Aging and heat stress: From rest to exercise

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THESIS ABSTRACT

The current thesis examined local and whole-body heat loss responses during heat stress at rest, exercise and/or pharmacological stimuli as a function of increasing age. The first study examined the effects of age on whole-body heat loss and heat storage during passive exposure to conditions representative of the upper temperature extremes in Canada. The results demonstrate that the cumulative change in body heat content after 2 h of rest was significantly greater in older adults in a hot-dry (older: 212±25; young: 131±27 kJ, P=0.018) and hot-humid (older: 426±37; young: 317±45 kJ, P=0.037) condition. The second study evaluated the maximal capacity of whole-body evaporative heat loss as a function of age and aerobic fitness. The findings demonstrate that whole-body evaporative heat loss was significantly lower in middle-aged untrained (Ex2: 426±34; Ex3:497±17 W) and older (Ex2: 424±38; Ex3: 485±44 W) compared to young (Ex2: 472±42; Ex3: 558±51 W) and middle-aged trained (Ex2: 474±21; Ex3: 552±23 W) males at the end of the last two exercise bouts (P<0.05). The third study assessed the maximal capacity of whole-body evaporative heat loss in females and found that whole-body evaporative heat loss was significantly lower (P=0.002) in the older (Ex2: 343±39 W; Ex3: 389±29 W) compared to the young (Ex2: 383±34 W; Ex3: 437±36 W) females at the end of the second and third exercise-induced heat loads of 325 and 400 W, while no differences were observed during recovery (P=0.693). The fourth study examined nitric oxide-dependent sweating during exercise/rest cycles in young and older adults. We showed that nitric oxide-dependent sweating during short bouts of exercise in the heat is observed in young males, but not in older adults. The fifth study examined: 1) the extent to which peripheral factors (i.e., sweat gland and skin vasodilatory function) contribute to the postexercise suppression of heat loss; and 2) whether age-related

differences exist in the mechanisms modulating postexercise heat loss. The findings demonstrate that there were no differences in sweat rate between the no exercise resting condition and a postexercise condition at either an acetylcholine (ACh) or methacholine (MCh) site for the young (ACh: P=0.992 and MCh: P=0.710) or older (ACh: P=0.775 and MCh: P=0.738) adults. However, older adults had a lower sweating response for both the no exercise resting condition (ACh: P=0.049 and MCh: P=0.006) and postexercise condition (ACh: P=0.050 and MCh: P=0.029) compared to their younger counterparts. Taken together, the current thesis shows true age-related impairments in the ability to dissipate heat exist during both a passive and exercise-induced heat stress aover a certain heat load threshold. Specifically, older adults have an impaired ability to dissipate heat compared to young adults during rest in hot-dry and hot-humid conditions. Additionally, middle-aged untrained and older adults have a reduced capacity to dissipate heat at an exercise-induced heat load of ≥400 W for males and ≥325 W for females, which becomes more pronounced at as the level of heat load increases. However, the age-related impairment in heat loss can be attenuated by maintaining a high level of aerobic fitness. Furthermore, age-related impairments in sweating may be associated with age-related reductions in nitric oxide-mediated sweating. However, there does not appear to be age-related differences in the modulation of heat loss postexercise.

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GLOSSARY

M – Rate of metabolic work load (W)

W – Rate of external workload (W)

(M-W) – Rate of metabolic heat production (W)

R - Rate of radiant heat exchange

C - Rate of convective heat exchange and

E or EHL - Rate of evaporative heat loss

THL – Rate of total heat loss (W)

DHL – Rate of dry heat loss (W)

S – Rate of heat storage (W)

ΔH_b – Change in body heat content (kJ)

E_{req} – required evaporative heat loss to attain heat balance

E_{max} – maximum evaporative capacity of a given environment

VO_{2max} – Rate of maximal oxygen consumption ($mL \cdot kg^{-1} \cdot min^{-1}$)

T_{es} – Esophageal temperature (°C)

T_{re} – Rectal temperature (°C)

T_{visc} – Visceral temperature (°C)

T_{Sk} – Mean skin temperature (°C)

T_b – Mean body temperature (°C)

b_m - body mass (kg)

C_p - specific heat capacity ($kJ \cdot kg^{-1} \cdot °C^{-1}$)

HR – Heart rate ($beats \cdot min^{-1}$)

Ex – Exercise

Rec – Recovery

RH – Relative humidity (%)

ACh - Acetylcholine

MCh - Methacholine

SNP – Sodium nitroprusside

L-NAME - N^G-nitro-L-arginine methyl ester

CHAPTER 1:

INTRODUCTION

1.1 Introduction

Epidemiological data indicate that older adults are at significantly higher risk for heat illness or death during heat waves compared with younger adults (Kenney & Hodgson, 1987; Kenny *et al.*, 2010b). The risks may even be greater when older individuals perform physical activity in a hot environment (Kenny *et al.*, 2010b). Although the physiological mechanisms underpinning the impaired heat tolerance in older adults are not well understood, aging is thought to be associated with deterioration in the body's ability to dissipate heat and therefore the ability to maintain core temperature at safe levels, especially during extreme heat events and/or physical activity in the heat. Some insight into the underlying mechanisms can be gleaned from the increasing evidence demonstrating that aging reduces the nitric oxide, a potent vasodilator, bioavailability in the skin (Holowatz *et al.*, 2003; Holowatz *et al.*, 2006b, a; Stanhewicz *et al.*, 2012; Stanhewicz *et al.*, 2013), skeletal muscle (Nyberg *et al.*, 2012), serum (Toprakci *et al.*, 2000), as well as arterial and venous blood (Nyberg *et al.*, 2012). Furthermore, some studies have reported attenuated sweating or skin vasodilation in older adults in response to pharmacological stimuli of methacholine or acetylcholine (Kenney, 1988; Inoue *et al.*, 1999a; Bruning *et al.*, 2012). However, this observation is not consistent (Holowatz *et al.*, 2005; Smith *et al.*, 2013a). To date, it remains unclear to what extent impairments in the control of heat loss responses can lead to a greater risk of dangerous increases in body heat content in older adults during passive exposure and/or physical activity in the heat. In light of the fact that the population is rapidly aging and the number of older adults 65 years of age or older in North America is expected to double by 2030 (Noe *et al.*, 2012), combined with the increased likelihood of extreme heat events over the next few decades (Marto, 2005; Pengelly *et al.*, 2007; O'Neill *et al.*, 2009), the need to

advance our understanding of the impacts of heat stress in older adults is becoming more critical than ever.

Many studies have evaluated the age-related changes in the body's physiological ability to dissipate heat between younger and older individuals. Previous studies measured local heat loss responses (i.e., sweating and skin blood flow) during local or whole-body passive heating, with the application of pharmacological agents or during exercise. Some studies have found differences in sweating (Drinkwater & Horvath, 1979; Anderson & Kenney, 1987; Kenney & Fowler, 1988; Buono *et al.*, 1991; Inoue *et al.*, 1991; Tankersley *et al.*, 1991; Inoue, 1996; Inoue & Shibasaki, 1996; Inbar *et al.*, 2004) or skin blood flow (Tankersley *et al.*, 1991; Inoue & Shibasaki, 1996; Kenney *et al.*, 1997; Minson *et al.*, 2002), while others have not observed such differences (Davies, 1979; Kenney, 1988; Pandolf *et al.*, 1988; Smolander *et al.*, 1990; Havenith *et al.*, 1995; Kenny *et al.*, 2010a; de Paula Viveiros *et al.*, 2012). The disparity in results between studies may be due to a number of factors. These include 1) regional variations in sweating and skin blood flow, 2) differences in physical characteristics between subjects, 3) different rates of metabolic heat production employed during exercise, 4) the heat load (metabolic ± environmental) employed did not exceed their physiological capacity to dissipate, and/or 5) the exercise model used (i.e., continuous versus intermittent).

With respect to regional variations, most studies have examined the effects of age on local heat loss responses at only one or at most four skin sites. This may be problematic given the evidence demonstrating varied responses of sweating and skin blood flow across different regions of the body, even in individuals of similar ages (Inoue *et al.*, 1991; Inoue & Shibasaki, 1996; Inoue *et al.*, 1998; Inoue *et al.*, 1999b; Dufour & Candas, 2007; Smith *et al.*, 2013a; Smith *et al.*, 2013b). Recently, Smith *et al.* (Smith *et al.*, 2013a) observed

reduced sweating during whole-body heating at rest in older compared to young adults at the arm, abdomen, thigh and lower back, with greatest impairment observed on the abdomen. However, during perfusion of acetylcholine via intradermal microdialysis, skin blood flow and sweat rates at the same regions of the body were similar between groups (Smith *et al.*, 2013a). Consequently, localized responses may not provide a reliable estimate of whole-body changes in sudomotor and vasomotor activity, thereby leading to erroneous conclusions when responses are compared between different groups.

When comparing thermoregulatory responses between independent groups it is important to control for biophysical factors (e.g. body mass, body surface area, body fatness, fitness, activity level) which may affect the body's capacity to dissipate heat (Gisolfi & Robinson, 1969; Baum *et al.*, 1976; Havenith & van Middendorp, 1990; Cheung & McLellan, 1998; Selkirk & McLellan, 2001; Gagnon *et al.*, 2008a; Cramer *et al.*, 2012b). However, this becomes difficult when comparing older and younger adults since factors such as body composition and level of aerobic fitness (for the purpose of this thesis is defined by maximal aerobic capacity, VO_{2max}) are known to deteriorate as a function of age (Rogers *et al.*, 1990; Trappe *et al.*, 1996; Wilson & Tanaka, 2000; Faulkner *et al.*, 2008). In many studies examining age-related differences in thermoregulatory function, exercise is performed at a percentage of VO_{2max} (Anderson & Kenney, 1987; Kenney & Anderson, 1988; Smolander *et al.*, 1990; Tankersley *et al.*, 1991; Inoue *et al.*, 1999a; Inbar *et al.*, 2004) in order to match age groups for exercise intensity, and therefore the level of physical effort. However, since older adults usually have a lower VO_{2max}, the rate of metabolic heat production for a given percentage of VO_{2max} is also lower compared to younger adults with a greater absolute maximal aerobic capacity. Given that the level of whole-body evaporative heat loss required to achieve heat balance (E_{req}) during exercise, particularly in the heat, is

determined by the sum of metabolic and environmental (dry heat gain from the environment) heat load (Gagge & Gonzalez, 1996; Jay *et al.*, 2011; Cramer *et al.*, 2012a; Gagnon *et al.*, 2013b), lower heat loss responses observed in older individuals may be simply due to the lower rate of heat production rather than true age-related differences. For example, Tankersley *et al.* (1991) recruited three groups of men to exercise in a warm ambient condition (30°C and 55% RH). One group was younger (24 – 32 y) and normally fit (VO_{2max} = 44.0 ± 2.7 $mL \cdot kg^{-1} \cdot min^{-1}$). Two groups of older (58 – 74 y) men were recruited: one highly fit (VO_{2max} = 46.4 ± 2.1 $mL \cdot kg^{-1} \cdot min^{-1}$) and one normally fit ($VO_{2max}$ = 32.9 ± 2.1 $mL \cdot kg^{-1} \cdot min^{-1}$). The younger and older high fit groups were matched for VO_{2max}, while all groups were matched for body surface area. During 20 min of recumbent cycling at 67% of the individual's pre-determined VO_{2max}, the older, normally fit group exhibited lower sweating at 10 min of exercise and lower forearm blood flow throughout the exercise compared to the younger group, with the responses of the older highly fit group being intermediate. While the authors concluded the increase in age and reduction in aerobic fitness were responsible for the attenuated heat loss responses, the differences were just as likely due to the lower heat production employed in the older, normally fit group.

To date, it is unclear if true age-related differences in the body's ability to dissipate heat exist when exercise is performed at a fixed rate of heat production. Employing a fixed rate of heat production would ensure a similar heat load is used when comparing independent groups. One of the challenges when employing this experimental model to compare young and older adults is the differences in aerobic fitness. Comparing responses based on a fixed rate of heat production would most likely result in the older adults working at much higher percentage of their VO_{2max} thereby leading to an earlier onset of fatigue in the older adults.

While matching young and older adults for VO_{2max} would eliminate this problem, matching older and younger subjects for VO_{2max} brings about its own issues. Matching young and older adults for both level of physical activity and aerobic capacity would lead to the recruitment of older individuals who are exceptional in these regards, making it difficult to generalise the results to the average aging population.

Based on previous research, it remains to be determined at what levels of heat stress (i.e., metabolic heat production ± environmental heat load) impairments in heat loss are evident in an older population. The lack of difference between young and older adults reported in some previous studies (Pandolf *et al.*, 1988; Smolander *et al.*, 1990; Havenith *et al.*, 1995; Kenny *et al.*, 2010a; de Paula Viveiros *et al.*, 2012) may simply be due to the fact the level of heat load was insufficient to demonstrate a difference as the amount of evaporative heat loss required to achieve heat balance (i.e., E_{req}) fell well below their maximal capacity to dissipate heat. Moreover, it is possible that the high level of aerobic fitness of the individuals tested offset any age-related impairment in heat dissipation. One study observed the achievement of heat balance (when the rate of metabolic heat production is equivalent to the rate of whole-body heat loss) for the different combinations of metabolic heat production and environmental heat load under these study conditions (exercise performed on separate days at 290 W in 30°C, 35°C and 40°C) (Kenny *et al.*, 2010a), demonstrating that the level of heat stress (and therefore rate of heat gain), did not exceed the physiological capacity of this population group to dissipate heat**.** It is likely, however, that difference in the physiological capacity to dissipate heat may be evident at higher levels of heat stress which would require a greater rate of whole-body heat loss to achieve heat balance, which has recently been shown in a study examining sex-related differences (Gagnon & Kenny, 2011b). Consequently, it is unknown if impairments in heat loss

responses in older individuals become evident above a certain level of heat stress and if the magnitude of impairment increases as a function of progressively greater increases in heat load.

Previous exercise studies have used a continuous model to examine heat loss responses during a single bout of exercise (Anderson & Kenney, 1987; Kenney & Anderson, 1988; Tankersley *et al.*, 1991; Kenney *et al.*, 1997; Inoue *et al.*, 1999a; Kenny *et al.*, 2010a). However, it remains to be determined the extent to which age-related reductions in heat loss responses during thermal transients (i.e., intermittent exercise) affect whole-body temperature regulation and ultimately changes in body heat content. Intermittent exercise is associated with a more rapid increase in whole-body heat loss (primarily due to changes in the rate of evaporative heat loss) following the initial exercise bout which ultimately results in a decrease in net heat gain during successive exercise cycles (Kenny *et al.*, 2009). This concept is termed 'the priming effect'. Furthermore, the body's ability to dissipate heat following exercise is compromised as evidenced by a rapid decrease in local and whole-body heat loss which is paralleled by a marked sustained increase in body heat storage and therefore core and muscle tissue temperature (Journeay *et al.*, 2004; Jay *et al.*, 2007b; Gagnon *et al.*, 2008b; Jay *et al.*, 2008; Kenny *et al.*, 2008b). The underlying mechanism for this postexercise disturbance in thermal balance is thought to be the result of a prevailing influence of nonthermal factors associated with postexercise blood pressure regulation (i.e., baroreceptors) (Carter *et al.*, 2002; Jackson & Kenny, 2003; Journeay *et al.*, 2004; Wilson *et al.*, 2004; Journeay *et al.*, 2007; Jay *et al.*, 2008). For a more detailed discussion on this topic, the reader is referred to reviews on this topic (Mekjavic & Eiken, 2006; Jay *et al.*, 2007b; Kondo *et al.*, 2010). Recently, studies have shown a progressively greater rate of heat storage in older adults during subsequent exercise/rest cycles was due to impaired heat loss

during exercise as no differences in heat loss were observed during recovery in older and middle-age adults over 2-hours of intermittent exercise (Larose *et al.*, 2013a; Larose *et al.*, 2013c; Larose *et al.*, 2013d). Yet, it remains unknown if age-related impairment in heat loss following exercise would occur at higher levels of heat stress and whether or not mechanisms governing postexercise thermoregulatory control differ as a function of age. Given that many tasks are intermittent in nature, it is essential to examine age-related differences in whole-body heat loss and body heat storage to determine the consequences of repeated exercise bouts on local and whole-body heat loss.

1.2 Rationale and statement of the problem

Literature examining differences in thermoregulatory function between young and older adults is confounded by the fact that many studies assessed local heat loss responses (i.e., sweating and skin blood flow) during local and/or passive heating only. Fewer studies, however, have examined age-related differences in heat loss during and following one or repeated exercise bouts. In light of the fact that studies on the effects of aging and the ability to dissipate heat have been limited to local measures of sweating and skin blood flow and/or changes in core temperature, little is known about the consequence of age-related changes on whole-body heat loss. Recent studies demonstrate large regional variations in local heat loss responses. Thus, local responses of sweating or skin blood flow may not directly parallel whole-body heat dissipation (Inoue *et al.*, 1991; Inoue & Shibasaki, 1996; Gagnon & Kenny, 2012b). Additionally, measurements of core and skin temperature do not accurately represent the amount of heat stored within the body such that heat storage is significantly underestimated during work in the heat (Jay *et al.*, 2007a; Jay & Kenny, 2007; Jay *et al.*, 2007b). As a result, this can lead to the misguided conclusion that differences in heat storage

as estimated by changes in body core temperature may not exist when in fact marked differences as measured by direct calorimeter show otherwise.

Examining heat loss responses between young and older individuals during exercise has many significant challenges. Age-related decrements in VO_{2max} have not always been considered when conducting exercise studies to compare the body's physiological capacity to dissipate heat. As a result, conclusions remain somewhat limited as to whether or not true age-related differences exist between young and older individuals in their ability dissipate heat during a heat stress. By considering differences between age groups in aerobic fitness, body mass and surface area we can discern the true effects of age on the capacity to dissipate heat. Furthermore, by incorporating local heat loss measures in combination with whole-body direct calorimetry (a device for making very accurate measurements of the heat emitted by the body) to measure whole-body heat loss, and therefore the amount of heat stored in the body, the proposed studies will advance our understanding of the extent to which age affects the body's ability to dissipate heat.

1.3 Objectives

The main objective of this thesis was to examine the physiological capacity to dissipate heat in older adults during challenges to human heat balance associated with a) environmental heat load, b) metabolic rate during physical activity and, c) the combination of the two. A secondary objective was to elucidate some of the mechanisms governing the impaired heat loss response in older adults. To achieve these objectives a series of studies have been conducted. These are as follows:

- The first study compared whole-body and local heat loss responses at rest between young and older individuals matched for body surface area and activity level. These

responses were compared under two different environmental heat loads (hot, dry and hot, humid) (**Study 1**).

- The second study assessed the effects of age as well as aerobic fitness in the context of aging on the physiological capacity to dissipate heat by comparing whole-body heat loss responses in young, middle-aged (trained and untrained) and older males during incremental increases in heat load (increasing rates of metabolic heat production) with successive exercise cycles (**Study 2**).
- The third study investigated the physiological capacity for heat dissipation between young and older females during intermittent exercise employing incremental increases in heat load (increasing rates of metabolic heat production) (**Study 3**).
- The fourth examined heat loss mechanisms, specifically the role of nitric oxide, during thermal transients associated with intermittent exercise performed at a fixed rate of metabolic heat production in the heat in young and older individuals (**Study 4**).
- The fifth study evaluated the extent to which peripheral factors (i.e., sweat gland and skin vasodilation function) contribute to the postexercise suppression of heat loss responses and whether there are differences in the mechanisms modulating postexercise heat loss as a function of age (**Study 5**).

1.4 Hypotheses

1.4.1 General hypothesis of the thesis

We hypothesized that, after accounting for factors which could influence heat dissipation and body heat storage, such as differences in metabolic heat production and body

size, older adults would demonstrate a reduced capacity to dissipate heat relative to their younger counterparts.

1.4.2 Specific hypotheses of the thesis

Specifically, it was hypothesized that:

- Whole-body evaporative heat loss would be reduced in older adults thereby leading to a greater rate of body heat storage for a given heat load during a passive heat exposure in hot, dry and hot, humid environments compared to their younger counterparts (Study 1).
- Middle-age untrained and older males would show a significantly lower whole-body evaporative heat loss response, and therefore whole-body sweat production, relative to their younger and middle-aged trained counterparts. Furthermore, this impairment would occur at some heat load threshold and the magnitude of difference would be greater as the heat load increases during exercise performed at progressive increments in fixed rates of metabolic heat production in a hot environment (Study 2).
- Older females would have a reduced physiological capacity to dissipate heat which would occur at a certain threshold for heat load whereby the magnitude of difference would be greater with progressive increases in heat load. This impairment capacity would be evident by significantly lower rates of whole-body evaporative heat loss in older compared to young females during exercise performed at progressive increments in fixed rates of metabolic heat production in a hot environment (Study 3).

- Nitric oxide-dependent sweating would be reduced in older adults compared to their younger counterparts during thermal transients associated with intermittent exercise (Study 4).
- Older individuals would have an attenuated responsiveness of the sweat glands and cutaneous vasculature to the administration of the pharmacological agonists compared to their younger counterparts, but to a similar extent prior to and following a single bout of exercise (Study 5).

1.5 Relevance

The results of this thesis provide a greater understanding of thermoregulatory control among aged humans during heat stress. By accounting for the many confounding factor associated with aging (i.e., VO_{2max}, body surface area, body composition) the findings of the studies of the thesis provide significant advancements regarding our understanding of age-related changes in the body's capacity to dissipate heat and at what level of heat stress differences occur The examination of age-related differences in the ability to dissipate heat performed at rest, in conditions representative of the upper environmental extremes experienced in Canada and during exercise at increasing levels of heat load advance our current knowledge on the level of heat load in which older adults may be at risk of suffering from heat-related illness and/or injuries. Furthermore, the results may be used to better understand the mechanisms of impaired sweating and cutaneous vasculature in older compared to young adults. Additionally, the findings may have practical implications for the development and expansion of health and safety guidelines specifically for older workers to mitigate the risk of heat stress and/or injury in the industrial, military and sporting industries.

Moreover, understanding the mechanisms associated with impaired heat loss in older adults could aid in finding potential countermeasures and/or treatments to reduce risk of heat-related illness and/or injury.

CHAPTER 2:

REVIEW OF LITERATURE

2.1 Human thermoregulation

The human body is a highly controlled system whereby core body temperature is tightly regulated around 37°C via neural feedback mechanisms. Temperature sensitive receptors (thermoreceptors) are located throughout the surface of the skin and the body's core. During a thermal challenge (i.e., exposure to hot ambient conditions, exercise, or a combination of the two) the balance between heat gain and heat loss will prevent increases in core temperature and maintain thermal homeostasis. However, when heat gained within the body exceeds the rate of heat lost from the body (i.e., passive heat exposure) and/or when the rate of metabolic heat production exceeds the rate of total heat loss (i.e., exercise), the resulting imbalance causes increases in muscle, core and/or skin temperatures and therefore body heat storage. The dynamic balance between heat transfer with the external environment and heat production from the body (i.e., during physical work) is described by the heat balance equation (Gagge & Gonzalez, 1996):

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

Where: S is the rate of body heat storage
M is the rate of metabolic energy expenditure
W is the rate of external work
R is the rate of radiant heat exchange
C is the rate of convective heat exchange and
E is the rate of evaporative heat exchange (All values in W or $W \cdot m^{-2}$)

When challenges to the heat balance equation associated with exposure to hot ambient conditions and/or exercise occur, heat balance is re-achieved by adjusting the rate of heat production ($M \pm W$) to match the rate of whole-body loss ($R \pm C - E$), or vice versa, in which case core temperature will once again achieve a stable value. A mismatch between the

rate of heat production and rate of heat loss will result in either a negative or positive rate of body heat storage (*S*), depending on the conditions. A negative rate of body heat storage occurs when the rate of total heat loss exceeds heat production (i.e., recovery from exercise and/or exposure to a cold environment) and core temperature will begin to drop. On the other hand, a positive rate of heat storage occurs when the rate of heat production exceeds the rate of total heat loss (i.e., non-steady state period of exercise). Figure 1 depicts the relationship between heat production, total heat loss (THL) and changes in body heat content (ΔH_b).

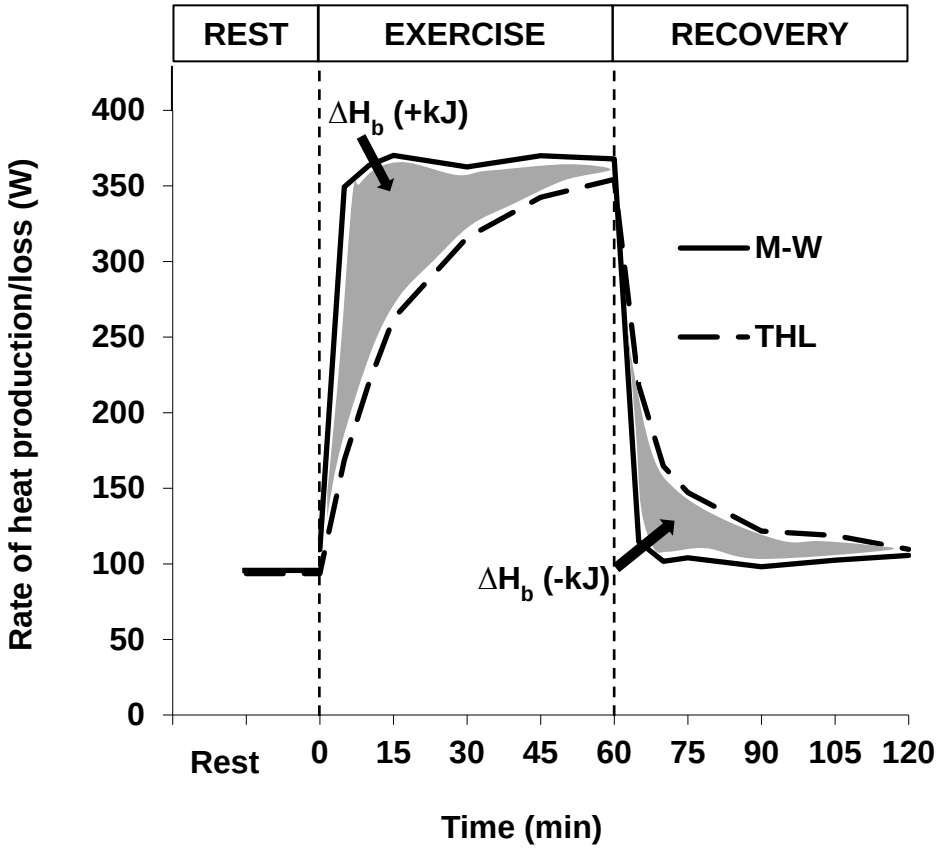

Figure 1. Rates of metabolic heat production and total heat loss during baseline rest, 60 min exercise bout, and 60 min recovery period. The shaded area between the rates of metabolic heat production and total heat loss represents the change in body heat content (ΔH_b) for the 60 min exercise and recovery period.

2.2 Activation of heat loss responses during heat stress

Changes in core and skin temperature are sensed by peripheral thermoreceptors located directly under the skin and by central thermoreceptors located in the spinal cord, abdominal cavity, major blood vessels, and the hypothalamus (Carter *et al.*, 1999; Romanovsky, 2007). Thereafter, all afferent input is integrated at the hypothalamus and efferent signals modulate skin vasculature (i.e., increase skin blood flow) and sweat gland activity (i.e., increase sweat production) to increase the rate of heat loss to the environment via evaporation, conduction and convection (Schepers & Ringkamp, 2009; Rhoades & Bell, 2013).

Skin blood flow and sweating are the main physiological thermoeffector mechanisms of heat transfer from the body to the environment (known as the active system). The onset threshold of thermoeffector responses represents a central modification of heat loss, whereas the thermosensitivity of the responses represents a peripheral modulation of temperature regulation (Nadel *et al.*, 1971). An increase in mean body temperature occurs before the activation (onset threshold) of heat loss responses (i.e., skin blood flow and sweating). Thereafter, sweating and skin blood flow increase proportionally to the rise in mean body temperature, the linear portion of which represents the thermosensitivity of the response (Gagnon & Kenny, 2012a). Once the heat loss response reaches maximal values, a flattening of the line is observed, whereby no further increase in heat loss responses will occur despite an increasing mean body temperature (Johnson & Park, 1981). Therefore, changes in either the onset threshold or thermosensitivity of the skin blood flow/sweating-mean body temperature relationship have been used to identify altered thermal control (Figure 2). Delays in the onset threshold or decreases in the thermal sensitivity of the heat loss responses can determine whether responses are centrally or peripherally altered, or a combination of both.

Altered heat loss during thermal challenges will result in greater heat storage, and therefore larger increases in core temperature for a given level of heat stress.

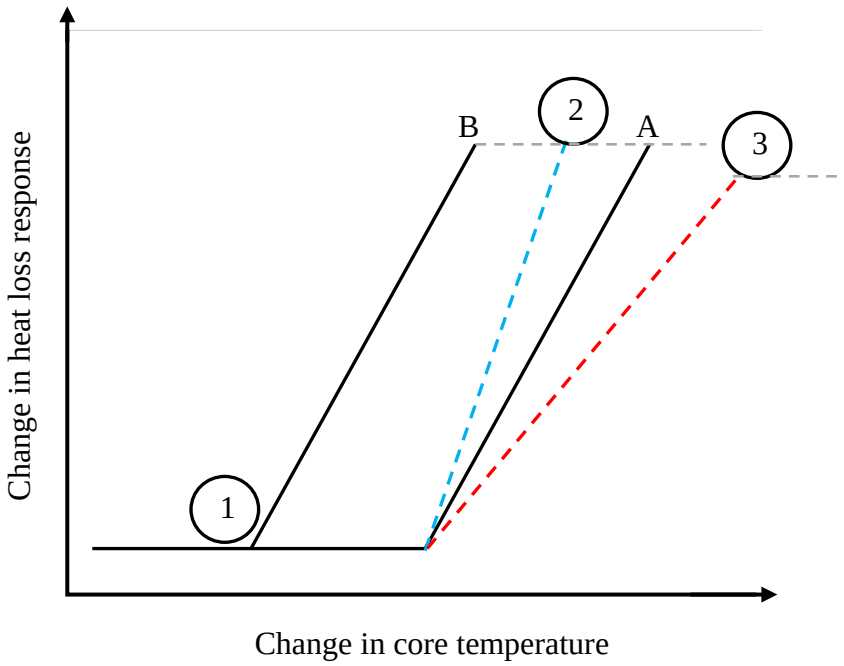

Figure 2. Schematic illustrating the relationship of the heat loss responses (i.e., skin blood flow and sweating) to changes in core temperature used to identify altered thermal control. [1] The onset threshold is represented here in condition B by a significant increase in SkBF/sweating, which occurs at an earlier core temperature than condition A. [2] An increase in the sensitivity of the heat loss response to changes in core temperature is illustrated here compared to A, while [3] represents a decrease in sensitivity. The dashed grey lines represent the plateau of the heat loss response. Figure adapted with permission from (Gagnon & Kenny, 2012a).

2.2.1 *Skin blood flow*

Non-glabrous skin (head, limbs and trunk) is dually innervated meaning that it is controlled by separate vasoconstrictor and vasodilator sympathetic neural pathways (Gisolfi *et al.*, 1993). The vasoconstrictor system maintains vasomotor tone responsible for

preservation of normal body temperature at rest in non-heat stressed (thermoneutral) environments. Under these conditions, skin blood flow is ~500 mL/min (5-10% cardiac output), which allows for heat dissipation (80-90 kcal/h) to equal that of metabolic heat production (Johnson & Proppe, 1996). The vasoconstrictor system is able to maintain core temperature at rest in thermoneutral environments from small changes in skin blood flow resulting in large changes in heat dissipation (Charkoudian *et al.*, 2003). In contrast, the large increase in skin blood flow observed during heat stress (hyperthermia) (6–8 L/min or 60-70% of cardiac output) is mostly due to the sympathetic active vasodilator system (80–90%) (Johnson & Proppe, 1996), while inhibition of the vasoconstrictor system plays a minor role (Charkoudian *et al.*, 2003). Blood is redistributed from renal and splanchnic vascular beds (Rowell, 1974) to the skin through cutaneous vasodilation which aids in transferring heat from the body's core to the surface of the skin where it can then be transferred to the environment (convective heat exchange, *C*), assist in the evaporation of sweat, or minimize heat gain from the environment when ambient temperature exceed skin temperature (Hammel, 1968; Charkoudian *et al.*, 2003).

Sympathetic cholinergic and adrenergic innervation of blood vessels under the skin nerves (Grant & Holling, 1938) are regulated by the anterior preoptic hypothalamus (Gisolfi *et al.*, 1993; Parsons, 2003; Mack, 2004). This closed-loop feedback control center receives afferent information from core and skin temperatures, integrates this information, and adjusts the level of skin perfusion accordingly. During heat stress, an initial rise in skin blood flow is attributed to a release of vasoconstrictor tone, primarily via the adrenergic system, through the binding of norepinephrine to α_1- and α_2-adrenergic receptors (Kellogg, 2006). Further heating causes active vasodilation via cholinergic activity with many studies demonstrating a co-transmission of acetylcholine with other transmitters (Kellogg *et al.*, 1995; Kellogg *et al.*,

2010, 2012). In addition, skin vasodilation is in part (~30-45%) through nitric oxide (NO)-dependent mechanisms (Shastry *et al.*, 1998; Holowatz *et al.*, 2003; Kellogg *et al.*, 2012; Brunt *et al.*, 2013; Swift *et al.*, 2013; Wong, 2013). Nitric oxide, a vasodilator, is produced in the vascular endothelium from the amino acid L-arginine by the enzyme nitric oxide synthase (Takamata *et al.*) and is necessary for full expression of skin vasodilation (Kellogg *et al.*, 1998; Kellogg *et al.*, 1999; Shibasaki *et al.*, 2002; Kellogg, 2006) in healthy individuals. Overall, increases in skin blood flow during passive heating or exercise are proportional to increases in core temperature until a steady state is reached as defined by an elevated plateau in core temperature, or until maximal increases in skin blood flow are obtained.

2.2.2 *Sweating*

Similar to the control of skin blood flow, sweating is also a reflex response to an elevation in core and skin temperature controlled centrally by the hypothalamus and sweat glands are innervated by sympathetic cholinergic and adrenergic nerves (Uno, 1977). While administration of acetylcholine induces profuse sweating, adrenergic agents cause minimal sweating (Sato, 1977). Also, blocking muscarinic receptors with atropine abolishes sweating during heat stress at rest (Kellogg *et al.*, 1995) providing sound evidence that acetylcholine released from cholinergic nerves is responsible for sweating during heat stress. Although skin blood flow can provide adequate heat dissipation during mild heat stress, sweating becomes increasingly important during exercise and exposure to high ambient temperatures. The high latent heat of vaporization (2426 J $\cdot$ g sweat^{-1}) provides a large potential for heat to be lost through evaporation (Wenger, 1972). For these reasons, the evaporation of sweat

from the human body is the only heat loss mechanism during exercise performed in the heat (i.e., ambient temperature > skin temperature).

The human body contains two distinct types of sweat glands. Apocrine sweat glands are located in the axilla and perianal areas and contribute very little to regulating the internal temperature of the body. Eccrine glands, on the other hand, are distributed almost entirely over the whole body and eccrine sweating is vital for whole-body human temperature regulation (Machado-Moreira *et al.*, 2008). As mentioned above, eccrine sweating is controlled by the autonomic nervous system, specifically the sympathetic nervous system. The pathway from the pre-optic anterior hypothalamus to the eccrine gland responsible for sweating is not entirely understood. The proposed pathway is as follows: the central and peripheral thermoreceptors sense an increase in core and skin temperature and send afferent signals to the hypothalamus where efferent signals then travel from the hypothalamus to the pons and the medullary raphe regions via the tegmentum. The signal then travels to the intermediolateral cell column of the spinal cord where neurons developing from the ventral horn pass through the white ramus communicans where they synapse in the sympathetic ganglion. Postganglionic non-myelinated C-fibers pass through the gray ramus communicans and combine with peripheral nerves that travel to the eccrine sweat gland along with nerve fibers encompassing the periglandular tissue of the gland (Shibasaki & Crandall, 2010). Unlike in some animals, sweat glands are unique to the sympathetic nervous system in humans given that they are one of two glands where the innervating postganglionic neurons release acetylcholine, as opposed to norepinephrine, for the activation of muscarinic receptors, although some sweating can also occur when stimulated by α- or β-adrenergic agonists (Sato, 1977). Acetylcholinesterase rapidly degrades acetylcholine to acetate and choline and therefore capable of regulating sweat rate under mild heat stress (Kimura *et al.*,

2007). More recently, studies show that nitric oxide is involved in the sweating response to the muscarinic-receptor agonist, methacholine (Lee & Mack, 2006) or during exercise in a hot environment (Welch *et al.*, 2009).

The amount of heat lost through sweating will depend on the amount of sweat secreted, the number of activated sweat glands and the conditions of the ambient environment. Kondo *et al*. (2001) demonstrated an abrupt increase in sweat rate during the first 8 min of exercise and passive heating (Kondo *et al.*, 2001). This initial increase in sweating was found to be a result of both increased density of activated sweat glands and increased sweat output per gland. As sweating begins to plateau, sweat rate results from increases in the amount of sweat output per gland only. Although sweating increases rapidly at the start of passive heating and exercise, it must evaporate to serve as a functional heat loss mechanism. It has been suggested that delays in the evaporation of sweat can be 2-5 min for a range of ambient conditions (Saltin *et al.*, 1970). In addition, measurements of whole-body evaporative heat loss by direct calorimetry demonstrate that following the onset of exercise the rate of evaporative heat loss lags significantly behind increases in metabolic heat production. Consequently, there is a pronounced increase in body heat storage and core temperature begins to increase at the start of exercise (Kenny *et al.*, 2008b; Kenny *et al.*, 2009). The slow response, reflected by a larger time constant (τ) value, also known as the thermal inertia or temporal dissociation (Kenny *et al.*, 2009). Furthermore, evaporation is determined by the water vapour pressure gradient between the skin and the environment. If humidity is high in a hot environment, then the water vapour pressure gradient between the moist skin and surrounding environment is greatly reduced, impeding the ability to dissipate heat through evaporation of sweat.

2.3 Heat stress and thermoregulation

2.3.1 Passive heat stress

Passive heat stress results in heat gained from the external environment. At rest, any imbalance between the rates of heat production and heat dissipation results in a rate of body heat storage (S) and a cumulative change in body heat content. Over time, exposure to hot ambient conditions results in an increase in skin temperature which then stimulates an effector response. Sweat production and cutaneous vasodilation will commence for heat exchange via evaporation, conduction and convection. As heat balanced is attained, the rate of heat storage will approach zero (i.e., $S = 0$) and core temperature will remain at an elevated steady-state value unless additional heat is added to the system. Increasing skin temperature will also have profound effects on the cardiovascular system. Using a water-perfused suit, Rowell (1974) increased skin temperature to 40.5°C and observed resting cardiac output to increase by nearly twice as much, a slight increase in stroke volume, and a redistribution of blood flow from the splanchnic and renal circulations to the skin, while mean arterial pressure was well maintained (Rowell, 1974). As such, during passive heat stress, blood volume is redistributed from the central regions of the body to peripheral circulations for the purpose of aiding thermoregulation.

2.3.2 Exercise

During exercise, the primary stimulus governing the effector response is the rapid increase in body heat storage, and therefore core temperature, associated with the immediate elevated rate of heat production ($M \pm W$). The rapid increase in the rate of metabolic heat production is primarily due to the inefficiency of energy extraction. Typically 70 to 95% of energy (depending upon the type of physical activity performed) is released as heat to the

surrounding tissues within the body via the blood stream. This response is followed by a slower increase in the rate of total heat loss ($\pm R \pm C - E$). As long as the increase in the rate of total heat loss is sufficient to match the rate of metabolic rate production, heat storage will be zero. However, if the rate of metabolic heat production exceeds the rate of total heat loss (i.e. $M \pm W > \pm R \pm C - E$), a progressive increase in body core temperature will occur. The increase can be more pronounced when exercise is performed in hot and/or humid ambient conditions due to the elevated rate of dry heat gain and/or reduction in evaporative cooling. Whole-body evaporative heat loss (and therefore sudomotor activity) during exercise, depends upon the required evaporation for heat balance (E_{req}) (Gagge & Gonzalez, 1996; Bain *et al.*, 2011; Jay *et al.*, 2011; Cramer *et al.*, 2012a; Gagnon *et al.*, 2013b). E_{req} is defined by the sum of metabolic heat production and dry heat exchange. Where maximal evaporation within a given environment (E_{max}) is possible (i.e., low humidity), the condition is compensable (i.e., $E_{max} \geq E_{req}$) and the individual is able to achieve heat balance. However, when environmental conditions and metabolic heat production are uncompensable (i.e., $E_{max} < E_{req}$), the individual will not be able to achieve heat balance placing them at a greater risk for suffering from heat-related illness and/or injury.

2.3.3 Recovery

Thermoregulatory control during recovery from dynamic exercise differs drastically from control mechanisms during exercise. At the cessation of dynamic exercise, metabolic heat production rapidly declines to pre-exercise levels. Parallel to this response, there is sudden decrease in local sweating and skin blood flow which occurs within ~20 minutes following the cessation of exercise (Thoden *et al.*, 1994; Kenny *et al.*, 2006; Kenny *et al.*, 2009). The rapid reductions in heat loss occur despite elevated core and muscle temperatures

above pre-exercise baseline values (Kenny *et al.*, 2006; Kenny & Jay, 2007; Kenny & Journeay, 2010). Kenny *et al.* (1996) demonstrated that postexercise thermoregulatory control likely results from residual effects of the exercise bout as opposed to an increase in body heat content (Kenny *et al.*, 1996). Subjects were heated in hot water (42°C) to increase end-immersion esophageal temperature to a similar extent to that of 15-min moderate intensity exercise. Following whole-body water immersion, no sustained increase in esophageal temperature was measured unlike that observed following dynamic exercise. Furthermore, exercise intensity can influence the magnitude of the sustained elevation in core and muscle temperatures (Kenny & Niedre, 2002; Kenny *et al.*, 2003). This is evidenced by greater increase in the onset threshold for sweating and greater magnitude and prolonged elevations in core and muscle temperatures above baseline resting values with higher intensity.

It has been suggested that nonthermal baroreflex activity is the primary factor responsible for the modulation of heat loss responses following exercise (Carter *et al.*, 2002; Jackson & Kenny, 2003; Wilson *et al.*, 2004; Journeay *et al.*, 2006; Kenny *et al.*, 2006; Jay *et al.*, 2007b; Kenny *et al.*, 2008a). At the termination of exercise, there is a drop in mean arterial pressure compared to pre-exercise levels (i.e., postexercise hypotension.). One study examined the relationship between postexercise hypotension and heat loss using the application of lower body positive pressure to reduce the effect of venous pooling in the legs following exercise, and therefore unloading the baroreceptors (Jackson & Kenny, 2003). Jackson & Kenny (2003) demonstrated a decrease of the elevated onset temperature threshold for sweating and cutaneous vasodilation following exercise (Jackson & Kenny, 2003) suggesting a role for baroreceptor activity in the control of local heat loss following exercise.

2.3.4 Intermittent exercise

Activities of daily living as well as many occupational, recreational and sporting activities are likely to be performed intermittently rather than continuously. As such, it is essential to understand how the thermoregulatory system responds to repeated bouts of exercise and recovery. Continuous and intermittent exercise may result in similar increases in core temperature if exercise periods are long and not performed in under extreme heat (Belding *et al.*, 1966; Kraning & Gonzalez, 1991; McLellan *et al.*, 1993; Drust *et al.*, 2000; Gagnon & Kenny, 2011a). Some studies, however, have shown that intermittent exercise leads to greater cumulative heat stress compared to continuous exercise (Ekblom *et al.*, 1971; Kraning & Gonzalez, 1991; Mora-Rodriguez *et al.*, 2008; Kenny *et al.*, 2009; Gagnon & Kenny, 2011a). For example, one study showed that a 1.5 min cycling bout at 90% VO_{2max} followed by 4.5 min cycling at 50% VO_{2max} for a total duration of 90 min resulted in greater heat storage and increases in core temperature compared to 90 min continuous exercise at 60% VO_{2max} despite total work output being similar between exercise sessions (Mora-Rodriguez *et al.*, 2008). Most studies have compared continuous vs. intermittent exercise by examining core temperature responses (Belding *et al.*, 1966; Gavhed *et al.*, 1991; Kraning & Gonzalez, 1991; McLellan *et al.*, 1993; Drust *et al.*, 2000). However, the effects on whole-body heat storage are less clear. One study by Gagnon & Kenny (2011) examined the effects of exercise/rest cycles on whole-body heat loss during four 120-min trials in 35°C at a workload of 130 W: 1) 60-min cycling and 60-min recovery; 2) 3 x 20-min cycling separated by 20-min recovery; 3) 6 x 10-min cycling separated by 10-min recovery, and; 4) 12 x 5-min cycling separated by 5-min recovery (Gagnon & Kenny, 2011a). No differences were reported in the rates of whole-body heat loss between any of the 4 different exercise/recovery

conditions thereby leading to similar changes in body heat content and increases in core temperature. The authors concluded that the capacity to dissipate was not altered by differences in short to moderate duration exercise/rest cycles when exercise was performed at a fixed rate of metabolic heat production (Gagnon & Kenny, 2011a).

Of particular note, Kenny *et al.* (2009) observed progressively quicker increases in the rate of heat loss following the first exercise bout, which they termed the "priming" effect (Kenny *et al.*, 2009). However, despite the cumulative increases in residual heat storage and core temperature, similar reductions in the rate of heat loss were observed during each recovery period. The authors suggest that the progressive increase in core temperature resulted from a possible re-setting of the skin blood flow and sweating–esophageal temperature relationship postexercise and, as described above, governed by nonthermal factors such as baroreceptors (Jay *et al.*, 2007b; Kenny *et al.*, 2009). These studies examining the effects of intermittent exercise on thermoregulatory function were performed on young, healthy individuals. A better understanding of how older adults respond to intermittent-type activities in the heat is warranted and will allow for the development of health and safety guidelines specifically to help mitigate the risk of heat stress and/or injury in this population.

2.4.4 *Effects of aerobic fitness*

Studies have generally reported that a high level of training improves exercise heat tolerance (Gisolfi & Robinson, 1969; Gisolfi, 1973; Baum *et al.*, 1976; Gisolfi & Cohen, 1979; Cheung & McLellan, 1998; Thomas *et al.*, 1999; Selkirk & McLellan, 2001; Ichinose *et al.*, 2009). For example, large increases in VO_{2max} has been shown to improve the sweating response during exercise at a given relative exercise intensity, as well as during passive heat stress (Araki, 1976; Henane *et al.*, 1977). In addition, higher levels of aerobic

fitness is associated with a lower core temperature threshold at which the onset of sweating occurs and a higher slope of the sweat rate-core temperature relationship (Araki, 1976; Baum *et al.*, 1976; Henane *et al.*, 1977; Irion, 1987; Yamazaki *et al.*, 1994; Stapleton *et al.*, 2010). Such factors can lead to an increase in heat tolerance during exercise as higher core temperatures have been shown to be the limiting factor in exercise performance (Baum *et al.*, 1976; Nielsen *et al.*, 1993). Moreover, attenuated increases in core temperature during work in the heat, through a greater ability for heat dissipation and lower resting core temperatures, are proven to be superior in trained compared to untrained individuals (McLellan, 2001; Selkirk & McLellan, 2001; Mora-Rodriguez *et al.*, 2010; Stapleton *et al.*, 2010).

Earlier studies have demonstrated that short-term exercise training in previously sedentary individuals improves exercise heat tolerance by lowering baseline and exercise core temperatures, lowering exercise heart rates, and increasing VO_{2max}, plasma volume and sweat rate (Gisolfi & Robinson, 1969; Gisolfi, 1973; Henane *et al.*, 1977; Convertino *et al.*, 1983). Similar findings have been observed with more recent studies (Ichinose *et al.*, 2009; Stapleton *et al.*, 2010). However, it is unclear if the improved heat loss responses (i.e., increased sweat rates) were due to training alone, or if they were based on the fact individuals working at a set percentage of VO_{2max} during the exercise-induced heat load, therefore a higher rate of metabolic heat production following the exercise training program. As heat load (metabolic ± environmental) determines the requirement for heat loss (Gagge & Gonzalez, 1996; Gagnon *et al.*, 2013b), working at a greater rate of metabolic heat production would elicit greater sweating and skin blood flow responses. Stapleton *et al.* (2010) verified that short-term exercise training does lower resting and exercise core temperatures; nonetheless, there was no difference in the change from baseline when working at a similar rate of metabolic heat production pre- and post-training. This can be

explained by the fact that during the post-training heat stress test, individuals were working at a lower relative percentage of VO_{2max} as compared with pre-training. However, since the rate of metabolic heat production was the same during the pre-training and post-training heat stress tests, similar rates of whole-body heat loss were observed, and therefore similar changes in body heat content were measured during the 90-min exercise bout in warm ambient conditions (30°C and 30% RH). In spite of this, improvements the thermoregulatory function were observed as lower onset thresholds for both sweating and skin blood flow due to the lowering of resting core temperature (Stapleton *et al.*, 2010). Consequently, these findings suggest that even after a short-term exercise program, trained individuals will be able to tolerate working for a longer duration compared to untrained individuals as it will take the trained individuals longer to reach the critical core temperature at which exhaustion occurs (Baum *et al.*, 1976). In addition, due to the regular exercise regime continuously raising core temperature above resting, trained individuals are more able to tolerate working in the heat at a high core temperature (Selkirk & McLellan, 2001).

When comparing untrained with trained individuals, factors such as VO_{2max}, metabolic heat production, body mass, and body surface area prove to be problematic due to the difficulties in matching individuals for each physical characteristic. Since the study by Stapleton *et al.* (2010) only elicited a ~10% increase in VO_{2max}, it remained unclear if a greater training stimulus would result in greater adaptations to the thermoregulatory system. Consequently, Jay *et al.* (2011) recruited fourteen males, 7 with a high VO_{2peak} (60.1 ± 4.5 $ml \cdot kg^{-1} \cdot min^{-1}$) and 7 with a low VO_{2max} (40.3 ± 2.9 $ml \cdot kg^{-1} \cdot min^{-1}$), but otherwise matched for body mass and body surface area (Jay *et al.*, 2011). Each participant cycled for 60 minutes at either: 1) a fixed rate heat production (~540 W) or 2) a similar relative exercise intensity (60% VO_{2peak}) at 24.8 ± 0.6°C and 26 ± 10% RH. The researchers showed that even

a ~50% difference in VO_{2max} between groups matched for body mass and body surface area resulted in similar changes in core temperature and sweating response during exercise at the same rate of heat production (Jay *et al.*, 2011). Thus, individuals with a high training status have improved tolerance to work in the heat compared to individuals with a low training status based on the fact that the highly trained individuals will have a lower core temperature onset threshold for heat dissipation mechanisms as well as lower starting core temperatures and ability to work at higher body temperatures. Caution should be taken when predicting an individual's heat tolerance based solely on VO_{2max}, as VO_{2max} may not be a reliable indicator of exercise-heat tolerance (Pandolf *et al.*, 1988). Rather, the quantity of regular physical activity may be a better indicator of the presence of training-induced adaptations to exercise heat tolerance (Avellini *et al.*, 1982).

2.4 Aging and thermoregulation

Studies show that the ability to dissipate heat during exercise and/or exposure to the heat declines with increasing age (Wagner *et al.*, 1972; Davies, 1979; Anderson & Kenney, 1987; Kenney & Anderson, 1988; Kenney & Fowler, 1988; Buono *et al.*, 1991; Inoue *et al.*, 1991; Tankersley *et al.*, 1991; Dufour & Candas, 2007; Larose *et al.*, 2013a; Larose *et al.*, 2013c; Larose *et al.*, 2013d). However, results are equivocal as to whether age alters thermoregulatory function or whether the attenuated heat loss responses are due to physical rather than physiological confounding factors such as current level of aerobic fitness, training status, body surface area, body composition etc. (Gisolfi & Robinson, 1969; Austin & Lansing, 1986; Havenith & van Middendorp, 1990; Anderson, 1999; Cheung *et al.*, 2000; Havenith, 2001). The examination of sweating and cutaneous vasodilation in older individuals has been assessed using various techniques such as pharmacological stimuli,

local and/or whole-body heating or during exercise. Some studies have suggested that decreases in heat loss responses are due to decrements in VO_{2max}, rather than age, per se (Dill & Consolazio, 1962; Davies, 1979; Drinkwater & Horvath, 1979; Pandolf *et al.*, 1988). For example, one study showed that when matched for VO_{2max}, younger and older subjects exhibit similar heat loss responses during exercise at a similar percentage of VO_{2max} (Tankersley *et al.*, 1991). On the other hand, another study showed a decreased sweat output in response to cholinergic stimuli in older compared to younger males with similar levels of aerobic fitness and body composition suggesting impairment to the actual sweat gland with increasing age (Kenney & Fowler, 1988). As such, despite similar levels of aerobic fitness, impaired sweating may result as a function of age although a number of studies still report sweat rate is strongly related to VO_{2max}, not age (Drinkwater *et al.*, 1982; Smolander *et al.*, 1990; Havenith *et al.*, 1995).

2.4.1 Skin blood flow

Decreased active vasodilation in the aged skin results from a decrease in sensitivity to increasing core temperature coupled with structural changes to the cutaneous vasculature (i.e., reduced elasticity) (Tankersley *et al.*, 1991; Kenney *et al.*, 1997), independent of changes to vasoconstrictor tone (Kenney *et al.*, 1991) or hydration effects (Kenney *et al.*, 1990; Kenney & Havenith, 1993). While maximum skin blood flow has been reported to be reduced in aged skin (Martin *et al.*, 1995; Minson *et al.*, 2002; Hodges *et al.*, 2010), the percentage of maximum skin blood flow attained during exercise is similar in older adults compared to younger individuals (Kenney *et al.*, 1997). Thus, overall skin blood flow has been shown to be reduced in older individuals. This may potentially lead to a reduction in

heat transfer from the body's core to the skin's surface for heat exchange thereby leading to potentially unsafe increases in core temperature.

During local heating of the skin, age-related changes in both axon reflex-mediated and nitric oxide-mediated vasodilation contribute to the attenuated cutaneous vasodilator responses in older adults (Minson *et al.*, 2002). During whole-body heating, attenuated reflex cutaneous vasodilation in older adults is likely due to diminished vascular responsiveness of the active vasodilator system (Kenney *et al.*, 1997; Holowatz *et al.*, 2010). Consequently, older adults must rely primarily on nitric oxide-dependent mechanisms to increase skin blood flow during heat stress (Holowatz *et al.*, 2003). However, there is evidence to suggest that nitric oxide is diminished with advanced age, including data showing a reduction in the nitric oxide precursor L-arginine and the nitric oxide metabolites nitrate and nitrite (Reckelhoff *et al.*, 1994). This concept is further strongly supported by the results showing that interventions which help to increase nitric oxide bioavailability improve cutaneous vasodilation in older individuals (Holowatz *et al.*, 2006b, a; Stanhewicz *et al.*, 2012; Stanhewicz *et al.*, 2013). More research is needed to understand the mechanisms of the active vasodilator system in humans and how they may be altered with advanced age (Holowatz *et al.*, 2003).

Whether lower skin blood flow during exercise in older individuals is associated with a lower VO_{2max} (Rogers *et al.*, 1990; Wilson & Tanaka, 2000) rather than aging per se is a matter of debate. Although not always possible, matching younger and older individuals for maximum aerobic capacity allows for the comparison of heat loss responses during exercise as well as the determination as to whether or not the differences are due to aerobic capacity since exercising at a similar level of VO_{2max} will also produce a similar level of metabolic heat production. The general consensus among studies is that attenuated skin blood flow is a

function of age (Kenney, 1988; Kenney *et al.*, 1990; Tankersley *et al.*, 1991; Kenney & Havenith, 1993; Havenith *et al.*, 1995; Ho *et al.*, 1997; Thomas *et al.*, 1999). For example, Kenney *et al.* (1988) recruited younger (19-30 years) and older (55-68 years) males and females matched for aerobic capacity (Kenney, 1988). During 75 minutes of cycling exercise at ~40% VO_{2max} in a hot environment (37°C and 60% RH), forearm blood flow, used as an index of skin blood flow, was significantly reduced in addition to an attenuated forearm blood flow-rectal temperature slope (Kenney, 1988). However, it has also been shown that maintaining vigorous exercise training throughout life can attenuate the age-related decreases in skin blood flow during exercise in older individuals (Tankersley *et al.*, 1991; Best *et al.*, 2012).

2.4.2 *Sweating*

Similar to skin blood flow, some studies report lower sweat rates in older adults (Shoenfeld *et al.*, 1978; Inoue *et al.*, 1991; Inoue, 1996; Inoue & Shibasaki, 1996; Inoue *et al.*, 1998; Inoue *et al.*, 1999b; Dufour & Candas, 2007), whereas others found no differences (Drinkwater *et al.*, 1982; Pandolf *et al.*, 1988; Sagawa *et al.*, 1988; Miescher & Fortney, 1989; Armstrong & Kenney, 1993). Drinkwater *et al.* (1982) showed a significant correlation between VO_{2max} and local sweat rate (Drinkwater *et al.*, 1982). Inoue (1996) observed a higher mean body temperature at the onset of sweating during whole-body heating (leg immersion in 42°C water bath in combination with ambient temperature of 35°C) (Inoue & Shibasaki, 1996). This response occurred at the thigh only. No differences in the temperature onset threshold for sweating occurred on the back. A shift in the mean body temperature onset threshold suggests possible altered central mechanisms. In contrast, other studies have found no differences in the core temperature at which the onset of sweating occurred but

older individuals did have a lower thermosensitivity (Inoue *et al.*, 1991; Inoue *et al.*, 1999b; Dufour & Candas, 2007) suggesting altered peripheral mechanisms (Nadel *et al.*, 1971). More research is required to determine the role of centrally and peripherally mediated sweating during whole-body heating or during increases in environmental heat load.

Sweat rate has been shown to be decreased in older as compared to younger individuals during exercise (Anderson & Kenney, 1987; Tankersley *et al.*, 1991; Inoue *et al.*, 1999a). Several factors may contribute to the attenuated sweat rate during exercise in older adults such as a lower cholinergic sensitivity (Kenney & Fowler, 1988; Inoue *et al.*, 1999a), decreased thermal sensitivity (Natsume *et al.*, 1992) as well as age-related structural changes to the gland itself (Anderson & Kenney, 1987). Reductions in sweating reflect a diminished output per sweat gland rather than a decrease in the number of sweat glands recruited (Anderson & Kenney, 1987). Given that aging impairs nitric oxide-dependent cutaneous vasodilation during heat stress, as mentioned earlier, and that nitric oxide has been implicated in sweating (Lee & Mack, 2006; Welch *et al.*, 2009), it is conceivable that the attenuated sudomotor control in older individuals during heat stress is at least partly nitric oxide-dependent; however, this has yet to be examined.

As with attenuated skin blood flow responses in older individuals, it remains unclear as to whether reduced sweating is due to decreases in VO_{2max} seen with age, rather than aging itself (Davies, 1979; Anderson & Kenney, 1987; Kenney & Anderson, 1988; Kenney & Fowler, 1988; Sagawa *et al.*, 1988; Smolander *et al.*, 1990; Buono *et al.*, 1991; Tankersley *et al.*, 1991; Havenith *et al.*, 1995). Smolander *et al.* (1990) compared the responses of young (28-37 years) and older (55-60 years) individuals, matched for aerobic capacity, during exercise at 30% VO_{2max} under thermoneutral (21°C and 43% RH), hot-dry (40°C and 20% RH), and hot-humid (30°C and 80% RH) conditions (Smolander *et al.*, 1990). They observed

similar sweating responses between the two groups. In the hot-dry environment, the evaporative rate (determined from changes in clothed body mass) increased more slowly in older adults compared to the younger group, suggesting an attenuation of sudomotor activity, but there were no overall differences in rectal temperatures or performance times between the younger and older men (Smolander *et al.*, 1990). Another study examined the relative influence of age on thermoregulatory responses during 60 min of cycling at a work output of 60 W (low intensity) in 35°C and 80% RH (Havenith *et al.*, 1995). A multiple regression analysis performed on the influence of age, aerobic fitness, activity level, body size and composition revealed that age has no significant influence on sweat loss, along with rectal temperature, or body heat storage, during exercise in the heat (Havenith *et al.*, 1995). Thus, it appears that sweating may be more closely related to VO_{2max}.

Regional differences exist in the attenuated local sweat rate response observed in older adults. Some studies show lower sweating response on the thigh and upper back, but no differences on the forehead, chest and forearm when compared with younger matched counterparts (Inoue *et al.*, 1991; Inoue & Shibasaki, 1996). Furthermore, one study showed lower sweat rates on the forehead and calf, but not on the thigh or chest, which was also related to a decreased thermal sensitivity in those impaired areas (Dufour & Candas, 2007). In a longitudinal study following the same group of older men in the sixth and seventh decade of their life, over a 5-year period there was a significant reduction in the sweating response to a 60-min exposure to a hot environment observed on the back, but not on the thigh (Inoue, 1996). A recent study has confirmed regional variations of sweating in older adults and have provided evidence that the lower sweating in older compared to young adults is not due to differences in cholinergic sensitivity (Smith *et al.*, 2013a). However, due to the low concentrations of acetylcholine used to assess cholinergic sensitivity, it is unclear if

responsiveness to the pharmacological agent would be evident at higher concentrations. Taken together, it is clear that measuring sweat rate at certain skin sites may not represent whole-body sweating or the total level of impairment in evaporative heat loss in aged humans. Therefore, it is yet to be determined if age-related impairments in sudomotor activity occur across the entire body thereby restricting the body's ability to dissipate heat leading to a greater amount of heat stored during exercise.

2.4.3 *Whole-body heat loss*

Given the variability in local heat loss measures across regions of the body in young and older adults (Inoue *et al.*, 1991; Inoue & Shibasaki, 1996; Inoue *et al.*, 2004; Smith *et al.*, 2013a; Smith *et al.*, 2013b), whole-body direct calorimetry assesses incorporates measurements from 2-4 million sweat glands and a large number of vascular beds across the whole body to measure whole-body heat loss, and can be used to give accurate measures of heat loss capacity in various population groups. One study compared rates of whole-body heat loss and changes in body heat content between physically active younger (18–25 years) and middle-aged (40-50 years) adults during 90 min of cycling (rate of metabolic heat production of 290 W) at increasing levels of environmental heat load (30°C, 35°C and 40°C) on separate days (Kenny *et al.*, 2010a). Although no differences in the rate of whole-body heat loss, and therefore change in body heat content was observed between groups in any of the environmental conditions, it is unclear if impairments would be evident at greater heat loads (i.e., greater metabolic and/or environmental heat load). More recently, a series of studies by Larose *et al.* (Larose *et al.*, 2013a; Larose *et al.*, 2013c; Larose *et al.*, 2013d) showed age-related impairments in whole-body heat loss during repeated exercise bouts at a fixed rate of metabolic heat production (400 W). The impairments occurred as early as the

40th decade and led to greater amounts of heat stored in middle-aged and older adults relative to their younger counterparts. Moreover, while impairments in whole-body heat loss were clear during exercise, no differences between age groups were observed during postexercise recovery. Furthermore, lower levels of whole-body heat loss and greater changes in body heat content were observed to a similar degree in both males (Larose *et al.*, 2013a; Larose *et al.*, 2013d) and females (Larose *et al.*, 2013c). However, it is unclear if similar decrements to the ability to dissipate heat would occur at lower (~300 W) or even higher (~500 W) levels of metabolic heat production.

CHAPTER 3:

ARTICLES

3.1 Thesis article #1 – published in Applied Physiology, Nutrition and Metabolism, 39(3): 292-298, 2014 – see Appendix A

Do older adults experience greater thermal strain during heat waves?

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Abstract: Heat waves are the cause of many preventable deaths around the world, especially among older adults and in countries with more temperate climates. In the present study, we examined the effects of age on whole-body heat loss and heat storage during passive exposure to environmental conditions representative of the upper temperature extremes experienced in Canada. Direct and indirect calorimetry measured whole-body evaporative heat loss and dry heat exchange, as well as the change in body heat content. Twelve young (21±3 years) and 12 older (65±5 years) adults with similar body weight (young: 72.0±4.4; older: 80.1±4.2 kg) and body surface area (young: 1.8±0.1; older: 2.0±0.1 m^2) rested for 2 hours in a hot-dry [36.5°C, 20% relative humidity (RH)] or hot-humid (36.5°C, 60% RH) environment. In both conditions, evaporative heat loss was not significantly different between groups (dry: P=0.758; humid: P=0.814). However, the rate of dry heat gain was significantly greater (by ~10 W) for older adults relative to young adults during the hot-dry (P=0.032) and hot-humid exposure (P=0.019). Consequently, the cumulative change in body heat content after 2 h of rest was significantly greater in older adults in the hot-dry (older: 212±25; young: 131±27 kJ, P=0.018) as well as the hot-humid condition (older: 426±37; young: 317±45 kJ, P=0.037). These findings demonstrate that older individuals store more heat during short exposures to dry and humid heat, suggesting that they may experience increased levels of thermal strain in such conditions than people of younger age.

Keywords: Aging, passive heat stress, calorimetry, heat waves

Introduction

Changes in climate towards more frequent and extreme heat events, which are longer in duration, have posed a significant health risk around the world. In Europe, 70 000 people died during the 2003 heat wave as a result of prolonged exposure to extreme heat. Countries with generally mild summertime temperatures were especially affected (Poumadere *et al.*, 2005; Fouillet *et al.*, 2006; Rey *et al.*, 2007; Robine *et al.*, 2008). In France for example, ~15 000 deaths were attributed to the exceptional heat wave where temperatures exceeded 40°C on 7 consecutive days (Poumadere *et al.*, 2005; Fouillet *et al.*, 2006; Rey *et al.*, 2007). More recently, an estimated 55,000 people died in Russia during the summer 2010 heat wave (Osborn, 2010), including ~11,000 deaths in Moscow (Barriopedro *et al.*, 2011). In Canada, summers are generally mild, with mid-summertime temperatures averaging ~25°C in many parts of the country (2013a). Canadian summers also tend to be humid with relative humidity typically ranging between 50-70% (2013a). The temperate weather in addition to the large fluctuations in temperature throughout the year can make it difficult for individuals to become heat-acclimatized. Therefore it is possible that people residing in these climates may be more susceptible to heat-related illness during periods of above-normal temperatures as a result of low levels of heat adaptation compared to people living in warmer places year round.

The number of days with temperatures ≥30°C and humidity index values exceeding 40°C is expected to increase in Canada (Maxwell *et al.*, 2006). Such extremes can have a considerable impact on the health of many people (Pengelly *et al.*, 2007; 2011), and older adults may be especially vulnerable to heat-related illness in these conditions (Minson *et al.*, 1998; Kenny *et al.*, 2010b; Frumkin *et al.*, 2012; Bustinza *et al.*, 2013). A number of studies have demonstrated important age-related differences in local heat loss responses (e.g., sweat

rate) and/or core temperature during passive heat exposure of 60 to 240 min in duration (Fennell & Moore, 1973; Crowe & Moore, 1974; Sagawa *et al.*, 1988; Miescher & Fortney, 1989; Dufour & Candas, 2007). In some cases, significant impairments in local heat loss or core temperature responses were evident after just 30 min of resting in the heat (Fennell & Moore, 1973; Crowe & Moore, 1974; Sagawa *et al.*, 1988; Miescher & Fortney, 1989; Dufour & Candas, 2007). However, whether these differences in local indices of heat strain are meaningful from a whole-body perspective is currently unclear. Moreover, thermoregulatory responses as a function of age were observed under ambient air temperatures ranging between 40-45°C. During extreme heat events, temperatures rarely rise above ~36-37°C in Canada. For example, the hottest day of the year in Toronto, one of the largest Canadian metropolitan cities, was 36.7°C during the 2012 North American heat wave (2013b). This is considerably lower than temperatures recorded in southern parts of the United States where daily temperatures were as high as 42.8 – 45.0°C during this particular heat wave (Burt, 2012). Nevertheless, humidity index readings, which indicate the approximate temperature when heat and humidity are considered, have been recorded upwards of 45°C during extreme heat events in Canada (2013a).

In the present study, we used conditions representative of the upper temperature extremes in Canada to assess the potential public health risk to older adults living in mild climates during heat waves. We examined whole-body heat loss and changes in body heat content in young (21±3 years) and older adults (65±5 years) during 2 hours of passive exposure to a hot-dry environment (temperature: 36.5°C, relative humidity: 20%, equivalent humidity index: 38°C) and a hot-humid condition (temperature: 36.5°C, relative humidity: 60%, equivalent humidity index: 51°C). It was hypothesized that whole-body heat loss

would be reduced in older adults compared to their younger counterparts thereby leading to a greater change in body heat content over 2 hours of exposure in both heat stress conditions.

Materials and methods

Ethical approval

The experimental protocol was approved by the University of Ottawa Health Sciences and Science Research Ethics Board and The Health Canada and Public Health Agency of Canada Research Ethics Board in accordance with the Declaration of Helsinki. Volunteers provided written informed consent before participating in the study.

Participants

Twelve young (21±3 years, 4 females and 8 males) and twelve older (65±5 years, 3 females and 9 males) adults who were not overly physically active (i.e., no more than 3 days per week of continuous exercise of ≤20 min in duration) participated in the study. Participants were non-smokers, and free of known respiratory or cardiovascular disease. Young and older participants had similar height (young: 1.7±0.1 m; older: 1.8±0.1 m), weight (young: 72.0±4.4 kg; older: 80.1±4.2 kg), body surface area (young: 1.8±0.1 m^2; older: 2.0±0.1 m^2) and body mass index (young: 24.7±1.2 kg/m^2; older: 25.8 ± 1.1 kg/m^2).

Experimental design

Participants volunteered for one preliminary session and two experimental test sessions. During the preliminary session, measurements of height and body weight were obtained. Body surface area was subsequently calculated from the measurements of height

and body mass (DuBois & DuBois, 1989). We also inquired about physical activity pattern using a quantitative (3 month) and seven day physical activity recall questionnaire proposed by Kohl et al. (Kohl *et al.*, 1988).

The experimental sessions involved two tests which were performed on separate days with a minimum of 72 hours between sessions. Participants were instructed to consume a light meal before their arrival and to avoid major thermal stimuli on their way to the laboratory. Participants were also asked to refrain from engaging in strenuous physical activity and consuming alcohol for 24 hours as well as caffeine for up to 12 hours before the testing sessions. To ensure euhydration, participants were instructed to drink 250 mL of water before bed, in the morning of and within 2 hours of the experimental trial. No fluid was ingested for the duration of the experimental protocol. There were no differences between groups in hydration status, according to urine specific gravity measurements, at the start of the hot-dry (young: 1.017±0.002; older 1.012±0.002) and hot-humid sessions (young: 1.018±0.003; older 1.012±0.002). The experimental sessions were performed in a whole-body direct air calorimeter, without prior laboratory acclimation sessions. Each session involved 120 min of passive heat exposure. During one experimental session, participants were seated in a whole-body calorimeter (a device for making very accurate measurements of the amount of heat emitted by the human body) regulated to 36.5°C and 20% relative humidity (RH), whereas during the other session, the environmental conditions were set to 36.5°C and 60% RH. The order of the sessions was random; an equal amount of participants performed the hot-dry session first followed by the hot-humid session and vice versa. Participants wore a light pair of athletic shorts and sandals during the sessions, and female participants also wore a sports bra.

Measurements

A detailed explanation of how direct calorimetry measures whole-body heat loss and heat storage has been described in a previous publication (Kenny & Jay, 2013). Also, a full technical description of the fundamental principles and performance characteristics of the Snellen calorimeter is available (Reardon *et al.*, 2006). In summary, direct calorimetry measured whole-body evaporative loss and dry heat exchange (radiation, conduction, convection), yielding an accuracy of ±2.3 W for the measurement of total heat loss. Indirect calorimetry was used to measure metabolic heat production. The change in body heat content (ΔH_b) was subsequently calculated by subtracting the total amount of heat production and heat loss over the 2 hour sessions. The amount of evaporation required to achieve heat balance (E_{req}) was calculated from the sum of the rate of metabolic heat production and dry heat loss.

Local sweat production was measured using the ventilated capsule technique. A 3.8 cm^2 plastic capsule was attached to three skin sites (upper trapezius, forearm and thigh) with an adhesive ring and topical skin glue (Collodion HV, Mavidon Medical products, Lake Worth, FL, USA). Compressed dry air was passed through the capsule at a rate of 1 $L \cdot min^{-1}$. Water content of the effluent air was measured using high precision dew point mirrors (model 473, RH systems, Albuquerque, NM, USA). Local sweat rate was calculated using the difference in water content between effluent and influent air multiplied by the flow rate and normalized for the skin surface area under the capsule.

Laser-Doppler velocimetry was employed for measuring skin blood flow (PeriFlux System 5000, Main control unit; PF5010 LDPM). A laser-Doppler probe (Perimed integrating probe 413, Järfälla, Sweden) was affixed to the forearm's surface in an area which did not seem overly vascular upon visual inspection and provided stable readings at

rest. To measure maximal skin blood flow, the heater housing the laser-Doppler probe was heated to 44°C until maximal skin vasodilation was achieved (~40 minutes) (Taylor *et al.*, 1984). Cutaneous vascular conductance was subsequently calculated as the ratio of skin blood flow perfusion units to mean arterial pressure and expressed as a percentage of maximum.

Visceral temperature was measured using a telemetric pill (VitalSense ingestible capsule thermometer is a Class II Medical Device according to 21 DFR 8982.1845; Mini Mitter Company Inc.) which moves freely and unobstructed through the digestive tract and is generally eliminated within 48 hours of ingestion (McKenzie & Osgood, 2004). The telemetric pill provides an estimate of internal body temperature.

Mean skin temperature was calculated as the weighted average of 4 skin temperature measurements: bicep 30%, chest 30%, quadriceps 20%, and back calf 20% (Ramanathan, 1964).

Systolic and diastolic blood pressures were determined using a Finometer (Finapres Medical Systems, Amsterdam, the Netherlands) from the beat-to-beat recording of the right middle finger arterial pressure waveform with the volume-clamp method (Penaz, 1973) and physiological criteria (Wesseling *et al.*, 1995). Blood pressure measurements were used to calculate mean arterial pressure (diastolic blood pressure + 1/3 × pulse pressure).

Heart rate was monitored, recorded continuously, and stored using a Polar coded WearLink and transmitter, Polar RS400 interface, and Polar ProTrainer 5 software (Polar Electro Oy, Finland).

Urine samples and body weight were obtained prior to the start and immediately following the experimental sessions. Urine specific gravity was determined using a handheld total solids refractometer (model TS400, Reichter Inc., Depew, NY, USA). Venous blood

samples were also obtained prior to and immediately following each session, while participants remained seated upright, via a single venipuncture (Becton, Dickinson and Company [BD], Franklin Lakes, NJ, USA). The samples were transferred directly into serum with no additive and plasma K_2EDTA 5.4 mg BD Vacutainer® tubes (BD, Franklin Lakes, NJ, USA). The K_2EDTA blood was mixed by inversion and used to measure hematological parameters (Beckman Coulter, Miami, FL, USA). Haemoglobin and hematocrit values were used to estimate the percent changes in plasma and blood volumes according to the method of Dill and Costill (Dill & Costill, 1974).

Thermal discomfort was evaluated using the ASHRAE 7-point scale (TS; Scale '0 = Neutral' to '7 = Very, Very Hot') and was recorded every 10 minutes.

Statistical Analysis

All dependent variables were compared between groups for each experimental condition (dry and humid) separately using a two-way analysis of variance (ANOVA) with the repeated factor of time (levels: 30, 60, 90 and 120 min) and non-repeated factor of age (levels: young and older). Independent *t* tests were used for post hoc analysis when a main effect of group was observed. The values at 30, 60, 90 and 120 min were obtained by averaging the last minute of data collected during that time period. Independent *t* tests were also used to compare participant characteristics and the cumulative change in body heat content measured over the 120 min exposure. The level of significance for all analyses was set at $p \leq 0.05$. Analyses were performed using commercially available statistical software (GraphPad Prism 6.0, GraphPad Software, La Jolla, CA, USA). All values are reported as mean ± standard error (SE). Note: visceral temperature was not measured in one of the

young participants in the hot-humid condition as well as in two older participants in both conditions; skin blood flow was only measured in ten young and eight older participants.

Results

Young versus Older Adults – Hot-Dry Ambient Condition

Calorimetry

Metabolic heat production, total heat loss, evaporative heat loss, E_{req} and dry heat exchange during the hot-dry condition are presented in Figure 1A and 1B. Metabolic heat production remained fairly consistent (P=0.219) in both groups (P>0.999) during the dry heat exposure. Total heat loss, which combines evaporative heat loss and dry heat exchange, increased progressively (P<0.001) for both groups (P=0.584), mainly due to a progressive reduction in dry heat gain (P <0.001). Moreover, the rate of dry heat gain significantly differed between groups (P=0.032). At 60, 90 and 120 min, the rate of dry heat gain was 11 W (P =0.022), 10 W (P=0.021) and 12 W (P=0.008) greater in older adults compared to young adults. The difference in dry heat gain at 30-min fell just short of significance (P=0.056). Evaporative heat loss on the other hand, remained constant throughout (P=0.291) and was almost identical between groups (P=0.758), averaging 104±7 W in young and 103±7 W in older adults. E_{req}, calculated as the sum of metabolic heat production and dry heat exchange, remained stable during the dry heat exposure session (P=0.139) and did not significantly differ between groups (P=0.328). However, due to the greater rate of dry heat gain measured in older adults, E_{req} (and therefore the net heat load) was higher by an average of ~11 W in older adults. Following the 2 h exposure, the cumulative change in body heat

content was significantly greater in the older age group relative to the young group (P=0.018) (Figure 2B).

Local heat loss responses

Data from local sweat rates measured on the upper back, forearm and thigh are presented in Table 1, whereas cutaneous vascular conduction is presented in Table 2. Local sweat rates did not significantly increase during the session (all P>0.400), however, sweat rate was significantly different between groups on the upper back (p=0.011). Older adults had a greater sweat rate at 30 (P=0.035), 60 (P=0.012), 90 (P=0.005) and 120 min (P=0.003). Local sweating on the forearm (P=0.066) and thigh (P=0.194) were not different between groups. Cutaneous vascular conductance (CVC) remained the same throughout the session (P=0.182). There was a difference between groups (P=0.019), whereby CVC was higher in older adults at 60 (P=0.003), 90 (P=0.010) and 120 min (P=0.015).

Core and skin temperatures

Visceral temperature during the hot-dry exposure is presented in Figure 2A while mean skin temperature data is presented in Table 1. Both visceral (P<0.001) and skin (P=0.006) temperatures increased over the 2 hours but the differences between groups were not statistically significant (P=0.107 and P=0.381, respectively).

Cardiovascular responses and hydration status

Heart rate and mean arterial pressure data are presented in Table 2. Neither of these variables changed over the course of the exposure (heart rate p=0.163; mean arterial pressure P=0.248) as well, young and older adults appeared to experience similar levels of

cardiovascular strain (heart rate P=0.210; mean arterial pressure P=0.964). The changes in blood volume (young: -2.5±0.5%; older: -3.9±0.6%, P=0.077), plasma volume (young: -4.5±0.8%; older: -6.6±1.1%, P=0.101) and urine specific gravity (young: 0.004±0.001; older: 0.007±0.001, P=0.219) were not significantly different between groups. There was a trend for change in body weight to differ between groups (P=0.057). Older adults had a slightly greater change in weight (-0.63±0.05 kg) relative to young adults (-0.51±0.04 kg).

Thermal discomfort

Participants were asked to indicate their level of thermal discomfort throughout the exposure. While perceived thermal discomfort did not increase or decrease during the session (p=0.245), there was significant difference between groups (P=0.051). Older adults consistently rated their thermal sensation higher than that of young adults and this was significantly different at 60 (3.1±0.4 vs. 2.2±0.3, P=0.044) and 120 min (3.1±0.4 vs. 1.9±0.3, P=0.008).

Young versus Older Adults: Hot-Humid Ambient Condition

Calorimetry

We present the rates of metabolic heat production, total heat loss, evaporative heat loss, E_{req} and dry heat exchange during the hot-humid experimental session in Figure 1C and 1D. Similar to the hot-dry session, metabolic heat production remained constant (P=0.139) and was not different between groups (P=0.535). Total heat loss increased as the session progressed (P<0.001). This was partly driven by a reduced rate of dry heat gain in both age groups (P<0.001). Older adults had a significantly greater rate of dry heat gain compared to young adults (P=0.019). Significant differences between groups were observed at 30 (+11

W, *P*=0.011), 60 (+10 W, *P*=0.032), 90 (+12 W, *P*=0.032) and 120 min (+12 W, *P*=0.021). In contrast to the hot-dry session, evaporative heat loss increased significantly over the course of the humid heat exposure (*P*<0.001), albeit this was similar between groups (*P*=0.814). As a result of a greater rate of dry heat gain, the average E_{req} in older adults exceeded that of young adults by ~13 W, although the difference between groups did not reach statistical significance (*P*=0.109). The net amount of heat that was stored (and therefore the change in body heat content) over the 2 hour hot-humid session was significantly greater in older adults relative to the young groups (*P*=0.037) (Figure 2D).

Local heat loss responses

Local sweat rate on the upper back was significantly different between groups (*P*=0.025); older adults had a greater sweat rate at 30 (*P*=0.016), 60 (*P*=0.012), 90 (*P*=0.016) and 120 min (*P*=0.004). Sweating on the forearm fell short of being significantly greater in older adults (*P*=0.056). Finally, local sweating on the thigh was significantly greater in young adults (*P*=0.010). Significant differences occurred at 60 (*P*=0.030), 90 (*P*=0.018) and 120 min (p=0.031). Cutaneous vascular conductance remained significantly higher in older adults (*P*=0.042) in the hot-humid condition compared to young adults. CVC was significantly different between groups at 60 (p=0.031), 90 (0.016) and 120 min (*P*=0.005).

Core and skin temperatures

Both visceral (*P*<0.001, Figure 2C) and skin (*P*=0.004) temperatures increased as the session progressed. Whereas skin temperature did not significantly differ between groups (*P*=0.213), the increase in visceral temperature was significantly greater in older adults

(*P*=0.019). Differences were observed at 30 (*P*=0.040), 60 (*P*=0.023), 90 (p=0.011) and 120 min (*P*=0.001).

Cardiovascular responses and hydration status

Heart rate became significantly higher as the session progressed (*P*=0.010), although this was similar in young and older adults (*P*=0.242). No differences in mean arterial pressure were measured over the course of the session (*P*=0.236) and responses were similar between groups (*P*=0.390). Young and older adults had similar changes in blood volume (young: -2.7±0.5%; older: -2.5±0.6%, *P*=0.441), plasma volume (young: -4.9±0.9%; older: -3.9±0.7%, *P*=0.464) and urine specific gravity (young: 0.005±0.002; older: 0.004±0.002, *P*=0.442). The difference in weight before and after the session fell short of statistical significance but tended to be more elevated in older adults (young: -0.49 ± 0.02 kg, older: -0.59 ± 0.04 kg, *P*=0.057).

Thermal discomfort

Thermal discomfort became increasingly greater over the course of the hot-humid exposure (*P*=0.002) and older adults reported a higher thermal sensation (*P*=0.033). This was significantly different at 30 (3.0±0.2 vs. 2.1±0.2, *P*=0.004), 60 (3.2±0.3 vs. 2.2±0.2, *P*=0.007) and 90 min (3.1±0.3 vs. 2.3±0.3, *P*=0.043).

Discussion

In the present study, older adults had a greater change in body heat content compared to young adults during short exposures to a hot-dry (36.5°C, 20% RH) and hot-humid

(36.5°C, 60% RH) environment. This was in large part due to the fact that heat load was more elevated in older adults during the exposures, owing to a greater rate of dry heat gain. This added heat load was not compensated for by a greater rate of evaporative heat loss, causing a greater rate of heat storage throughout the sessions which resulted in a progressively greater change in body heat content among older adults.

A number of studies reported decrements in thermoregulatory function in older adults during passive exposure to extreme ambient conditions ranging from 40-90°C (Shoenfeld *et al.*, 1978; Drinkwater *et al.*, 1982; Sagawa *et al.*, 1988; Miescher & Fortney, 1989; Armstrong & Kenney, 1993; Dufour & Candas, 2007). In the present study, we were concerned with investigating whole-body heat loss and heat storage under a lower environmental heat load since Canada does not typically experience such extreme temperatures, yet heat-related injuries and/or deaths are still prevalent (Pengelly *et al.*, 2007; 2011)). The results from our study indicate that during exposure to a hot environment, with relatively low humidity, whole-body evaporative heat loss was similar between young and older adults. Even so, older adults stored 38% more heat than their younger counterparts. It is important to note that older adults had a greater rate of dry heat gain (~10 W), owing to the slightly lower mean skin temperature (and therefore greater air to skin temperature gradient). As shown in Figure 1A, the amount of evaporative heat loss required to achieve balance (determined as the sum of metabolic heat load ± dry heat exchange) was actually more elevated in older adults compared to the young group throughout the session. The average rate of evaporative heat loss required for older adults to achieve heat balance was ~133 W whereas for young adults, this was only ~122 W. Despite the slightly greater heat load, we did not observe a concomitant increase in the rate of whole-body evaporative heat loss in

older adults. Evaporative heat loss accounted for ~85% of the evaporative heat loss required for heat balance in young adults, but only ~77% in older adults. The net effect over the 2 hour exposure was a greater cumulative increase in body heat content.

Canadian climates tend to be very humid in the summer. In fact, it is not uncommon for humidity indices to reach 45°C with some extreme readings recorded as high as 51-53°C during heat waves (2013a). Such extreme conditions can severely impede thermoregulatory control by reducing the amount of heat that can be dissipated to the environment through evaporative heat loss; the primary physiological mechanism for cooling (McLellan *et al.*, 1996; Taylor, 2006). In the present study, evaporative heat loss was reduced to a similar extent (~26%) in the hot-humid relative to the hot-dry condition in both young and older adults. Consequently, heat storage increased by 59% in young and 50% in older adults, compared to the amount of heat that was stored during the hot-dry condition. The change in body heat content remained higher in older adults (by 26%) compared to young adults, again largely due to a greater rate of dry heat gain (~10W). Interestingly, local sweat rate was greater by ~55% and 49% (average from the three sites measured) for young and older adults respectively in the humid condition compared to the dry environment, yet both groups stored more heat. Considering that local sweat rate was somewhat more elevated in older adults (0.38 ± 0.04 vs. 0.31 ± 0.05 $mg \cdot cm^{-2} \cdot min^{-1}$) but that they still stored more heat would suggest that sweating efficiency was reduced to a greater degree in older individuals, thereby leading to increased thermal strain (Candas *et al.*, 1979).

Core temperature measurements are commonly used to assess thermal stress among individuals in a variety of scenarios involving exposure to the heat. There is growing evidence from many population groups that core temperature measurements can severely underestimate whole-body heat storage as core temperature is only indicative of heat content

within a specific region of the body (Kenny *et al.*, 2010b; Kenny & Jay, 2013). Our results indicate that core temperature increased progressively in young (+0.09±0.06°C) and older adults (+0.22±0.08°C) during the dry heat condition. In the hot-humid condition, the increase in visceral temperature was significantly more elevated in older adults (+0.32±0.06°C) than young adults (+0.12±0.04°C). The change in core temperature, however, does not adequately reflect the much greater change in body heat content, and therefore the level of thermal strain experienced in each group. In fact, the small change in visceral temperature under both environmental conditions could lead to the misguided conclusion that the risk of developing a heat injury is relatively low. Conversely, the change in mean body temperature can more accurately be estimated using the change in body heat content measured by direct calorimetry with the equation: $\Delta T_b = \Delta H_b/(b_m * C_p)$ where ΔT_b is the change in mean body temperature, ΔH_b is the change in body heat content, b_m is body mass and C_p is specific heat of the participant. Based on this equation, the estimated increase in mean body temperature would be ~0.52 and ~0.76°C in young and older adults respectively during the hot-dry exposure. For the hot-humid condition, the change in mean body temperature would actually be as high as ~1.34 (young) and ~1.58°C (older) after just 2 h. So while visceral temperature measurements may suggest minimal health risks in both groups under the conditions tested, it is important to keep in mind that mean whole-body temperature is much more elevated, with the greatest increase observed in older adults.

Perspectives

The main question behind the present study was whether or not exposure to extreme environmental conditions, which are representative of Canadian climates, poses a greater

threat to the health of older adults relative to younger counterparts. Increases in heat waves could be particularly concerning in places like Canada where summertime temperatures are generally mild and temperatures fluctuate considerably throughout the year. As a result, individuals residing in such climates do not tend to be acclimatized to hot weather conditions and are likely more susceptible to the potential harmful effects of extreme heat compared to those living in countries and/or cities where high summertime heat and humidity are common. In the present study, 2 hours of exposure to a dry and humid heat stress resulted in greater body heat storage among older adults, suggesting a greater level of thermal strain compared to younger individuals. This is supported by the fact that older adults reported a higher level of thermal discomfort after only 60 and 30 minutes for the hot-dry and hot-humid environments respectively. Nonetheless, there are a few things to consider when interpreting our study findings. First, the older adults in this study were healthy and not taking any medications. Thus, older adults with chronic medical conditions (e.g. diabetes, cardiovascular disease, respiratory disease, etc.) and/or taking medication may be more vulnerable than the older adults who participated in the present study (Kenny *et al.*, 2010b; Kenny & Jay, 2013). Moreover, the experimental sessions were conducted in a laboratory setting and did not factor in additional heat that could be gained through solar radiation. Also, the temperature selected to mimic the upper temperature extremes in Canada (i.e., 36.5°C) may not be representative of extreme temperatures in large metropolitan cities, which could be even higher as a result of the "heat island effect" (Kovats & Hajat, 2008; Lundgren *et al.*, 2013). Finally, the exposure was purposely selected to be short. This was the first time that we used the direct calorimeter to assess whole-body heat loss and heat storage in a group of vulnerable older adults who had no prior experience in our laboratory. There are certain risks involved with doing these types of studies where participants are enclosed in

a capsule like device for an extended period of time. Given that previous studies had observed marked differences between young and older adults after just 30 min (Fennell & Moore, 1973; Crowe & Moore, 1974; Sagawa *et al.*, 1988; Miescher & Fortney, 1989; Dufour & Candas, 2007), we opted to only do a 2 h exposure. In reality, older adults may spend longer than 2 hours exposed to high temperatures and/or humidity during heat waves, hence our findings underline a critical need to examine age-related changes in the heat stress responses during prolonged exposure (i.e., >4 hours).

In summary, exposure to dry heat as well as humid heat posed a significantly greater thermal challenge for older adults as evidenced by greater changes in body heat content. Initiatives to increase awareness regarding the potential health risks associated with exposure to the heat, particularly when humidity is high, maybe necessary to protect older individuals against heat illness.

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Table 1. Local sweating rates and mean skin temperature during passive exposure to dry and humid heat.

	LSR ($mg \cdot cm^{-2} \cdot min^{-1}$)						T_{Sk} (°C)	
	Arm		Back		Leg			
Hot-dry	**Young**	**Older**	**Young**	**Older**	**Young**	**Older**	**Young**	**Older**
0	0.18±0.03	0.20±0.03	0.19±0.04	0.22±0.03	0.21±0.02	0.20±0.03	34.11±0.12	34.19±0.15
30	0.15±0.03	0.17±0.02	0.14±0.03	0.22±0.03*	0.18±0.02	0.17±0.02	34.47±0.13	34.36±0.18
60	0.14±0.03	0.21±0.02	0.14±0.03	0.24±0.03*	0.19±0.02	0.19±0.02	34.56±0.12	34.35±0.18
90	0.13±0.02	0.18±0.02	0.12±0.02	0.22±0.03*	0.21±0.02	0.15±0.01	34.57±0.11	34.40±0.19
120	0.11±0.02	0.20±0.03	0.12±0.02	0.23±0.03*	0.21±0.02	0.16±0.02	34.69±0.10	34.44±0.17
Hot-humid								
0	0.39±0.04	0.51±0.06	0.35±0.04	0.43±0.04*	0.48±0.04	0.39±0.03	34.41±0.10	34.28±0.07
30	0.33±0.03	0.43±0.04	0.30±0.04	0.41±0.05*	0.43±0.04	0.34±0.02	34.74±0.10	34.55±0.10
60	0.33±0.04	0.46±0.05	0.28±0.03	0.42±0.06*	0.42±0.03	0.31±0.02*	34.80±0.08	34.65±0.14
90	0.31±0.03	0.41±0.04	0.28±0.03	0.41±0.06*	0.39±0.03	0.30±0.02*	34.84±0.08	34.65±0.16
120	0.33±0.04	0.42±0.04	0.26±0.02	0.41±0.05*	0.40±0.03	0.31±0.02*	34.87±0.07	34.63±0.14

Values are presented as mean ± SE. LSR; local sweat rate, T_{Sk}; mean skin temperature. *denotes a significant difference between young and older adults.

Table 2. Cutaneous vascular conductance, heart rate and mean arterial pressure responses during passive exposure to dry and humid heat.

	CVC (% of max)		HR (beats·min^{-1})		MAP (mmHg)	
Hot-dry	**Young**	**Older**	**Young**	**Older**	**Young**	**Older**
0	18.4±1.4	21.0±1.9	78±4	77±3	85±2	95±3
30	19.0±1.8	23.7±1.8	80±4	78±3	94±3	95±5
60	17.6±1.3	24.5±1.6*	79±5	75±3	94±3	91±6
90	17.1±0.9	22.8±1.8*	84±5	74±2	94±2	97±6
120	17.2±1.0	22.6±1.9*	86±4	75±2	96±3	96±5
Hot-humid						
0	20.6±2.3	25.9±2.3	84±4	79±3	88±3	93±3
30	23.4±3.5	31.0±4.0	83±4	76±3	95±3	93±2
60	22.1±2.8	31.7±3.4*	86±4	81±4	95±3	93±2
90	21.6±3.0	33.8±3.7*	85±4	78±4	93±2	89±2
120	20.2±1.8	32.7±3.6*	86±5	80±3	94±2	92±2

Values are presented as mean ± SE. CVC; cutaneous vascular conductance, HR; heart rate, MAP; mean arterial pressure. * denotes a significant difference between young and older adults.

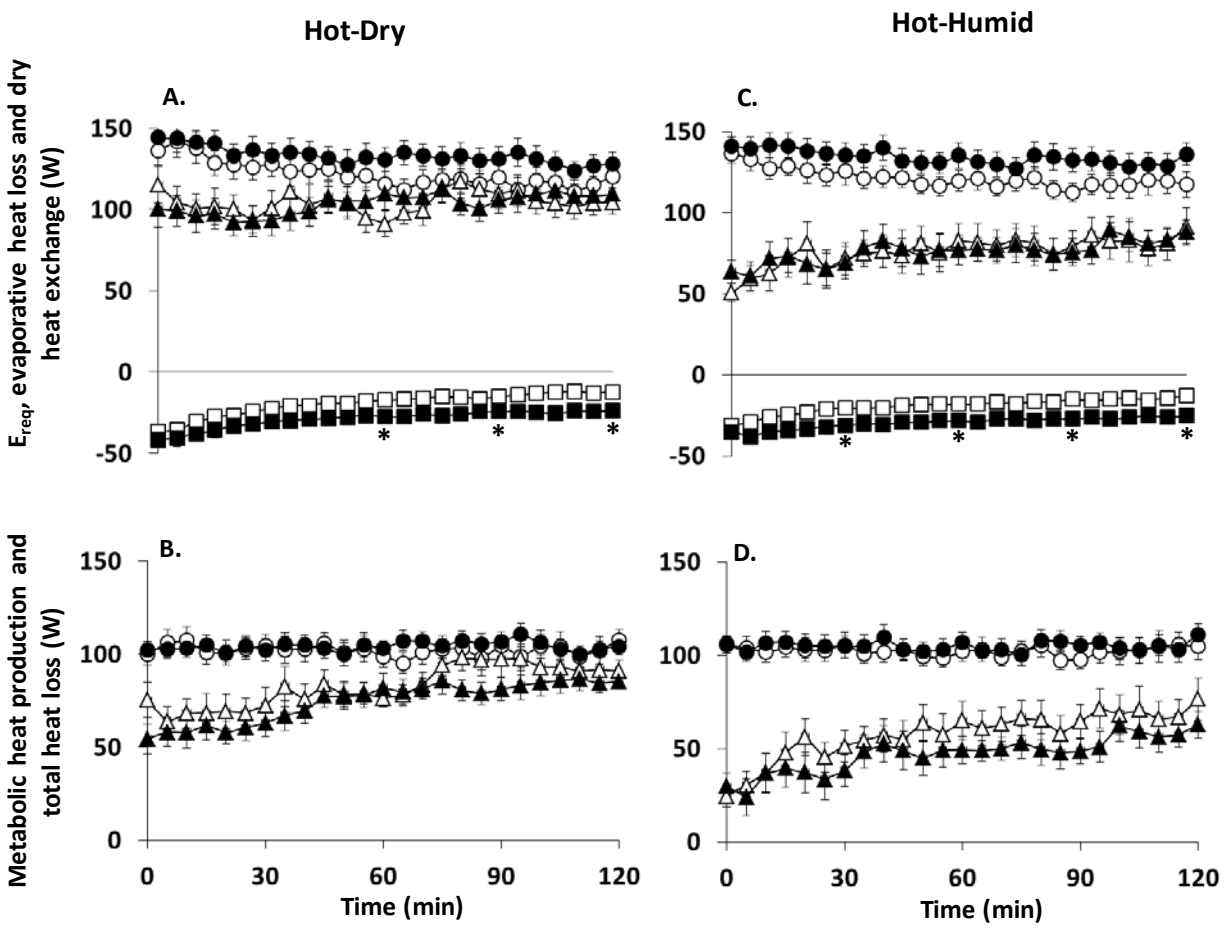

Figure 1. (**A & C**) Required amount of evaporative heat loss to achieve heat balance (E_{req}) (circles), evaporative heat loss (triangles) and dry heat exchange (squares); **(B & D)** Metabolic heat production (circles) and total heat loss (triangles); during passive exposure to a hot-dry (36.5°C, 20% relative humidity) and hot-humid (36.5°C, 60% relative humidity) condition for a duration of 2 h. Data for the young group is presented in open symbols, while data for the older group is presented in dark filled symbols. * denotes a significant difference between groups. All values are presented as means ± SE.

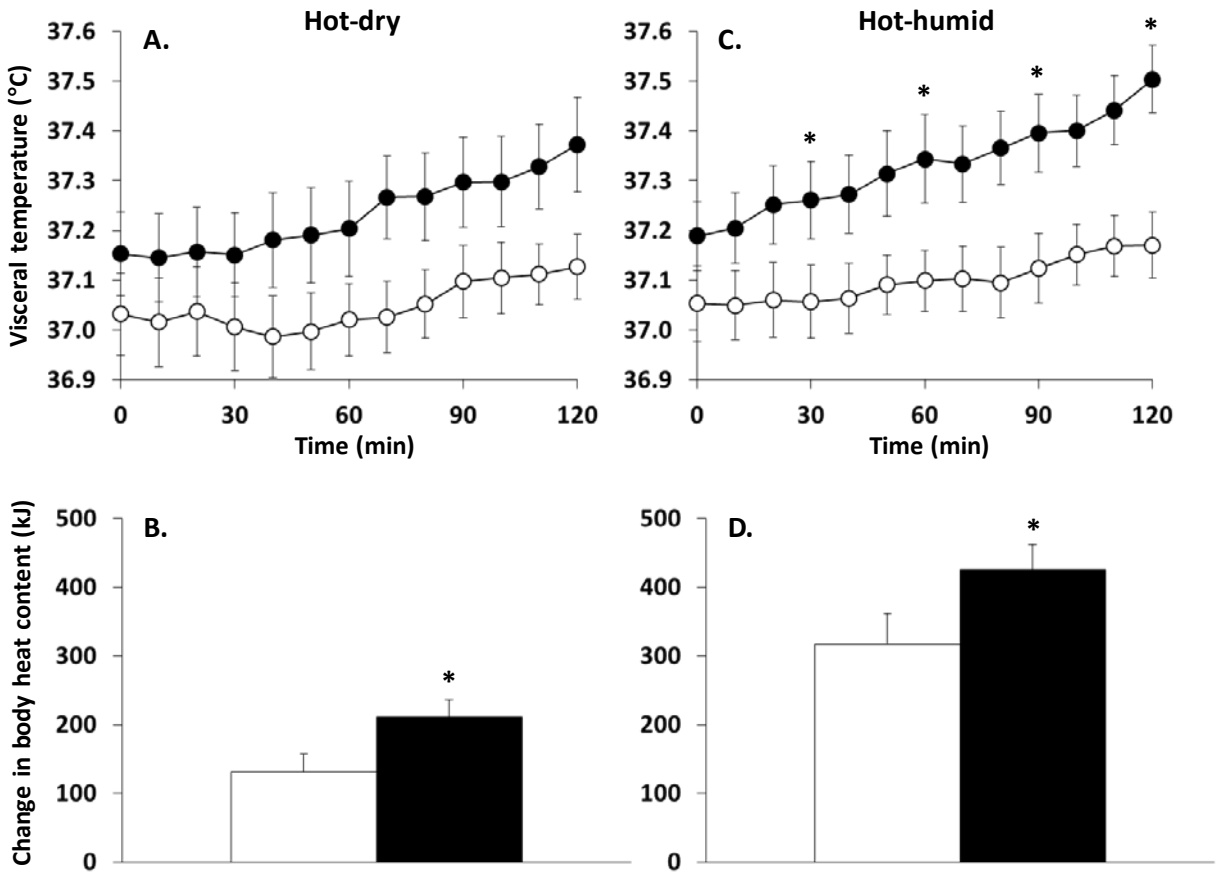

Figure 2. (**A & C**) Visceral temperatures; (**B & D**) Cumulative change in body heat content; during passive exposure to a hot-dry (36.5°C, 20% relative humidity) and hot-humid (36.5°C, 60% relative humidity) condition for a duration of 2 h. Data for the young group is presented in open symbols,, while data for the older group is presented in dark filled symbols.. * denotes a significant difference between groups. All values are presented as means ± SE.

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Aging impairs heat loss, but when does it matter?

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Running head: Aging and heat loss capacity

Author Contributions:

J.M.S., A.D.F., P.B., R.J.S., J.M., and G.P.K. conceived and designed the experiments. J.M.S. and M.P.P. contributed to data collection. J.M.S. performed the data analysis. J.M.S., M.P.P., A.D.F., P.B., R.J.S., J.M., and G.P.K. interpreted the experimental results. J.M.S. prepared the figures and drafted the manuscript. All authors edited and revised the manuscript and approved the final version of the manuscript.

ABSTRACT

Aging is associated with an attenuated physiological ability to dissipate heat. However, it remains unclear if age-related impairments in heat dissipation only occur above a certain level of heat stress and whether this response is altered by aerobic fitness. Therefore, we examined changes in whole-body evaporative heat loss (H_E) as determined using whole-body direct calorimetry, in young (n=10; 21±1 years), untrained middle-aged (n=10; 48±5 years) and older (n=10; 65±3 years) males matched for body surface area. We also studied a group of trained middle-aged males (n=10; 49±5 years) matched for body surface area with all groups and for aerobic fitness with the young group. Participants performed intermittent aerobic exercise (30-min exercise bouts separated by 15-min rest) in the heat (40°C and 15% RH) at progressively greater fixed rates of heat production equal to 300 (Ex1), 400 (Ex2) and 500 (Ex3) W. Results showed that H_E was significantly lower in middle-aged untrained (Ex2: 426±34; Ex3:497±17 W) and older (Ex2: 424±38; Ex3: 485±44 W) compared to young (Ex2: 472±42; Ex3: 558±51 W) and middle-aged trained (Ex2: 474±21; Ex3: 552±23 W) males at the end of the last two exercise bouts (P<0.05). No differences between groups were observed during recovery. We conclude that impairments in H_E in older and middle-aged untrained males occur at exercise-induced heat loads of ≥ 400 W when performed in a hot environment. These impairments in untrained middle-aged males can be minimized through regular aerobic exercise training.

Key words: calorimetry, evaporative capacity, sweating, skin blood flow, age, aerobic fitness

INTRODUCTION

Age-related reductions in whole-body and/or local heat loss and/or increased body heat storage during exercise in the heat have been reported in a number of studies (Anderson & Kenney, 1987; Tankersley *et al.*, 1991; Kenney *et al.*, 1997; Inoue *et al.*, 1999a; Inbar *et al.*, 2004; Larose *et al.*, 2013a; Larose *et al.*, 2013d; Larose *et al.*, 2014; Stapleton *et al.*, 2014). A recent study (Larose *et al.*, 2013d) reported that older males (63 ± 3 years) demonstrate a reduced rate of whole-body heat loss as early as 10 min after the onset of exercise at fixed rates of metabolic heat production (i.e., 400 W) in the heat [35°C, 20% relative humidity (RH)] relative to their younger counterparts (26 ± 2 years) (Larose *et al.*, 2013d). Furthermore, in the same study, the ability to dissipate heat in middle-aged males (43 ± 2 years) was intermediate to that measured in young and older males for the given heat load employed (Larose *et al.*, 2013d). The differences between young and older males were not the result of a change in the mean body temperature at which heat loss responses were activated (i.e., the onset threshold), but rather the result of a reduced rate of increase in heat loss (i.e., thermosensitivity) leading to greater amount of heat stored. While this study was the first to examine age-related differences in whole-body heat dissipation during exercise as assessed by direct calorimetry, it could not be determined if age-related differences occur at lower exercise intensities (and therefore heat loads) and if the age-related differences would be exacerbated at higher heat loads. This is due to the fact the study employed a single repeated short duration exercise-induced heat load. Moreover, all participants were matched for aerobic fitness (defined by peak oxygen uptake, VO_{2peak}). As a consequence, the effects of aerobic fitness in the context of aging could not be evaluated. Of note however, no differences in whole-body heat loss were observed when young (22 ± 2 years) and middle-aged (45 ± 4 years) males, matched for VO_{2peak} (~52 $mL \cdot kg^{-1} \cdot min^{-1}$), exercised for 90

min at a rate of metabolic heat production of 290 W (equivalent to ~22% VO_{2peak}) (Kenny *et al.*, 2010a). However, it is unclear if the similar responses were due to the fact that the heat load employed did not exceed the individual's physiological capacity to dissipate heat or if the middle-aged adults had a higher rate of heat dissipation due to the relatively higher aerobic fitness level.

Aerobic fitness level is an important determinant in the health status of individuals of any age, however, advanced aging is associated with a ~7% reduction in VO_{2peak} per decade (Wilson & Tanaka, 2000). Some studies showed that the level of aerobic fitness, associated with regular endurance-type exercise, can induce partial acclimation and thereby improve thermoregulatory control during exercise (Gisolfi & Robinson, 1969; Buono & Sjoholm, 1988; Havenith & van Middendorp, 1990; Cheung & McLellan, 1998; Selkirk & McLellan, 2001). While a study by Stapleton *et al.* (2010) found no improvements in heat dissipation following 8 weeks of acute exercise training in young adults (Stapleton *et al.*, 2010), the long-term effects (i.e., as a function of increasing age) of regular exercise training may help attenuate the age-related impairment in heat loss (Tankersley *et al.*, 1991; Inoue *et al.*, 1999a; Best *et al.*, 2012; de Paula Viveiros *et al.*, 2012). On the contrary, two other studies showed that heat loss was reduced in older adults irrespective of aerobic fitness (Anderson & Kenney, 1987; Larose *et al.*, 2013a). Thus, the separate and combined influence of age and aerobic fitness on the maximal capacity to dissipate heat remains to be elucidated.

Thus, we used an exercise model consisting of progressive increases in heat load (and therefore thermal drive) in young, middle-aged untrained and older males to examine the threshold at which age-related impairments in the body's physiological capacity to dissipate heat during exercise exist. Additionally, we examined the effects of aerobic fitness, in the context of

aging, on whole-body heat loss in a group of middle-aged trained males matched for VO_{2peak} with the young males. The rates of metabolic heat production were chosen to ensure that a near uncompensable heat stress condition was achieved during the first exercise bout, progressing to a fully uncompensable condition during the final exercise bout. Based on the disparity of age-related impairments in heat loss between studies, we hypothesized that differences in the capacity to dissipate heat between both middle-aged untrained and older males and their younger counterparts would occur at some heat load threshold, thereafter, the magnitude of difference would be greater with progressive increases in heat load. Additionally, we hypothesized that the heat load in which age-related differences occur would be higher in the middle-aged trained males compared to their middle-aged untrained counterparts.

MATERIALS AND METHODS

Ethical approval

The experimental protocol was approved by the University of Ottawa Health Sciences and Science Research Ethics Board, in accordance with the Declaration of Helsinki. Volunteers provided written informed consent before participating in the study.

Participants

Participant characteristics are presented in Table 1. We conducted a power analysis using 80% power and a significance level of 0.05 to calculate the minimum sample size required based on reported age-related differences in whole-body heat loss in young, middle-aged and older males during exercise in the heat at a moderate heat load (Larose *et al.*, 2013d). Assuming an effect of 30% and standard deviation of 25%, the calculated minimum sample size per group was

n = 6. Thus, forty males volunteered for the study and were divided into four groups of 10 young (21 ± 1 years), 10 middle-aged trained (49 ± 5 years), 10 middle-aged untrained (48 ± 5 years) and 10 older (65 ± 3 years) males. Participants were matched for height (P=0.436), body mass (P=0.895), and body surface area (P=0.762). We carefully matched our participants for physical characteristics by first recruiting an individual for one of the groups (i.e., young) and then selected a match for each of the other groups (i.e., middle-aged trained/untrained and older). However, there was a significant difference between groups for percent body fat (P<0.001) and VO_{2peak} (P<0.001). Specifically, the young males had a lower percent body fat compared to the middle-aged untrained and older males (P<0.05). Moreover, the young and middle-aged trained males had a similar VO_{2peak} (P>0.05) which was greater compared to the middle-aged untrained and older males (P<0.05). All participants were healthy and non-smokers. The Kohl physical activity questionnaire (Kohl *et al.*, 1988) was used to assess the participant's physical activity level (i.e., duration, frequency and mode) for a period of 3 months prior to the start of the experimental test session. The results from the questionnaire revealed that young, middle-aged untrained and older adults were performing a minimum of 30 min of exercise/day (i.e., walking, running, cycling) for 1 – 3 days per week (habitually active), while middle-aged trained males were highly endurance trained (≥30 min of exercise per/day of running, cycling or cross country skiing for 4 – 7 days per week)."

Experimental design

Each participant completed one preliminary and one experimental session. During the preliminary session, body height, mass, and density, as well as VO_{2peak} were determined. Body height was determined using a stadiometer (Detecto, model 2391, Webb City, MO, USA), while

body mass was measured using a digital high-performance weighing terminal (model CBU150X, Mettler Toledo Inc., Mississauga, ON, CAN). Body surface area was subsequently calculated from the measurements of body height and mass (DuBois & DuBois, 1989). Body density was measured using the hydrostatic weighing technique, and used to calculate body fat percentage (Siri, 1956). VO_{2peak} was measured on a recumbent cycle ergometer (Corival, Lode B.V., Groningen, Netherlands) during a progressive incremental exercise protocol which consisted of a 2-min warm-up at 40 W followed by 20 W increments every minute until the participant could no longer maintain a pedaling cadence of at least 60 rpm. For the older males, 12-lead ECG was monitored continuously throughout the maximal exercise test by a qualified technician to detect any abnormalities in heart activity. If detected, participants were excluded from the study and referred to their physician. No participants were excluded from the study based on this criterion.

The experimental protocol was performed in a whole-body direct air calorimeter outside of summer months (September to May only) to ensure that there was no potential confounding effect of acclimatization. An equal number of participants within each group performed the experimental protocol in the morning and in the afternoon. Participants consumed a standardized light meal or snack of dry toast and orange juice before their arrival (a minimum of 2 hours before testing) and avoided major thermal stimuli on their way to the laboratory (i.e., running, cycling, etc.). Strenuous activity and alcohol were avoided for 24 hours and caffeine for 12 hours before testing sessions. To ensure euhydration, participants drank ~250 mL of water before bed, in the morning of the experimental trial and within 2 hours of the start of the trial. No fluid was ingested for the duration of the experimental protocol.

The calorimeter was regulated to an ambient temperature of 40°C and 15% RH. Since some participants may not have been able to withstand high levels of exercise intensity, and

therefore high exercise-induced heat loads (i.e., > 600 W), the high ambient temperature (i.e., 40°C) was used to create an additional heat load (i.e., environmental heat load). All participants wore a light pair of athletic shorts and sandals. Following instrumentation, participants rested for a 30-min habituation period on an upright seated cycle ergometer in the calorimeter. Habituation was followed by three bouts of 30-min cycling exercise (Ex) at increasingly greater rates of metabolic heat production (Ex1: 300 W; Ex2: 400 W; Ex3: 500 W). Each exercise bout was followed by a 15-min recovery (Rec) period (Rec1, Rec2, and Rec3) in the calorimeter. The rates of metabolic heat production employed were equivalent to ~25% and ~33% VO_{2peak} for Ex1, ~35% and ~47% VO_{2peak} for Ex2 and ~44% and ~60% VO_{2peak} for Ex3 for young/middle-aged trained and older/middle-aged untrained, respectively.

Measurements

Whole-body evaporative loss and dry heat exchange as well as change in body heat content were quantified using the modified Snellen direct air calorimeter. A full technical description of the fundamental principles and performance characteristics of the Snellen calorimeter is available elsewhere (Reardon *et al.*, 2006). Data from the direct calorimeter were collected continuously at 8 s intervals during the experimental sessions. Real-time data were displayed and recorded on a personal computer with Lab-VIEW software (version 7.0, National Instruments, Austin, Texas, USA). The rate of evaporative heat loss was calculated from the calorimetry data using the following equation: (Massflow x ($Humidity_{out}$ – $Humidity_{in}$) x 2,426) / 60, where mass flow is the rate of air mass (kg air·s^{-1}); ($Humidity_{out}$ – $Humidity_{in}$) is the difference in absolute humidity (g water·kg air^{-1}) between the in and out flows of the calorimeter; and 2,426 is the latent heat of vaporization of sweat (J·g sweat-1). The rate of dry

heat loss, from radiation, convection and conduction was calculated from calorimetry data using the following equation: (Massflow x ($Temperature_{out}$ – $Temperature_{in}$) x 1,005) / 60, where mass flow is the rate of air mass (kg air·s^{-1}); ($Temperature_{out}$ – $Temperature_{in}$) is the difference in inflow-outflow air temperature (°C) of the calorimeter; and 1,005 is the specific heat of air [J·(kg air·°C)$^{-1}$]. Direct calorimetry measures whole-body evaporative loss and dry heat exchange (radiation, conduction, convection), yielding an accuracy of ±2.3 W for the measurement of total heat loss. Indirect calorimetry was used to measure metabolic heat production. Electrochemical gas analyzers located outside of the calorimeter (AMETEK model S-3A/1 and CD 3A, Applied Electrochemistry, Pittsburgh, PA, USA) were used to continuously determine the concentration of expired O_2 and CO_2 during testing sessions. To account for respiratory heat exchange, expired air was recycled back into the calorimeter. The change in body heat content was subsequently calculated by subtracting the total amount of heat production and heat loss over the experimental protocol. The amount of evaporation required to achieve heat balance (E_{req}) was defined as the combination of metabolic heat production and dry heat exchange.

Local sweat production was measured using the ventilated capsule technique. A 3.8 cm^2 plastic capsule was attached to three skin sites (upper back, chest and forearm) with an adhesive ring and topical skin glue (Collodion HV, Mavidon Medical products, Lake Worth, FL, USA). Compressed dry air was passed through the capsule at a rate of 1.0 L·min^{-1}. Water content of the effluent air was measured using high precision dew point mirrors (model 473, RH systems, Albuquerque, NM, USA). Local sweat rate was calculated using the difference in water content between effluent and influent air multiplied by the flow rate and normalized for the skin surface area under the capsule. Laser-Doppler velocimetry was employed to measure skin blood flow (PeriFlux System 5000, Main control unit; PF5010 LDPM). A laser-Doppler probe (Perimed

integrating probe 413, Järfälla, Sweden) was affixed to the forearm's surface in an area which did not seem overly vascular upon visual inspection and provided stable readings at rest. To measure maximal skin blood flow, the heater housing the laser-Doppler probe was heated to 44°C at the end of Rec3 until maximal skin vasodilation was achieved (~30-40 minutes) (Taylor *et al.*, 1984).

Esophageal temperature was measured using a thermocouple temperature probe (Mon-a-therm General Purpose Temperature Probe, Mallinckrodt Medical Inc., St-Louis, MO, USA). The esophageal probe was inserted 40 cm past the nostril entrance while the participants sipped water (100-300 mL) through a straw. Mean skin temperature was calculated as the weighted average of 4 skin temperature measurements: bicep 30%, chest 30%, quadriceps 20%, and front calf 20% (Ramanathan, 1964). Data were collected in 15-s intervals and were displayed and recorded in spreadsheet format using a HP Agilent data acquisition module (model 3497A; Agilent Technologies Canada Inc., Mississauga, ON, Canada) and a personal computer with LabVIEW software (Version 7.0, National Instruments, Austin, TX, USA).

Heart rate was monitored, recorded continuously, and stored using a Polar coded WearLink and transmitter, Polar RS400 interface, and Polar ProTrainer 5 software (Polar Electro Oy, Finland). Pre-experimental session urine specific gravity was determined in duplicate using a handheld total solids refractometer (model TS400, Reichter Inc., Depew, NY, USA).

Data and Statistical analysis

Minute averages for all variables were calculated and used to obtain values for the end of each exercise (Ex) bout and recovery (Rec) period. Baseline values were obtained by averaging the last 5–10 min of data during the 30-min baseline resting period. Mean body temperature was

calculated as: 0.9 × esophageal temperature + 0.1 × mean skin temperature (Shibasaki *et al.*, 2006). Whole-body evaporative heat loss was plotted against the corresponding mean body temperature. The onset threshold and thermosensitivity of whole-body evaporative heat loss during each exercise period was determined using the linear portion of each response and analyzed using a segmented regression analysis as described by Cheuvront *et al.* (2009) with aid of a computer algorithm (GraphPad Prism 6.0, GraphPad Software, La Jolla, CA, USA) (Cheuvront *et al.*, 2009). The onset threshold was determined by plotting evaporative heat loss over time and determining visually the point at which it increased over three consecutive measurements. The corresponding mean body temperature at that time point was selected as the onset threshold. The thermosensitivity was determined as the slope of the relationship between evaporative heat loss and mean body temperature for each exercise period. Time constant (τ, the time it takes to reach 63.2% of the total response) and amplitude (the difference between the evaporative heat loss value at the onset and at the end of each exercise bout) values were calculated for evaporative heat loss at each exercise and recovery heat load. Whole-body sweat production at the end of each Ex and Rec bout (in $g \cdot min^{-1}$) was calculated as evaporative heat loss (in W) multiplied by 60 s and divided by the latent heat of vaporisation of sweat (2426 $J \cdot g$ of $sweat^{-1}$).

Dependent variables of rates of metabolic heat production, total heat loss, evaporative heat loss, dry heat exchange and E_{req}, as well as esophageal and mean skin temperatures, local sweat rates, skin blood flow, and heart rate responses were analyzed using a two-way analysis of variance (ANOVA). The ANOVAs were performed with one factor of group (four levels: young, middle-aged trained, middle-aged untrained and older) and repeated factor of exercise time (three levels: Ex1, Ex2, Ex3) or recovery time (three levels: Rec1, Rec2, Rec3). Additionally,

changes in body heat content, physical characteristics, and baseline values were analyzed using a one-way ANOVA to identify differences between groups. When a significant main effect was observed, period- and group-specific *post hoc* comparisons were carried out using the Newman-Keuls procedure. The level of significance for all analyses was set at $P \leq 0.05$. When $0.05 \leq P \leq 0.10$, the effect size (eta squared, η^2) is reported. Statistical analyses were performed using commercially available statistical software (GraphPad Prism 6.0, GraphPad Software, La Jolla, CA, USA). All values are reported as mean ± standard deviation unless otherwise indicated.

RESULTS

Hydration status

On the day of the experimental session, all participants were well hydrated according to urine specific gravity, a measure of hydration status, with similar values between groups (young: 1.013 ± 0.010, middle-aged trained: 1.014 ± 0.011, middle-aged untrained: 1.016 ± 0.008 and older: 1.014 ± 0.009, P=0.907). For all groups, baseline urine specific gravity ranged between 1.002 and 1.020, which were below the cut-off value of >1.020 (Casa *et al.*, 2000).

Whole-body direct calorimetry

Baseline and Exercise

The rate of metabolic heat production at baseline was similar for young (117 ± 19 W), middle-aged trained (123 ± 9 W), middle-aged untrained (112 ± 11 W) and older (112 ± 13 W) males (P=0.212). By experimental design, the rate of metabolic heat production was similar between groups (P=0.758) during each of the exercise periods (average across groups: Ex1 = 305

± 18; Ex2: 404 ± 18; Ex3: 504 ± 17 W). The rates of evaporative heat loss and E_{req} during exercise are shown in Figure 1. The rate of evaporative heat loss at baseline was similar for young (146 ± 42 W), middle-aged trained (150 ± 46 W), middle-aged untrained (132 ± 40 W) and older (125 ± 34 W) males (P=0.516). However, a main effect of group on the rate of evaporative heat loss (P=0.002) was detected during exercise, whereby, the rate of evaporative heat loss was greater for the young and middle-aged trained compared to the middle-aged untrained and older males at the end of Ex2 (P<0.05) and Ex3 (P<0.05). Rates of dry heat exchange during baseline were not different between the young (-77 ± 16 W), middle-aged trained (-83 ± 12 W), middle-aged untrained (-79 ± 11 W) and older (-78 ± 12 W) males (P=0.696). Likewise, there were no differences in dry heat exchange between groups at the end of each exercise bout, an indication of the amount of heat gained by the body from the environmental heat load (average between groups: Ex1 = -105 ± 14; Ex2: -114 ± 17; Ex3: -117 ± 20 W, P=0.384). As a consequence, the total heat load for each exercise (rate of metabolic heat production and dry heat gain or E_{req}) was similar between groups (Ex1 = 410 ± 28; Ex2: 518 ± 25; Ex3: 621 ± 25 W; P=0.443). Whole-body sweat rate was significantly different between groups during exercise (P=0.001), whereby, whole-body sweat rate was significantly greater in the young and middle-aged trained groups compared to the older and middle-aged untrained groups at the end of Ex2 (P<0.05) and Ex3 (P<0.05) (Table 3).

The changes in body heat content are presented in Figure 2. There was a main effect of group on change in body heat content during exercise (P<0.001). The young males had a lower change in body heat content for all three exercise periods (Ex1-3) compared to the middle-aged untrained (percent difference from young = Ex1: 48%; Ex2: 55%; Ex3: 57%) and older males (percent difference from young = Ex1: 47%; Ex2: 57%; Ex3: 54%, P<0.05). Additionally, the

middle-aged trained males had a lower change in body heat content for Ex3 (P<0.05) compared to the middle-aged untrained (percent difference from middle-aged trained for Ex3 = 44%) and older (percent difference from middle-aged trained for Ex3 = 41%) males.

Recovery

There was a rapid decline in the rate of metabolic heat production following each exercise bout which occurred to a similar extent between groups (P=0.454). The rates of evaporative heat loss and E_{req} for recovery are presented in Figure 1. There was no main effect of group on evaporative heat loss (P=0.326) at the end of the recovery periods. However, there was a main effect of group on the rate of dry heat exchange (P=0.046) such that the young males (-78 ± 8 W) had a lower rate of dry heat exchange compared to both the middle-aged trained (-102 ± 14 W) and untrained males (-97 ± 16 W) at the end of Rec3 (P<0.05). Nevertheless, there was no difference in E_{req} (P=0.065, η^2=0.20) between groups. Moreover, there was no main effect of group on whole-body sweat rate (P=0.271) during recovery (Table 3).

The changes in body heat content during recovery are presented in Figure 2. The change in body heat content was similar between groups during recovery (P=0.255). However, there was a main effect of group on the residual change in body heat content (Ex plus Rec) (P=0.001). As such, the middle-aged untrained and older males had 61% and 53% significantly greater cumulative changes in body heat content respectively, compared to the young adults. Furthermore, the cumulative changes in body heat content were 35% and 28% in the middle-aged untrained and older males, respectively, compared to the middle-aged trained males (P<0.05). There were no differences between the young and middle-aged trained groups (P>0.05).

Onset thresholds and thermosensitivities, time constant and amplitude values for whole-body evaporative heat loss

Exercise

Mean body temperature onset thresholds, thermosensitivities, time constant and amplitude values for whole-body evaporative heat loss during each exercise bout are presented in Table 2. The mean body temperature onset threshold for whole-body evaporative heat loss did not differ between groups (P=0.294). However, a main effect of group on thermosensitivity was observed (P=0.001). For Ex1, the young males had a significantly greater thermosensitivity for the whole-body evaporative heat loss response compared to the other three groups (P<0.05). Additionally, during the third exercise bout, the young and middle-aged trained males had a significantly greater thermosensitivity compared to both the middle-aged untrained and older males (P<0.05). Furthermore, there was a significant difference between groups in time constant values for the rate of evaporative heat loss during exercise (P=0.045). This was demonstrated by the young having a shorter time to reach 63.2% of the evaporative heat loss response during the third exercise bout compared to the other three groups. Likewise, the amplitude for evaporative heat loss was different between groups (P=0.036) such that the young and middle-aged trained males had a significantly greater amplitude for Ex3 compared to both the middle-aged untrained and older males.

Recovery

Time constant and amplitude values during recovery for whole-body evaporative heat loss during each exercise bout are presented in Table 2. There was a significant difference

between groups in time constant values for the rate of evaporative heat loss during recovery (P<0.001). The young had a shorter time to reach 63.2% of the evaporative heat loss response during Rec2 compared to the other three groups, and during Rec3 compared to the middle-aged untrained and older males. Similarly, the amplitude for evaporative heat loss was different between groups (P=0.050). As such, the young had larger amplitude values for Rec2 relative to the older males and the young and middle-aged trained males had larger amplitude compared to the middle-aged untrained and older males for Rec3.

Esophageal and mean skin temperatures

Baseline and Exercise

Esophageal and mean skin temperatures are presented in Figure 3. Esophageal temperatures during baseline were not different between the young (36.77 ± 0.40°C), middle-aged trained (36.72 ± 0.22°C), middle-aged untrained (36.83 ± 0.24°C) and older (36.83 ± 0.26°C) males (P=0.787). However, there was a main effect of group on esophageal temperature during exercise (P=0.013). This was evidenced by the young and middle-aged trained males having lower esophageal temperatures at the end of Ex3 compared to middle-aged untrained and older males (P<0.05). Baseline values for mean skin temperature were not different between the young (35.33 ± 0.66°C), middle-aged trained (35.26 ± 0.44°C), middle-aged untrained (35.16 ± 0.37°C) and older (35.26 ± 0.48°C) males (P=0.882). Likewise, there was no difference between groups for mean skin temperature (P=0.765) during exercise. Similarly, when values were examined from a change from baseline, a main effect of group on esophageal temperature during exercise (P=0.016) was observed. The young and middle-aged trained males demonstrated smaller changes in esophageal temperatures at the end of Ex3 (young: 0.65°C, middle aged

trained: 0.57°C) compared to middle-aged untrained (1.10°C) and older (0.95°C) males (P<0.05). However, there was no difference in the change in mean skin temperature (P=0.357) during exercise between groups.

Recovery

Esophageal and mean skin temperatures at the end of recovery are presented in Figure 3. There was no main effect of group on esophageal temperature during recovery (P=0.061, η^2=0.24). Likewise, mean skin temperature was not different between groups during recovery (P=0.256). When values were compared from a change in baseline, there remained no differences in esophageal (P=0.073, η^2=0.21) or mean skin temperature (P=0.137) between groups during the recovery period.

Local heat loss and heart rate responses

Baseline and Exercise

Local sweat rate (chest, back, forearm), skin blood flow (percentage of maximum), and heart rate values are presented in Table 3. There were no differences in baseline sweat rates between groups on the chest (young: 0.16 ± 0.13; middle-aged trained: 0.18 ± 0.08; middle-aged untrained: 0.15 ± 0.09; older: 0.14 ± 0.06 $mg \cdot min^{-1} \cdot cm^{-2}$, P=0.840), back (young: 0.14 ± 0.09; middle-aged trained: 0.18 ± 0.09; middle-aged untrained: 0.14 ± 0.09; older: 0.16 ± 0.08 $mg \cdot min^{-1} \cdot cm^{-2}$, P=0.818) or forearm (young: 0.13 ± 0.08; middle-aged trained: 0.15 ± 0.07; middle-aged untrained: 0.15 ± 0.09; older: 0.18 ± 0.10 $mg \cdot min^{-1} \cdot cm^{-2}$, P=0.693). Similarly, sweat rates were similar between groups during exercise at the chest (P=0.734), back (P=0.404), forearm (P=0.903). Likewise, local skin blood flow was similar between groups at baseline

(young: 32.9 ± 10.7; middle-aged trained: 41.3 ± 9.5; middle-aged untrained: 29.6 ± 15.1; older: 41.0 ± 14.4% of max, P=0.129) and for all three exercise bouts (P=0.219). There was a tendency for heart rate to be different between groups (P=0.057, η^2=0.86) at the end of the exercise bouts such that the middle-aged trained males had a lower heart rate compared to the middle-aged untrained males at the end of Ex2 (P<0.05) and Ex3 (P<0.05).

Recovery

Local sweat rates, skin blood flow and heart rate values for recovery are presented in Table 3. Sweat rates were similar between groups at the chest (P=0.109) and back (P=0.443) during recovery, however a main effect of group was measured on local forearm sweating (P=0.014). At the forearm local sweat rate site, sweating was lower for the young and middle-aged trained males compared to middle-aged untrained and older males at the end of Rec3 (P<0.05). In contrast, no differences in local skin blood flow (P=0.176) or heart rate (P=0.060, η^2=0.54) were measured between groups during each of the recovery periods.

DISCUSSION

The main finding of the present study is that aging alters the physiological capacity to dissipate heat. This was evident by the fact that older males exhibited a reduced whole-body evaporative heat loss compared to young males at exercise-induced heat loads as low as 400 W. Furthermore, middle-aged untrained males had a similar level of impairment compared to the older males, matched for aerobic fitness (i.e., VO_{2peak}). Conversely, we observed that middle-aged trained males maintain a higher rate of heat dissipation than their untrained counterparts, such that the rate of heat dissipation was similar to that measured in young males. As a result, the

rate of heat storage was markedly greater in older and middle-aged untrained males compared to young and middle-aged trained males.

Effects of age on the physiological capacity to dissipate heat

In the current study, we employed a unique incremental exercise model involving progressive increases in heat load (and therefore E_{req}) to determine the threshold at which age-related differences in the body's physiological capacity to dissipate heat occurs. Of note, when we factor in the heat gained from the environment (i.e., dry heat gain), the net heat loads, or E_{req}, were ~400 W for Ex1, ~500 W for Ex 2, and ~600 W for Ex 3 (Figure 1). In the present study, we showed that whole-body evaporative heat loss was reduced in both middle-aged untrained and older males compared to young males at the end of the two highest heat loads employed (Figure 1). Similar findings were reported by previous studies employing an exercise-induced heat load similar to that of Ex2 in our study (i.e., 400 W), albeit lower environmental stress (35°C and 20% RH), and therefore minimal dry heat gain (Larose *et al.*, 2013a; Larose *et al.*, 2013d). As such, heat loads greater than ~400 W, derived from a combination of metabolic and environmental heat, are sufficient to exceed the physiological capacity to dissipate heat of middle-aged as well as older adults matched for aerobic fitness. In line with our study hypothesis, as the heat load increased, the degree of impairment in whole-body evaporative loss was greater in both the middle-aged untrained and older adults. While the young adults were able to increase their rate of whole-body evaporative heat loss from Ex1 to Ex 2 by ~28% and Ex2 to Ex3 by ~18%, the older adults had an increase in whole-body evaporative heat loss from Ex1 to Ex2 of ~26% and from Ex2 to Ex3 of only ~14%. This translated into 69 and 95 kJ of greater body heat storage at the end of Ex2 and Ex3, respectively, in the older compared to the young

adults. Although, a true measure of maximal heat loss capacity would be observed if no increase in the rate of whole-body heat loss occurred from Ex2 to Ex3, our observations provide evidence to indicate that both the middle-aged untrained and older adults are approaching near maximal levels of heat dissipation.

Insight into whether impairment in heat loss occurs centrally (neural activity/integration) or peripherally (end organ) can be gleaned from examining the mean body temperature at which heat loss is activated (central) or from the rate of rise in the heat loss response relative to increasing mean body temperature (peripheral) (Nadel *et al.*, 1971; Gisolfi & Wenger, 1984). While the mean body temperature onset threshold of evaporative heat loss was not different in each group (Table 2), the thermosensitivity of the evaporative heat loss response was reduced in the middle-aged untrained and older males. These results are consistent with previous studies that have reported that older adults have a reduced rate of heat loss (i.e., sweating) for a given increase in mean body temperature (Anderson & Kenney, 1987; Inbar *et al.*, 2004; Larose *et al.*, 2013d), which is indicative of a peripheral modification (Nadel *et al.*, 1971). In our study, we observed the greatest level of impairment during Ex3 where the time constant of the evaporative heat loss response was lower in young (4.8 ± 1.4 min) compared to both middle-aged untrained (7.0 ± 2.6 min) and older (8.5 ± 2.3 min) males (Table 2). The lower thermosensitivities and greater time constants were coupled with reduced amplitude of evaporative heat loss during the third exercise bout. It has been suggested that the attenuated heat loss response in older adults during heat stress is due to a decrease in cholinergic sensitivity and lower sweat gland output, rather than fewer heat activated sweat glands ultimately resulting in a reduced capacity for heat dissipation in older adults (Anderson & Kenney, 1987; Kenney & Fowler, 1988). We showed that this may be true for middle-aged untrained males as well. As a consequence of the reduced

thermosensitivity and magnitude of evaporative heat loss, the change in body heat content was greater in middle-aged untrained and older males relative to their younger counterparts, respectively by 48 and 47% for Ex1, 55 and 57% for Ex2 and 57 and 54% for Ex3.

Effects of aerobic fitness on age-related impairments in heat loss

We observed that the middle-aged trained group had a significantly greater capacity for whole-body heat dissipation at the moderate (400 W) and high (500 W) heat load conditions compared to the age-matched untrained group (Figure 1). The differences in heat loss between the two middle-aged groups were solely due to the level of aerobic fitness, since the middle-aged trained males participated in regular endurance-type exercise training (i.e., running, cycling, cross country skiing), while the untrained males did not. Furthermore, participants were matched for physical characteristics such as body mass, body surface area, and body composition, which are known to influence heat dissipation and therefore body heat storage (Havenith & van Middendorp, 1990; Selkirk & McLellan, 2001). The increased capacity for heat dissipation in middle-aged trained males was the result of an increased thermosensitivity of the evaporative heat loss response combined with greater amplitude of increase in the rate of evaporative heat loss (Table 2). As such, evaporative heat loss reached significantly greater values for a given mean body temperature in the trained compared to untrained middle-aged counterparts in addition to the higher rate of evaporative heat loss at the last two exercise bouts. This finding is consistent with previous studies showing that endurance-trained adults demonstrate greater thermosensitivity of the sweating and/or skin blood flow response when compared to their untrained counterparts (Henane *et al.*, 1977; Tankersley *et al.*, 1991). It has been proposed by some that this response is in part attributed to partial acclimation caused by regular vigorous

exercise training (Gisolfi & Robinson, 1969; Buono & Sjoholm, 1988; Havenith & van Middendorp, 1990; Cheung & McLellan, 1998; Selkirk & McLellan, 2001). In the present study, the greater rate of heat dissipation measured in the middle-aged trained adults translated into a 20% and 44% reduction in the amount of heat stored in the moderate and high heat load exercise bouts (i.e., Ex2 and Ex3), respectively, relative to the middle-aged untrained group. Future studies should be conducted to assess differences between non-trained adults with a low versus high VO_{2peak} to discern if the level of VO_{2peak} per se (likely genetically determined) may influence this response.

Improvements in thermoregulatory capacity due to high levels of aerobic fitness are not always observed. Jay *et al.* (2011) examined two groups of young (19 – 24 y) males matched for body mass and surface area during exercise for 60 min at a fixed rate of metabolic heat production (540 W) in a thermoneutral environment (Jay *et al.*, 2011). Jay *et al.* (2011) reported no differences in rectal temperature, whole-body sweat rate or local sweat rate (upper back) between the two groups despite the large differences in VO_{2peak} (~40 vs. 60 $mL \cdot kg^{-1} \cdot min^{-1}$). Thus, while aerobic fitness may not be a factor affecting heat loss in young males when exercising at moderate levels of heat production, we showed that maintaining a higher level of aerobic fitness in middle-aged males promotes a greater capacity to dissipate heat. Furthermore, consistent with previous studies, when older untrained are compared with older trained adults during exercise (35-67% VO_{2peak}), heat loss responses of sweating and skin blood flow are reported to be lower in the older untrained adults (Tankersley *et al.*, 1991; Inoue *et al.*, 1999a). However, it is unknown if results would have been the same if both groups had exercised at the same rate of heat production, thereby providing a similar thermal drive (Gagnon *et al.*, 2013b).

Therefore, further research is required to examine the effect of aerobic fitness with increasing age (i.e., >60 years).

Whole-body calorimetry vs. core temperature and local heat loss responses

An interesting finding of the current study is that we only observed a significantly greater increase in esophageal temperature at the end of the third exercise bout (Figure 3), despite the greater change in body heat content measured at all heat loads in the older and middle-aged untrained males compared to their younger counterparts. Ultimately, direct calorimetry allows us to precisely measure the amount of heat that is stored in the body irrespective of differences in tissue heat distribution that may occur as a result of age-related changes in tissue blood distribution during and following exercise. While changes in esophageal temperature provide us with an indication of where some of the heat is stored, it cannot be used as a reliable measure to quantify whole-body heat storage (Kenny & Jay, 2013). By the same logic, the reverse is correct. The calorimeter can only tell us how much heat is stored in the body, but not where it is stored. Of note, the measurement of esophageal temperature is affected by the powerful vascular changes that occur in this region. This effect is clearly observed in our study findings such that, as noted above, we only observed a significantly greater increase in esophageal temperature by the end of the third exercise bout between the middle-aged untrained and older group compared to both the young and middle-aged trained group. In contrast, we observed significant differences in heat storage by Ex1 for the middle-aged untrained and older males relative to the young and middle-aged trained males. From a practical standpoint, measurement of body core temperature using esophageal temperature may underestimate the level of thermal strain

experienced by a middle-age untrained and older adult exercising in the heat especially when exercise is of short duration and/or low intensity.

In the present study, age-related differences in whole-body evaporative heat loss (and therefore sweat production) were not paralleled by similar differences in local sweat rate at the three skin sites. Previous studies have reported within-group regional differences in sweating during passive heating and/or exercise within groups (Inoue *et al.*, 1991; Inoue & Shibasaki, 1996; Gagnon & Kenny, 2012b; Smith *et al.*, 2013a; Smith *et al.*, 2013b). However, the measurement of whole-body evaporative heat loss by direct calorimetry allows us to precisely measure the net consequences of aging per se on whole-body sweat production without the confounding effects of regional differences in sweating. While age-related differences in local skin blood flow responses have been observed (Kenney *et al.*, 1997; Holowatz *et al.*, 2003; Holowatz *et al.*, 2005), the pattern of response can differ between skin sites (Inoue *et al.*, 1991; Inoue & Shibasaki, 1996; Gagnon & Kenny, 2012b; Smith *et al.*, 2013a; Smith *et al.*, 2013b). In the present study, we only measured skin blood flow at one site, thus it is plausible that differences might have been observed at other sites. Taken together, our findings demonstrate that caution should be used when employing surrogate measures of body heat storage (i.e., core temperature) and local heat loss responses for the purpose of assessing age-related differences in the body's physiological capacity to dissipate heat, especially at low to moderate heat loads. These thermoregulatory measures can underestimate the extent to which aging may alter the body's physiological capacity to dissipate heat.

Postexercise response

We observed a rapid reduction in whole-body evaporative and local heat loss in all groups at the cessation of exercise despite sustained elevations in esophageal temperature, which is the typical pattern of response postexercise (Kenny *et al.*, 2007; Kenny & Jay, 2013). The rapid reductions in heat loss has been attributed to nonthermal factors overriding the thermal control of heat loss during postexercise recovery in young adults (Kenny *et al.*, 2007; Kenny & Jay, 2013), thus altering the body's ability to dissipate heat during recovery. In the present study, we observed lower amplitude of change in evaporative heat loss for the third recovery period in the middle-aged untrained and older males compared to their young and middle-aged trained counterparts (Table 2). Furthermore, it took a longer amount of time to achieve ~63% of the evaporative heat loss response for Rec3 in older males. These findings may imply that the level of influence of nonthermal factors on heat loss postexercise may be reduced in middle-aged untrained and older adults as a result of the greater thermal drive, associated with the progressively greater heat storage during exercise. Moreover, we see that the rates of whole-body heat loss are slightly elevated in the middle-aged untrained and older males compared to the young and middle-aged trained males during the third recovery period. It is possible that the greater rates of heat loss during recovery in the middle-aged untrained and older groups are due to some compensatory mechanism to try to offset the greater amounts of heat stored during exercise. Therefore, it brings into question if these differences would become more pronounced had we extended the recovery time period. Despite the few previous studies examining heat loss during the postexercise recovery period in older adults (Larose *et al.*, 2013d; Larose *et al.*, 2014; Stapleton *et al.*, 2014), future research is required to examine the potential influence of thermal

and nonthermal factors on heat loss responses postexercise as a function of age and aerobic fitness.

Limitations

Due to technical limitations associated with performing measurements of whole-body heat loss in a calorimeter (i.e., subject must remain isolated in the calorimeter, access in and out of temperature chamber housing the calorimeter is restricted during the trial), we were unable to measure changes in hydration status during the experimental trial. As a consequence, we were unable to determine the potential effects of age-related differences in fluid distribution on whole-body heat dissipation as a function of increases in exercise-induced heat loads. However, by design we employed an incremental exercise model that required that the young, middle-aged and older adults exercise at the same fixed absolute heat load and therefore requirement for heat loss. This experimental model was used to ensure that any differences in sweating (and therefore fluid loss) would be the result of age-related differences and not due to differences in the requirement for heat loss. Additional research is required to determine how changes in hydration status may influence this pattern of response as a function of increasing heat loads.

Perspectives

Advanced aging is associated with adverse changes to the thermoregulatory system, but when do these alterations matter? The results from this study provide important insight into the level of heat load in which differences in the capacity to dissipate heat occur as a function of age and aerobic fitness. We showed for the first time that the impairment in thermoregulatory function in older as well as middle-aged untrained males is heat load dependent. Thus, our

results can be used by health care providers, exercise specialists, health and safety managers, sporting event organizers (i.e., marathons, soccer tournaments, etc.) and others to better understand the level of heat stress in which dangers associated with exercise in hot conditions occur and decide when it is safe to perform physical activity in the heat. As such, it is critical that the previously mentioned groups re-evaluate heat exposure thresholds and adjust these limits to include information based on the age as well as level of aerobic fitness of the individual to reduce the risk of heat-related illness/injury for all adults. However, during a work shift, it is possible that older adults self-pace differently relative to their younger counterparts as a way to mitigate the level of physical/thermal strain they experience. Thus, while older adults, and/or those who are less physically active, might have a reduced capacity to dissipate heat during work in the heat, they may experience similar levels of thermal strain as their younger and/or endurance-trained counterparts when allowed to self-pace. Furthermore, the participants in the present study were relatively healthy. Therefore, older adults with health conditions such as cardiovascular disease, diabetes etc. may be at an even greater risk of heat-related illness and/or injury during work in the heat.

Summary

Our findings demonstrate that age-related impairments in whole-body evaporative heat loss in middle-aged untrained and older relative to young males are evident at exercise-induced heat loads as low as 400 W. This impairment in the capacity to dissipate heat is characterized by a reduced thermosensitivity of the evaporative heat loss response as well as a lower level of whole-body heat loss achieved during exercise. Ultimately, the inability to dissipate heat to the same extent as younger adults led to a 1.5 to 1.6-fold greater increase in heat storage in the older

and middle-aged untrained males, respectively. However, the age-related impairments in the body's physiological capacity to dissipate heat can be minimized through maintaining a high level of aerobic fitness (as defined by VO_{2peak}).

ACKNOWLEDGEMENTS

We would like to thank all the participants who volunteered for the present study. The authors would like to thank Mr. Michael Sabino of Can-Trol Environmental Systems Limited (Markham, ON, Canada) for their support. The authors would also like to thank all the members of the Human and Environmental Physiology Research Unit who assisted with data collection.

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DISCLOSURES

The authors disclose that they have no conflicts interests.

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FIGURES

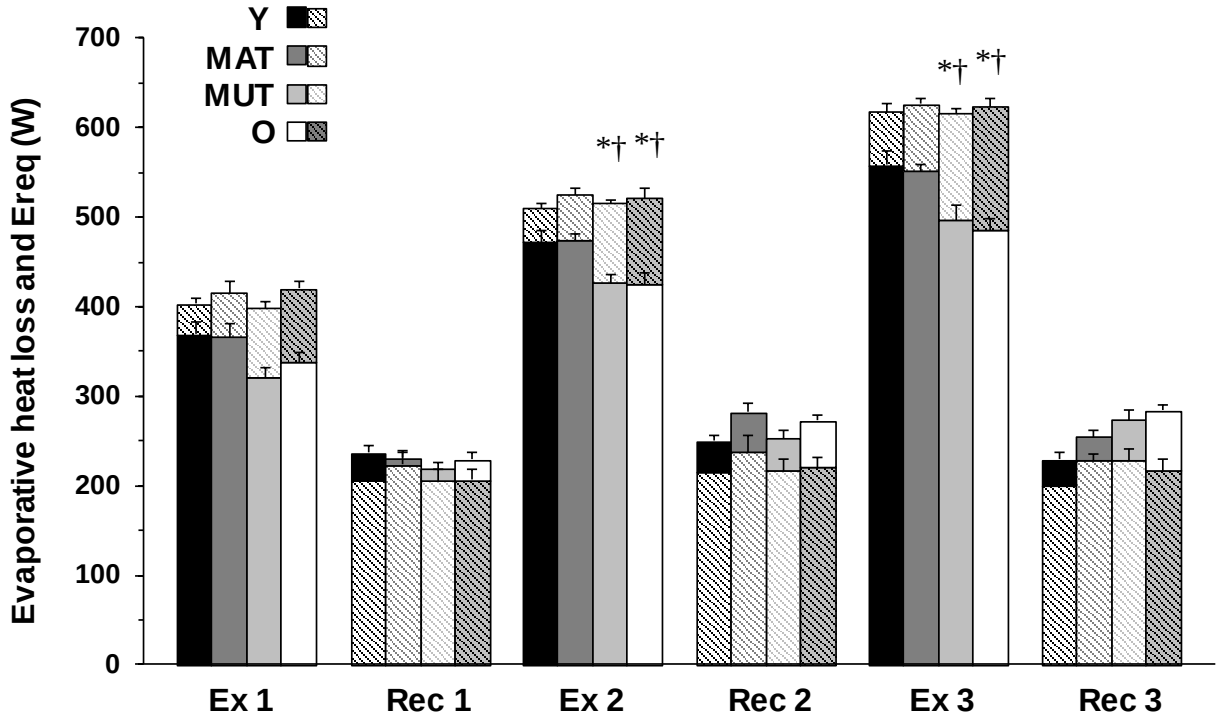

Figure 1. Mean ± standard error values for evaporative heat loss (solid bars) and the required amount of evaporation for heat balance (E_{req}, striped bars) measured over three 30-min exercise bouts (Ex 1, Ex 2 and Ex 3) and three 15-min recovery bouts (Rec 1, Rec 2 and Rec 3) in a hot, dry (40°C, 15% RH) environment. The black bars/stripes represent the young (Y) group, the dark grey bars/stripes represent the middle-aged trained (MAT) group, the light grey bars/stripes represent the middle-aged untrained (MUT) group and the white bars/grey and black stripes represent the older (O) group. There were no differences in the required amount of evaporation for heat balance between groups. Significant difference in evaporative heat loss from young is denoted by an asterisk (*). Significant difference in evaporative heat loss from middle-aged trained is denoted by a cross (†). Significance level was set at $P \leq 0.05$.

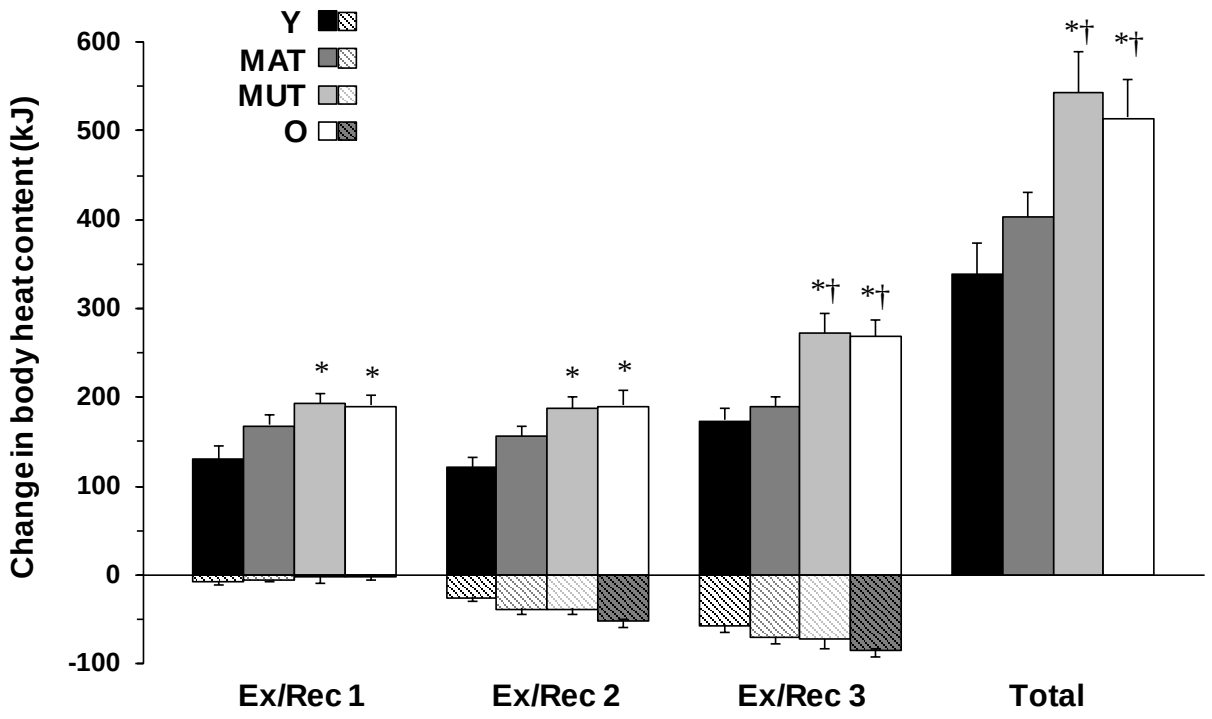

Figure 2. Mean ± standard error values for changes in body heat content during each exercise/recovery cycle as well as the total change in body heat content over the exercise protocols in a hot, dry (40°C, 15% RH) environment. The solid bars represent changes in body heat content during exercise and the striped bars represent the changes in body heat content during recovery. The black bars/stripes represent the young (Y) group, the dark grey bars/stripes represent the middle-aged trained (MAT) group, the light grey bars/stripes represent the middle-aged untrained (MUT) group and the white bars/grey and black stripes represent the older (O) group. Significantly different from young is denoted by an asterisk (*). Significantly different from middle-aged trained is denoted by a cross (†). Significance level was set at $P \leq 0.05$.

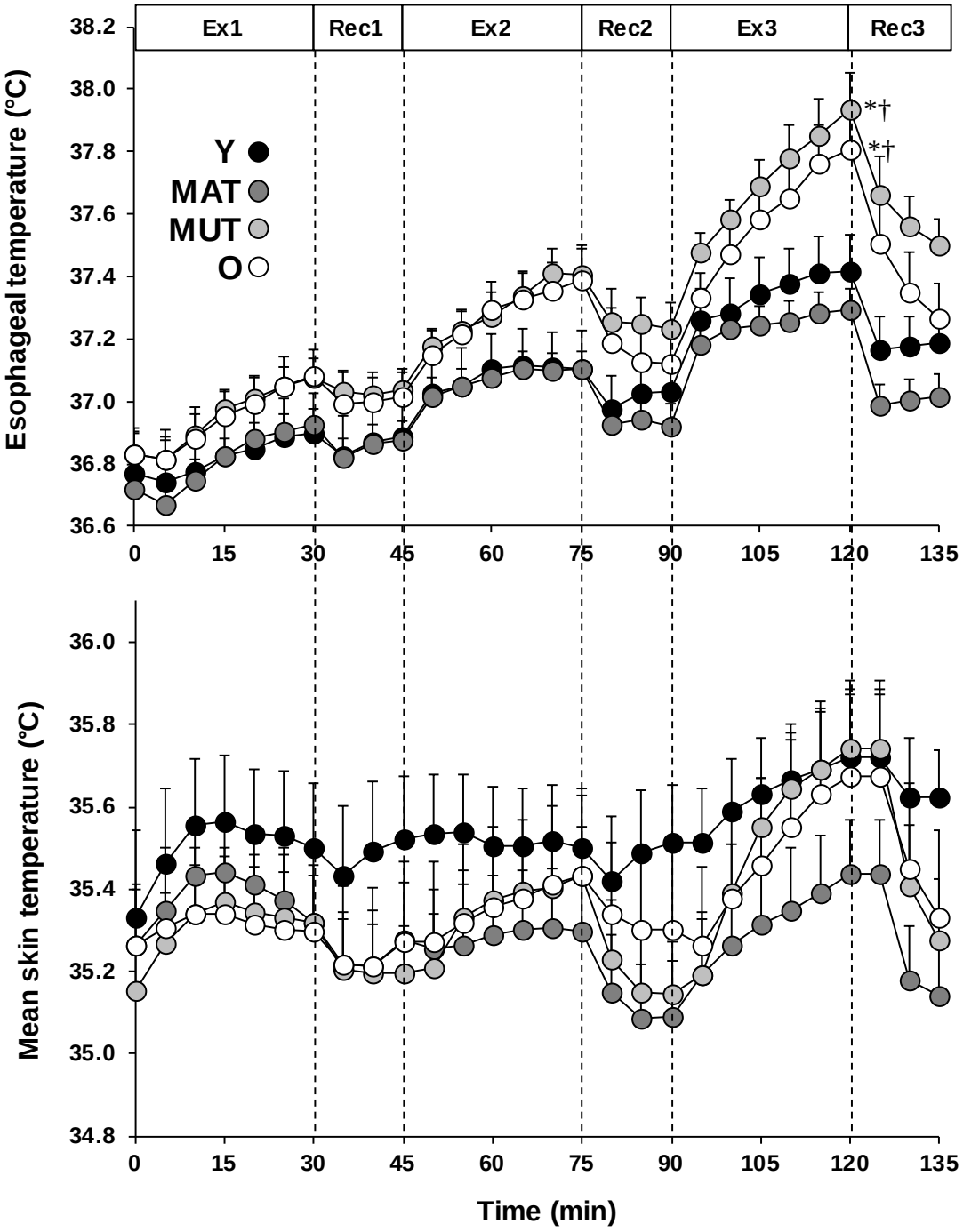

Figure 3. Mean ± standard error values for esophageal and mean skin temperatures during each exercise/recovery cycle in a hot, dry (40°C, 15% RH) environment. The black circles represent the young (Y) group, the dark grey circles represent the middle-aged trained (MAT) group, the light grey circles represent the middle-aged untrained (MUT) group and the white circles represent the older (O) group. Significantly different from young is denoted by an asterisk (*). Significantly different from middle-aged trained is denoted by a cross (†). Analysis was performed at the end of each exercise/recovery period. Significance level was set at $P \leq 0.05$.

TABLES

Table 1. Participant characteristics.

	Age (years)	Height (cm)	Mass (kg)	BSA (m^2)	VO_{2peak} ($mL \cdot kg^{-1} \cdot min^{-1}$)	Body fat (%)
Y	21 ± 1	179 ± 6	79 ± 9	1.98 ± 0.12	50.0 ± 3.7	13.4 ± 3.3
MAT	49 ± 5*	181 ± 5	82 ± 8	2.02 ± 0.11	51.0 ± 6.8	19.1 ± 4.3
MUT	48 ± 5*	179 ± 8	82 ± 12	2.00 ± 0.18	37.3 ± 3.5*†	23.1 ± 6.3*
O	65 ± 3*	176 ± 5	80 ± 7	1.96 ± 0.06	37.9 ± 7.8*†	21.2 ± 5.6*

Values are mean ± standard deviation. Y, young. MAT, middle-aged trained. MUT, middle-aged untrained. O, older. BSA, body surface area. VO_{2peak}, peak oxygen uptake. *Significant difference from young males. †Significant difference from middle-age trained males.

Table 2. Time constants, amplitudes, onset thresholds and thermosensitivities of evaporative heat loss for each exercise/recovery bout.

τ, min	**Ex1**	**Rec1**	**Ex2**	**Rec2**	**Ex3**	**Rec3**
Y	11.0±10.4	2.99±1.67	6.5±3.1	3.09±0.74	4.8±1.4	2.87±1.03
MAT	16.4±5.4	5.69±4.70	8.0±5.2	5.39±1.22*	7.9±3.1*	3.93±1.08
MUT	21.9±14.1	3.98±1.85	6.9±2.9	5.84±0.91*	7.0±2.6*	5.51±2.10*
O	14.0±6.1	5.09±2.90	8.7±3.2	7.33±2.60*	8.5±2.3*	7.09±4.66*
Amplitude, W						
Y	172±43	119±71	198±52	223±54	268±51	255±53
MAT	175±46	145±52	207±51	185±51	265±69	272±29
MUT	148±56	110±50	179±46	177±28	215±61*†	201±66*†
O	158±28	129±44	180±31	161±48	189±50*†	189±69*†
Onset threshold of evaporative heat loss, °C						
Y	36.63±0.40		36.82±0.37		36.94±0.33	
MAT	36.57±0.21		36.72±0.20		36.83±0.26	
MUT	36.91±0.70		36.88±0.14		37.08±0.21	
O	36.64±0.28		36.88±0.31		36.97±0.31	
Thermosensitivity of evaporative heat loss, W/°C						
Y	1575±1160		1217±559		1295±629	
MAT	1022±270*		1141±536		1031±352	
MUT	879±415*		833±604		513±238*†	
O	721±318*		694±424		411±263*†	

Values are mean ± standard deviation. Y, young. MAT, middle-aged trained. MUT, middle-aged untrained. O, older. τ, time constant. *Significant difference from young males. Mean body temperature was used to calculated the onset threshold and thermosensitivity. †Significant difference from middle-age trained group.

Table 3. Local heat loss, whole-body sweat rate and heart rate responses during each exercise (Ex)/recovery (Rec) cycle.

LSR -Chest, $mg \cdot min^{-1} \cdot cm^{-2}$	Ex1	Rec1	Ex2	Rec2	Ex3	Rec3
Y	0.37±0.19	0.18±0.11	0.50±0.20	0.18±0.14	0.66±0.26	0.20±0.16
MAT	0.44±0.15	0.29±0.13	0.60±0.26	0.26±0.09	0.79±0.38	0.34±0.15
MUT	0.37±0.16	0.23±0.12	0.55±0.27	0.29±0.14	0.68±0.31	0.40±0.21
O	0.38±0.19	0.22±0.11	0.51±0.17	0.26±0.12	0.58±0.22	0.39±0.25
LSR – Back, $mg \cdot min^{-1} \cdot cm^{-2}$						
Y	0.44±0.24	0.23±0.11	0.54±0.22	0.22±0.10	0.67±0.22	0.27±0.12
MAT	0.44±0.18	0.28±0.14	0.59±0.22	0.26±0.11	0.80±0.25	0.31±0.11
MUT	0.30±0.14	0.23±0.10	0.48±0.20	0.30±0.15	0.60±0.25	0.36±0.17
O	0.39±0.16	0.28±0.13	0.56±0.17	0.35±0.11	0.67±0.15	0.39±0.13
LSR – Arm, $mg \cdot min^{-1} \cdot cm^{-2}$						
Y	0.36±0.13	0.16±0.07	0.53±0.16	0.20±0.10	0.82±0.14	0.24±0.13
MAT	0.40±0.18	0.23±0.07	0.52±0.08	0.25±0.10	0.77±0.23	0.25±0.12
MUT	0.34±0.14	0.29±0.11	0.53±0.22	0.38±0.12	0.77±0.21	0.41±0.15*†
O	0.43±0.17	0.26±0.10	0.55±0.14	0.30±0.10	0.68±0.16	0.43±0.14*†
WBSR, $g \cdot min^{-1}$						
Y	9.1±1.1	5.9±1.1	11.7±1.0	6.2±1.2	13.8±1.3	5.7±0.7
MAT	9.1±1.1	5.7±1.1	11.7±0.5	7.0±1.4	13.7±0.6	6.3±0.6
MUT	8.0±0.9	5.5±1.0	10.5±0.8*†	6.3±1.0	12.3±1.3*†	6.1±2.3
O	8.3±0.9	5.7±0.9	10.5±0.9*†	6.8±0.8	12.0±1.1*†	7.0±1.0
SkBF, % of max						
Y	43.2±12.9	33.9±9.5	44.3±12.8	32.8±9.3	49.1±12.7	30.9±9.0
MAT	49.4±9.9	39.7±16.2	54.4±14.0	44.3±16.9	62.2±15.7	41.0±9.8
MUT	44.6±15.7	32.3±14.5	52.5±16.0	31.8±14.6	54.7±14.9	36.9±13.5
O	51.9±12.0	42.4±9.5	55.1±14.1	41.9±8.9	60.2±16.1	40.2±8.5
HR, beats/min						
Y	95±8	81±10	111±11	84±12	131±14	90±7
MAT	85±14	70±15	99±13	74±14	117±18	79±17
MUT	99±11	85±11	119±18†	92±14	143±23†	101±17
O	93±11	76±8	109±17	84±10	135±14	94±14

Values are mean ± standard deviation. Y, young. MAT, middle-aged trained. MUT, middle-aged untrained. O, older. LSR, local sweat rate. WBSR, whole-body sweat rate. SkBF, skin blood flow. HR, heart rate. *Significant difference from young males. †Significant difference from middle-age trained males.

3.3 Thesis article #3 – at the time of submission of thesis, paper was under second round of reviews with PloOne (reference id: PONE-D-14-44230)

At what level of heat load are age-related impairments in the ability to dissipate heat evident in females?

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Short title: Older females and heat loss capacity

ABSTRACT

Studies have reported that older females have impaired heat loss responses during work in the heat compared to young females. However, it remains unclear at what level of heat stress these differences occur. Therefore, we examined whole-body heat loss [evaporative (H_E) and dry heat loss, via direct calorimetry] and changes in body heat storage (ΔH_b, via direct and indirect calorimetry) in 10 young (23±4 years) and 10 older (58±5 years) females matched for body surface area and aerobic fitness (VO_2peak) during three 30-min exercise bouts performed at incremental rates of metabolic heat production of 250 (Ex1), 325 (Ex2) and 400 (Ex3) W in the heat (40°C, 15% relative humidity). Exercise bouts were separated by 15 min of recovery. Since dry heat gain was similar between young and older females during exercise (p=0.52) and recovery (p=0.42), differences in whole-body heat loss were solely due to H_E. Our results show that older females had a significantly lower H_E at the end of Ex2 (young: 383±34 W; older: 343±39 W, p=0.04) and Ex3 (young: 437±36 W; older: 389±29 W, p=0.008), however no difference was measured at the end of Ex1 (p=0.24). Also, the magnitude of difference in the maximal level of H_E achieved between the young and older females became greater with increasing heat loads (Ex1=10.2%, Ex2=11.6% and Ex3=12.4%). Furthermore, a significantly greater ΔH_b was measured for all heat loads for the older females (Ex1: 178±44 kJ; Ex2: 151±38 kJ; Ex3: 216±25 kJ, p=0.002) relative to the younger females (Ex1: 127±35 kJ; Ex2: 96±45 kJ; Ex3: 146±46 kJ). In contrast, no differences in H_E or ΔH_b were observed during recovery (p>0.05). We show that older habitually active females have an impaired capacity to dissipate heat compared to young females during exercise-induced heat loads of ≥325 W when performed in the heat.

Key words: calorimetry; thermoregulation; women; sweating; skin blood flow; aging

INTRODUCTION

A number of studies have shown that thermoregulatory function (i.e., heat loss through sweating and skin blood flow) during exercise is compromised in older compared to younger males (Lind *et al.*, 1970; Smolander *et al.*, 1990; Tankersley *et al.*, 1991; Inbar *et al.*, 2004; Larose *et al.*, 2013a; Larose *et al.*, 2013d; Larose *et al.*, 2014). However, there have been very few studies that have examined the effects of aging on the body's ability to dissipate heat during exercise in females (Drinkwater & Horvath, 1979; Anderson & Kenney, 1987; Kenney & Anderson, 1988; Larose *et al.*, 2013c), all of which showed that older females have impaired tolerance to work in the heat. While these studies consistently demonstrated that aging in females is associated with impairments in heat dissipation, it remains unclear if the impairment in heat loss only occurs above a certain level of heat load (defined as the sum of metabolic heat production and dry heat exchange) and therefore requirement for heat loss.

At the onset of dynamic exercise, there is an instant and rapid elevation in the rate of metabolic heat production. However, this immediate increase in heat production is not initially offset by an increase in the rate of whole-body heat loss. Thus, the thermal imbalance caused by the lag in the activation of thermoefferent activity (i.e., sweating and skin blood flow) relative to the rapid gain in heat production causes pronounced increases in body heat storage during the early stages of exercise. A decrease in thermosensitivity and/or delay in the onset threshold, as previously shown to occur in older adults (Hellon & Lind, 1956; Tankersley *et al.*, 1991; Kenney *et al.*, 1997; Inbar *et al.*, 2004), would increase the duration of this heat imbalance resulting in a greater change in body heat storage. This is consistent with a recent study by Larose *et al.* (Larose *et al.*, 2013c) who observed marked reductions in whole-body evaporative heat loss in older compared to young females after the first 10 min of exercise. This resulted in greater heat

storage during four short intermittent (i.e., 15 min) exercise bouts at a fixed rate of metabolic heat production of 300 W [equivalent to ~44% peak oxygen uptake (VO_2peak)] in the heat [35°C and 20% relative humidity (RH)] (Larose *et al.*, 2013c).

While the aforementioned study by Larose *et al.* (Larose *et al.*, 2013c) demonstrated age-related differences in the body's physiological ability to dissipate heat occurs in the early stages of exercise during a moderate heat load (metabolic plus environmental heat load equivalent to ~320 W), it was not possible to discern if these age-related differences are observed with extended periods of exercise. As such, it is unknown if older females are able to achieve heat balance (i.e., rate of heat production matched with rate of heat loss), and therefore a stable core temperature, as exercise continues. A sustained reduction in whole-body heat loss would result in a progressive increase in body heat storage. If left unchecked, this could result in a heat-related injury or even death. In fact, previous studies reported that older females demonstrate attenuated local sweat rates as early as 30-min during a 2-h exercise protocol (35–40% VO_2peak) in a hot, dry environment (48°C, 10% RH) compared to younger females matched for aerobic fitness, body surface area, and body adiposity (Anderson & Kenney, 1987; Kenney & Anderson, 1988). A reduction in the level of sudomotor activity achieved for a given heat load, and therefore requirement for heat loss, would result in a greater heat storage and core temperature response as observed in these previous studies (Anderson & Kenney, 1987; Kenney & Anderson, 1988). While these findings provide some evidence to suggest that age-related impairments are sustained with prolonged exercise, it remains unclear if age-related differences in the physiological capacity to dissipate heat remain intact or increase as the heat load becomes greater.

In light of the above knowledge gaps, the purpose of this study was to determine if age-related differences in whole-body heat loss are only evidenced above a certain heat load, and therefore requirement for heat loss, and if the degree of impairment augments with increases in heat load. To achieve this objective, we compared whole-body heat loss as assessed using direct calorimetry in young and older females during three 30-min intermittent exercise bouts performed at increasing levels of metabolic heat production of 250, 325 and 400 W, each separated by 15-min of recovery, under a constant environmental heat load (40°C and 15% RH). These rates of metabolic heat production were chosen to ensure that a compensable heat stress condition was achieved during the first exercise bout, progressing to a fully uncompensable condition during the final exercise bout for the older females. Based on previous studies showing differences in heat loss between young and older females at moderate levels of heat load (i.e., ~320 W or ~40% VO_2peak), we hypothesized that differences in the capacity to dissipate heat between young and older females would occur at or near this threshold and that the magnitude of difference would become greater with progressive increases in heat load. As a consequence, older females would increasingly store more heat with elevated levels of heat stress.

METHODOLOGY

Ethics statement

The experimental protocol was approved by the University of Ottawa Health Sciences and Science Research Ethics Board, in accordance with the Declaration of Helsinki. Volunteers provided written informed consent before participating in the study.

Participants

Twenty females volunteered for the study and were divided into two groups of 10 young (23 ± 4 years) and 10 older (58 ± 5 years) females. Participants were matched for height (young: 1.66 ± 0.02; older: 1.66 ± 0.04 m, p=0.73), body mass (young: 63.6 ± 5.9; older: 60.0 ± 4.9 kg, p=0.16), body surface area (young: 1.70 ± 0.07; older: 1.67 ± 0.08 m^2, p=0.23), body fat percentage (young: 23.9 ± 5.2; older: 24.3 ± 5.7%, p=0.76) and VO_2peak (young: 39.7 ± 8.0; older: 39.0 ± 7.7 $mL \cdot kg^{-1} \cdot min^{-1}$, p=0.80). All participants were non-smokers and did not report any history of hypertension, heart disease, diabetes or autonomic disorders. The young female participants had not taken medications except monophasic oral contraceptive, which provided 30–35 µg of ethinyl estrogen and low dose progestin for 21 days and placebo for 7 days. To control for hormonal effects, the younger females were tested in the early to mid-follicular phase (1-9 days after the onset of menstruation). All older females were postmenopausal; however two of the older females were on hormone replacement therapy. A 3-month recall physical activity questionnaire (Kohl *et al.*, 1988) revealed that all participants were habitually active (i.e., 3-4 days of continuous exercise of 30-60 min in duration).

Experimental design

Each participant completed one preliminary and one experimental session. During the preliminary session, body height, mass, and density, as well as VO_2peak were determined. Body height was determined using a stadiometer (Detecto, model 2391, Webb City, MO, USA), while body mass was measured using a digital high-performance weighing terminal (model CBU150X, Mettler Toledo Inc., Mississauga, ON, Canada). Body surface area was subsequently calculated from the measurements of body height and mass (DuBois & DuBois, 1989). Body density was

measured using the hydrostatic weighing technique, and body fat percentage was calculated using the Siri equation (Siri, 1956). VO_2peak was measured during an incremental exercise protocol performed on a cycle ergometer (Corival, Lode B.V., Groningen, Netherlands) which consisted of a 2-min warm-up at 40 W followed by 20 W increments every minute until the participant could no longer maintain a pedaling cadence of at least 60 rpm. For the older females, a 12-lead ECG was monitored throughout the maximal exercise test by a qualified technician to detect any abnormalities in heart activity. If abnormalities were detected, participants were excluded from the study and referred to their physician; however, no abnormalities were detected in the participants screened.

The experimental protocol was performed in a whole-body direct air calorimeter regulated to an ambient temperature of 40°C and 15% RH. An equal number of young and older females performed the experimental protocol in the morning and in the afternoon. Participants consumed a light meal or snack before their arrival (approximately 2 hours before testing) and were asked not to run or bike on their way to the laboratory to avoid any thermal stimuli. Additionally, strenuous activity and alcohol were avoided for 24 hours and caffeine for 12 hours before the experimental session. Participants were instructed to drink ~250 mL of water before going to bed the night before the experimental session as well as in the morning of and within 2 hours of the start of the experimental session. Thereafter, no fluid was ingested.

All participants wore a light pair of athletic shorts, sports bra and sandals. Following the placement of sweat capsules, skin blood flow probe, heart rate monitor and core temperature probe/pill, participants rested for a 30-min baseline period on an upright seated cycle ergometer located in the calorimeter. Baseline rest was followed by three bouts of 30-min cycling exercise (Ex) at increasingly greater rates of metabolic heat production of 250 W (Ex1), 325 W (Ex2),

and 400 W (Ex3). Each exercise bout was followed by a 15-min recovery period in the direct calorimeter. The workloads were equivalent to a 35.9 ± 5.5 and 37.9 ± 7.8 for Ex1, 47.1 ± 7.8 and 50.6 ± 10.4 for Ex2, and 58.7 ± 9.6 and 62.9 ± 12.8 for Ex3 percentage of VO_2peak for the young and older females, respectively or a 36 ± 6 and 35 ± 7 W for Ex1, 60 ± 5 and 57 ± 8 W for Ex2 and 80 ± 8 and 76 ± 9 W for Ex3 external workload for young and older females, respectively.

Measurements

Whole-body evaporative heat loss and dry heat exchange as well as change in body heat storage were quantified using the modified Snellen direct whole-body air calorimeter. A detailed explanation of how direct calorimetry measures whole-body heat loss and heat storage has been described in a previous publication (Kenny & Jay, 2013). Also, a full technical description of the fundamental principles and performance characteristics of the Snellen calorimeter is available (Reardon *et al.*, 2006). In summary, direct calorimetry measured whole-body evaporative loss and dry heat exchange (radiation, conduction, convection), yielding an accuracy of ±2.3 W for the measurement of whole-body heat loss while indirect calorimetry was used to measure metabolic heat production. Electrochemical gas analyzers located outside of the calorimeter (AMETEK model S-3A/1 and CD 3A, Applied Electrochemistry, Pittsburgh, PA, USA) were used to determine the concentration of expired O_2 and CO_2 during experimental sessions and subsequently the respiratory exchange ratio (RER). Using the energy equivalent for the full oxidation of carbohydrates (19.63 kJ per L of O2 consumed) and fats (21.13 kJ per L of O2 consumed), metabolic heat production can be subsequently calculated (Kenny & Jay, 2013). To account for respiratory heat exchange, expired air was recycled back into the calorimeter. The

change in body heat storage was subsequently calculated by subtracting the total amount of heat produced and heat dissipated over the experimental protocol. The amount of evaporation required to achieve heat balance was calculated by combining the rates of metabolic heat production and dry heat exchange.

Local sweat production was measured using the ventilated capsule technique. A 3.8 cm^2 plastic capsule was attached to three skin sites (upper back, chest and forearm) with an adhesive ring and topical skin glue (Collodion HV, Mavidon Medical products, Lake Worth, FL, USA). Compressed dry air was passed through the capsule at a rate of 1.0 $L \cdot min^{-1}$. Water content of the effluent air was measured using high precision dew point mirrors (model 473, RH systems, Albuquerque, NM, USA). Local sweat rate was calculated using the difference in water content between effluent and influent air multiplied by the flow rate and normalized for the skin surface area under the capsule.

Laser-Doppler velocimetry was employed for measuring skin blood flow (PeriFlux System 5000, Main control unit; PF5010 LDPM). A laser-Doppler probe (Perimed integrating probe 413, Järfälla, Sweden) was affixed to the skin on the surface of the left forearm in an area which did not seem overly vascular upon visual inspection and provided stable readings at rest. To measure maximal skin blood flow, the heater housing the laser-Doppler probe was heated to 44°C until maximal skin vasodilation was achieved (~40 min) (Taylor *et al.*, 1984).

Esophageal temperature was measured with a thermocouple temperature probe (Mon-a-therm General Purpose Temperature Probe, Mallinckrodt Medical Inc., St-Louis, MO, USA). The esophageal probe was inserted 40 cm past the nostril entrance while the participants sipped water (100 - 300 mL) through a straw. Visceral temperature was measured using a telemetric pill (VitalSense ingestible capsule thermometer is a Class II Medical Device according to 21 DFR

8982.1845; Mini Mitter Company Inc.) which moves freely and unobstructed through the digestive tract and is generally eliminated within 48 hours of ingestion (McKenzie & Osgood, 2004). The telemetric pill provides an estimate of internal body temperature. Mean skin temperature was calculated as the weighted average of 4 skin temperature measurements: upper trapezius 30%, chest 30%, quadriceps 20%, and back calf 20% (Ramanathan, 1964). Data were collected in 15-s intervals and were displayed and recorded in spreadsheet format using a HP Agilent data acquisition module (model 3497A; Agilent Technologies Canada Inc., Mississauga, ON, Canada) and a personal computer with LabVIEW software (Version 7.0, National Instruments, Austin, TX, USA).

Heart rate was monitored, recorded continuously, and stored using a Polar coded WearLink and transmitter, Polar RS400 interface, and Polar ProTrainer 5 software (Polar Electro Oy, Finland).

Statistical analysis

For all variables, minute averages were calculated to carry out the statistical analyses. Baseline values were obtained by averaging the last 5–10 min of data during the 30-min baseline resting period. Mean body temperature was calculated as: 0.9 × esophageal temperature + 0.1 × mean skin temperature (Shibasaki *et al.*, 2006). Whole-body evaporative heat loss was plotted against the corresponding mean body temperature. Thereafter, the onset threshold and thermosensitivity of whole-body evaporative heat loss during each exercise period was determined using the linear portion of each response and analyzed using a segmented regression analysis as described by Cheuvront *et al.* (2009) with aid of a computer algorithm (GraphPad Prism 6.0, GraphPad Software, La Jolla, CA, USA) (Cheuvront *et al.*, 2009). The onset threshold

corresponded to the intercept of the regression line with the evaporative heat loss values at rest, while the thermosensitivity was defined as the slope of the regression line. The time constant (τ, time it takes to reach 63.2% of the total response) and amplitude (difference between evaporative heat loss at the onset and at the end of each exercise bout) of the evaporative heat loss response was calculated for each exercise bout.

Physical characteristics, baseline values and cumulative changes in body heat storage were analyzed using independent samples *t* tests. Dependent variables of rates of metabolic heat production, whole-body evaporative heat loss and dry heat exchange, evaporative requirement for heat loss, changes in body heat storage, as well as esophageal, visceral and mean skin temperatures, local sweat rates and skin blood flow, heart rate responses, evaporative heat loss onset thresholds, thermosensitivities, time constants, and amplitudes were analyzed to compare responses as a function of increasing heat loads (primary analysis). For this purpose, we used a two-way analysis of variance (ANOVA) performed with one factor of age (2 levels: young and older) and the repeated factor of either end exercise (three levels: Ex1, Ex2, Ex3) or end recovery (three levels: Rec1, Rec2, Rec3). We conducted a secondary analysis to further examine whole-body evaporative heat loss by determining at what time point differences in whole-body evaporative heat loss between young and older females occur during each exercise heat load separately. To do so, we used a two-way ANOVA to compare whole-body evaporative heat loss between groups for each heat load separately with a repeated factor of time (six levels: 5, 10, 15 20, 25 and 30 min) and a non-repeated factor of group (two levels: young and older). When a significant main effect was observed, *post hoc* comparisons were carried out using the Bonferroni procedure. The level of significance for all analyses was set at $p \leq 0.05$. Statistical

analyses were performed using commercially available statistical software (GraphPad Prism 6.0, GraphPad Software, La Jolla, CA, USA). All values are reported as mean ± standard deviation.

RESULTS

Responses as a function of increasing heat loads

Whole-body direct calorimetry

Baseline and Exercise

There were no differences (p=0.28) in baseline rates of metabolic heat production between the young (94 ± 14 W) and older (87 ± 12 W) females. By experimental design, the rate of metabolic heat production increased from Ex1 to Ex2, Ex1 to Ex3 and Ex2 to Ex3 in both young and older females (p<0.001), but was similar between age groups for Ex1 (young: 251 ± 7; older: 253 ± 17 W), Ex2 (young: 328 ± 7; older: 330 ± 6 W) and Ex3 (young: 404 ± 8; older: 406 ± 8 W) (p=0.45). Furthermore, no differences were observed for the rate of dry heat exchange at baseline (young: -65 ± 14; older: -62 ± 14 W, *P*=0.59) or during exercise (p=0.52) between groups, but did change over time (p=0.004) such that the rate of dry heat gain was greater at the end of Ex2 compared to Ex1 for the older females (p<0.05). The rates of dry heat exchange at the end of each exercise bout were: young: -79 ± 11 and older: -75 ± 19 W for Ex1; young: -82 ± 9 and older: -79 ± 19 W for Ex2 and young: -85 ± 9 and older: -80 ± 19 W for Ex3. Consequently, the evaporative requirement for heat loss did not differ between age groups at baseline (young: 159 ± 16; 149 ± 13 W, p=0.14) or during exercise (p=0.78), but did increase from Ex1 to Ex 2, Ex1 to Ex3 and Ex2 to Ex3 (p<0.001). As a result, the net heat load for each exercise bout was 329 ± 24 W (Ex1), 409 ± 17 W (Ex2) and 488 ± 16 W (Ex3).

Whole-body heat loss during exercise was solely due to evaporative heat loss as the negative rate of dry heat exchange resulted in a net dry heat gain. Rates of whole-body evaporative heat loss and the evaporative requirement for heat loss during baseline and exercise are presented in Figure 1. The rate of evaporative heat loss at baseline was lower in the older compared to young females (young: 115 ± 25; older: 88 ± 24 W, $p=0.02$). Additionally, despite exercising at similar requirements for heat loss, a main effect of age on the rate of whole-body evaporative heat loss was observed ($p=0.02$) such that the rate of evaporative heat loss was greater for the young compared to the older females at the end of Ex2 and Ex3. Furthermore, the rate of evaporative heat loss increased from Ex1 to Ex2, Ex1 to Ex3 and Ex2 to Ex3 in both groups ($p<0.001$), but was 10.2%, 11.6% and 12.4% lower in the older compared to young females at the end of Ex1, Ex2 and Ex3, respectively.

The changes in body heat storage are presented in Figure 2. The amount of heat stored in the body increased significantly between each exercise bout in both the young and older females ($p<0.001$). Additionally, there was a main effect of age on change in body heat storage during exercise ($p=0.002$), whereby the young females had a lower amount of heat stored for all three exercise periods (Ex1-3) compared to the older females.

Recovery

Rates of whole-body evaporative heat loss and the evaporative requirement for heat loss during recovery are presented in Figure 1. There were no age-related differences in the rate of metabolic heat production during any of the recovery periods ($p=0.18$), but the rate of metabolic heat production was significantly lower ($p=0.03$) for Rec3 relative to Rec1 and Rec2 in the older females. Furthermore, there was no main effect of age on the rate of whole-body evaporative

heat loss ($p=0.55$), although evaporative heat loss increased from Rec1 to Rec2 and Rec1 to Rec3 in both young and older females. Moreover, dry heat exchange ($p=0.42$) and the evaporative requirement for heat loss ($p=0.48$) were similar between age groups at the end of the recovery periods and did not change over time ($p>0.05$).

The changes in body heat storage during recovery and total heat storage (Ex plus Rec) are presented in Figure 2. The amount of body heat dissipated (and therefore resulting in a decrease in body heat storage) was significantly greater compared to the previous recovery period in both young and older females ($p<0.001$) due to the greater amount of heat stored during exercise, but did not differ between age groups during any of the recovery periods ($p=0.79$). However, there was a main effect of group on the total body heat storage ($p=0.02$), whereby the young females stored significantly less heat by the end of the three exercise/recovery cycles compared to the older females.

Onset thresholds, thermosensitivities, time constants and amplitudes for whole-body evaporative heat loss

Mean body temperature onset thresholds, thermosensitivities, time constants and amplitudes for whole-body evaporative heat loss during each exercise bout are presented in Table 1. The mean body temperature onset threshold for whole-body evaporative heat loss increased from Ex1 to Ex2 in the older females and from Ex2 to Ex3 in both the young and older females ($p<0.001$). Moreover, the onset threshold for whole-body evaporative heat loss was significantly different between groups ($p=0.02$) such that the onset of evaporative heat loss occurred at a greater mean body temperature for Ex2 and Ex3 for the older females compared to the young females. Also, the thermosensitivity of evaporative heat loss was significantly lower

for Ex3 compared to both Ex1 and Ex2 in the older females ($p>0.05$). However, there was no significant difference in thermosensitivity between age groups ($p=0.10$). Furthermore, the time constant for the rate of evaporative heat loss decreased from Ex1 to Ex2 and Ex1 to Ex3 in both young and older females, but was not different between young and older females ($p=0.41$) during exercise. In contrast, a main effect of age was evident for the amplitude of evaporative heat loss ($p=0.05$) such that the older females had significantly lower amplitudes for Ex2 and Ex3, and increased from Ex1 to Ex2 and Ex1 to Ex3 in the young females only ($p<0.05$).

Esophageal, visceral mean skin temperatures

Baseline and Exercise

Esophageal, visceral and mean skin temperatures during exercise are presented in Table 1. Baseline values for esophageal (young: 37.14 ± 0.26; older: 37.18 ± 0.18°C), visceral (young: 37.22 ± 0.25; older: 37.25 ± 0.22°C) and mean skin (young: 35.44 ± 0.35; older: 35.54 ± 0.26°C) temperatures were similar between groups (all $p>0.05$). Esophageal and visceral temperature increased between each exercise bout (Ex1 to Ex3) in both the young and older females ($p<0.001$) while mean skin temperature was greater for Ex2 and Ex3 relative to Ex1 in the young ($p=0.04$) for Ex3 relative to Ex1 in the older females ($p<0.001$). However, there was no main effect of age on esophageal ($p=0.09$), visceral ($p=0.89$) or mean skin ($p=0.81$) temperatures during exercise. Similar findings were observed when core and mean skin temperatures were presented as a change from baseline; that is, there was no main effect of age on esophageal ($p=0.15$), visceral ($p=0.91$) or mean skin ($p=0.73$) temperatures from baseline resting values.

Recovery

Recovery values for esophageal, visceral and mean skin temperatures are presented in Table 1. Esophageal and visceral temperatures were significantly greater at the end of Rec3 relative to Rec1 in both young and older females and to Rec2 in the older females only ($p<0.001$), but mean skin temperature was greater for Rec3 relative to Rec2 in both groups ($p<0.001$). In addition, there was a main effect of age on esophageal temperature during recovery ($p=0.04$), such that the older females had a significantly greater esophageal temperature at the end of Rec3 compared to the young females ($p<0.05$). In contrast, visceral ($p=0.41$) and mean skin ($p=0.40$) temperatures were not different between age groups during recovery.

Local heat loss and heart rate responses

Baseline and Exercise

Local sweat rate (chest, back and forearm), local skin blood flow (forearm) and heart rate values are presented in Table 2. There were no differences between groups in local sweat rates during baseline resting for sweat rate measured on the chest (young: 0.14 ± 0.06; older: 0.14 ± 0.06 $mg \cdot min^{-1} \cdot cm^{-2}$, $p=0.98$), back (young: 0.20 ± 0.16; older: 0.19 ± 0.06 $mg \cdot min^{-1} \cdot cm^{-2}$, $p=0.78$) and forearm (young: 0.16 ± 0.12; older: 0.13 ± 0.02 $mg \cdot min^{-1} \cdot cm^{-2}$, $p=0.36$) skin sites. Local sweat rate at all three sites increased significantly from one exercise bout to the next in both the young and older females ($p<0.001$). On the other hand, sweat rates were similar during exercise between age groups at the chest ($p=0.78$), back ($p=0.99$) and forearm ($p=0.61$) skin sites. Furthermore, local skin blood flow did not differ between age groups at baseline (young: 43.3 ± 17.1; older: 34.9 ± 21.1%, $p=0.37$) or during any of the three exercise bouts ($p=0.28$), but did increase from Ex1 to Ex2 in the older females ($p=0.009$). Heart rate was significantly greater

at the end of Ex2 relative to Ex1 and at the end of Ex3 relative to Ex2 in both the young and older females ($p<0.001$) but did not differ between age groups during exercise when presented as either absolute ($p=0.07$) or as a percentage of maximum ($p=0.80$).

Recovery

Local sweat rate, skin blood flow and heart rate values during recovery are presented in Table 2. Local sweat rate was greater for Rec2 compared to Rec1 on the chest in the young and greater for Rec3 compared to both Rec1 and Rec2 on the chest and forearm in the older females ($p<0.05$), but were otherwise stable during recovery. Furthermore, local sweat rates were similar between groups at the chest ($p=0.75$), back ($p=0.99$) and forearm ($p=0.61$) skin sites during recovery. Moreover, no differences in local skin blood flow were measured over recovery time ($p>0.05$) or between age groups ($p=0.96$) during recovery. Heart rate was significantly greater at the end of Rec2 and Rec3 compared to Rec1 in both the young and older females and was also greater at the end of Rec3 compared to Rec2 in the young females ($p>0.05$), but was not different between age groups during recovery for absolute ($p=0.21$) or percentage of maximum ($p=0.81$) values.

Time dependent changes of evaporative heat loss for each heat load

When each heat load was analyzed separately, a main effect of age on rates of whole-body evaporative heat loss was measured for Ex1 ($p=0.05$), Ex2 ($p=0.02$) and Ex3 ($p=0.009$). This was evidenced by the rate of whole-body evaporative heat loss being lower in the older females at 5 and 10 min for Ex1 ($p<0.05$), which was most likely the result of the lower rate of evaporative heat loss in the older females during baseline. Moreover, the rate of evaporative heat

loss was lower in the older females at 20, 25 and 30 min for Ex2 and at 10, 15, 20, 25 and 30 min for Ex3 when compared to young females ($p<0.05$).

DISCUSSION

We showed that the maximal rate of whole-body evaporative heat loss achieved during the moderate (Ex2, 325 W) and highest (Ex3, 400 W) heat load employed was significantly lower in older compared to young females matched for aerobic fitness, body surface area and body composition. Furthermore, we showed for the first time that the degree of impairment in evaporative heat loss between the young and older females is greater as the requirement for heat loss increases. Moreover, we showed that the onset threshold for the activation of whole-body evaporative heat loss was delayed in older females relative to their younger counterparts for the second and third heat loads. This was further exacerbated by a significantly more pronounced decrease in the thermosensitivity of the response measured during the successive exercise bouts in the older females. When combined with the attenuated rate of whole-body evaporative heat loss measured throughout the exercise bout, the older females stored significantly more heat than their younger counterparts and the magnitude of increase was more pronounced with increases in the level of heat stress and therefore requirement for heat loss. Of particular note, these impairments in whole-body evaporative heat loss and body heat storage were not paralleled by differences in local heat loss responses of sweating and skin blood flow or increases in core temperature assessed by esophageal and visceral temperature. Finally, no age-related differences in heat loss or heat storage were observed during any of the recovery periods with the exception of esophageal temperature being greater in the older females at the end of the final recovery period.

Capacity for whole-body evaporative heat loss

Keeping in line with our study hypothesis, we observed that the capacity for whole-body evaporative heat loss was significantly reduced in older compared to young females at the two highest requirements for heat loss (Figure 1). Although the time constant of the evaporative heat loss response was similar between the young and older females during the exercise bouts, the younger females exhibited greater amplitudes of change in evaporative heat loss for Ex2 and Ex3 (Table 1). Thus, the younger females achieved greater levels of evaporative heat loss within the same amount of time as the older females during Ex2 and Ex3. Impairments in the capacity to dissipate heat in older females have also been reported by Anderson & Kenney (Anderson & Kenney, 1987) who observed a reduction in sudomotor activity in older relative to young females at heat loads similar to that of Ex2 (i.e., exercise at 40% VO_2peak and 48°C, 15% RH). In the present study, we observed that older females attained a lower level of evaporative heat loss at the end of the second and third heat load, while no difference was measured at the end of the first heat load which suggests that sudomotor activity is impaired in older females above a heat load threshold equivalent to ~330 W. Furthermore, our exercise model of progressive increases in the evaporative requirement for heat loss allowed us to evaluate if the magnitude of difference in the body's physiological capacity to dissipate heat becomes greater with progressive increases in heat load. We observed that the separation in the rate of evaporative heat loss between the young and older females was more pronounced with each exercise heat load (Ex1 = 10.2%, Ex2 = 11.6% and Ex3 = 12.4%) despite the same increases in the requirement for heat loss. Altogether, these results suggest that the maximal level of whole-body evaporative heat loss achieved was lower in the older compared to young females for the two highest heat

loads employed, and the magnitude of impairment became more pronounced as the heat load increased.

In order to determine whether the age-related impairments in heat loss are due to central and/or peripheral modulations of sudomotor function, the onset threshold and thermosensitivity of whole-body evaporative heat loss can be examined in the context of the observed age-related changes whole-body evaporative heat loss (Nadel *et al.*, 1971). Previous studies have reported that older adults have an increased mean body temperature threshold for the onset of sweating during heat stress (Fennell & Moore, 1973; Crowe & Moore, 1974; Foster *et al.*, 1976; Sagawa *et al.*, 1988). However, this finding is not always consistent whereby some studies observed no differences in the mean body temperature at which sweating began (Hellon & Lind, 1956; Collins *et al.*, 1977; Inoue *et al.*, 1991; Inoue *et al.*, 1999b). As noted earlier, we observed an increase in the onset threshold for evaporative heat loss in the older females for Ex2 and Ex3 (Table 1) which is reflective of the greater heat storage for the older females over time. Furthermore, this was exacerbated by a significant reduction in the thermosensitivity of the response measured between Ex2 and Ex3 in the older females only. Taken together, our findings demonstrate that whole-body sudomotor activity is attenuated with progressive increases in body heat storage in older females. Given that the onset threshold represents a central modulation whereas the thermosensitivity of the response represents a peripheral modulation (Nadel *et al.*, 1971), our findings indicate that the age-related impairments in whole-body sudomotor activity in older females may be attributed to both a central and peripheral modulation of heat loss; a response which we show to be heat load dependent.

Time-dependent changes in whole-body evaporative heat loss

In addition to comparing the maximal rate of whole-body sudomotor capacity achieved during exercise in older relative to young females, we also assessed the time-dependent changes in whole-body evaporative heat loss for each exercise heat load. Despite observing no significant differences in the maximal level of evaporative heat loss achieved at the end of the first exercise bout between age groups, impairments in whole-body evaporative heat loss were evident during the first 10 min of Ex1, but were similar between groups thereafter. Given that the onset and thermosensitivity for whole-body evaporative heat loss did not differ between groups for Ex1, the lower rate of evaporative heat loss measured for the older females during the early stages of Ex1 was likely due to their lower rate of evaporative heat loss at baseline. In contrast to Ex1, age-related differences in whole-body evaporative heat loss occurred as early as 20 min into Ex2 and 10 min into Ex3 and remained lower for the duration of the 30-min exercise bout. This time dependent decrease in evaporative heat loss can be attributed to a delayed activation of the response in the older females which was paralleled by a progressively greater reduction in thermosensitivity measured during the successive exercise bouts in the older females only. Consequently, the impaired ability to dissipate heat in the older females resulted in a 40, 57 and 47% greater change in body heat storage compared to young females for Ex1, Ex2 and Ex3, respectively. While Anderson & Kenney (Anderson & Kenney, 1987) reported lower sweat rates after 30 min of exercise at 40% VO_2peak in a hot, dry environment (48°C, 10% RH) and Larose *et al.* (Larose *et al.*, 2013c) observed a lower rate of whole-body evaporative heat loss in older females as early as 10 min after the onset of exercise at a moderate heat load (i.e., ~320 W), we showed that as the heat load increases, the separation in whole-body evaporative heat loss between young and older females occurs earlier into exercise, thereby causing a greater amount

of heat to be stored in the body. Our findings demonstrate that age-related impairments in the physiological capacity to dissipate heat are exacerbated with increases in the level of heat stress, and therefore as the requirement for heat loss becomes greater.

Core temperature and local heat loss responses

In contrast to the greater heat storage measured for the older females for each exercise bout, differences in esophageal temperature responses were not evident until the very end of the intermittent exercise protocol (end of Rec3) (Table 1). Furthermore, no differences between groups for visceral temperature were observed at any stage of exercise or recovery. Whole-body calorimetry provides an accurate measure of changes in whole-body heat content whereas surrogate measures of body core temperature only represent regional changes in heat content. Regional tissue temperature at any point in time is the result of regional differences in metabolic rate, conductive heat loss to adjacent tissues, and deep and peripheral convective blood flow (Kenny & Jay, 2013). Thus, the disparity between body heat storage and core body temperatures can be ascribed to regional variations in tissue blood flow leading to differences in heat transfer/distribution between internal tissues, which occurs to a greater extent in older adults (Kenney & Munce, 2003). Of note however, the cumulative amount of heat stored by the end of the exercise-rest protocol was 42% greater in the older compared to young females, which was comparable to the 41% greater increase in esophageal temperature. This was also a similar finding to Larose *et al.* (Larose *et al.*, 2013c) who showed that the cumulative change in body heat storage and net change in rectal temperature were both ~63% greater in older females at the end of the exercise-rest cycles. Thus, despite the differences in heat distribution/storage at the

beginning of the Ex/Rec cycles, it appears that core temperature gradually increases to reflect the changes in total body heat storage.

In the present study, we measured whole-body heat loss in parallel with local sweat rates at the chest, upper back and forearm as well as skin blood flow on the forearm. Despite observing marked differences in whole-body evaporative heat loss between young and older females at the end of the second and third exercise bouts, no differences were measured for local heat loss responses at the end of any of the exercise bouts (Table 2). To date, several studies have observed age-related decrements in sweating which occurred at different rates across various regions of the body (Inoue *et al.*, 1991; Inoue & Shibasaki, 1996; Dufour & Candas, 2007; Smith *et al.*, 2013a). Moreover, a high degree of heterogeneity has been observed between different local measurement sites on the body for both sudomotor activity and skin blood in adults of a similar age (Inoue *et al.*, 2004; Smith & Havenith, 2012; Smith *et al.*, 2013a; Smith *et al.*, 2013b). These previous observations are consistent with our observation that sweat rates measured on the upper back were greater than those measured on the chest and forearm for both the young and older females (Table 2). Likewise, Smith *et al.* (Smith *et al.*, 2013a) also observed higher sweat rates on the back compared to both the arm and abdomen in young and older adults and Inoue *et al.* (Inoue *et al.*, 1991) observed higher sweat rates on the back compared to the thigh in both young and older males during whole-body heating at rest. Furthermore, although we only measured skin blood flow at one site and therefore are unable to make conclusions on regional variations of skin blood flow from our data, skin blood flow has been reported to be lower on the chest and thigh in older compared to young males, with no apparent differences between groups on the forehead (Inoue & Shibasaki, 1996). Ultimately, the discrepancy between local and whole-body heat loss responses may lead to inaccurate conclusions regarding the effect

that aging can have on the body's physiological capacity to dissipate heat in both males and females. Whole-body direct calorimetry provides an accurate assessment of the combined response of the 2-4 million sweat glands across the entire surface of the body which can be used to examine differences in the body's capacity to dissipate heat between groups.

Postexercise heat loss responses

In the present study, core temperature and body heat storage remained elevated above baseline levels after each exercise bout (Figure 1 and Table 2), while local and whole-body heat loss responses rapidly declined. This pattern of response has been observed in previous studies in young adults (Kenny *et al.*, 2007; Kenny & Jay, 2013) and has been attributed to nonthermal factors overriding the thermal control of heat loss during postexercise recovery (Kenny *et al.*, 2007; Kenny & Jay, 2013). Furthermore, despite the greater end-exercise heat storage with successive exercise bouts in the older females, the rates of whole-body heat loss, and subsequently changes in body heat storage, during the postexercise recovery periods were similar between the young and older females. In fact, 7, 47 and 47% of the heat stored during each exercise bout was lost during Rec1, Rec2 and Rec3, respectively in the young females whereas only 6, 30 and 36% of the heat stored during each exercise bout was lost during Rec1, Rec2 and Rec3, respectively in the older females. As such, it is possible that the level of influence of nonthermal factors on heat loss postexercise may be even greater in older compared to young females due to the greater heat storage at the end of each exercise bout compared to the young females but similar heat loss responses postexercise. Further research is required to establish the actual contribution of thermal and nonthermal mechanisms governing heat loss postexercise in young and older females.

Considerations

The older females who volunteered for this study were of similar aerobic fitness as the young females. This is consistent with the studies by Anderson and Kenney (Anderson & Kenney, 1987) and Larose *et al.* (Larose *et al.*, 2013c) who also observed impaired heat loss in older relative to young females of similar aerobic capacity (Anderson & Kenney, 1987; Larose *et al.*, 2013c). Thus, the decrements in local and/or whole-body heat loss in older females appears to be unrelated to aerobic capacity, but instead may reflect alterations in central and/or peripheral control of sweating associated with advanced aging. It is important to consider that the similar VO_2peak values between groups in the present study, and others, may indicate that the older females were more physically active than the average older female. In fact, the older females in our study were in the 80 to 90th percentile while the younger females were in the 70 to 80th percentile for aerobic capacity. Since high levels of aerobic fitness associated with regular endurance-type exercise, can improve thermoregulatory control during exercise (Gisolfi & Robinson, 1969; Buono & Sjoholm, 1988; Havenith & van Middendorp, 1990; Cheung & McLellan, 1998; Selkirk & McLellan, 2001), less fit older females may have an even further attenuated capacity to dissipate heat compared to both young and older, habitually active females.

Given that whole-body heat loss responses between groups were compared using a progressive increase in the exercise-induced heat load, it remains unclear if the pattern and/or magnitude of difference in whole-body heat loss, and therefore body heat storage, observed between groups would differ had each exercise condition been performed on separate days. It is known that there is a greater activation of whole-body evaporative heat loss response following

an initial exercise bout, termed the priming effect, and this response occurs irrespective of the age of the individual (Larose *et al.*, 2013c; Larose *et al.*, 2013d). While we cannot determine how the prior exercise bout might have affected the rate of whole-body evaporative heat loss, and therefore the magnitude of difference in body heat storage between groups, we consistently observed an attenuated rate of heat dissipation in older females relative to their young counterparts irrespective the greater thermal drive associated with the successively greater exercise-induced heat loads. Future studies are required to determine how a prior exercise bout might influence the extent to which these age-related impairments may attenuate whole-body evaporative heat loss, and therefore body heat storage, when exercise is performed at the higher exercise-induced loads where we show the greatest differences between groups to occur.

Finally, another point to consider is that reproductive hormones may influence the thermoregulatory system, whereby estrogen and progesterone levels are reported to alter baseline core temperatures (Tankersley *et al.*, 1992; Brooks *et al.*, 1997). In the present study, two of the older females were on hormone replacement therapy (HRT). There were no significant differences in baseline esophageal temperature between the young females (37.14 ± 0.26°C, n=10) and older females not on HRT (37.15 ± 0.16°C, n=8). However, those who were taking HRT had a slightly elevated esophageal temperature (37.32 ± 0.21°C, n=2). Despite the elevated core temperature at baseline, there were no differences in changes in esophageal temperature (older no HRT: 37.90 ± 0.43°C vs. HRT: 37.96 ± 0.44°C) or body heat storage (older no HRT: 430 ± 174 kJ vs. HRT: 418 ± 10 kJ) between females who were taking or not taking HRT by the end of the exercise/recovery protocol. Furthermore, these values are in sharp contrast to younger females whose change in body heat storage was 247 ± 155 kJ. Thus, HRT did not appear to affect the clear age-related impairments in whole-body heat dissipation causing greater amounts

of heat stored in the body of the older compared to young females. Nevertheless, further studies are warranted to fully examine the effects of HRT on whole-body heat storage.

Summary

We showed that older females demonstrated a lower capacity for whole-body evaporative heat loss compared to young females when exercising in the heat at a rate of metabolic heat production of ≥325 W. Further, we observed a greater separation between age groups with progressive increases in the requirements for heat loss. These increasingly greater impairments in whole-body sudomotor capacity between young and older females were the result of a greater onset threshold and gradual decrease in thermosensitvity for the older females. Ultimately, the impaired capacity to dissipate heat led to greater heat storage in older females which was more pronounced with increases in the level of heat stress.

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FIGURES

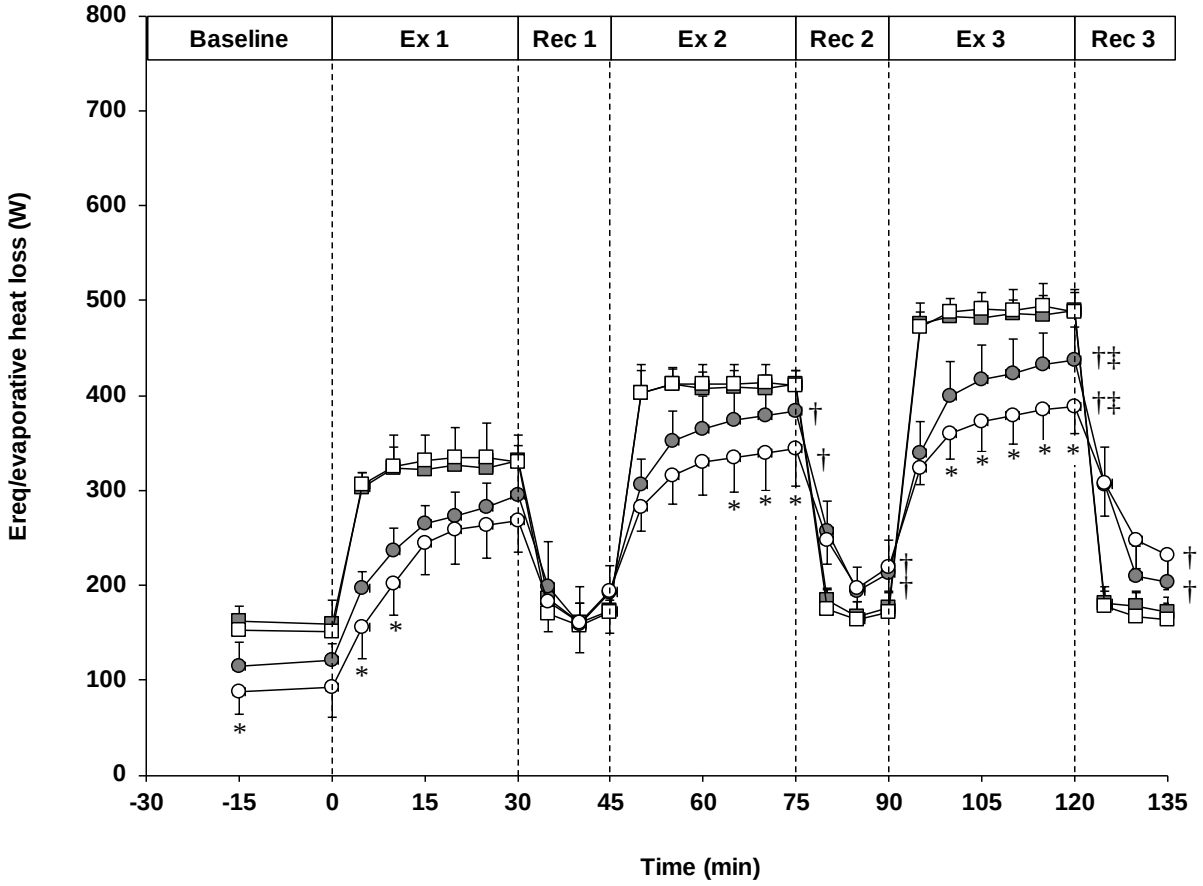

Figure 1. Mean ± standard deviation rates of evaporative heat loss (circles) and the required amount of evaporation for heat balance (E_{req}, squares) measured at baseline and over three 30-min exercise bouts (Ex 1, Ex 2 and Ex 3) and three 15-min recovery bouts (Rec 1, Rec 2 and Rec 3) in a hot, dry (40°C, 15% RH) environment in young (grey) and older (white) females. There were no differences in the required amount of evaporation for heat balance between groups. Significant difference ($p \leq 0.05$) in evaporative heat loss from young is denoted by an asterisk (*). Significant difference from Ex1/Rec1 is denoted by a cross (†). Significant difference from Ex2/Rec2 is denoted by a double cross (‡).

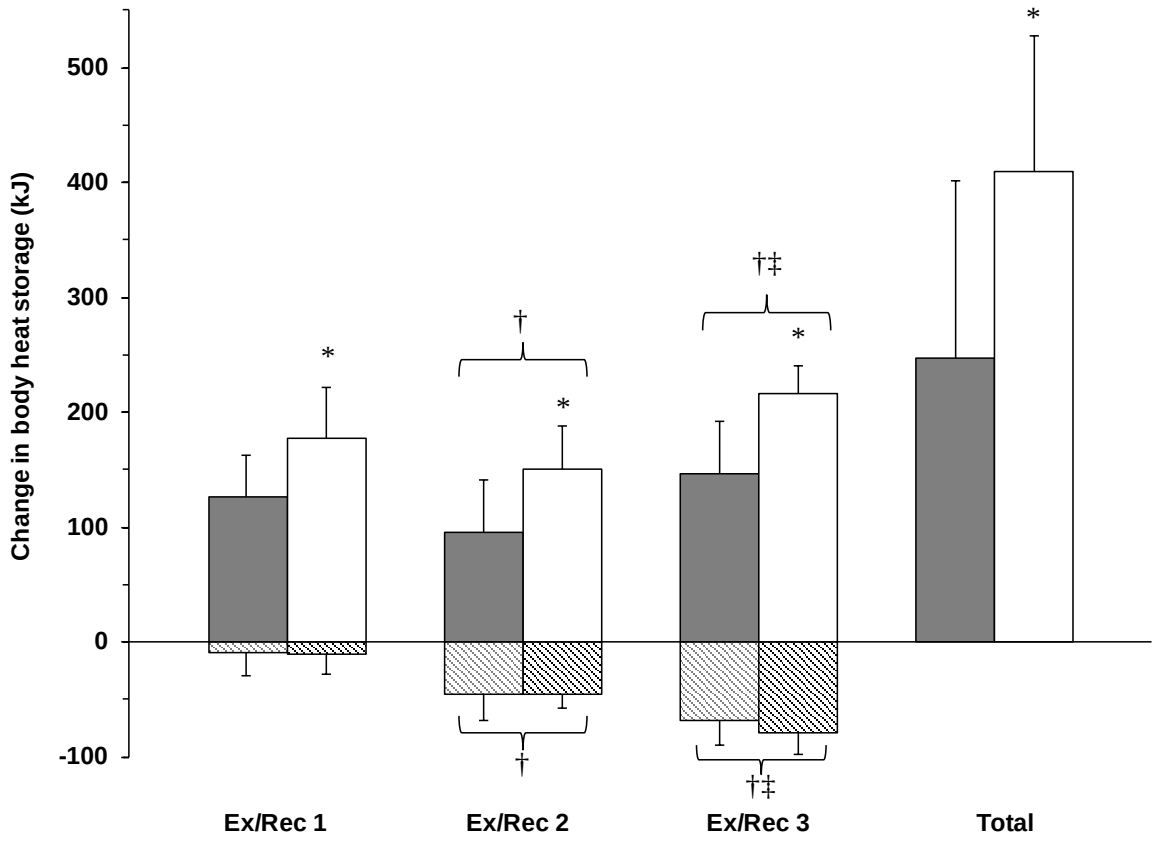

Figure 2. Mean ± standard deviation values for changes in body heat storage during each exercise/recovery cycle as well as the total change in body heat storage over the exercise protocols in a hot, dry (40°C, 15% RH) environment. The solid bars represent changes in body heat storage during exercise and the striped bars represent changes in body heat storage during recovery. The grey bars/stripes represent the young group and the white bars/black stripes represent the older group. Significantly different ($p \leq 0.05$) from young is denoted by an asterisk (*).Significant difference from Ex1/Rec1 is denoted by a cross (†). Significant difference from Ex2/Rec2 is denoted by a double cross (‡).

TABLES

Table 1. Esophageal, visceral and mean skin temperature responses during each exercise (Ex)/recovery (Rec) cycle and onset thresholds, thermosensitivities, time constants and amplitudes of evaporative heat loss for each exercise bout.

$T_{es,}$ °C	Ex1	Rec1	Ex2	Rec2	Ex3	Rec3
Young	37.44±0.23	37.33±0.24	37.74±0.32†	37.42±0.19	38.12±0.42†‡	37.56±0.29†
Older	37.59±0.15	37.51±0.20	37.96±0.18†	37.65±0.29	38.33±0.22†‡	37.91±0.41*†‡
$T_{visc,}$ °C						
Young	37.55±0.26	37.49±0.12	37.94±0.28†	37.77±0.22†	38.17±0.21†‡	37.85±0.14†
Older	37.65±0.28	37.60±0.27	37.90±0.22†	37.76±0.21	38.15±0.31†‡	38.00±0.35†‡
$T_{Sk,}$ °C						
Young	35.65±0.20	35.59±0.27	35.80±0.28	35.63±0.29	35.92±0.40	35.78±0.33‡
Older	35.64±0.28	35.53±0.25	35.76±0.41	35.48±0.33	35.08±0.47†‡	35.64±0.38‡
Onset threshold of evaporative heat loss, °C						
Young	36.92±0.30		37.06±0.19		37.20±0.20†	
Older	37.04±0.15		37.33±0.19*†		37.50±0.26*†‡	
Thermosensitivity of evaporative heat loss, $W \cdot °C^{-1}$						
Young	569±152		495±144		425±220	
Older	570±222		392±243		246±146†‡	
τ, min						
Young	10.4±2.6		5.0±1.3†		4.9±1.0†	
Older	10.6±3.8		7.0±3.3†		4.7±1.9†	
Amplitude, W						
Young	153±33		191±27†		214±31†	
Older	167±24		134±57*		160±70*	

Values are mean ± standard deviation. T_{es}, esophageal temperature. T_{visc}, visceral temperature. T_{Sk}, mean skin temperature. τ, time constant. Mean body temperature was used to calculated the onset threshold and thermosensitivity. *Significant difference from young females. †Significant difference from Ex1/Rec1. ‡Significant difference from Ex2/Rec2.

Table 2. Local heat loss and heart rate responses during each exercise (Ex)/recovery (Rec) cycle.

LSR – Chest, mg·min^{-1}·cm^{-2}	**Ex1**	**Rec1**	**Ex2**	**Rec2**	**Ex3**	**Rec3**
Young	0.37±0.07	0.20±0.04	0.51±0.12†	0.26±0.09†	0.62±0.15†‡	0.27±0.11†
Older	0.40±0.13	0.22±0.08	0.55±0.17†	0.26±0.12	0.63±0.22†‡	0.34±0.11†‡
LSR – Back, mg·min^{-1}·cm^{-2}						
Young	0.52±0.27	0.26±0.16	0.69±0.27†	0.31±0.14	0.84±0.40†‡	0.30±0.11
Older	0.54±0.16	0.32±0.10	0.71±0.22†	0.39±0.17	0.82±0.24†‡	0.47±0.23
LSR – Arm, mg·min^{-1}·cm^{-2}						
Young	0.39±0.19	0.19±0.10	0.55±0.19†	0.25±0.13	0.69±0.29†‡	0.25±0.11
Older	0.38±0.13	0.24±0.10	0.53±0.19†	0.25±0.08	0.57±0.21†‡	0.34±0.13
SkBF, % of max						
Young	52.1±15.9	43.7±16.4	56.5±13.5	45.3±17.7	57.0±17.3	45.5±16.1
Older	57.1±14.2	41.7±20.2	65.1±14.5†	46.2±17.8	65.1±10.6	44.5±21.3
HR, beats·min^{-1}						
Young	117±18 64±9	87±17 48±10	140±22† 77±12†	95±18† 52±10†	161±19†‡ 88±10†‡	105±20†‡ 58±11†‡
Older	103±10 64±5	82±8 51±4	123±9† 77±5†	85±9† 53±6†	146±8†‡ 91±5†‡	91±14†‡ 56±8†

Values are mean ± standard deviation. LSR, local sweat rate. SkBF, skin blood flow. HR, heart rate. Heart rate is presented as absolute (first row) as well as percentage of maximum (second row). †Significant difference from Ex1/Rec1. ‡Significant difference from Ex2/Rec2.

3.4 Thesis article #4 – published in Experimental Physiology, 99(6): 921-931, 2014 – see Appendix B

Diminished nitric oxide-dependent sweating in older males during intermittent exercise in the heat

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Running title: Nitric oxide and local sweat rate in older males

Key words: thermal stress, sudomotor activity, physical activity

Word count: 6,021

Subject area: Human/environmental and exercise physiology

NEW FINDINGS

- What is the central question of this study? Sweating during exercise in the heat is, in part, mediated through nitric oxide-dependent mechanisms. It is unclear if aging reduces nitric oxide-dependent sweating during exercise in the heat.

- What is the main finding and its importance? Nitric oxide-dependent sweating during short bouts of exercise in the heat was observed in young males, but not in older adults. These findings show that age-related impairment in sweating may be associated with age-related reductions in nitric oxide-mediated sweating.

ABSTRACT

Nitric oxide (NO) is a signalling molecule which contributes to the control of many physiological pathways including the heat loss response of skin vasodilation. Recently, NO has been implicated in the control of sweating during exercise in young adults. We tested the hypothesis that aging reduces NO-dependent sweating during exercise in the heat. Ten young (23 ± 3 years) and older (64 ± 5 years) males, matched for body surface area, performed 3 successive 15-min bouts of exercise (Ex) at the same rate of metabolic heat production (300 $W \cdot m^{-2}$) in the heat (35ºC, 20% relative humidity). Each exercise was interspersed with a 15-min recovery period. Local sweat rate (ventilated capsule) was measured on two forearm skin sites which were continuously perfused via intradermal microdialysis with: 1) 0.9% saline as control (CON) or 2) 10 mM N^G-nitro-L-arginine methyl ester (L-NAME), a non-selective NO synthase inhibitor. Local sweat rate at the end of Ex1 was lower at the CON condition in the older versus young males (0.69±0.19 vs. 0.90±0.17 $mg \cdot min^{-1} \cdot cm^{-2}$, P=0.018). In the young males, local sweat rate was reduced at the L-NAME treated condition compared to the CON condition at the end of Ex1 (0.67±0.14 vs. 0.90±0.17 $mg \cdot min^{-1} \cdot cm^{-2}$, P=0.004), Ex2 (0.78±0.20 vs. 1.03±0.20 $mg \cdot min^{-1} \cdot cm^{-2}$, P=0.013) and Ex3 (0.78±0.20 vs. 1.03±0.21 $mg \cdot min^{-1} \cdot cm^{-2}$, P=0.014). In the older males, there was no main effect of treatment condition on local sweat rate (P=0.537) such that local sweat rate at the L-NAME treated and CON conditions were similar (Ex1: 0.65±0.20 vs. 0.69±0.19 $mg \cdot min^{-1} \cdot cm^{-2}$; Ex2: 0.80±0.27 vs. 0.91±0.29 $mg \cdot min^{-1} \cdot cm^{-2}$; and, Ex3: 0.84±0.31 vs. 0.94±0.38 $mg \cdot min^{-1} \cdot cm^{-2}$). We conclude that aging attenuates the influence of NO in the control of local forearm sweating observed in young adults during short 15 min bouts of exercise in the heat. This mechanism may in part, explain the age-related impairments in sweating.

INTRODUCTION

Evaporation of sweat promotes heat loss from the human body. Although the degree of evaporative heat loss from sweat production is affected by the ambient medium, temperature and water-vapour pressure; sweat evaporation is the main avenue for heat loss when ambient temperature exceeds mean skin temperature (~34°C) and the evaporative capacity of the environment does not exceed the required evaporation for heat balance (i.e., compensable conditions) (Gisolfi & Wenger, 1984; Parsons, 2003). Compared with young adults, sweating has been shown to be reduced in older adults during exercise in the heat (Tankersley *et al.*, 1991; Inoue *et al.*, 1999a; Larose *et al.*, 2013b; Larose *et al.*, 2013c; Larose *et al.*, 2013d). Consequently, older individuals may be at an elevated risk of experiencing heat-related injuries during exercise in the heat. However, the underlying mechanism(s) for age-related impairments in sweating during exercise in the heat remains unclear.

Eccrine sweat glands possess both adrenergic and cholinergic receptors (Robertshaw, 1977; Schotzinger & Landis, 1988; Weihe *et al.*, 2005). However, recent studies in humans revealed that there is no contribution of adrenergic receptors to the sweating response (Buono *et al.*, 2011; Machado-Moreira *et al.*, 2012). For example, local β-adrenergic receptor blockade with propranolol did not affect the sweating response during exercise in the heat (Buono *et al.*, 2011). Also, psychological sweating, which was originally thought to be mediated by non-cholinergic mechanisms (e.g., adrenergic mechanisms), was completely abolished using the systemic muscarinic receptor blockade atropine (Machado-Moreira *et al.*, 2012). Further, local and systemic muscarinic receptor blockade with atropine abolished thermal sweating (Kellogg *et al.*, 1995; Machado-Moreira *et al.*, 2012). It is therefore conceivable that acetylcholine (ACh) released from cholinergic nerves may be a key

neurotransmitter in the control of sweating during heat stress. Additionally, a recent study by Welch *et al.* (Welch *et al.*, 2009) showed sweating was lower in skin infused with N^G-nitro-L-arginine methyl ester [L-NAME; a non-selective nitric oxide (NO) synthase (NOS) inhibitor] through intradermal microdialysis, compared to a control skin site infused with 0.9% saline during continuous cycling exercise (~47% VO_{2peak}) performed in a hot environment. This finding suggests that NO may also be an important modulator of the exercise-induced increases in sweating.

There is increasing evidence demonstrating that aging reduces the NO bioavailability in the skin (Holowatz *et al.*, 2003; Holowatz *et al.*, 2006b, a; Stanhewicz *et al.*, 2012; Stanhewicz *et al.*, 2013), skeletal muscle (Nyberg *et al.*, 2012), serum (Toprakci *et al.*, 2000), as well as arterial and venous blood (Nyberg *et al.*, 2012). It is therefore plausible that NO-dependent sweating during exercise in the heat may be compromised in older adults. Elucidating mechanisms of age-related differences in sweating during exercise-induced heat stress, and specifically assessing the potential role of NO in this response, would provide us with important new information about the regulation of sweating. This knowledge could be used to define potential countermeasures and/or treatments to improve heat dissipation in older adults engaged in exercise, thus mitigating the risk of heat-related injuries.

Rate of metabolic heat production increases immediately upon the onset of exercise and is not initially offset by an increase in the rate of total heat loss [known as thermal inertia (Murgatroyd *et al.*, 1993) or temporal dissociation (Webb & Annis, 1966)], thus giving rise to a pronounced increase in body heat storage during the early stages of exercise (Kenny & Jay, 2013). Hence, any delay associated with the activation of the sweating response, such as may occur with aging as recently demonstrated by Larose e*t al.* (Larose *et al.*, 2013d) where marked differences in whole-body evaporative heat loss were evident as early as ~10 min of

exercise, would lead to more pronounced increases in body heat storage and therefore core temperature during exercise (Kenny & Jay, 2013). This would be exacerbated by the rapid attenuation of heat loss following cessation of exercise, which has been shown to be similar in both young and older adults despite the fact that older adults stored more heat during exercise (Larose *et al.*, 2013d). While both young and older adults exhibited a greater evaporative heat loss with successive exercise bouts, the age-related impairments remained intact despite a greater increase in body heat content, and therefore thermal drive, with successive exercise bouts (Kenny *et al.*, 2009; Larose *et al.*, 2013d). Thus, the purpose of this study was to test the hypothesis that: 1) NO-dependent sweating during exercise is reduced in older adults compared to their younger counterparts and this response remains intact with successive exercise bouts; and, 2) the relative influence of NO may be less evident during recovery irrespective of the increase in core temperature observed with successive exercise bouts.

METHODS

Ethical Approval

This study was approved by the University of Ottawa Health Sciences and Science Research Ethics Board, in accordance with the Declaration of Helsinki. Written, informed consent was obtained from all participants prior to their involvement in the study.

Participants

Twenty males volunteered for the study and were divided into two groups of 10 young (23 ± 3 years) and 10 older (64 ± 5 years) males. All participants were healthy, non-smoking, physically active males free from cardiovascular disease or diabetes. Young and

older males were matched for physical activity behavior (i.e., 3-4 days/week of continuous exercise of 30-60 min in duration). In addition, all experimentation occurred between the months of October and April to avoid the effects of natural acclimatization.

Experimental Procedures

Each participant completed one preliminary and one experimental session. During the preliminary session, body height, mass, and density, as well as maximum oxygen uptake (VO_2max) were determined. Body height was determined using a stadiometer (Detecto, model 2391, Webb City, MO, USA), while body mass was measured using a digital high-performance weighing terminal (model CBU150X, Mettler Toledo Inc., Mississauga, ON, CAN). Body surface area was subsequently calculated from the measurements of body height and mass (DuBois & DuBois, 1989). Body density was measured using the hydrostatic weighing technique with a correction of 100 mL of trapped gas volumes in the gastrointestinal tract (Adams & Beam, 2008), and used to calculate body fat percentage (Siri, 1956).

VO_2max was determined by indirect calorimetry (MOXUS system, Applied Electrochemistry, Pittsburgh, PA, USA) during a progressive incremental exercise protocol (Canadian Society for Exercise Physiology, 1986) performed on an upright constant-load cycle ergometer (Corival, Lode B.V., Groningen, Netherlands) to volitional fatigue. During the VO_2max test, the older participants were monitored by electrocardiogram under supervision of a qualified technician.

During the experimental session, participants reported to the laboratory and changed into shorts and running shoes. The participants were asked to drink 500 mL of water the night before, as well as ~2 hours prior to the experimental session. They were also asked to

refrain from alcohol, caffeine, and exercise 24 hours prior to experimentation. Upon arrival at the laboratory, the participants provided a urine sample and a baseline body mass was measured. They subsequently rested quietly at an ambient room temperature of 24°C. During this time, two microdialysis fibers (MD 2000, Bioanalytical Systems, West Lafayette, IN, USA) (30KDa cutoff, 10 mm membrane) were placed in the dermal space of the forearm under aseptic conditions. To place the fiber, a 25-gauge needle was first inserted into the dermal space of the dorsal aspect of the left forearm and then the needle exited 20–25 mm away from the point of entry. The fiber was then inserted through the lumen of the needle. The needle was withdrawn, leaving the fiber in place. Each fiber was separated by at least 4.0 cm. After insertion, the fibers were perfused with a 0.9% saline solution at a rate of 2 $\mu L \cdot min^{-1}$ via a micro infusion pump (CMA/400, CMA Microdialysis, Solna, Sweden).

Sixty minutes after the fiber placement (to allow for hyperemia associated with probe insertion trauma to subside), the participant entered a thermal chamber regulated to an ambient air temperature of 35°C and a relative humidity of 20%. The participant rested for an additional 60 minute baseline period in the upright seated posture while the rest of the instrumentation was placed. During the second hour, 10 mM L-NAME (Sigma Aldrich, Oakville, ON), which has previously been used to non-selectively inhibit NOS in eccrine sweat glands (Lee & Mack, 2006; Welch *et al.*, 2009), dissolved in saline solution was infused through one of the microdialysis fibers while the second fiber continued to receive 0.9% saline as a control at a rate of 2 $\mu L \cdot min^{-1}$. The infusions continued for the remainder of the experimental protocol. Although it has been suggested that L-NAME has anti-muscarinic effects, as shown in rat diaphragmatic microcirculation (Chang *et al.*, 1997), a more recent study inferred minimal anti-muscarinic effect of using 10 mM L-NAME on sweating in

humans exercising in a hot environment (Welch *et al.*, 2009). This confirmation is important since the sweating response during heat stress requires muscarinic receptor activation (Kellogg *et al.*, 1995).

After the second 60 minute baseline period, the participant was moved to an upright bike, where they rested for a 10 minute baseline period followed by performing three 15 minute bouts of cycling, each separated by 15 minutes of recovery. By design, each exercise was performed at a similar fixed rate of metabolic heat production of 300 ± 2 $W \cdot m^{-2}$ (equivalent to 51.7 ± 7.4 and 69.6 ± 9.6% of VO_2max for young and older males, respectively) to ensure a similar heat load between groups. Thus, any observed differences in the sweating response between groups would be attributed to the effects of aging as opposed to differences in heat load and therefore thermal drive (Jay *et al.*, 2011; Gagnon *et al.*, 2013b). Following the intermittent exercise protocol, a post urine sample and body mass were measured.

Measurements

The ventilated capsule technique was employed for the purpose of measuring local sweat rate. Local sweat rate was measured from a 3.8 cm^2 plastic capsule placed directly over the center of the microdialysis membranes. The sweat capsules were attached to the forearm skin with adhesive rings and topical skin glue (Collodion HV, Mavidon Medical products, Lake Worth, FL, USA). Dry compressed air was passed through each capsule at a rate of 1.0 $L \cdot min^{-1}$. Water content of the effluent air was measured using high precision dew point mirrors (model 473, RH systems, Albuquerque, NM, USA). The gas tanks and tubes used for the measurement of sweat rate were placed inside the environmental chamber regulated at 35°C. Long 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 trial (i.e., 35°C). Local sweat rate was calculated every one second using the difference in water content between effluent and influent air multiplied by the flow rate and normalized for the skin surface area under the capsule ($mg \cdot min^{-1} \cdot cm^{-2}$).

Esophageal temperature (T_{es}) was measured with a general purpose thermocouple temperature probe (Mallinckrodt Medical Inc., St-Louis, MO, USA). The probe was inserted 40 cm past the entrance of the nostril while the participants sipped water through a straw. Rectal temperature (T_{re}) was measured using a general purpose thermocouple temperature probe (Mallinckrodt Medical Inc., St-Louis, MO, USA) inserted to a minimum of 12 cm past the anal sphincter. Skin temperature was measured at 4 sites using thermocouples (Concept Engineering, Old Saybrook, CT, USA) attached to the skin with surgical tape. Mean skin temperature was calculated using the four skin temperatures weighted to the following regional proportions: upper trapezius 30%, chest 30%, quadriceps 20%, and back calf 20% (Ramanathan, 1964). Temperature data were collected using a HP Agilent data acquisition module (model 3497A; Agilent Technologies Canada Inc., Mississauga, ON, Canada) at a rate of one sample every 15 seconds and simultaneously displayed and recorded in spreadsheet format on a personal computer with LabVIEW software (Version 7.0, National Instruments, Austin, TX, USA).

Pre- and postexercise urine specific gravities were determined in duplicate using a handheld total solids refractometer (model TS400, Reichter Inc., Depew, NY, USA).

Statistical analyses

The values for each exercise (Ex) and recovery (R) were obtained by averaging the last minute of the exercise or recovery period. T_{es}, T_{re}, and mean skin temperature were also

presented as a change from baseline (ΔT_{es}, ΔT_{re}, and Δmean skin temperature). Dependent variables were analysed using a two-way analysis of variance (ANOVA). The ANOVAs were performed with one factor of exercise time (three levels: Ex1, Ex2, Ex3) or recovery time (three levels: R1, R2, R3) and the second factor of treatment condition (two levels: CON and L-NAME) or age (two levels: young and older). When a significant main effect was observed, *post hoc* comparisons were carried out using dependent or independent samples *t* tests corrected for multiple comparisons using the Bonferroni procedure. Additionally, physical characteristics, baseline values for all variables, and local sweat rates as a function of increases in T_{es} were analyzed using an independent samples *t* test to identify age-related differences.

We evaluated the age-related difference in sweat rate by comparing responses: 1) at end exercise for each of the three exercise bouts; and, 2) as a function of an equivalent T_{es} (i.e., thermal drive) as defined by the end exercise response in young adults. Mean body temperature was calculated as: $0.8 \times T_{es} + 0.2 \times$ mean skin temperature. The onset threshold and thermosensitivity of local sweat rate during each exercise period was determined from local sweat rate plotted against mean body temperature with a segmented regression analysis as described by Cheuvront *et al.* (Cheuvront *et al.*, 2009) with aid of a computer algorithm (GraphPad Prism 6.0, GraphPad Software, La Jolla, CA, USA). The level of significance for all analyses was set at $P \leq 0.05$. Statistical analyses were performed using commercially available statistical software (GraphPad Prism 6.0, GraphPad Software, La Jolla, CA, USA). All values are reported as mean ±standard error (SE) unless otherwise indicated as standard deviation (SD).

RESULTS

Participant characteristics

Participant characteristics are presented in Table 1. There were no differences in height (P=0.348), body mass (P=0.169) and body surface area (P=0.225) between groups. Furthermore, the younger males had a greater VO_2max, both in absolute value (P=0.002) and relative to body mass (P=0.010). On the day of the experimental session prior to Ex1, all participants were well hydrated according to urine specific gravity, a measure of hydration status, with similar values between groups (Young: 1.014 ± 0.007 vs. Older: 1.016 ± 0.007, P=0.627). In addition, the absolute change before and after exercise in urine specific gravity (Young: 0.004 ± 0.005 vs. Older: 0.003 ± 0.006, P=0.820) and weight (Young: -1.13 ± 0.20 vs. Older: -1.12 ± 0.24 kg, P=0.892) did not differ between groups.

Local sweat rate

Baseline rest

Baseline resting local sweat rate was similar between young and older males (Figure 1A and B) and between treatment conditions (Figure 2A and B) (all P>0.05).

Exercise

There was a main effect of age on local sweat rate during exercise at the CON condition (P=0.038) but not at the L-NAME treated condition (P=0.817). Local sweat rate at the CON condition was greater for the young males compared to the older males at the end of Ex1 (P=0.018), but not at the end of Ex2 (P=0.291), and Ex3 (P=0.519) (Figure 1A). There was a main effect of treatment condition on local sweat rate in the young males (P=0.031), but not in the older males (P=0.537). In the young males, L-NAME resulted in a

lower local sweat rate compared to the CON condition at the end of Ex1 (P=0.004), Ex2 (P=0.013) and Ex3 (P=0.014) (Figure 2A). We found a main effect of time on local sweat rate at the CON and L-NAME treated conditions (both P<0.001). At the CON condition, in both young and older males, local sweat rate increased from end of Ex1 to end of Ex2 (both P<0.01), but not from end of Ex2 to end of Ex3 (both P>0.05) (Figure 1A). At the L-NAME treated condition, local sweat rate increased from end of Ex1 to end of Ex 2 in the older males (P=0.004), but not in the young males (P=0.098), and it did not increase from end of Ex2 to end of Ex 3 in both groups (both P>0.05, Figure 1B).

When comparing the level of sweat rate for a similar increase in T_{es} between groups with successive exercise (Figure 3), the young group had a greater end exercise sweat rate for a ΔT_{es} of 0.54°C (P=0.004) and 0.76°C (P=0.013) as measured in Ex1 and Ex 2 respectively, but not for a ΔT_{es} of 0.81°C (P=0.188) as measured in Ex3 at the CON condition. There were no differences between groups at the L-NAME treated condition (all P>0.05).

Recovery

During recovery, there was no main effect of age on local sweat rate at the CON (P=0.893) or the L-NAME (P=0.227) treatment conditions. There was no main effect of treatment condition on local sweat rate in the young (P=0.177) or older males (P=0.856). We found a main effect of time on local sweat rate at the CON condition (P=0.006), but not at the L-NAME treated condition (P=0.245). However, at the CON condition, local sweat rate was not different between end of R1 and R2 and between end of R2 and R3 in both young and older males (all P>0.05, Figure 1A).

Core and skin temperatures

Baseline rest

Compared to the young males, the older males had a lower baseline T_{es} at rest (Young: 37.09 ± 0.22 vs. Older: 36.90 ± 0.17°C, *P*=0.047). However, there were no between-group differences in baseline T_{re} (Young: 37.17 ± 0.24 vs. Older: 37.07 ± 0.29°C, *P*=0.422) and mean skin temperature (Young: 34.94 ± 0.32 vs. Older: 34.77 ± 0.56°C, *P*=0.435).

Exercise

There was no main effect of age on T_{es} (*P*=0.817), T_{re} (*P*=0.800) and mean skin temperature (*P*=0.146). There was a main effect of time on T_{es}, T_{re} and mean skin temperatures (all *P*<0.001). In both young and older males, T_{es}, T_{re} and mean skin temperature at the end of Ex2 were higher than those at the end of Ex1 (all *P*<0.05) (Table 2). In both young and older males, T_{es}, and mean skin temperature were not different between end of Ex2 and end of Ex3 (all *P*>0.05), however, T_{re} increased from end of Ex2 to end of Ex3 (*P*<0.001) (Table 2). When core and mean skin temperatures were compared as a change from baseline, the results were similar. There was no main effect of age on ΔT_{es} (*P*=0.067), ΔT_{re} (*P*=0.273), and Δmean skin temperature (*P*=0.217). There was a main effect of time on ΔT_{es}, ΔT_{re}, and Δmean skin temperature (all *P*<0.001). In both young and older males ΔT_{es}, ΔT_{re}, and Δmean skin temperature at the end of Ex2 were higher than those at the end of Ex1 (all *P*<0.05) (Table 3). In both young and older males, ΔT_{es} and and Δmean skin temperature were not different between end of Ex2 and end of Ex3 (all *P*>0.05), however, ΔT_{re} increased from end of Ex2 to end of Ex3 (*P*<0.001) (Table 3).

Recovery

There was no main effect of age on T_{es} (P=0.674), T_{re} (P=0.245) and mean skin temperature (P=0.416). We found a main effect of time on T_{re}, and mean skin temperature (both P<0.001), but not on T_{es} (P>0.05). In both young and older males, T_{re} increased from end of R1 to end of R2 (P<0.001), but not from end of R2 to end of R3 (P<0.05) (Table 2). In the young and older males, mean skin temperature did not change from end of R1 to end of R2 (P<0.05) (Table 2). Mean skin temperature decreased from the end of R2 to the end of R3 in the older males (P<0.05). No differences were measured in the young males (both P>0.05) (Table 2). Likewise, there was no main effect of age on ΔT_{es} (P=0.062) and Δmean skin temperature (P=0.980). There was, however, a main effect of age on ΔT_{re} (P=0.040). The older males had a greater ΔT_{re} at the end of R2 (P=0.002) and R3 (P=0.001) compared to the younger males. We found a main effect of time on ΔT_{re} and Δmean skin temperature (both P<0.001), but not on ΔT_{es} (P>0.05). In both young and older males, ΔT_{re} increased from end of R1 to end of R2 (P<0.001), but not from end of R2 to end of R3 (P<0.05) (Table 3). In the young and older males, Δmean skin temperature did not change from end of R1 to end of R2 (P<0.05) (Table 3). ΔMean skin temperature decreased from end of R2 to end of R3 in the older males (P<0.05). No differences were measured in the young males (P>0.05) (Table 3).

Mean body temperature onset threshold and sensitivity for local sweat rate

There was no main effect of age or treatment condition on the mean body temperature onset threshold or on the thermosensitivity for local sweat rate (all P>0.05, Table 4). Therefore, onset threshold for local sweat rate was not different between young and older adults at the CON condition and at the L-NAME treated condition. Also, no differences

in thermosensitivity between young and older adults for local sweat rate were found at the CON condition and at the L-NAME treated condition. There was a main effect of time on the mean body temperature onset threshold for local sweat rate for both the CON and L-NAME treated conditions (both $P<0.001$), but not for thermosensitivity at the both treated conditions (both $P>0.05$). Irrespective of group or treatment condition, mean body temperature at the onset threshold for local sweat rate increased from Ex1 to Ex2 (all $P<0.05$), but not from Ex2 to Ex3 (all $P>0.05$). When mean body temperature threshold was expressed as a change from baseline, there was still no main effect of age or treatment condition (all $P>0.05$, Table 4).

DISCUSSION

The present study is the first to examine age-related changes in the role of NO on local forearm sweat rate during intermittent exercise in the heat. Older males had a lower local sweat rate at the end of the first exercise bout compared to the young males, but thereafter local sweat rate was similar between groups for the subsequent two exercise/recovery cycles. We showed that while NOS inhibition with L-NAME reduced sweating at the end of each exercise bout relative to the control condition in the young males, no effect of L-NAME was evident in the older males. Additionally, local sweat rate at the end of each recovery was not affected by L-NAME compared to the control condition in either the young or older males. We show that aging diminishes NO-dependent sweating during short bouts of exercise in the heat.

Age-related reduction in sweating

By design, we used a similar fixed rate of metabolic heat production for the young and older adults (Jay *et al.*, 2011; Gagnon *et al.*, 2013b). In the present study, local sweat

rate measured at the CON condition at the end of the first exercise bout was lower in the older males compared to the young males (Figure 1A). However, given that end exercise core temperature differed between groups it was not possible to clearly define the extent to which differences in thermal drive may have influenced the sweating response. When we compared the sweat rates based on an equivalent core temperature response, we show that older adults demonstrate a reduced capacity to dissipate heat as evidenced by a markedly lower sweat rate. Therefore, our results show that true age-related impairments in sweating are evident and remain intact with successive exercise bouts.

In contrast to exercise, there were no differences in sweat rate measured during the recovery period between the young and older adults. In fact, we show that the young and older adults demonstrate a rapid reduction in sweating followed by a plateau at near baseline resting levels which is maintained for the duration of the recovery period. These findings are consistent with recent reports demonstrating that whole-body evaporative heat loss is attenuated to a similar extent in young and older adults despite the fact that older adults stored more heat at the end of exercise (Larose *et al.*, 2013d). As our study findings demonstrate, the pattern of the postexercise sweating response cannot be attributed to age-related differences in NO-dependent influences on sweating. It could be argued, however, that the control of sweating postexercise in both young and older adults may be mediated by factors of nonthermal origin. The latter mechanism is discussed in greater detail in the subsequent sections.

NO-dependent sweating in young males

Our finding of a diminished local sweat rate at the L-NAME treated condition in comparison to the CON condition in the young males (Figure 2A) demonstrates that

increases in NO augments sweat production during exercise. A similar result was also shown in a previous study of young males during 30 min of continuous moderate intensity exercise (~47% VO_{2peak}) (Welch *et al.*, 2009). Additionally, it is important to note that the attenuation in local sweat rate measured at the L-NAME treated condition relative to the CON condition was not limited to the first exercise bout. In fact we observed a similar attenuation during the second and third exercise bouts despite the greater increase in core temperature and therefore thermal drive.

While this study was not specifically designed to assess the underlying mechanisms for the NO-dependent influences on sweating, some insight may be gleaned from previous studies. First, potassium (K^+) and chloride (Cl^-) channels are involved in sweat secretion (Sato, 1993). NO activates Cl^- channels as demonstrated in human lung epithelial cells (Kamosinska *et al.*, 1997). Thus, NO may activate Cl^- channels in human sweat glands, contributing to the sweating response in young adults. Secondly, calcium-activated potassium (KCa) channels are found in equine sweat gland epithelial cells (Huang *et al.*, 1999) and NO activates KCa channels in the aortal smooth muscle of rabbit (Bolotina *et al.*, 1994). Therefore, it is possible that a similar response may occur in human sweat glands, contributing to the sweat response in young adults. Taken together, one or more of these mechanisms may explain the role of NO in sweating in young adults. Alternatively, it is equally possible that none of these mechanisms may explain the observed pattern of response. Further studies are required to examine these potential mechanisms.

In the present study, L-NAME did not reduce local sweat rate at the end of each recovery compared with the CON condition in our younger males, suggesting that unlike during exercise, there is no role of NO on the sweat response during recovery. Alternatively, it is possible that the role of NO is less evident at low sweat rates similar to those levels

measured during recovery (i.e., ~0.2 to 0.4 $mg \cdot min^{-1} \cdot cm^{-2}$ for our younger males). In keeping with our findings, Kellogg *et al*. (Kellogg *et al.*, 1998) reported no effect of NOS inhibition on local sweat rates below 0.40 $mg \cdot min^{-1} \cdot cm^{-2}$ during a passive heat stress in young males. Similarly, we showed no role of NO on sweating in the beginning of exercise when local sweat rates were below ~0.4 $mg \cdot min^{-1} \cdot cm^{-2}$, however, a role of NO on sweating was observed at the end of each exercise bout where local sweat rate exceeded ~0.9 $mg \cdot min^{-1} \cdot cm^{-2}$ in the young males (Figure 2A). Also, Welch *et al.* (Welch *et al.*, 2009) reported no role of NO on sweating in young males during the beginning of continuous exercise in the heat where local sweat rate was below 1.3 $mg \cdot min^{-1} \cdot cm^{-2}$ but thereafter they observed a clear NO contribution to sweating. As such, it is possible that NO-dependent sweating in young adults only occurs above a certain sweat rate. Alternatively, it is plausible that other factors of nonthermal origin may have exerted a more pronounced effect in the control of sweating in the postexercise period. Numerous studies have reported that whole-body evaporative heat loss (i.e., sweating) as well as skin blood flow is rapidly attenuated following dynamic exercise despite sustained elevations in core and muscle temperature in young adults (Thoden *et al.*, 1994; Kenny *et al.*, 2006; Kenny *et al.*, 2009). This has been ascribed to a nonthermal baroreflex-mediated response associated with postexercise hypotension (Kenny & Jay, 2013). While we cannot confirm that the lack of NO-dependent sweating is a result of a greater nonthermal-mediated suppression of sweating, the pattern of response observed in this study is consistent with previous reports of nonthermal modulation of sweating.

Diminished NO-dependent sweating in older males

We found that local sweat rate at the end of each exercise remained similar between the CON and L-NAME treated conditions in the older adults (Figure 2B), and the pattern of

response remained intact with successive exercise bouts. These findings suggest that the bioavailability of NO is reduced in aged skin, possibly diminishing NO-dependent sweating. Currently, the precise mechanism by which aging impairs NO-dependent sweating remains unclear. However, studies examining skin blood flow during whole-body heat stress at rest show that the bioavailability of NO is reduced in aged skin (Holowatz *et al.*, 2003; Holowatz *et al.*, 2006b, a; Stanhewicz *et al.*, 2012; Stanhewicz *et al.*, 2013). This is thought to be due to age-related differences in oxidative stress (Holowatz *et al.*, 2006a) and/or arginase activity (Holowatz *et al.*, 2006b). Further studies are required to assess these mechanisms.

We show that sweat rate at the L-NAME treated condition was significantly increased from the end of Ex1 to the end of Ex2 (P=0.004). This was equivalent to a ~19% increase in sweating (Figure 1B). In view of the fact that sweat rate was comparable between the CON and L-NAME treated conditions, this suggests that increases in sweat rate may be mediated through NO-independent mechanisms in older adults. An *in vitro* study showed that prostaglandin E_1 (PGE_1), which is produced by cyclooxygenase (COX)-1 and -2, can directly increase sweat rate (Sato, 1977). Further, an age-dependent COX-2 up-regulation was reported in the mesenteric artery of rats (Ramos-Alves *et al.*, 2012), thereby modulating the production of PGE_1 which ultimately increases sweating. Although speculative, up-regulation of COX-2 may also occur in the eccrine sweat glands of older adults, increasing PGE_1 and therefore sweat rate.

Similar to the response measured in young adults, there were no differences in sweat rate at the CON or L-NAME treated condition during recovery in the older adults. Moreover, the level of sweating achieved during recovery was similar between young and older adults despite the marked differences in end exercise sweating. As discussed above, nonthermal

factors have been shown to have a pronounced influence on heat dissipation following cessation of exercise. In light of the similar sweating responses observed between young and older adults following cessation of exercise, it is plausible that modulation of the sweating response may be influenced by nonthermal factors. To date, little is known about the relative contribution of thermal and nonthermal factors in the regulation of postexercise heat loss in older adults. However, given the similar postexercise sweating response measured in both the young and older adults, it is conceivable that as reported in the case of young adults (Kenny & Gagnon, 2010), the attenuation in sweating may be linked to a baroreceptor-mediated suppression of heat dissipation in older adults.

Limitations

It is important to note that in the current study local sweat rate was only measured at the forearm. Age-related decrements in sweating may not occur at an even rate across the body (Inoue *et al.*, 1991; Inoue & Shibasaki, 1996; Dufour & Candas, 2007; Smith *et al.*, 2013a) and have been found to vary between individuals of similar ages (Inoue *et al.*, 2004; Smith & Havenith, 2012; Smith *et al.*, 2013b). Recently, Smith *et al.* (Smith *et al.*, 2013a) observed reduced sweating during whole-body heating at rest in older compared to young adults at the arm, abdomen, thigh and lower back, with greatest impairment observed on the abdomen. Therefore, it is conceivable that a more pronounced age-related difference in the sweating response might have been observed in other areas such as the abdomen. It is possible that L-NAME did not diffuse to all of the skin area covered by the sweat capsule (3.8 cm^2). However, we show that the relative reduction in sweat rate during exercise measured at the L-NAME treated condition (~30%) in the present study was similar to the findings reported by Welch *et al.* (2009) (~25%) who used a smaller sized sweat capsule

(0.567 cm^2). Taken together, these finding suggest that the use of sweat capsules ranging from 0.567-3.8 cm^2 do not influence the effectiveness of L-NAME in the regulation of sweating. Additionally, in the present study we examined age-related differences in the sweating response in males only. Recent studies have found sex-related differences in the sweat response (Gagnon *et al.*, 2008a; Gagnon *et al.*, 2013b). As such, our findings cannot be generalized to young or older females.

Conclusions

We show that NOS inhibition with L-NAME significantly attenuates local forearm sweat rate relative to the control condition during each of the 15 min exercise bouts despite progressively greater increases in thermal drive in young adults. In contrast, there was no significant difference in sweating between the CON and L-NAME treated conditions in the older adults. Local forearm sweat rate during each recovery period was not significantly different between the CON and L-NAME treated conditions in both young and older adults, and the pattern of response was similar between groups. In summary, NO-dependent sweating during intermittent exercise is impaired in older adults.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

Jill M. Stapleton and Glen P. Kenny contributed to the conception and design of the experiment, to the collection analysis and interpretation of data and drafting the article and revising it critically for important intellectual content. Naoto Fujii and Michael Carter contributed to the collection analysis and interpretation of data and drafting the article and revising it critically for important intellectual content. We confirm that all authors approved the final version of the manuscript and all authors qualify for authorship.

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TABLES

Table 1. Participant characteristics.

Group	Age (years)	Height (cm)	Body mass (kg)	Body surface area (m^2)	Body fat (%)	VO_2max ($L \cdot min^{-1}$)	VO_2max ($mL \cdot kg^{-1} \cdot min^{-1}$)
Young	23 ± 3*	179.9 ± 6	81.6 ± 7.7	2.01 ± 0.11	17.6 ± 5.0	4.1 ± 0.8*	50.2 ± 9.1*
Older	64 ± 5	177.4 ± 5.3	75.7 ± 10.3	1.95 ± 0.12	19.5 ± 7.1	3.0 ± 0.6	39.5 ± 7.5

VO_2max, maximum oxygen consumption. A significant difference between young and older males is denoted by an asterisk (*). All values are presented as means ± SD.

Table 2. Core and mean skin temperature responses at the end of successive exercise and recovery periods.

Time	T_{es} (°C)		T_{re} (°C)		Mean skin temperature (°C)	
	Young	**Older**	**Young**	**Older**	**Young**	**Older**
Ex1	37.66 ± 0.37	37.61 ± 0.23	37.51 ± 0.27	37.37 ± 0.22	35.61 ± 0.32	35.42 ± 0.55
Ex2	37.87 ± 0.38	37.93 ± 0.23	37.89 ± 0.30	37.84 ± 0.29	35.75 ± 0.36	35.52 ± 0.51
Ex3	37.92 ± 0.38	38.05 ± 0.30	38.05 ± 0.33	38.20 ± 0.28	35.76 ± 0.40	35.36 ± 0.54
R1	37.38 ± 0.26	37.35 ± 0.17	37.60 ± 0.29	37.56 ± 0.29	35.29 ± 0.42	35.08 ± 0.46
R2	37.50 ± 0.29	37.48 ± 0.22	37.80 ± 0.33	38.01 ± 0.33	35.30 ± 0.43	35.05 ± 0.47
R3	37.49 ± 0.38	37.39 ± 0.29	37.88 ± 0.31	38.16 ± 0.35	34.99 ± 0.53	34.64 ± 0.58

Ex, exercise; R, recovery; T_{es}, esophageal temperature; T_{re}, rectal temperature. No significant differences were observed between age groups. All values are presented as means ± SD.

Table 3. Relative changes in core and mean skin temperature responses at the end of successive exercise and recovery periods.

Time	ΔT_{es} (°C)		ΔT_{re} (°C)		ΔMean skin temperature (°C)	
	Young	**Older**	**Young**	**Older**	**Young**	**Older**
Ex1	0.57 ± 0.25	0.71 ± 0.24	0.35 ± 0.08	0.31 ± 0.09	0.67 ± 0.19	0.61 ± 0.16
Ex2	0.78 ± 0.29	1.03 ± 0.25	0.72 ± 0.16	0.77 ± 0.14	0.82 ± 0.27	0.69 ± 0.22
Ex3	0.84 ± 0.31	1.12 ± 0.38	0.89 ± 0.17	1.07 ± 0.18	0.82 ± 0.32	0.62 ± 0.27
R1	0.29 ± 0.09	0.46 ± 0.12	0.43 ± 0.11	0.49 ± 0.12	0.35 ± 0.31	0.35 ± 0.31
R2	0.41 ± 0.14	0.58 ± 0.20	0.63 ± 0.16	0.91 ± 0.17	0.36 ± 0.33	0.36 ± 0.33
R3	0.40 ± 0.29	0.49 ± 0.31	0.71 ± 0.16	1.07 ± 0.20	0.20 ± 0.39	0.37 ± 0.09

Ex, exercise; R, recovery; ΔT_{es}, change in esophageal temperature; ΔT_{re}, change in rectal temperature. No significant differences were observed between age groups. All values are presented as means ± SD.

Table 4. Absolute and relative mean body temperature onset thresholds and thermosensitivities for each exercise period.

Time	Absolute onset threshold for sweat rate (°C)		Relative onset threshold for sweat rate (°C)		Thermosensitivity of sweat rate ($mg \cdot min^{-1} \cdot cm^{-2}/°C$)	
	Young	**Older**	**Young**	**Older**	**Young**	**Older**
CON						
Ex1	36.79 ± 0.24	36.58 ± 0.27	0.11 ± 0.13	0.07 ± 0.14	0.63 ± 0.83	0.90 ± 0.58
Ex2	36.99 ± 0.25	36.92 ± 0.19	0.30 ± 0.13	0.41 ± 0.16	1.58 ± 0.91	1.01 ± 0.43
Ex3	37.08 ± 0.26	36.99 ± 0.31	0.40 ± 0.15	0.48 ± 0.30	1.90 ± 1.42	0.76 ± 0.47
L-NAME						
Ex1	36.81 ± 0.30	36.55 ± 0.27	0.12 ± 0.18	0.06 ± 0.16	1.28 ± 0.69	1.00 ± 0.82
Ex2	37.03 ± 0.26	36.90 ± 0.24	0.35 ± 0.11	0.39 ± 0.15	1.24 ± 0.64	0.86 ± 0.49
Ex3	37.11 ± 0.24	37.03 ± 0.28	0.43 ± 0.14	0.51 ± 0.25	1.42 ± 0.85	0.75 ± 0.50

Ex, exercise; CON, control; L-NAME, N^G-nitro-L-arginine methyl ester. No significant differences were observed between age groups. All values are presented as means ± SD.

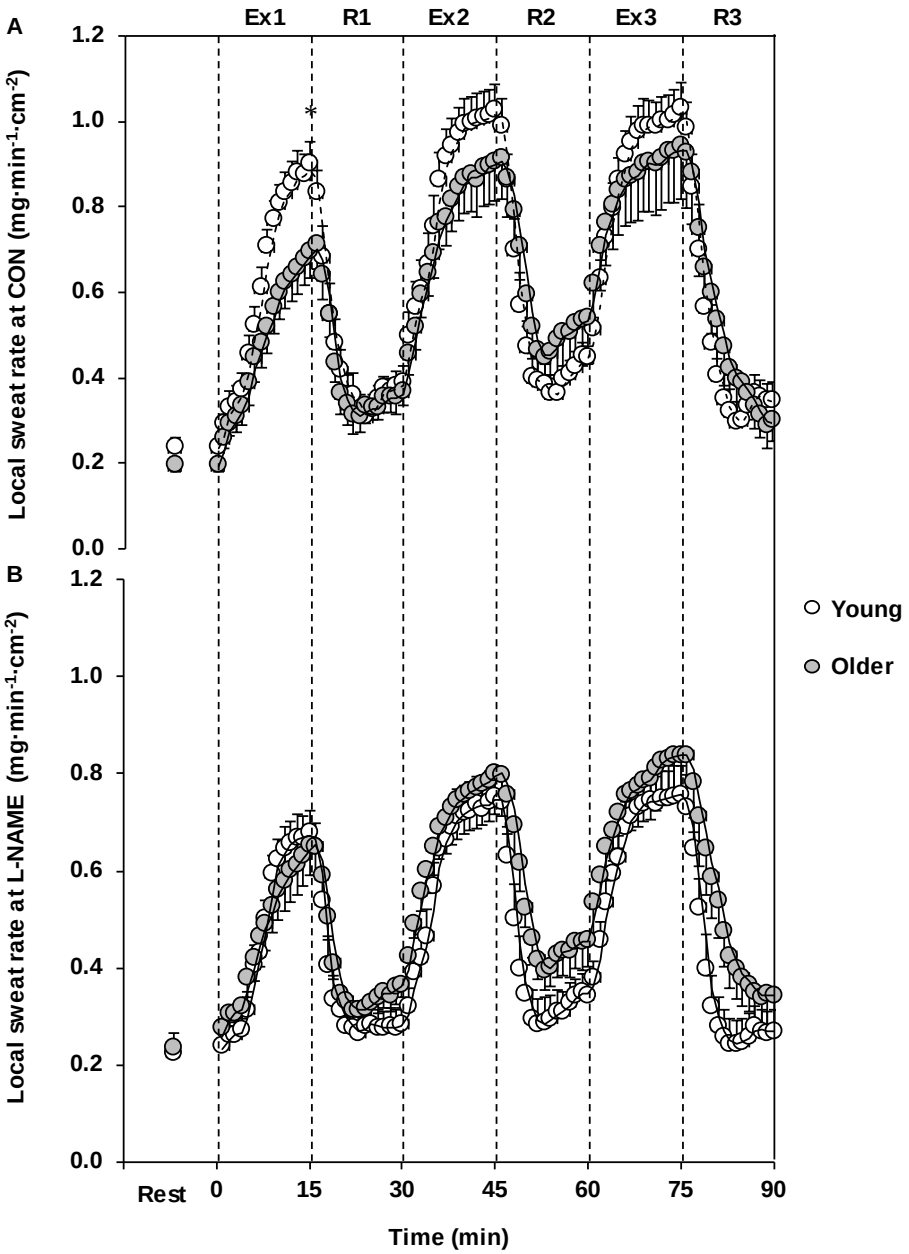

Figure 1. Local sweat rates at CON (panel A) and L-NAME treated (panel B) conditions during exercise (Ex) and recovery (R) in the heat (35.0°C, 20% relative humidity). Data from young males are presented in white circles while data from older males are presented in grey circles. All values are presented as means ± SE. A significant difference between age groups is denoted by an asterisk (*).

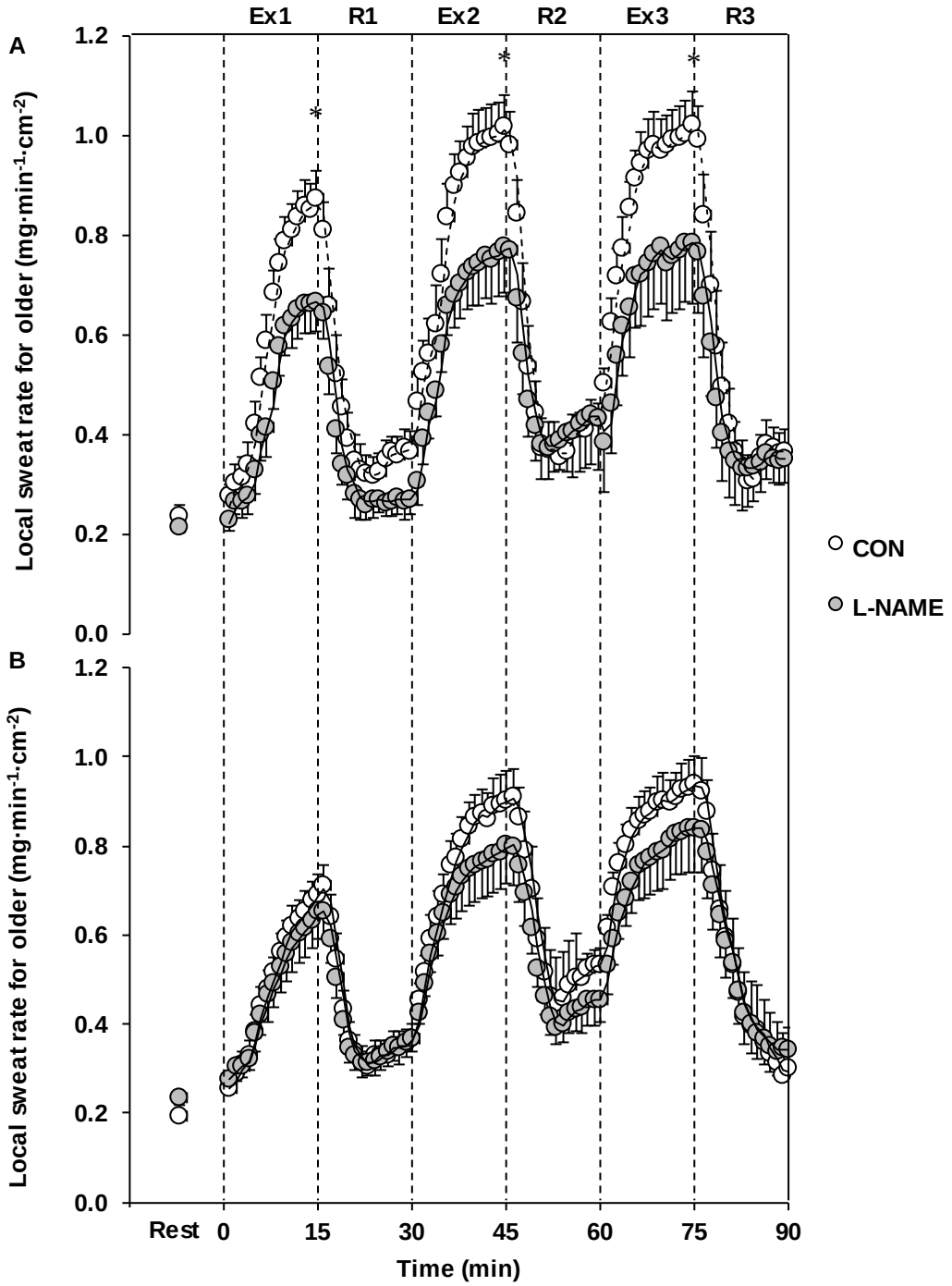

Figure 2. Local sweat rates for young (panel A) and older (panel B) males during exercise (Ex) and recovery (R) in the heat (35.0°C, 20% relative humidity). Data from CON condition are presented in white circles while data from L-NAME treated condition are presented in grey circles. All values are presented as means ± SE. A significant difference between treatment condition is denoted by an asterisk (*).

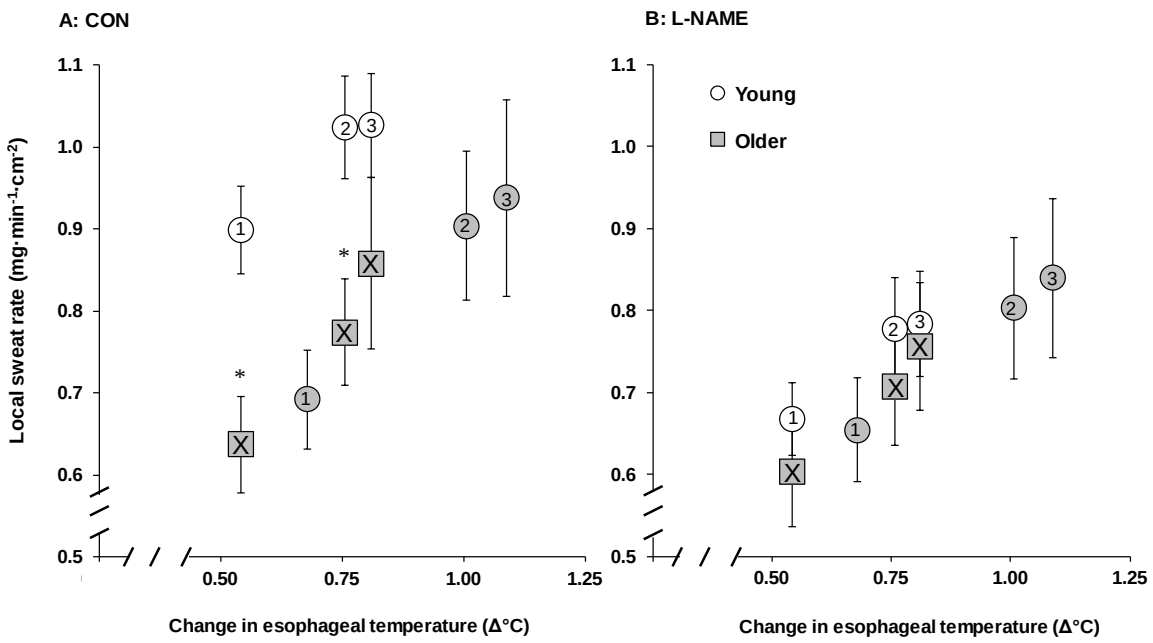

Figure 3. Local sweat rates at the end of each exercise bout for young (white circles) and older (light grey circles) males as a function of the successive exercise bouts at the CON (panel A) and L-NAME (panel B) treated condition. The squares with an 'X' represent the sweat rate achieved by the older males as a function of a similar increase in esophageal temperature measured at end exercise for the young adults. The numbers represent the first (1), second (2) and third (3) exercise bouts, respectively. All values are presented as means ± SE. A significant difference between young and older males at the similar increase in esophageal temperature is denoted by an asterisk (*).

3.5 Thesis article #5 – published in Physiological Reports, 2(7): pii: e12078, 2014 – see Appendix C

Age-related differences in post-synaptic increases in sweating and skin blood flow postexercise

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Running title: Postexercise thermoeffector activity and aging

Key words: exercise, dose response, heat loss, skin perfusion, sweat rate, nonthermal factors

Word count: 6,685

Subject area: Human/environmental and exercise physiology

NEW FINDINGS

- What is the central question of this study? Centrally-mediated factors of nonthermal origin modulate the control of heat loss following exercise in young adults. It is unclear if peripheral factors also play a role and whether the mechanisms modulating the postexercise suppression of heat loss differ as a function of age.

- What is the main finding and its importance? Peripheral factors do not modulate postexercise sweating and skin blood flow in both young and older adults. End organ function of sweating is impaired in older adults, but to a similar extent pre and postexercise.

ABSTRACT

The influence of peripheral factors on the control of heat loss responses (i.e., sweating and skin blood flow) in the postexercise period remains unknown in young and older adults. Therefore, in 8 young (22 ± 3 years) and 8 older (65 ± 3 years) males, we examined dose-dependent responses to administration of acetylcholine (ACh) and methacholine (MCh) for sweating (ventilated capsule), as well as to ACh and sodium nitroprusside (SNP) for cutaneous vascular conductance (CVC, laser-Doppler flowmetry, % of max). In order to assess if peripheral factors are involved in the modulation of thermoeffector activity postexercise, pharmacological agonists were perfused via intradermal microdialysis on two separate days: 1) at rest (**DOSE**) and 2) following a 30-min bout of exercise (**Ex+DOSE**). No differences in sweat rate between the DOSE and Ex+DOSE conditions at either ACh or MCh were observed for the young (ACh: P=0.992 and MCh: P=0.710) or older (ACh: P=0.775 and MCh: P=0.738) adults. Similarly, CVC was not different between the DOSE and Ex+DOSE conditions for the young (ACh: P=0.123 and SNP: P=0.893) or older (ACh: P=0.113 and SNP: P=0.068) adults. Older adults had a lower sweating response for both the DOSE (ACh: P=0.049 and MCh: P=0.006) and Ex+DOSE (ACh: P=0.050 and MCh: P=0.029) conditions compared to their younger counterparts. These findings suggest that peripheral factors do not modulate postexercise sweating and skin blood flow in both young and older adults. Additionally, sweat gland function is impaired in older adults, albeit the impairments were not exacerbated during postexercise recovery.

INTRODUCTION

Thermoregulatory control of sweating and skin blood flow during postexercise recovery is altered such that at the cessation of dynamic exercise, there is a rapid decrease in sweating and skin blood flow despite a significant residual heat load (Kenny & Jay, 2013). As a result, the rate of whole-body heat loss is reduced and consequently is paralleled by a prolonged elevation in core and muscle temperatures above pre-exercise baseline levels for 60 to 90 min (Kenny *et al.*, 2006; Kenny *et al.*, 2007; Kenny & Gagnon, 2010). It has been suggested that centrally-mediated factors of nonthermal origin (i.e., baroreceptor loading status) can modulate the control of heat loss following exercise in young adults (Carter *et al.*, 2002; Jackson & Kenny, 2003; Wilson *et al.*, 2004; Journeay *et al.*, 2006; Kenny *et al.*, 2006; Jay *et al.*, 2007b; Kenny *et al.*, 2008b). However, it has yet to be determined whether or not peripheral factors, such as sensitivity of the effector organ (i.e., sweat glands and/or skin vessels), contribute to the control of heat loss postexercise.

Peripheral factors modulating heat loss postexercise can be assessed by examining changes in sweat production and skin vasodilation to increasing doses of pharmacological agonists. For example, exogenously administering incremental doses of acetylcholine (**ACh**) and methacholine (**MCh**) can be employed to examine sweat gland function (Kenney & Fowler, 1988; Inoue *et al.*, 1999a; Lee & Mack, 2006; Kimura *et al.*, 2007; Gagnon *et al.*, 2013a; Smith *et al.*, 2013a; Metzler-Wilson *et al.*, 2014). Further, differences in sweat rate observed between ACh (hydrolyzed by acetylcholinesterase, **AChE**) and MCh (resistant to AChE) can allude to whether or not the response is mediated by AChE enzyme activity (Kimura *et al.*, 2007). Likewise, perfusion of endothelium-dependent (ACh) and/or endothelium-independent (sodium nitroprusside, **SNP**) agonists in an incremental manner can be utilized to examine skin vascular function (Lee & Mack, 2006; Medow *et al.*, 2008;

Bruning *et al.*, 2012; Gagnon *et al.*, 2013a; Smith *et al.*, 2013a). To the best of our knowledge, no study has examined if peripheral mechanisms contribute to the disturbance of postexercise heat loss responses of sweating and skin blood flow.

To date, much of our limited understanding of the underlying mechanisms governing the control of postexercise heat loss responses is based on findings obtained in young adults. Human aging is associated with attenuated sweating and skin vasodilation during exercise (Anderson & Kenney, 1987; Kenney & Anderson, 1988; Tankersley *et al.*, 1991; Inoue *et al.*, 1999a; Larose *et al.*, 2013a; Larose *et al.*, 2013c; Larose *et al.*, 2013d). However, the extent to which these age-related impairments in the thermoeffector activity may influence heat dissipation during the postexercise recovery period remains unclear. Some insight may be gleaned from a recent study by Larose *et al.* (Larose *et al.*, 2013d) who examined local and whole-body heat loss responses in young and older adults during intermittent exercise in the heat (Larose *et al.*, 2013d). They found that despite greater heat storage during each of the four 15-min exercise bouts in the older adults, the magnitude of the postexercise suppression in whole-body evaporative heat loss, as measured by direct calorimetry, was similar between the young and older males (Larose *et al.*, 2013d). A similar pattern was measured for the local responses of sweating and skin blood flow. These findings suggest the likely possibility that the underlying factors affecting postexercise heat dissipation may be of similar origin for young and older adults.

Previous studies have compared sweat rates and skin vasodilation between young and older adults at rest with the use of pharmacological stimulation and have yielded conflicting results. While some studies have reported an attenuated sweating response in older adults as assessed by a subcutaneous injection of 5 mM of MCh (Kenney, 1988; Inoue *et al.*, 1999a), others observed no differences using intradermal microdialysis to administer increasing

doses (1 x 10^{-7} to 0.1 M) of ACh (Smith *et al.*, 2013a). Further, Bruning *et al.* (Bruning *et al.*, 2012) reported an attenuated ACh-induced skin vasodilation in middle-aged (53 ± 1 years) compared with younger (23 ± 1 years) adults at the highest concentration employed (0.1 M) while infusing ACh via intradermal microdialysis in a dose response manner (Bruning *et al.*, 2012). Others however, have not observed any differences in skin blood flow between young and older adults receiving doses of ACh from 1 x 10^{-7} to 0.1 M (Holowatz *et al.*, 2005; Smith *et al.*, 2013a). To date, no study has evaluated if age-related differences in sweating or skin vasodilation exist at higher doses of ACh or MCh (>0.1 M). It remains to be determined if older adults have an attenuated responsiveness to the administration of pharmacological agonists (>0.1 M) compared to their younger counterparts and whether or not the same pattern of response exists postexercise.

Thus, the purpose of this study was two-fold: to examine 1) the extent to which peripheral factors (i.e., sweat gland and skin vasodilatory function) contribute to the postexercise suppression of heat loss responses; and 2) whether there are differences in the mechanisms modulating postexercise heat loss as a function of age. We hypothesized that: 1) peripheral factors would not modulate the postexercise suppression of heat loss as determined by local measurements of sweating and skin blood flow and, 2) the mechanisms for postexercise suppression of heat loss would not differ as a function of age, but older adults would have an attenuated responsiveness to the administration of the pharmacological agonists compared to their younger counterparts.

METHODS

Ethical Approval

This study was approved by the University of Ottawa Health Sciences and Science Research Ethics Boards, in accordance with the Declaration of Helsinki. Written, informed consent was obtained from all participants prior to their involvement in the study.

Participants

Sixteen males volunteered for the study and were divided into two groups of 8 young (18-25 years) and 8 older (61-70 years). All participants were healthy, non-smoking, physically active males free from cardiovascular disease and diabetes. Physical characteristics of the participants are presented in Table 1.

Experimental Procedures

Each participant completed one preliminary and two experimental sessions. The experimental sessions were performed in a random order and on separate days with a minimum of 72 hours and maximum of 2 weeks between sessions. During the preliminary session, body height, mass, and density, as well as maximum oxygen uptake (VO_2max) were determined. Body height was determined using a stadiometer (Detecto, model 2391, Webb City, MO, USA), while body mass was measured using a digital high-performance weighing terminal (model CBU150X, Mettler Toledo Inc., Mississauga, ON, Canada). Body surface area was subsequently calculated from the measurements of body height and mass (DuBois & DuBois, 1989). Body density was measured using the hydrostatic weighing technique, and body fat percentage was calculated using the Siri equation (Siri, 1956). VO_2max was measured during a progressive cycle ergometer protocol which consisted of a 2-min warm-

up at 40 W followed by 20 W increments every min until the participant could no longer maintain a pedaling cadence of at least 60 rpm. Continuous electrocardiographic monitoring was used for the older males during the maximal exercise test under supervision of a qualified technician.

Participants performed the experimental sessions at the same time of day and were asked to drink 500 mL of water the night prior to, as well as the morning of the experimental session. They were also asked to refrain from alcohol, caffeine, and exercise 24 hours prior to experimentation. Upon arrival at the laboratory, the participants provided a urine sample and a baseline body mass was measured. They subsequently rested quietly in an upright semi-recumbent posture on a bed in a room set to an ambient temperature of 24°C and 20% relative humidity. During this time, three microdialysis fibers (MD 2000, Bioanalytical Systems, West Lafayette, IN, USA) were placed in the dermal space of the forearm under aseptic conditions. To place the fibers, a 25-gauge needle was inserted into the dermal space of the lateral mid-anterior aspect of the left forearm and then exited the skin 20–25 mm away from the point of entry. The microdialysis fiber was inserted through the lumen of the needle. The needle was subsequently withdrawn, leaving the semi-permeable membrane (30KDa cutoff, 10 mm membrane) in place under the skin. After insertion, the fibers were perfused with lactated Ringer's solution at a rate of 2 $\mu L \cdot min^{-1}$ via a perfusion pump (CMA/400, CMA Microdialysis, Solna, Sweden).

For one of the experimental conditions (**DOSE**), participants remained resting in an upright semi-recumbent posture on the bed in a non-heat stress environment (i.e., ambient temperature of 24°C and 20% relative humidity) for 60 to 90 min after the fiber placement (to allow for hyperemia associated with fiber insertion trauma to subside) (Anderson *et al.*,

1994). Baseline resting data were obtained for 10 min following the hyperemia response. Subsequently, increasing doses of methacholine (**MCh,** *site 1*) and acetylcholine (**ACh,** *site 2*) (Sigma Aldrich, Oakville, ON, Canada) were infused in a dose-dependent manner at two mid-anterior forearm skin sites to assess the sweating response. The ACh infusion at site 2 as well as infusion of sodium nitroprusside (**SNP**, *site 3*) was used in a dose-dependent manner to assess skin vasodilation (Sigma Aldrich). All pharmacological agonists were infused in 10-fold increments, from 1 x 10^{-6} M to 1 M for MCh and ACh, and from 5 x 10^{-6} M to 5 x 10^{-2} M for SNP (Gagnon *et al.*, 2013a). Each dose was initially primed through the microdialysis membrane at an infusion rate of 100 $\mu L \cdot min^{-1}$ for ~1 min, thereafter, each dose was infused for 8 min at a rate of 2 $\mu L \cdot min^{-1}$. This amount of time ensured a plateau in sweat rate or skin blood flow was reached at each concentration of the agonists. A higher dose of ACh and MCh (1.5 M) was infused at the end for an additional 25 min while the maximum dose of SNP (5 x 10^{-2} M) continued to be infused to ensure a steady-state maximal response to the highest concentration employed.

For the second experimental condition (**Ex+DOSE**), after fiber placement, the participants entered a thermal chamber regulated to an ambient air temperature of 30°C and 20% relative humidity. The participants rested for 60 to 90 min on a semi-recumbent cycle ergometer (Corival, Lode B.V., Groningen, Netherlands) while the remainder of the instrumentation was placed. Once the instrumentation was placed and the hyperemia response had subsided, baseline resting data were obtained for 10 min after which the participants performed a 30-min exercise bout. To ensure that both groups received a similar heat load they exercised at the same constant rate of metabolic heat production of 247 ± 17 $W \cdot m^{-2}$ (equivalent to 46.3 ± 4.7% VO_2max for the young and 55.7 ± 6.9% VO_2max for the older males). Following the exercise bout, participants rested for 15 min to allow local sweat

rate and skin blood flow to return to baseline resting values (Kenny *et al.*, 2007). At this point, the dose response relationships for sweating and skin vasodilation were assessed in the same manner as for the DOSE experimental session.

Measurements

The ventilated capsule technique was employed for the purpose of measuring local sweat rate. Sweat rate was measured from 3.8 cm^2 plastic 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 placed directly over the fiber membrane of each agonist sites (i.e., skin sites 1 and 2). The sweat capsule at the ACh site (*site 2*) also housed the laser-Doppler flow probe (see details below), allowing for the simultaneous measurement of local sweat rate and skin blood flow. Compressed dry nitrogen was passed through each capsule at a rate of 0.5 $L \cdot min^{-1}$. Long 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 trial. Water content of the effluent air was measured using high precision dew point mirrors (model 473, RH systems, Albuquerque, NM, USA) or capacitance hygrometers (Vaisala, Woburn, WA, USA). Both instruments offer precise, quality measures of changes in humidity at the skin (RH systems: dew point accuracy = ±0.2°C and Viasala: absolute humidity accuracy =~1.08 g/m^3). Local sweat rate was calculated using the difference in water content between effluent and influent air multiplied by the flow rate and normalized for the skin surface area under the capsule. Local skin blood flow was estimated at 32 Hz using laser-Doppler velocimetry (PeriFlux System 5000, Perimed AB, Stockholm, Sweden). A laser-Doppler probe (integrating probe 413, Perimed AB, Stockholm, Sweden) was placed directly over the microdialysis membrane at the ACh

(*site 2*) and SNP (*site 3)* sites. Cutaneous vascular conductance (CVC) was subsequently calculated as the ratio of skin blood flow perfusion units to mean arterial pressure and expressed as a percentage of maximum.

Systolic and diastolic blood pressures were determined manually using brachial auscultation at the end of each 8 min infusion during the DOSE condition. Mean arterial pressure was then calculated as diastolic blood pressure + 1/3 × pulse pressure (difference between systolic and diastolic pressure). Additionally, mean arterial pressure was measured continuously using a Finometer (Finapres Medical Systems, Amsterdam, the Netherlands) from the beat-to-beat recording of the left middle finger arterial pressure waveform with the volume-clamp method (Penaz, 1973) and physiocal criteria (Wesseling *et al.*, 1995) during the Ex+DOSE condition. The left middle finger was supported at heart level for calibration and for the duration of the experimental protocol. Blood pressures were verified during the Ex+DOSE condition by manual brachial auscultation.

Rectal temperature was measured during the Ex+DOSE condition using a general purpose thermocouple temperature probe (Mallinckrodt Medical Inc., St-Louis, MO, USA) inserted to a minimum of 12 cm past the anal sphincter. Rectal temperature data were collected using a HP Agilent data acquisition module (model 3497A; Agilent Technologies Canada Inc., Mississauga, ON, Canada) at a rate of one sample every 15 s and simultaneously displayed and recorded in spreadsheet format on a personal computer with LabVIEW software (Version 7.0, National Instruments, TX, USA).

A pre-experimental test urine sample was obtained to ensure all participants were in a euhydrated state. Urine specific gravity was determined in duplicate using a handheld total solids refractometer (model TS400, Reichter Inc., Depew, NY, USA).

Data analysis

To determine the concentration of the agonist causing 50% of the maximal response (EC_{50}), dose response curves were created by plotting local sweat rate and CVC as a function of the log concentration of the agonist and fitted using a nonlinear regression analysis with a Hill slope of 1 (GraphPad Prism 6.0, GraphPad Software, La Jolla, CA, USA) (Davis *et al.*, 2007; Kimura *et al.*, 2007). The log EC_{50} is an indicator of sensitivity of the end organ to the agonist where a negative log EC_{50} closer to 0 indicates a lower sensitivity. Baseline resting data for both the DOSE and Ex+DOSE conditions were obtained by averaging the final 5 min of the 10-min baseline resting period. Postexercise data were obtained for the Ex+DOSE condition only by averaging the final min of the 15-min postexercise recovery period. Sweat rate and CVC averages were obtained during the final min of each 8 min dose. The dose response curves were compared within age groups for the DOSE and Ex+DOSE conditions to assess whether peripheral factors influence heat loss responses postexercise. Additionally, the dose response curves were compared between age groups for the DOSE and Ex+DOSE conditions separately to assess the effect of age on end-organ function and on the mechanism of the postexercise suppression of heat loss responses.

Statistical analysis

Sweating, CVC, mean arterial pressure and rectal temperature (Ex+DOSE only) data were analyzed using a two-way repeated measures ANOVA protocol using the repeated factor of agonist concentration (8 levels: 10-fold increments from 1×10^{-6} M to 1 M and 1.5 M for MCh and ACh and from 5×10^{-6} M to 5×10^{-2} M for SNP) and the non-repeated factor of test condition (DOSE vs. Ex+DOSE) and age (2 levels: young and older), separately. When a significant main effect was observed for test condition (DOSE vs.

Ex+DOSE), post-hoc comparisons were carried out using Student's paired two-tailed *t* tests. Likewise, when a significant main effect was observed for age (young vs. older), post-hoc comparisons were carried out using Student's unpaired two-tailed *t* tests. Additionally, physical characteristics as well as baseline resting, end exercise (Ex+DOSE only) and 15-min postexercise (Ex+DOSE only) values, the log EC_{50} and absolute maximal CVC values were analyzed using Student's unpaired two-tailed *t* tests. Within group comparisons (i.e., baseline resting vs. 15-min postexercise values), were analyzed using Student's paired two-tailed *t* tests. The level of significance for all analyses was set at $P \leq 0.05$. Analyses were performed using commercially available statistical software (GraphPad Prism 6.0, GraphPad Software, La Jolla, CA, USA). All values are reported as mean ± standard deviation unless otherwise indicated as standard error.

RESULTS

Participant characteristics

Participant characteristics are presented in Table 1. There were no differences in height (P=0.460), body mass (P=0.459) and body surface area (P=0.534) between groups. However, the younger males had a greater maximum oxygen consumption relative to body mass (P<0.001). On the day of both experimental sessions, baseline urine specific gravity did not significantly differ between groups (DOSE: young = 1.026 ± 0.006 vs. older = 1.019 ± 0.005, P=0.229 and Ex+DOSE: young = 1.017 ± 0.007 vs. older = 1.017 ± 0.004, P=0.967). During the Ex+DOSE condition, young and older adults exercised at a fixed rate of heat production which was kept similar between the young (257 ± 12 $W \cdot m^{-2}$) and older (240 ± 18 $W \cdot m^{-2}$, P=0.114) adults.

Post-synaptic sweating during no exercise resting (DOSE) and postexercise recovery (Ex+DOSE)

Sweating responses at baseline rest, 15-min postexercise (Ex+DOSE only) and to incremental doses of ACh and MCh for the young and older adults are presented in Figure 1A and B, respectively. Baseline resting sweat rate was similar between the DOSE and Ex+DOSE conditions in both young and older adults for the ACh (young: P=0.614, older: P=0.105) and MCh (young: P=0.123, older: P=0.666) skin sites. For the Ex+DOSE condition, there were no differences in sweat rates between baseline rest and 15-min postexercise in either young or older adults for both the ACh (young: P=0.453, older: P=0.301) and MCh (young: P=0.348, older: P=0.152) sites. Likewise, there was no main effect of the experimental condition such that sweating responses were similar between DOSE and Ex+DOSE in young and older adults during the incremental doses of both ACh (young: P=0.992, older: P=0.775) and MCh (young: P=0.710, older: P=0.738).

Age-related effects on post-synaptic sweating

*No–exercise resting condition (**DOSE**)*

There were no significant differences in baseline resting sweat rate between young and older males at both the ACh (P=0.230) and MCh (P=0.276) skin sites. Sweating increased as a function of increasing concentrations for both ACh and MCh (both $P \leq 0.001$). Further, there was a main effect of age on local sweat rate for ACh (P=0.049) and MCh (P=0.006). For ACh, sweat rate did not differ between age groups at the lower concentrations (i.e., 1×10^{-6} to 1×10^{-1} M), but was greater in the young compared to the older males at the two higher concentrations (1 and 1.5 M) employed (both $P<0.05$). For MCh, sweat rate was greater in the young compared to the older males at 1×10^{-6} and $1 \times 10^{-}$

^{4} to 1.5 M (all $P<0.05$). The log EC_{50} did not differ between age groups for ACh ($P=0.814$), but was lower (i.e., further away from 0) for the young compared to the older adults for MCh ($P=0.035$).

*Postexercise resting recovery condition (**Ex+DOSE**)*

There were no significant differences in baseline resting sweat rate between the young and older adults at both the ACh ($P=0.351$) and MCh sites ($P=0.934$). Likewise, there were no differences observed between groups in sweat rate 15-min postexercise for ACh ($P=0.121$) or MCh ($P=0.246$). Sweating increased as a function of increasing concentrations of both ACh and MCh (both $P<0.001$). There was a main effect of age on local sweat rate for ACh ($P=0.05$) and MCh ($P=0.029$). For ACh, sweat rate did not differ between age groups at the lower concentrations (i.e., 1×10^{-6} to 1×10^{-1} M), but was greater in young compared to older males at the two highest concentrations (1 and 1.5 M) employed (both $P<0.05$). For MCh, sweat rate was greater in young compared to older males at 1×10^{-2} to 1.5 M (all $P<0.05$). However, the log EC_{50} did not differ between age groups for ACh ($P=0.483$) or MCh (P=0.362).

Post-synaptic skin vasodilation during no exercise resting (DOSE) and postexercise recovery (Ex+DOSE)

CVC responses at baseline rest, 15-min postexercise (Ex+DOSE only) and to incremental doses of ACh and SNP for the young and older adults are presented in Figure 2A and B, respectively. Baseline resting values for CVC were similar between in both young and older adults for the DOSE and Ex+DOSE conditions for both ACh (young: $P=0.308$, older: $P=0.113$) and SNP (young: $P=0.949$, older: $P=0.068$) skin sites. For the Ex+DOSE

condition, CVC returned to pre-exercise baseline levels such that there was no difference between baseline rest and 15-min postexercise levels in young and older adults for both the ACh (young: P=0.826, older: P=0.853) and SNP (young: P=0.187, older: P=0.883) sites. Additionally, CVC responses were not different in either young or older adults at the ACh (young: P=0.123, older: P=0.832) or SNP (young: P=0.893, older: P=0.360) sites between the DOSE and Ex+DOSE conditions during the infusion of the incremental doses.

Age-related effects on post-synaptic skin vasodilation

*No–exercise resting condition (**DOSE**)*

There were no significant differences in baseline resting CVC between young and older males at both the ACh (P=0.864) and SNP (P=0.507) skin sites. CVC increased as a function of increasing concentrations of both ACh and SNP (both $P \leq 0.001$), but there was no main effect of age on CVC for ACh (P=0.127) or SNP (P=0.131). In contrast, the log EC_{50} was lower for the young compared to the older adults for SNP (P=0.041), but not for ACh (P=0.087). Additionally, maximal absolute CVC values did not differ between young (ACh: 1.65 ± 0.46 and SNP: 1.78 ± 0.48 perfusion units·mmHg^{-1}) and older (ACh: 1.78 ± 0.83 and SNP: 1.97 ± 0.62 perfusion units·mmHg^{-1}) adults (both P>0.10). Mean arterial pressure did not change throughout the protocol (P=0.120) and there was no main effect of age (P=0.873) between young (average: 87 ± 10 mmHg) and older (average: 87 ± 8 mmHg) adults.

*Postexercise resting recovery condition (**Ex+DOSE**)*

There were no significant differences in pre-exercise baseline resting CVC between the young and older males at both the ACh (P=0.401) or SNP (P=0.191) skin sites.

Likewise, there were no differences between groups observed in CVC 15-min postexercise at the ACh (P=0.425) or SNP (P=0.530) sites. CVC increased as a function of increasing concentrations of both ACh and SNP (both $P \leq 0.001$). There was a main effect of age on CVC for ACh (P=0.014). CVC was greater in the young at 1 x 10^{-4} to 1 x 10^{-2} M compared to the older males (all $P<0.05$). In contrast, there was no main effect of age on CVC for SNP (P=0.573). Consequently, the log EC_{50} was lower for the young compared to older adults for ACh (P=0.044), but was not different between groups for SNP (P=0.665). Additionally, maximal absolute CVC values did not differ between young (ACh: 1.91 ± 0.53 and SNP: 1.37 ± 0.70 perfusion units·mmHg^{-1}) and older (ACh: 1.63 ± 0.85 and SNP: 1.23 ± 0.44 perfusion units·mmHg^{-1}) adults (both $P>0.10$).

Age-related effects on rectal temperature and mean arterial pressure responses

There were no differences between age groups in baseline resting (P=0.835), end-exercise (P=0.572), or 15-min postexercise (P=0.933) rectal temperatures (Table 2). Additionally, rectal temperature decreased as a function of time from end of exercise to end of the dose response protocol ($P<0.001$), but was not different between age groups (P=0.354, Table 2). However, the older adults had a greater change in rectal temperature from pre-exercise baseline resting values at the end of the dose response protocol (0.50 ± 0.28°C) compared to the young adults (0.23 ± 0.13°C, P=0.041). There were no age group differences at baseline resting (P=0.336), end exercise (P=0.253) or 15-min postexercise in mean arterial pressure (P=0.551, Table 2). However, the older adults had a significantly lower mean arterial pressure at 15-min postexercise compared to pre-exercise baseline resting values (Table 2, P=0.049). No differences were observed in the young adults

(P=0.369). Mean arterial pressure did not differ over time during the agonist infusion protocol (P>0.10) and was not different between age groups (P=0.217, Table 2).

DISCUSSION

A key finding of the present study was our observation that the dose response relationships with incremental pharmacological agonists (ACh, MCh and SNP) were similar between the no-exercise resting condition (DOSE) and the postexercise resting recovery period (Ex+DOSE) for both sweating and skin vