide production, and thereby suppress sweating during the postexercise period.

The purpose of this study was to evaluate the mechanisms by which baroreceptor loading status modulates the suppression in heat loss during recovery from exercise in the heat. High intensity intermittent exercise (2 bouts) in the heat was incorporated into this study to achieve progressively greater levels of hyperthermia. Furthermore, we induced changes in baroreceptor loading status during each postexercise period through the application of lower body positive pressure (LBPP) or negative pressure (LBNP) as commonly employed (Kellogg *et al.*, 1990; Mack *et al.*, 1995a; Crandall *et al.*, 1996; Mack *et al.*, 2001a; Journeay *et al.*, 2004a). We hypothesized that compared to a Control condition: 1) LBPP would increase cutaneous blood flow and sweating via nitric oxide-dependent mechanisms whereas LBNP would only suppress cutaneous blood flow to a greater extent; 2) changes in baroreceptor loading status would also modulate cutaneous blood flow via noradrenergic vasoconstrictor activity and adenosine receptor activation evidenced by similar responses between pressure conditions; and 3) that the baroreceptor influence of cutaneous blood flow and sweating would be attenuated at greater levels of hyperthermia.

MATERIALS AND METHODS

Ethical approval

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

Participants

Eight young males from the university community volunteered for the current study. Participants were healthy, non-smoking, and normotensive with no history of respiratory, metabolic, or cardiovascular disease. Age, height, body mass, body surface area, and maximal oxygen consumption for the participants were (mean ± standard deviation): 23 ± 4 years, 177 ± 7 cm, 81 ± 9 kg, 1.98 ± 0.14 m^2, and 49 ± 5 ml kg^{-1} min^{-1}, respectively. All participants reported being physically active (≥30 min of structured moderate-to-vigorous physical activity for at least 3 sessions per week).

Experimental design

Participants volunteered for one screening visit and three experimental sessions. For both the screening visit and the experimental sessions, participants were asked to abstain from alcohol, caffeine, and severe or prolonged physical activities for at least 24 hours. Participants were instructed to eat a small breakfast (i.e., toast and orange juice) 2 hours prior to arriving at the laboratory and to drink 500 mL of water the night before as well as the morning of each session. All experimental sessions were performed in a randomized

order, started between 0830 h and 1030 h (at the same time for a given participant) and were separated by at least 72 hours.

The screening visit consisted of measurements of body height and mass as well as maximal oxygen consumption. Body height was determined using a stadiometer (Detecto, model 2391, Webb City, MO, USA), while body mass was determined using a digital high-performance weighing terminal (model CBU150X, Mettler Toledo Inc., Mississauga, ON, Canada). Body surface areas was subsequently calculated from the measurements of body height and mass (Du Bois & Du Bois, 1916). Maximum oxygen uptake was determined by indirect calorimetry (MCD Medgraphics Ultima Series, Sun Tech Medical, Morrisville, NC, USA) during a progressive incremental exercise protocol performed on an upright seated constant-load cycle ergometer (Corival, Lode B.V., Groningen, Netherlands). The participants were instructed to pedal at a cadence of 80-90 r.p.m. at 100 W for the first minute and the external workload was increased by 20 W every minute thereafter until the required cadence could no longer be maintained.

Upon their arrival for the experimental sessions, participants provided a urine sample and voided their bladder before a nude body mass measurement. Urine specific gravity was assessed using a refractometer (Reichert TS 400 total solids refractometer, Reichert Inc., Depew, NY, USA). The participants then rested in the semi-recumbent position on a bed for a 30 min instrumentation period at an ambient room temperature of 25ºC and 30% relative humidity. Four microdialysis fibres (30 kDa cutoff; MD 2000, Bioanalytical Systems Inc., West Lafayette, IN, USA) were inserted in the dermal layer of the skin on the right dorsal forearm under aseptic conditions with each fibre separated by at least 4 cm. A 25 gauge needle was first inserted into the dermal space of the skin with entry and exit points ~25 mm

apart. The microdialysis fibre was then threaded through the lumen of the needle which was subsequently withdrawn to leave a 10 mm dialysis membrane in the dermal space (Bioanalytical Systems Inc.). Following instrumentation, all microdialysis fibres were first perfused with lactated Ringer solution (Baxter, Deerfield, IL, USA) at a rate of 2 μl min^{-1} via a micro perfusion pump (model 400, CMA Microdialysis, Solna, Sweden) for at least 60 min to allow for the resolution of the local hyperemic response.

The participants were then moved to an environmental chamber regulated at 35ºC and 20% relative humidity and were seated in the upright position. At this point, each microdialysis site was instrumented for the simultaneous measurement of local sweat rate and cutaneous blood flow (see below for details). During this time, the microdialysis fibres were perfused at a rate of 2 μl min^{-1} with either: *1*) lactated Ringer's solution, serving as the control skin site (**Ringer's**); *2*) 10 m_M N^G-nitro-$_L$-arginine methyl ester (Sigma-Aldrich, St Louis, MO, USA), a non-selective inhibitor of nitric oxide synthase and thus nitric oxide production (**$_L$-NAME**); *3*) 4 m_M theophylline (Sigma-Aldrich), a non-selective competitive adenosine (A_1/A_2) receptor inhibitor (**Theophylline**); and *4*) 10 m_M bretylium tosylate (Finetech Industry Limited, London, United Kingdom), an inhibitor of the presynaptic release of vasoconstrictor neurotransmitters (e.g., noradrenaline and neuropeptide Y) (**Bretylium**). These concentrations were chosen based on those used in previous studies employing intradermal microdialysis for $_L$-NAME (Minson *et al.*, 2001; Fieger & Wong, 2010, 2012; McGinn *et al.*, 2014; Swift *et al.*, 2014), Theophylline (Fieger & Wong, 2010, 2012; McGinn *et al.*, 2014; Swift *et al.*, 2014), and Bretylium (Wilson *et al.*, 2004; McGinn *et al.*, 2014). Drug infusion was maintained for 45 min to ensure the establishment of each blockade (Minson *et al.*, 2001; Wilson *et al.*, 2004; Fieger & Wong, 2010).

Prior to the baseline period, a cold-pressor test was performed by immersing the left hand into an ice bath for ~3 min in order to verify that Bretylium would block reflex vasoconstriction observed by comparison to the Ringer's site (Pergola *et al.*, 1994). Once cutaneous blood flow had returned to resting levels, 10 min of baseline data collection ensued. Thereafter, participants were transitioned to an upright cycle ergometer (Corival, Lode B.V.) and performed 15 min of exercise at 85% of their pre-determined maximal oxygen consumption (***Exercise 1***). Immediately after the cessation of cycling, participants were moved to a pressure box sealed at the waist (<5 min) and rested for 45 min of recovery in the upright seated posture (***Recovery 1***). During the final 30 min of recovery, one of three experimental conditions was employed: *1*) no pressure (**Control**); *2*) lower body positive pressure (+45 mmHg; **LBPP**); or *3*) lower body negative pressure (-20 mmHg; **LBNP**). Participants donned a loose-fitting neoprene suit below the waist to minimize the effects of air flow and air was also circulated into the unsealed box during the Control condition to simulate air flow during the pressure conditions. The pressure was verified every minute manually by a pressure gauge. At the end of Recovery 1, participants were moved back to the upright seated cycle ergometer (<5 min) for a second 15 min exercise bout at 85% of their maximal oxygen consumption (***Exercise 2***). Finally, participants were transitioned back to the pressure box sealed at the waist (<5 min) for a second 45 min recovery period in the upright seated position (***Recovery 2***). The experimental condition employed during Recovery 1 was repeated during the final 30 min of Recovery 2. At the end of the experimental session, participants remained in the upright seated position for a period of local perfusion of sodium nitroprusside (50 mM, Hospira, Lake Forest, IL, USA) at 6 μl min^{-1} for ~25-30 min to determine maximum cutaneous blood flow. Perfusion continued until a stable plateau of

cutaneous blood flow was achieved for at least 2 min. At this time mean arterial pressure was measured by manual auscultation for the determination of maximal cutaneous vascular conductance (CVC). A final nude body mass measurement and urine sample were obtained at the end of the experimental session.

Measurements

Oesophageal temperature was measured continuously using a pediatric thermocouple probe of ~2 mm in diameter (Mon-a-therm, Mallinckrodt Medical, St Louis, MO, USA) inserted through the nose ~40 cm past the entrance of the nostril. Mean skin temperature was calculated according proportions determined by Hardy and Dubois (1938) based on local skin temperature at ten sites (forehead [7%], upper back [8.75%], chest [8.75%], bicep [9.5%], abdomen [8.75%], lower back [8.75%], quadriceps [9.5%], hamstring [9.5%], and front calf [20%]). Temperature data were collected using a data acquisition module (model 3497A, Agilent Technologies Canada Inc., Mississauga, ON, Canada) at a sampling interval of 15 s and simultaneously displayed and recorded in spreadsheet format on a personal computer with LabVIEW software (version 7.0; National Instruments, Austin, TX, USA).

Heart rate was recorded throughout each experimental session, and stored every 15 s using a Polar coded WearLink and transmitter, Polar RS400 interface, and Polar Trainer 5 software (Polar Electro, Oy, Finland). Mean arterial pressure was estimated continuously before exercise and during each recovery using a Finometer (Finapres Medical Systems, Amsterdam, Netherlands) and was measured by manual auscultation during the final minute of each exercise. Finometer measurements were derived from the beat-to-beat recording of the left middle finger arterial pressure waveform via the volume-clamp method (Penaz,

1973). The Finometer was calibrated using upper arm return-to-flow systolic pressure detection (Bos *et al.*, 1996) and physiocal criteria (Wesseling *et al.*, 1995) following brachial artery pressure reconstruction (Gizdulich *et al.*, 1996; Gizdulich *et al.*, 1997). The right arm was supported at heart level for calibration as well as the measurement periods prior to and following each exercise.

The ventilated capsule technique was employed for the purpose of measuring local sweat rate. Forearm sweat rate was measured from 3.8 cm^2 capsules attached to the skin with adhesive rings and topical skin glue (Collodion HV, Mavidon Medical Products, Lake Worth, FL, USA). The sweat capsules were custom built to house the laser-Doppler flow probe (see below) and allowed for the simultaneous measurement of local sweat rate and cutaneous blood flow. Compressed anhydrous nitrogen was passed through each capsule at a rate of 0.75 l min^{-1}. Long vinyl tubes were used to supply the dry gas to and from the ventilated capsules to ensure optimal equilibration with ambient environmental conditions for the experimental sessions. Water content of the effluent air was measured using capacitance hygrometers (Model HMT333, Vaisala, Woburn, WA, USA). Forearm sweat rate was calculated using the difference in water content between the effluent and influent air multiplied by the flow rate and normalized for the skin surface area under the capsule (in mg min^{-1} cm^{-2}).

Forearm cutaneous blood flow was estimated at 32 Hz using laser-Doppler velocimetry (PeriFlux System 5000, Perimed AB, Stockholm, Sweden). A laser-Doppler probe integrated with a 7 laser array (integrating probe 413, Perimed AB) was placed directly over each microdialysis membrane and was secured for the entire experimental session. Cutaneous vascular conductance was subsequently calculated as the ratio of

cutaneous blood flow (perfusion units) to mean arterial pressure and expressed as a percentage of maximum as determined during the maximal cutaneous blood flow protocol.

Data Analysis

In order to detect the mechanisms governing the baroreceptor mediated suppression in postexercise heat loss, CVC and sweat rate at each treatment site were calculated and presented at baseline, end of each exercise, and at 5 min intervals throughout recovery. Subsequently, CVC and sweat rate at the Ringer's site were compared to the $_{L}$-NAME, Theophylline, and Bretylium sites to evaluate the time-dependent modulators of postexercise CVC and sweating. Additionally, CVC and sweat rate at each site were compared independently as a function of the three pressure conditions in order to evaluate the mechanisms underlying the baroreceptor-mediated modulation of CVC and sweat rate. Secondary variables including oesophageal and mean skin temperatures, heart rate, and mean arterial pressure were presented as a 5 min average at baseline, end of each exercise (note only one measurement for mean arterial pressure), and every 5 min during each recovery period. It is important to note that for all variables in both recovery periods the time point at 5 min was omitted to account for the transition from the cycle ergometer to the pressure box (<5 min) and subsequent calibration of the Finometer (~3 min). Finally, maximal CVC was calculated as an average of cutaneous blood flow (perfusion units) once a plateau was achieved (~2 min), divided by the corresponding mean arterial pressure, and multiplied by 100 to be expressed as perfusion units per mmHg.

Statistical Analysis

A three-way repeated measures analysis of variance (ANOVA) was used for CVC and sweat rate which were analysed with factors of time (nineteen levels: baseline, end of the first and second exercise, and min 10, 15, 20, 25, 30, 35, 40 and 45 of the first and second recovery), treatment site (four levels: Ringer's, $_{L}$-NAME, Theophylline, and Bretylium), and pressure condition (three levels: Control, LBPP, and LBNP). Cold pressor test CVC as well as that during the maximal vasodilation protocol were analysed with a separate two-way ANOVA with a factor of treatment site (cold pressor test: two levels [Ringer's and Bretylium]; maximal CVC: four levels) and pressure condition (three levels). In addition, all secondary variables (oesophageal and mean skin temperatures, heart rate, and mean arterial pressure) were each analysed separately using a two-way ANOVA with a factor of time (nineteen levels) and pressure condition (three levels). In order to ensure participants were similarly hydrated in each of the three experimental sessions, a two-way ANOVA was conducted for urine specific gravity with factors of time (two levels: pre and post) and pressure condition (three levels) as well as a one-way ANOVA with the factor of pressure condition (three levels) for pre-mass and the percent change in body mass. Finally, a two-way ANOVA with factors of exercise period (two levels) and pressure condition (three levels) was performed for external workload and the percentage of maximal oxygen consumption to ensure similar intensities were achieved during the final 10 min of each exercise. When a significant main effect was observed, *post hoc* comparisons were carried out using Student's paired samples *t*-tests. The level of significance for all analyses was set at an alpha level of $P < 0.05$. All statistical analyses were completed using the software package SPSS 21.0 for Windows (IBM, Armonk, NY, USA). Values are presented as mean

± 95% confidence intervals unless otherwise indicated. Confidence intervals were calculated as 1.96 × standard error of the mean.

RESULTS

Experimental sessions

Participants achieved a similar level of hydration for each experimental session as there was no main effect of pressure condition for pre nude body mass ($P = 0.997$) or for the percent change in body mass during the experimental sessions ($P = 0.389$). Urine specific gravity exhibited a main effect of time ($P < 0.001$) such that there was an increase from the start (Control: 1.011 ± 0.005; LBPP: 1.010 ± 0.005; LBNP: 1.012 ± 0.004) to the end (Control: 1.021 ± 0.005; LBPP: 1.020 ± 0.006; LBNP: 1.021 ± 0.003) of each experimental session. However, urine specific gravity did not differ as a function of pressure condition ($P = 0.753$). The percentage of maximal oxygen consumption attained was similar between Exercise 1 (Control: 85 ± 2%; LBPP: 85 ± 1%; LBNP: 85 ± 1%) and Exercise 2 (Control: 86 ± 2%; LBPP: 85 ± 1%; LBNP: 86 ± 1%, $P = 0.544$) and did not vary between pressure conditions ($P = 0.822$). Similarly, the external workload required did not differ between pressure conditions ($P = 0.927$) whereas it was lower during Exercise 2 (Control: 173 ± 34 W; LBPP: 175 ± 31 W; LBNP: 179 ± 31 W) compared to Exercise 1 (Control: 179 ± 33 W; LBPP: 184 ± 33 W; LBNP: 184 ± 31 W, $P < 0.001$).

Cold pressor test

CVC at the Ringer's site was reduced following the cold pressor test to a similar extent in each pressure condition (Control: 22 ± 3%; LBPP: 23 ± 2%; LBNP: 22 ± 2%) compared to the corresponding baseline levels (Control: 33 ± 3%; LBPP: 34 ± 3%; LBNP: 32 ± 2%, $P < 0.001$). In contrast, baseline CVC at the Bretylium site (Control 32 ± 3%;

LBPP: 34 ± 2%; LBNP: 34 ± 3%) did not differ from CVC at the end of the cold pressor test (Control: 32 ± 3%; LBPP: 34 ± 2%; LBNP: 33 ± 3%, $P = 0.523$).

Hemodynamic responses

Heart rate responses are depicted in Fig. 1*A*. There was an interaction of time and pressure condition for heart rate ($P < 0.001$). Heart rate was not different between pressure conditions at baseline levels, at the end of each exercise, and up to 15 min into each recovery (all $P > 0.100$). However, the application of LBPP resulted in a progressive decrease compared to the Control condition by 14 ± 3 bpm and 16 ± 5 bpm at the end of Recovery 1 and 2, respectively. During the application of LBNP, heart rate was observed to increase compared to Control by 16 ± 5 bpm and 15 ± 4 bpm at the end of Recovery 1 and 2, respectively ($P < 0.001$).

Mean arterial pressure exhibited an interaction of time and pressure condition ($P < 0.001$; Fig. 1*B*). Baseline levels and end-exercise measurements of mean arterial pressure were not different between pressure conditions ($P > 0.354$). Likewise, postexercise mean arterial pressure was reduced from baseline levels by 5 ± 0 and 9 ± 1 mmHg in all conditions at 15 min into recovery following both Exercise 1 ($P < 0.001$) and Exercise 2 ($P < 0.001$) respectively. Noteworthy, the magnitude of postexercise hypotension was greater during Recovery 2 compared to Recovery 1 (all $P < 0.020$). In addition, mean arterial pressure was increased for the final 30 min of each recovery during LBPP compared to Control by 10 ± 1 mmHg ($P < 0.001$) whereas mean arterial pressure was not influenced by LBNP (all $P > 0.119$).

Temperature responses

Oesophageal temperature. An interaction of time and pressure condition was detected for oesophageal temperature ($P < 0.001$, Fig. 2*A).* Specifically, no differences were found for oesophageal temperature between conditions at baseline levels (all $P > 0.173$), at the end of Exercise 1 (all $P > 0.553$) or at the end of Exercise 2 (all $P > 0.077$). In addition, oesophageal temperature was similar in the Control and LBNP pressure conditions throughout Recovery 1 ($P = 0.898$) and Recovery 2 ($P = 0.754$). Conversely, oesophageal temperature decreased more rapidly during the LBPP condition such that it was lower than Control by 0.27 ± 0.10ºC at the end of Recovery 1 ($P = 0.032$) and for the final 20 min of Recovery 2 (all $P < 0.020$). Finally, oesophageal temperature was increased by 0.57 ± 0.15ºC at the end of Exercise 2 compared to Exercise 1 in all pressure conditions ($P < 0.001$).

Mean skin temperature. Mean skin temperatures exhibited an interaction of time and pressure condition ($P = 0.024$, Fig 2*B*). Mean skin temperature was similar at baseline levels (all $P > 0.117$) as well as at the end of Exercise 1 and Recovery 1 (all $P > 0.096$). Although mean skin temperature remained similar between the Control and LBPP conditions until the end of Recovery 2 (all $P > 0.075$), it was progressively lower than Control during the final 20 min of Recovery 2 such that end-recovery values were 0.83 ± 0.25ºC in the LBNP condition (all $P < 0.042$).

Heat loss responses

CVC – Effect of Pressure. An interaction of time and pressure condition was measured for CVC ($P < 0.001$, Fig. 3). CVC was similar between pressure conditions within

all treatment sites at the end of Exercise 1 (all $P > 0.251$) and Exercise 2 (all $P > 0.076$). Similarly, CVC did not differ during Recovery 1 within the $_{L}$-NAME, Theophylline, and Bretylium sites (all $P > 0.958$), whereas CVC was increased from Control during the LBPP condition at the Ringer's site only for the final 30 min of Recovery 1 (all $P < 0.028$). Conversely, CVC at the Ringer's site was similar between LBPP and Control conditions throughout Recovery 2. The LBNP condition resulted in lower CVC for the last 15 min of Recovery 2 at each treatment site compared to the Control condition (all $P < 0.011$). Finally, there was no main effect of pressure condition ($P = 0.904$) for maximal CVC as determined at the end of each experimental session (Table 1).

CVC – Effect of treatment site. An interaction of time and treatment was also measured for CVC ($P < 0.001$, Table 2). CVC was similar at baseline levels, as well as at the end of Exercise 1 and 2 at the Ringer's, Theophylline, and Bretylium sites in each pressure condition (all $P > 0.136$) whereas CVC at the $_{L}$-NAME site was lower than Ringer's at the end of each exercise period (all $P < 0.001$). During the Control condition, CVC at the Ringer's site paralleled that reported at the $_{L}$-NAME site throughout Recovery 1 (all $P > 0.087$) whereas CVC was elevated at the Ringer's site throughout Recovery 2 (all $P < 0.002$). In contrast, CVC at the Ringer's site was elevated during the LBPP and LBNP conditions relative to the $_{L}$-NAME site throughout Recovery 1 and 2 (all $P < 0.023$). Additionally, whereas CVC was reduced at the Ringer's site compared to the Theophylline and Bretylium sites throughout Recovery 1 and 2 of the Control and LBNP conditions (all $P < 0.047$), CVC was similar between these sites during the application of LBPP ($P > 0.067$). Finally, there was no main effect of treatment site detected for maximal CVC ($P = 0.259$, Table 1).

Sweating – Effect of pressure. There was no interaction of time and pressure condition ($P = 0.470$) for sweat rate (Fig. 4). Specifically, sweat rate within each treatment site was not different between pressure conditions at baseline levels and remained similar between pressure conditions for the entire experimental session ($P = 0.963$). However, there was a main effect of time for sweat rate ($P < 0.001$) such that sweating increased during each exercise bout and decreased towards baseline levels during each recovery period.

Sweating – Effect of treatment site. Sweat rate did exhibit an interaction of time and treatment site ($P < 0.001$; Table 3). Specifically, sweat rate at the $_{L}$-NAME site was reduced from the Ringer's site in all pressure conditions at the end of Exercise 1 (all $P < 0.018$) and Exercise 2 (all $P < 0.012$). In addition, while sweat rate at the Ringer's site was similar to that at the $_{L}$-NAME site for the final 25 min of Recovery 1 (all $P > 0.068$), sweat rate throughout Recovery 2 was elevated at the Ringer's site (all $P < 0.015$) in all pressure conditions. Conversely, sweat rate did not differ throughout the experimental protocol in any pressure condition at the Theophylline or Bretylium sites compared to the Ringer's site (all $P > 0.078$).

DISCUSSION

In this study, we evaluated the mechanisms involved in the baroreceptor-mediated suppression of cutaneous blood flow and sweating during recovery from intermittent exercise. Consistent with our first hypothesis, LBPP was associated with increases in CVC from a Control condition and this increase was blunted by the non-selective inhibition of nitric oxide synthase. Furthermore, CVC was reduced with LBNP which was not affected by inhibition of noradrenergic vasoconstriction. Conversely, we did not observe any baroreceptor-mediated modulation of sweating during Recovery 1 or 2. However, a role for nitric oxide was detected during the early stages of Recovery 1 and throughout Recovery 2 as sweat rate at the Ringer's site was elevated from the $_{L}$-NAME site. Consistent with our second hypothesis, CVC did not differ between pressure conditions at the Theophylline or Bretylium sites thereby implicating a potential role for adenosine receptors and noradrenergic vasoconstriction in the baroreceptor-mediated suppression of cutaneous blood flow. Finally, in line with our third hypothesis, we observed a temperature-dependent influence of baroreceptor loading status on CVC shown by a blunted influence of LBPP during Recovery 2 relative to Recovery 1. Taken together, our findings indicate that baroreceptor loading status modulates cutaneous blood flow during postexercise recovery through nitric oxide, adenosine receptor, and noradrenergic vasoconstrictor-dependent mechanisms; however, an influence of baroreceptors on postexercise sweating was not observed.

Effects of pressure on postexercise hyperthermia

The influence of baroreceptor loading status on postexercise heat loss has been evidenced in numerous studies (Kenny *et al.*, 2003a, b; Journeay *et al.*, 2004a; Gagnon *et al.*, 2008). The reversal of baroreceptor unloading (i.e., blunting postexercise hypotension) has been shown to increase cutaneous blood flow and sweating (Journeay *et al.*, 2004a; Gagnon *et al.*, 2008), and this has been shown to translate into a more rapid decrease in core body temperature (Journeay *et al.*, 2004a). Consistent with the study by Journeay and colleagues (2004), we observed a more rapid reduction in postexercise core temperature such that oesophageal temperature was ~0.5°C lower in the LBPP condition relative to the Control condition at the end of Recovery 2 (Fig. 2). On the other hand, it is important to note that the postexercise core temperature response was similar during the LBNP and Control conditions during Recovery 1 and 2. This is consistent with Journeay *et al*. (2004) and with our heat loss data (discussed below) as differences during LBNP were not found for sweating during Recovery 1 or 2 and CVC was only reduced in the later stages of Recovery 2.

Effects of pressure on postexercise CVC

The effects of baroreceptor loading status on cutaneous blood flow have been well documented (Kellogg *et al.*, 1990; Mack *et al.*, 1995a; Crandall *et al.*, 1996; Journeay *et al.*, 2004a). In particular, it is believed that baroreceptor unloading associated with postexercise hypotension is involved in suppressing postexercise cutaneous blood flow (Kenny & Journeay, 2010). Our findings are consistent with this notion such that LBPP application resulted in an increase in CVC at the Ringer's site during Recovery 1. In addition, we demonstrated that this response is largely nitric oxide-dependent as no effect of LBPP was

observed at the $_{L}$-NAME site (Fig. 3). However, it is important to note that this increase in CVC associated with LBPP was only observed in Recovery 1 as CVC was similar between the LBPP and Control conditions during Recovery 2. Oesophageal temperature was also ~0.5°C higher in the Control condition at the end of Recovery 2 compared to Recovery 1. Thus, the elevation in CVC at the Ringer's site during Recovery 2 is likely a thermal-mediated response and is consistent with previous findings implicating a temperature-dependent influence of baroreceptor loading status on CVC during the postexercise period (Gagnon *et al.*, 2008). Interestingly, postexercise CVC was similar during Recovery 1 and 2 at the $_{L}$-NAME site, lending evidence to suggest that the mechanism involved in elevating CVC during Recovery 2 at the Ringer's site may also be nitric oxide-dependent.

A previous study has implicated adenosine receptors and noradrenergic vasoconstriction as modulators of postexercise CVC (McGinn *et al.*, 2014). Consistent with these findings, CVC was elevated in the present study throughout Recovery 1 and 2 at the Theophylline and Bretylium sites compared to the Ringer's site. Our observations extend the role for adenosine receptor and noradrenergic vasoconstrictor inhibition in modulating CVC during recovery following exercise in the heat as well as following repeated exercise bouts. Interestingly, we observed no effect of LBPP on CVC in Recovery 1 or 2 for the Theophylline or Bretylium sites. In fact, CVC at the Ringer's site was similar to CVC at the Theophylline and Bretylium sites during LBPP application. While the mechanism for these findings is unclear, some evidence has linked stress (e.g., pronounced hypotension or ischemia) to a greater release of ATP in the nucleus tractus solitarii in the rat (Van Wylen *et al.*, 1988; St Lambert *et al.*, 1997; Ichinose *et al.*, 2012). This response has not been studied in humans; however, it is known that ATP is rapidly catabolized by ectonucleotidases to

serve as the endogenous source of adenosine. Given that ATP is located in cutaneous sympathetic nerves (Burnstock, 1990), and the substantial role shown for adenosine in modulating postexercise CVC (McGinn *et al.*, 2014), it is plausible to suggest that marked baroreceptor unloading during the postexercise period results in a concomitant increase in cutaneous adenosine receptor activation in an effort to restore and/or sustain mean arterial pressure. While speculative, our observations at the Bretylium site may reflect a similar mechanism given that ATP is known to be co-localized and released with noradrenenaline and neuropeptide Y (Burnstock, 1990), albeit future research is needed to expand on these interactive mechanisms.

In the present study, a further reduction in mean arterial pressure during LBNP application in Recovery 1 and 2 was not observed which parallels findings by Journeay and colleagues (2004). However, we observed a higher heart rate throughout LBNP application during Recovery 1 and 2 compared to Control, thereby indicating a greater level of baroreceptor unloading (Fig. 1). Unlike LBPP application, we did not observe any differences in CVC at any treatment site during Recovery 1 of the LBNP condition which is also consistent with previous findings (Journeay *et al.*, 2004a). Conversely, we were able to detect a decrease in CVC associated with LBNP application at all treatment sites during Recovery 2 when the magnitude of hyperthermia and postexercise hypotension was greater. Although this is inconsistent with the baroreceptor influence on cutaneous blood flow being reduced at greater levels of hyperthermia (Gagnon *et al.*, 2008), it seems that combining the stress of Exercise 2 (as indicated by a greater magnitude of hyperthermia and hypotension) with LBNP during Recovery 2 placed a greater demand for the redistribution of blood from the skin to the central cavity. In line with studies using passive heat stress (Kellogg *et al.*,

1990; Crandall *et al.*, 1996) it is likely that the baroreceptors induced a greater withdrawal of cutaneous active vasodilator activity as the magnitude by which CVC was reduced with LBNP was similar at the Ringer's and Bretylium sites. However, we also noted that this decrease in CVC during Recovery 2 was markedly suppressed at the Theophylline site (by ~42%). These findings are consistent with our above statement concerning the potential link between baroreceptor unloading and adenosine release; however, our data alone cannot confirm or deny this hypothesis.

Effects of pressure on postexercise sweating

It is well established that sweating is the primary avenue for heat dissipation during exercise in the heat (Kenny & Journeay, 2010; Shibasaki & Crandall, 2010); however, sweat rate is suppressed in the early stages of recovery towards baseline levels (Kenny & Journeay, 2010). While some studies indicate that this suppression may involve a baroreceptor-mediated component (Kenny *et al.*, 2003b; Journeay *et al.*, 2004a; Gagnon *et al.*, 2008), other studies suggest that baroreceptor loading status does not impact sweating (Mack *et al.*, 2001a; Wilson *et al.*, 2001, 2005). Our findings indicate that changes in baroreceptor loading status do not alter sweating during recovery from exercise in the heat as evidenced by a similar level of sweating during Recovery 1 and 2 in each pressure condition at the Ringer's site. These results are not consistent with those by Journeay *et al*. (2004) who previously employed LBPP and LBNP conditions during the postexercise period. However, it is important to consider that the level of sweating during recovery in the present study was ~0.3 mg min^{-1} cm^{-2} greater, likely due to a combination of warmer ambient conditions and ~0.3 to 0.6ºC higher oesophageal temperature measured in our study. On the other hand, we

did report a lower oesophageal temperature in the LBPP condition and a lower mean skin temperature in the LBNP condition compared to the Control condition (reduced by ~0.5ºC and ~0.8ºC, respectively). Thus, a similar level of sweating occurred between all pressure conditions despite differences in thermal input which may implicate to some extent, although not conclusively, a baroreceptor-mediated component. On the other hand, we also show that inhibiting adenosine receptors or preventing the release of noradrenaline and neuropeptide Y from sympathetic nerves does not modulate sweating during or following exercise irrespective of baroreceptor loading status.

Recent studies have identified nitric oxide as a contributor to sweat production during exercise (Welch *et al.*, 2009; Stapleton *et al.*, 2014); however, its role was not evident during recovery as the level of sweating was similar between the $_{L}$-NAME and Ringer's sites (Stapleton *et al.*, 2014). Our findings are consistent with these prior reports during Exercise 1 and 2 such that we observed a reduction in sweating at the $_{L}$-NAME site relative to the Control site by ~0.2 mg min^{-1} cm^{-2}. However, unlike the results from Stapleton and colleagues (2014), we did demonstrate a role for nitric oxide during recovery. Specifically, we found sweat rate at the Ringer's site to be elevated from the $_{L}$-NAME site during the first 20 min of Recovery 1 and for the duration of Recovery 2. Sweat rate did not vary between pressure conditions which suggest a baroreceptor-mediated modulation of sweating is unlikely. In fact, Gagnon *et al*. (2008) indicated that baroreceptor modulation of sweating during the postexercise period was only apparent in the later stages of recovery when core temperature was ≤0.6ºC above baseline levels. Thus, at a level of hyperthermia exceeding 0.6ºC above baseline levels, thermal input appeared to override any baroreceptor-mediated input. Given that postexercise oesophageal temperature in the present study did not drop

below this threshold (~0.6ºC and ~1.0ºC above baseline at the end of Recovery 1 and 2 respectively), it seems that the elevated sweat rate at the Ringer's site was the result of thermal input that was nitric oxide-dependent. Taken together, our findings suggest that nitric oxide can modulate sweating during the postexercise period, but only at higher core temperatures.

Perspectives

In the present study we demonstrated a role for nitric oxide in modulating postexercise cutaneous blood flow and sweating. It is well known that there are three isoforms of nitric oxide synthase (NOS) found in humans (i.e., neuronal [nNOS], endothelial [eNOS], and inducible [iNOS]); however, due to the non-selective inhibitory nature of $_{L}$-NAME, we were unable to decipher which enzyme isoform is responsible for our observations. A recent study by Kellogg *et al*. (2008) reported that nNOS was responsible for nitric oxide generation during centrally mediated cutaneous active vasodilation during whole-body heat stress. In addition, it was postulated in this study that eNOS was not involved in the centrally-mediated active vasodilatory response (Kellogg *et al.*, 2008), whereas any role of iNOS was ruled out as it is typically found in such small concentrations in human skin (Wang *et al.*, 1996). Given that baroreceptor loading status is believed to represent a central modulation of heat loss (Kenny & Journeay, 2010), nNOS is the most probable isoform to account for the nitric oxide-dependent modulation of cutaneous blood flow and sweating during the postexercise period.

Finally, it is important to note that although we induced marked changes in baroreceptor loading status during Recovery 1 as evidenced by heart rate and mean arterial

pressure responses (Fig. 1), these cardiovascular as well as core body and skin temperature responses during Exercise 2 were similar between pressure conditions. Taken together, our results indicate that irrespective of any intervention applied to augment postexercise heat loss (i.e., lower body positive pressure application), the cardiovascular and body temperature responses to a subsequent exercise are unchanged. Considering that the progressive increase in the level of hyperthermia during repeated exercise bouts is reported to result from a sustained suppression of heat loss during the postexercise periods (Kenny & Gagnon, 2010), employing such interventions to elevate heat dissipation during recovery would be critical to prevent heat-related illness and/or injury. Specifically with regards to athletic and workplace conditions, such convenient and practical interventions (e.g., passive recovery such that the limbs are passively taken through the full range of motion and/or compression garments) need to be established and implemented in order to minimize the risk for adverse heat events.

CONCLUSION

In summary, the present study indicates that the postexercise suppression of cutaneous blood flow is modulated by baroreceptor loading status. Our findings implicate a nitric oxide-mediated component to postexercise vasodilation associated with LBPP; however, the involvement of adenosine receptors and noradrenergic vasoconstriction in contributing to this response cannot be discounted. In addition, the exacerbated suppression of cutaneous blood flow associated with LBNP was likely mediated by withdrawal of active vasodilation as we demonstrated this response to be intact with the inhibition of noradrenergic vasoconstriction. Conversely, we did not observe a baroreceptor-mediated suppression of sweating during the postexercise period. However, it is possible that the level of hyperthermia during recovery was such that thermal afferents superseded any influence of baroreceptors. Finally, we also found this thermal-mediated response of sweating during recovery to exhibit a nitric oxide-dependent component.

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ADDITIONAL INFORMATION

Competing Interests

The authors have no conflict of interest to declare.

Author Contributions

R.M. and G.P.K. contributed to the conception and design of the experiments. R.M., G.P., R.D.M., and N.F. contributed to the collection of data. R.M., G.P., R.D.M., N.F., and G.P.K. contributed to the analysis and interpretation of the data as well as the preparation of the manuscript. All authors approved the final version of the manuscript. All experiments took place at the Human and Environmental Physiology Research Unit located at the University of Ottawa.

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TABLES

Table 1. Absolute maximal cutaneous vascular conductance at each treatment as assessed at the end of each experimental session by infusion of 50 m_M sodium nitroprusside via microdialysis at 6 μl min^{-1}.

	Control	LBPP	LBNP
Ringer's	217 ± 58	255 ± 102	235 ± 49
$_L$-NAME	199 ± 55	221 ± 40	218 ± 53
Theophylline	225 ± 67	244 ± 36	268 ± 82
Bretylium	256 ± 81	237 ± 55	245 ± 67

Lower body negative pressure condition, LBPP; Lower body negative pressure condition, LBNP; $_L$-NAME, N^G-nitro-$_L$-arginine methyl ester. Values are mean ± 95% confidence intervals. Values were calculated from maximal cutaneous blood flow (perfusion units) averaged over 2 min, multiplied by 100 and divided by the corresponding mean arterial pressure. No main effect of treatment site (P = 0.157) or pressure condition (P = 0.904) was detected.

Table 2. Cutaneous vascular conductance responses for each pressure condition at each treatment site (Control, N^G-nitro-$_L$-arginine methyl ester [L-NAME], Theophylline [Theo], and Bretylium) at baseline (Rest), end of each exercise (End-Ex 1 and End-Ex 2), and at 15 minute intervals throughout each recovery (Recovery 1 and Recovery 2).

	Rest	End-Ex 1		Recovery 1		End-Ex 2		Recovery 2	
	0	15	30	45	60	75	90	105	120
					Control (% Max)				
Ringer's	33 ± 3	57 ± 7	36 ± 5	33 ± 4	33 ± 3	56 ± 6	41 ± 3	38 ± 3	36 ± 3
L-NAME	30 ± 3	43 ± 4*	33 ± 2	28 ± 3	28 ± 3	43 ± 4*	32 ± 3*	29 ± 4*	28 ± 3*
Theo	35 ± 3	54 ± 5	47 ± 4	46 ± 6	44 ± 5	56 ± 4	46 ± 3	46 ± 5	46 ± 4
Bretylium	32 ± 3	56 ± 6	46 ± 6	43 ± 4	40 ± 4	56 ± 5	47 ± 5	45 ± 3	42 ± 2
					Lower body positive pressure (% Max)				
Ringer's	34 ± 3	56 ± 4	39 ± 3	41 ± 2	39 ± 2	60 ± 3	40 ± 3	42 ± 2	40 ± 2
L-NAME	31 ± 3	43 ± 2*	32 ± 2*	30 ± 4*	29 ± 4*	46 ± 2*	33 ± 2*	31 ± 4*	30 ± 4*
Theo	38 ± 2	57 ± 3	45 ± 2*	44 ± 3	45 ± 3	59 ± 3	47 ± 2	46 ± 3	44 ± 4
Bretylium	34 ± 2	59 ± 4	44 ± 2*	43 ± 2	42 ± 2	61 ± 4	47 ± 3	41 ± 2	40 ± 2
					Lower body negative pressure (% Max)				
Ringer's	32 ± 2	57 ± 3	37 ± 2	33 ± 3	27 ± 3	57 ± 4	41 ± 2	32 ± 3	26 ± 4
L-NAME	31 ± 2	42 ±2*	32 ± 2*	26 ± 3*	26 ± 4*	43 ± 3*	32 ± 3*	27 ± 2*	22 ± 3*
Theo	34 ± 4	56 ± 2	45 ± 3*	42 ± 2*	41 ± 3*	55 ± 2	46 ± 2*	42 ± 3*	38 ± 4*
Bretylium	34 ± 2	56 ± 3	45 ± 2*	39 ± 5*	33 ± 5*	58 ± 3	45 ± 2*	38 ± 3*	31 ± 3*

Values presented a mean ± 95% confidence interval. * different from Ringer's in the same pressure condition ($P < 0.05$).

Table 3. Local sweat rate responses for each pressure condition at each treatment site (Control, N^G-nitro-$_L$-arginine methyl ester [L-NAME], Theophylline [Theo], and Bretylium) at baseline (Rest), end of each exercise (End-Ex 1 and End-Ex 2), and at 15 minute intervals throughout each recovery (Recovery 1 and Recovery 2).

	Rest	End-Ex 1		Recovery 1		End-Ex 2		Recovery 2	
	0	15	30	45	60	75	90	105	120
				Control (% Max)					
Ringer's	0.16 ± 0.05	1.28 ± 0.21	0.96 ± 0.20	0.58 ± 0.14	0.49 ± 0.11	1.25 ± 0.16	0.99 ± 0.21	0.80 ± 0.16	0.77 ± 0.17
L-NAME	0.14 ± 0.03	1.00 ± 0.15*	0.66 ± 0.14	0.42 ± 0.10	0.36 ± 0.07	0.92 ± 0.13*	0.72 ± 0.14*	0.60 ± 0.13*	0.57 ± 0.12
Theo	0.17 ± 0.04	1.22 ± 0.19	0.87 ± 0.19	0.58 ± 0.17	0.51 ± 0.11	1.21 ± 0.18	0.91 ± 0.21	0.75 ± 0.18	0.70 ± 0.15
Bretylium	0.16 ± 0.05	1.24 ± 0.16	0.88 ± 0.16	0.56 ± 0.17	0.49 ± 0.13	1.20 ± 0.17	0.95 ± 0.21	0.78 ± 0.18	0.74 ± 0.19
				Lower body positive pressure (% Max)					
Ringer's	0.18 ± 0.04	1.34 ± 0.14	1.01 ± 0.17	0.53 ± 0.07	0.53 ± 0.07	1.29 ± 0.17	1.13 ± 0.18	0.77 ± 0.15	0.65 ± 0.15
L-NAME	0.14 ± 0.03	1.02 ± 0.16*	0.73 ± 0.18	0.35 ± 0.07	0.38 ± 0.07	0.98 ± 0.17*	0.82 ± 0.18	0.56 ± 0.16	0.44 ± 0.12
Theo	0.14 ± 0.03	1.23 ± 0.21	0.87 ± 0.28	0.42 ± 0.15	0.43 ± 0.10	1.16 ± 0.22	0.97 ± 0.25	0.69 ± 0.24	0.53 ± 0.17
Bretylium	0.15 ± 0.03	1.19 ± 0.15	0.89 ± 0.21	0.41 ± 0.13	0.42 ± 0.09	1.22 ± 0.11	1.04 ± 0.15	0.74 ± 0.20	0.57 ± 0.15
				Lower body negative pressure (% Max)					
Ringer's	0.13 ± 0.03	1.27 ± 0.10	0.87 ± 0.14	0.55 ± 0.14	0.52 ± 0.08	1.25 ± 0.13	1.00 ± 0.12	0.75 ± 0.13	0.65 ± 0.13
L-NAME	0.14 ± 0.04	1.06 ± 0.13*	0.70 ± 0.15	0.43 ± 0.11	0.44 ± 0.11	0.92 ± 0.11*	0.78 ± 0.13	0.58 ± 0.12	0.49 ± 0.14
Theo	0.16 ± 0.04	1.21 ± 0.17	0.81 ± 0.23	0.54 ± 0.21	0.55 ± 0.14	1.15 ± 0.15	0.92 ± 0.18	0.61 ± 0.16	0.52 ± 0.14
Bretylium	0.16 ± 0.03	1.22 ± 0.07	0.92 ± 0.11	0.60 ± 0.13	0.50 ± 0.07	1.20 ± 0.06	1.01 ± 0.09	0.79 ± 0.10	0.68 ± 0.11

Values presented a mean ± 95% confidence interval. * different from Ringer's in the same pressure condition ($P < 0.05$).

FIGURE CAPTIONS

Figure 1. Heart rate (*A*) and mean arterial pressure (*B*; MAP) at baseline (Rest), end of exercise 1 and 2 (Ex 1 and Ex 2, respectively), and at 5 min intervals throughout the postexercise periods (Rec 1 and Rec 2) during a Control condition (i.e., no pressure; *open circles*), lower body positive pressure (LBPP; *closed squares*) and lower body negative pressure (LBNP; *grey triangles*). Each pressure condition was employed at 15 min into recovery (indicated by a down arrow) and lasted for the final 30 min of each recovery. The values at 5 min of each recovery were omitted to allow for the transfer of participants to a pressure box sealed at the waist. Values are mean ± 95% confidence intervals. * LBPP significantly different from Control ($P < 0.05$); † LBNP significantly different from Control ($P < 0.05$).

Figure 2. Oesophageal temperature (panel A) and mean skin temperature (panel B) at baseline (Rest), end of exercise 1 and 2 (Ex 1 and Ex 2, respectively), and at 5 min intervals throughout the postexercise periods (Rec 1 and Rec 2) during a Control condition (i.e., no pressure; *open circles*), and conditions applying lower body positive pressure (LBPP; *closed squares*), and lower body negative pressure (LBNP; *grey triangles*). Each pressure condition was employed at 15 min into recovery (indicated by a down arrow) and lasted for the final 30 min of each recovery. The values at 5 min of each recovery were omitted to allow for the transfer of participants to a pressure box sealed at the waist. Values are mean ± 95% confidence intervals. * LBPP significantly different from Control ($P < 0.05$); † LBNP significantly different from Control ($P < 0.05$).

Figure 3. Cutaneous vascular conductance (CVC) presented as a percentage of maximum at baseline (Rest), end of exercise 1 and 2 (Ex 1 and Ex 2, respectively) and at 5 min intervals throughout the postexercise periods (Rec 1 and Rec 2) at skin sites perfused continuously with Lactated Ringer's solution (*A*), 10 m_M N^G-nitro-$_L$-arginine methyl ester (*B*; $_L$-NAME), 4 m_M Theophylline (*C*), and 10 m_M Bretylium Tosylate (*D*). Responses are presented during a Control condition (i.e., no pressure; *open circles*), and conditions employing lower body positive pressure (LBPP; *closed squares*) and lower body negative pressure (LBNP; *grey triangles*). Each pressure condition was employed at 15 min into recovery (indicated by a down arrow) and lasted for the final 30 min of each recovery. The values at 5 min of each recovery were omitted to allow for the transfer of participants to a pressure box sealed at the waist. Values are mean ±95% confidence intervals. * LBPP significant different from Control ($P < 0.05$); † LBNP significantly different from Control ($P < 0.05$).

Figure 4. Local sweat rate presented at baseline (Rest), end of exercise 1 and 2 (Ex 1 and Ex 2, respectively) and at 5 min intervals throughout the postexercise periods (Rec 1 and Rec 2) at skin sites perfused continuously with Lactated Ringer's solution (*A*), 10 m_M N^G-nitro-$_L$-arginine methyl ester (*B*; $_L$-NAME), 4 m_M Theophylline (*C*), and 10 m_M Bretylium Tosylate (*D*). Responses are presented during a Control condition (i.e., no pressure; *open circles*), and conditions employing lower body positive pressure (LBPP; *closed squares*) and lower body negative pressure (LBNP; *grey triangles*).). Each pressure condition was employed at 15 min into recovery (indicated by a down arrow) and lasted for the final 30 min of each recovery. The

values at 5 min of each recovery were omitted to allow for the transfer of participants to a pressure box sealed at the waist and. Values are mean ±95% confidence intervals. No differences between pressure conditions were found at any skin site throughout the experimental protocol ($P > 0.05$).

Figure 1.

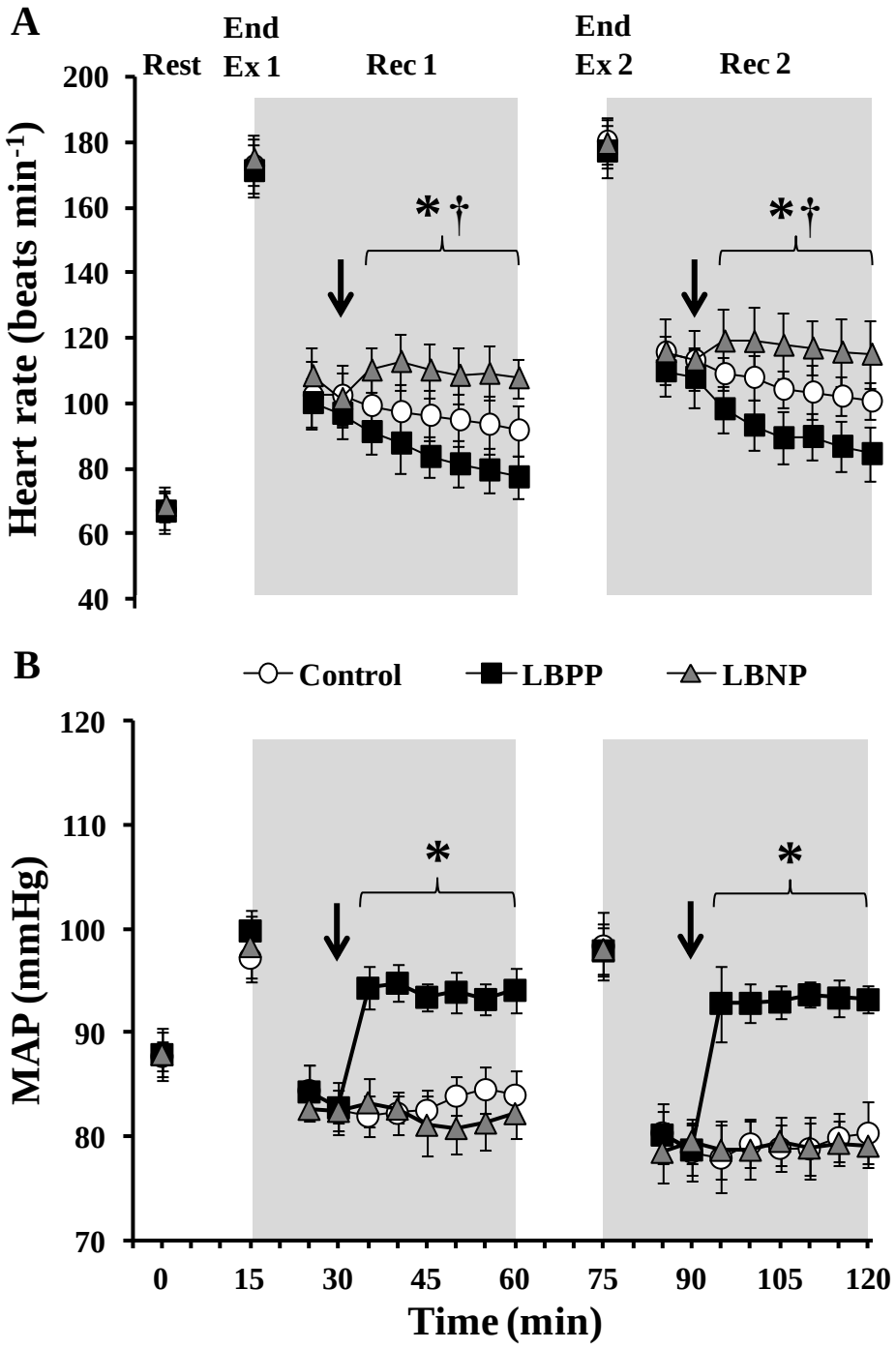

A
Rest
End Ex 1
Rec 1
End Ex 2
Rec 2
Heart rate (beats min-1)
200
180
160
140
120
100
80
60
40
* †
* †
B
Control
LBPP
LBNP
MAP (mmHg)
120
110
100
90
80
70
*
*
0
15
30
45
60
75
90
105
120
Time (min)

Figure 2.

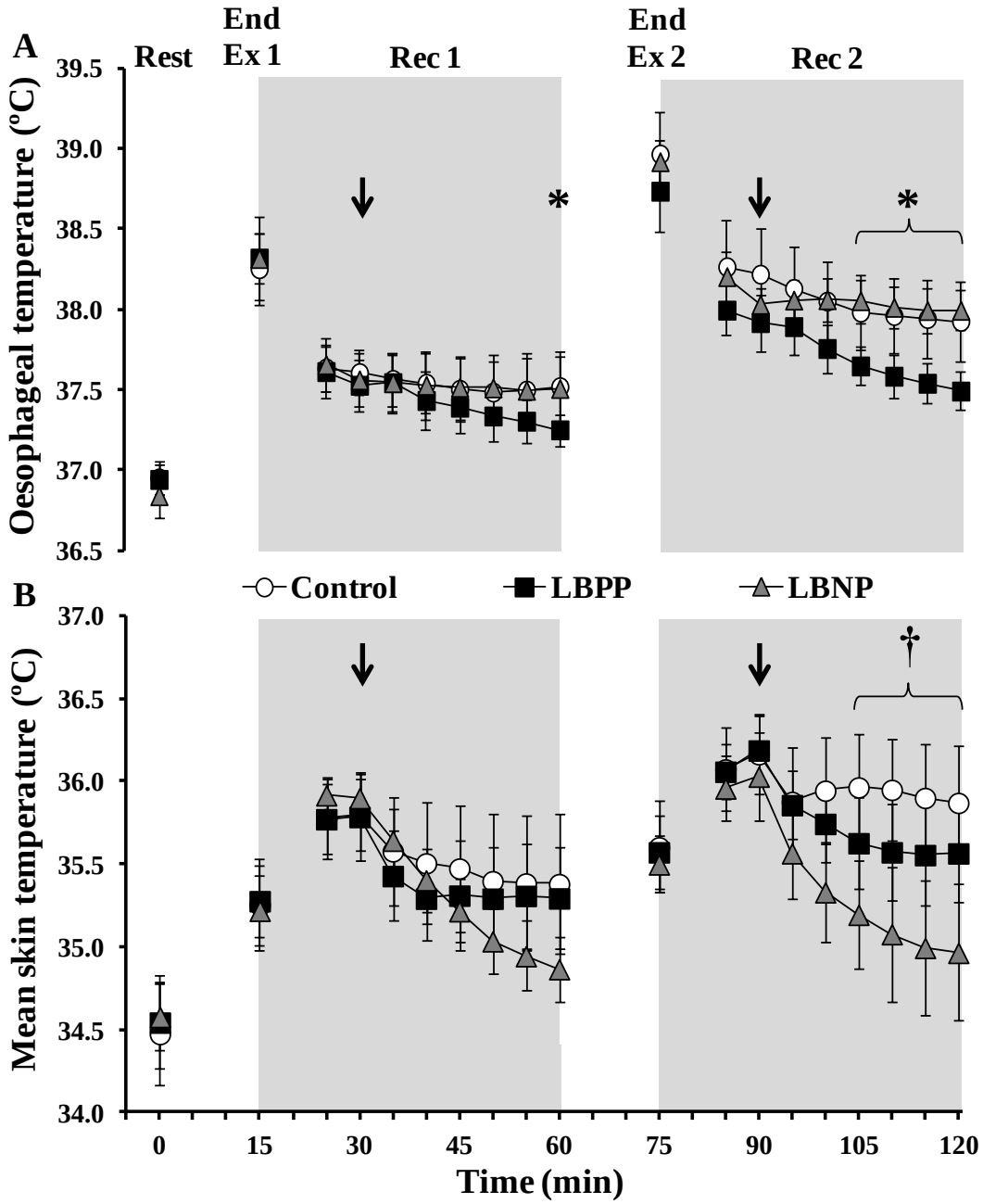

A
Rest
End Ex 1
Rec 1
End Ex 2
Rec 2
Oesophageal temperature (°C)
39.5
39.0
38.5
38.0
37.5
37.0
36.5
*
*
B
Control
LBPP
LBNP
Mean skin temperature (°C)
37.0
36.5
36.0
35.5
35.0
34.5
34.0
†
0
15
30
45
60
75
90
105
120
Time (min)

Figure 3.

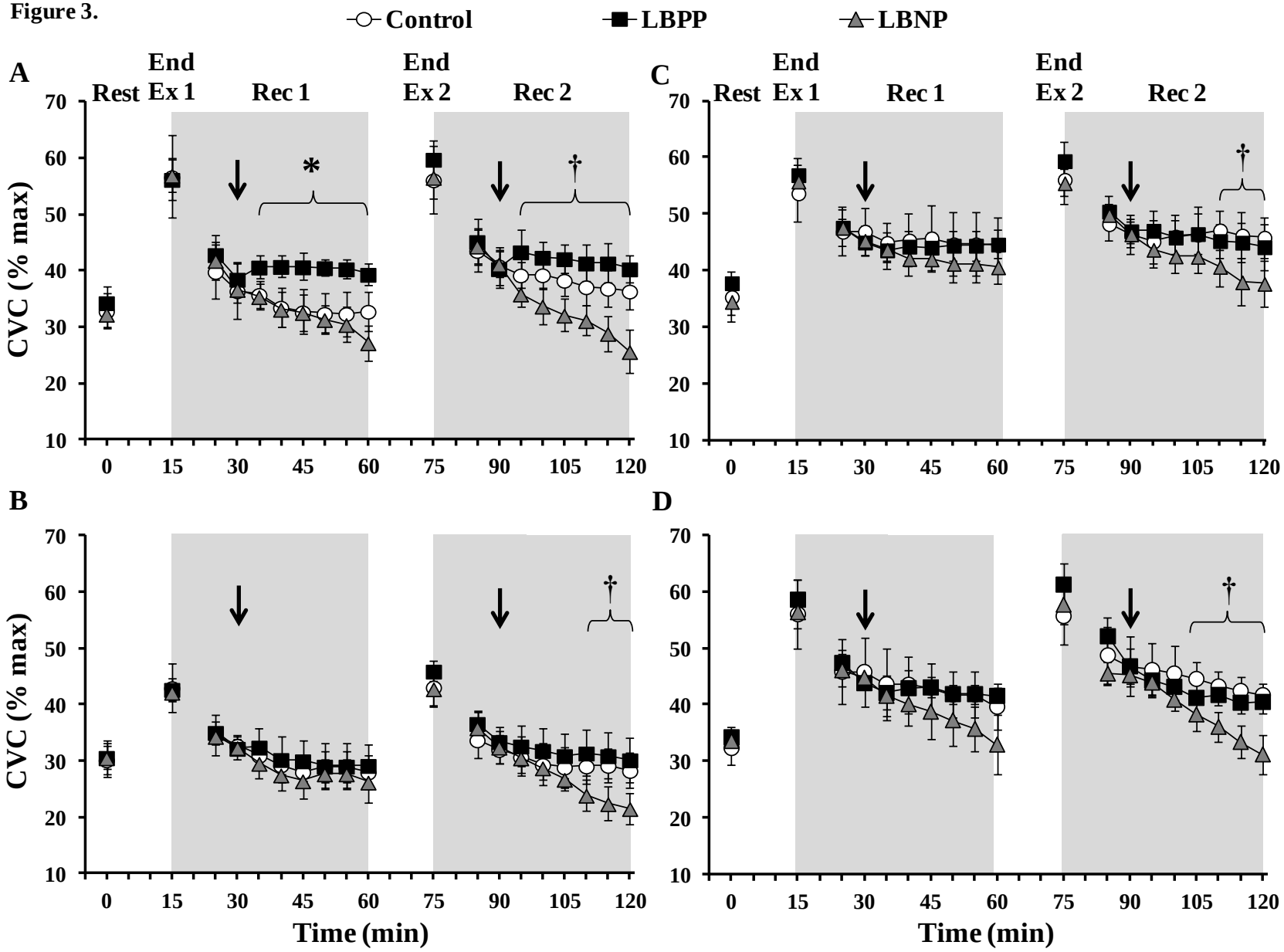
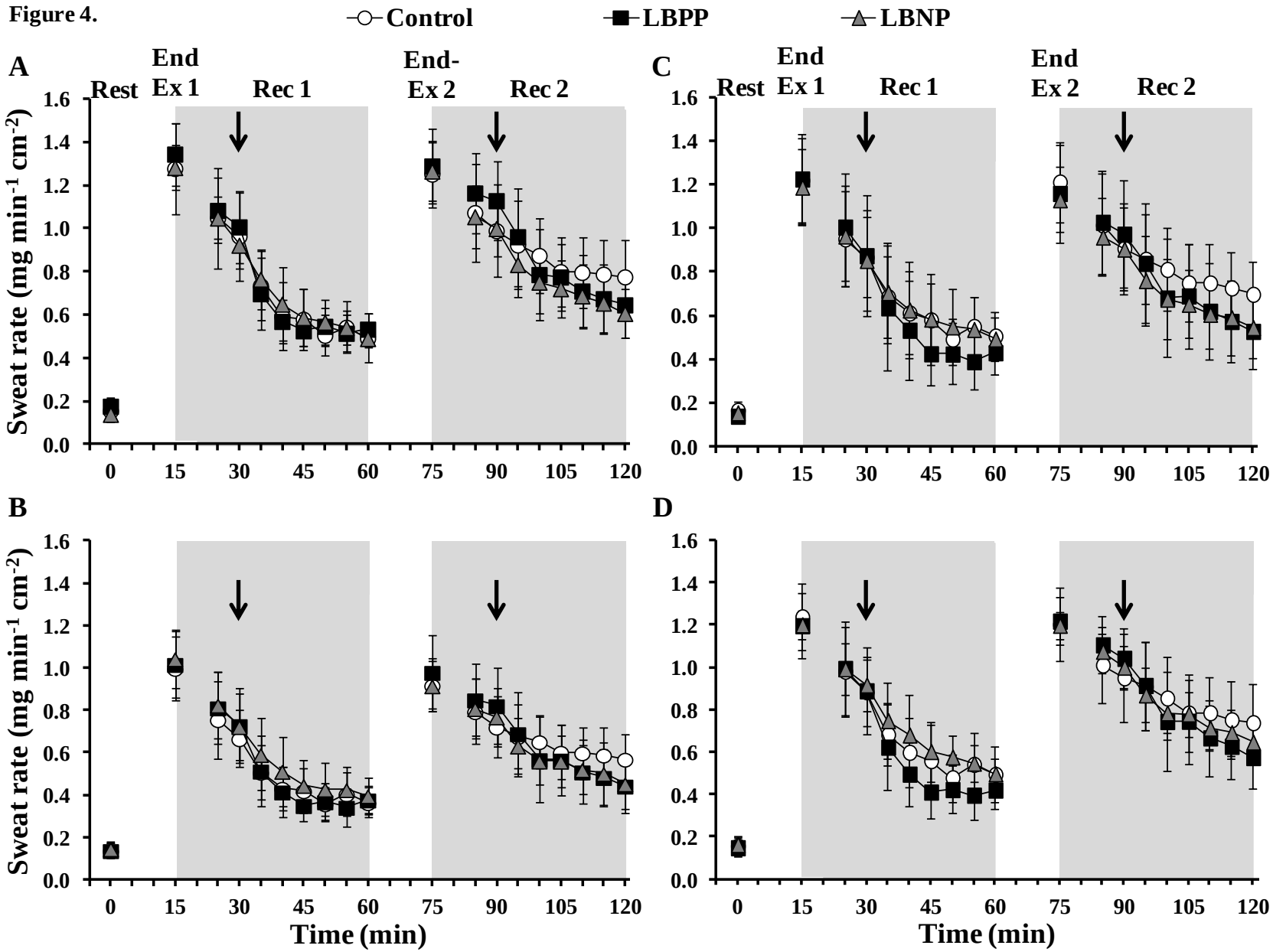

Control
LBPP
LBNP
A
B
C
D
Rest
End Ex 1
Rec 1
End Ex 2
Rec 2
CVC (% max)
Time (min)
*
†

Figure 4.


Control
LBPP
LBNP
A
B
C
D
Rest
End Ex 1
Rec 1
End-Ex 2
Rec 2
End Ex 2
Sweat rate (mg min-1 cm-2)
Time (min)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
0
15
30
45
60
75
90
105
120

PART THREE: GENERAL CONCLUSIONS OF THE THESIS

The current thesis examined the underlying mechanisms that explain the rapid suppression of heat loss following exercise despite pronounced hyperthermia. In particular, the results indicate that postexercise baroreceptor unloading associated with postexercise hypotension has an important role in the return of cutaneous blood flow, but not sweating, towards baseline resting levels. Importantly, the role of baroreceptor loading status exhibited a temperature-dependent effect on cutaneous blood flow such that higher core temperatures during recovery were associated with a reduced impact on cutaneous blood flow. In addition, at the level of the end-organ (i.e., skin vessels and sweat glands), the results implicated an important role for nitric oxide, adenosine receptors, and noradrenergic vasoconstriction in the suppression of cutaneous blood flow postexercise. Specifically, the combination of reduced nitric oxide production together with increased adenosine receptor activation and noradrenaline release likely explain a significant portion of the reduction in cutaneous blood flow. On the other hand, manipulation of nitric oxide, adenosine receptor or noradrenaline signaling pathways did not amount to any measurable effect on sweating during or following exercise. Therefore, this study demonstrates that postexercise hypotension exacerbates postexercise hyperthermia by suppressing heat loss via cutaneous blood flow. Furthermore, nitric oxide, adenosine receptors, and noradrenaline are involved as end-organ effectors in the reduction of cutaneous blood flow; however, the mechanisms underlying the rapid suppression of sweating remain to be elucidated.

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CHAPTER 5

APPENDIX

5.1 Physical Activity Readiness Questionnaire (Par-Q+)

CSEP approved Sept 12 2011 version

PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

Regular physical activity is fun and healthy, and more people should become more physically active every day of the week. Being more physically active is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

SECTION 1 - GENERAL HEALTH

	Please read the 7 questions below carefully and answer each one honestly: check YES or NO.	YES	NO
1.	Has your doctor ever said that you have a heart condition OR high blood pressure?	☐	☐
2.	Do you feel pain in your chest at rest, during your daily activities of living, OR when you do physical activity?	☐	☐
3.	Do you lose balance because of dizziness OR have you lost consciousness in the last 12 months? Please answer NO if your dizziness was associated with over-breathing (including during vigorous exercise).	☐	☐
4.	Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)?	☐	☐
5.	Are you currently taking prescribed medications for a chronic medical condition?	☐	☐
6.	Do you have a bone or joint problem that could be made worse by becoming more physically active? Please answer NO if you had a joint problem in the past, but it does not limit your current ability to be physically active. For example, knee, ankle, shoulder or other.	☐	☐
7.	Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐

If you answered NO to all of the questions above, you are cleared for physical activity.

Go to Section 3 to sign the form. You do not need to complete Section 2.

- Start becoming much more physically active – start slowly and build up gradually.
- Follow the Canadian Physical Activity Guidelines for your age (www.csep.ca/guidelines).
- You may take part in a health and fitness appraisal.
- If you have any further questions, contact a qualified exercise professional such as a CSEP Certified Exercise Physiologist® (CSEP-CEP) or CSEP Certified Personal Trainer® (CSEP-CPT).
- If you are over the age of 45 yrs. and NOT accustomed to regular vigorous physical activity, please consult a qualified exercise professional (CSEP-CEP) before engaging in maximal effort exercise.

If you answered YES to one or more of the questions above, please GO TO SECTION 2.

Delay becoming more active if:

- You are not feeling well because of a temporary illness such as a cold or fever – wait until you feel better
- You are pregnant – talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the PARmed-X for Pregnancy before becoming more physically active OR
- Your health changes – please answer the questions on Section 2 of this document and/or talk to your doctor or qualified exercise professional (CSEP-CEP or CSEP-CPT) before continuing with any physical activity programme.

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SECTION 2 - CHRONIC MEDICAL CONDITIONS

Please read the questions below carefully and answer each one honestly: check YES or NO.			YES	NO
1.	Do you have Arthritis, Osteoporosis, or Back Problems?		☐ If yes, answer questions 1a-1c	☐ If no, go to question 2
	1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	☐	☐
	1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	☐	☐
2.	Do you have Cancer of any kind?		☐ If yes, answer questions 2a-2b	☐ If no, go to question 3
	2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and neck?	☐	☐
	2b.	Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)?	☐	☐
3.	Do you have Heart Disease or Cardiovascular Disease? This includes Coronary Artery Disease, High Blood Pressure, Heart Failure, Diagnosed Abnormality of Heart Rhythm		☐ If yes, answer questions 3a-3e	☐ If no, go to question 4
	3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	3b.	Do you have an irregular heart beat that requires medical management? (e.g. atrial brillation, premature ventricular contraction)	☐	☐
	3c.	Do you have chronic heart failure?	☐	☐
	3d.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer YES if you do not know your resting blood pressure)	☐	☐
	3e.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	☐	☐
4.	Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes		☐ If yes, answer questions 4a-4c	☐ If no, go to question 5
	4a.	Is your blood sugar often above 13.0 mmol/L? (Answer YES if you are not sure)	☐	☐
	4b.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, and the sensation in your toes and feet?	☐	☐
	4c.	Do you have other metabolic conditions (such as thyroid disorders, pregnancy-related diabetes, chronic kidney disease, liver problems)?	☐	☐
5.	Do you have any Mental Health Problems or Learning Difficulties? This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome)		☐ If yes, answer questions 5a-5b	☐ If no, go to question 6
	5a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	5b.	Do you also have back problems affecting nerves or muscles?	☐	☐

Please read the questions below carefully and answer each one honestly: check YES or NO.			YES	NO
6.	Do you have a Respiratory Disease? This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure		☐ If yes, answer questions 6a-6d	☐ If no, go to question 7
	6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	6b.	Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	☐	☐
	6c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	☐	☐
	6d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	☐	☐
7.	Do you have a Spinal Cord Injury? This includes Tetraplegia and Paraplegia		☐ If yes, answer questions 7a-7c	☐ If no, go to question 8
	7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	7b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	☐	☐
	7c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	☐	☐
8.	Have you had a Stroke? This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event		☐ If yes, answer questions 8a-c	☐ If no, go to question 9
	8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	8b.	Do you have any impairment in walking or mobility?	☐	☐
	8c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	☐	☐
9.	Do you have any other medical condition not listed above or do you live with two chronic conditions?		☐ If yes, answer questions 9a-c	☐ If no, read the advice on page 4
	9a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months OR have you had a diagnosed concussion within the last 12 months?	☐	☐
	9b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	☐	☐
	9c.	Do you currently live with two chronic conditions?	☐	☐

Please proceed to Page 4 for recommendations for your current medical condition and sign this document.

PAR-Q+

If you answered NO to all of the follow-up questions about your medical condition, you are ready to become more physically active:

- It is advised that you consult a qualified exercise professional (e.g., a CSEP-CEP or CSEP-CPT) to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually – 20-60 min. of low- to moderate-intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate-intensity physical activity per week.
- If you are over the age of 45 yrs. and NOT accustomed to regular vigorous physical activity, please consult a qualified exercise professional (CSEP-CEP) before engaging in maximal effort exercise.

If you answered YES to one or more of the follow-up questions about your medical condition:

- You should seek further information from a licensed health care professional before becoming more physically active or engaging in a fitness appraisal and/or visit a or qualified exercise professional (CSEP-CEP) for further information.

Delay becoming more active if:

- You are not feeling well because of a temporary illness such as a cold or fever – wait until you feel better
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise profesional, and/or complete the PARmed-X for Pregnancy before becoming more physically active OR
- Your health changes - please talk to your doctor or qualified exercise professional (CSEP-CEP) before continuing with any physical activity programme.

SECTION 3 - DECLARATION

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The Canadian Society for Exercise Physiology, the PAR-Q+ Collaboration, and their agents assume no liability for persons who undertake physical activity. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.
- Please read and sign the declaration below:

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that a Trustee (such as my employer, community/fitness centre, health care provider, or other designate) may retain a copy of this form for their records. In these instances, the Trustee will be required to adhere to local, national, and international guidelines regarding the storage of personal health information ensuring that they maintain the privacy of the information and do not misuse or wrongfully disclose such information.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

For more information, please contact:
Canadian Society for Exercise Physiology
www.csep.ca

KEY REFERENCES

1. Jamnik VJ, Warburton DER, Makarski J, McKenzie DC, Shephard RJ, Stone J, and Gledhill N. Enhancing the effectiveness of clearance for physical activity participation: background and overall process. APNM 36(S1):S3-S13, 2011.
2. Warburton DER, Gledhill N, Jamnik VK, Bredin SSD, McKenzie DC, Stone J, Charlesworth S, and Shephard RJ. Evidence-based risk assessment and recommendations for physical activity clearance; Consensus Document. APNM 36(S1):S266-s298, 2011.

The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or BC Ministry of Health Services.

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5.2 Ethics 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	Fuji	Health Sciences / Physiotherapy	Co-investigator
Ryan	McGinn	Health Sciences / Human Kinetics	Co-investigator
Rob	Meade	Health Sciences / Human Kinetics	Co-investigator
Gabrielle	Paul	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.

Signature: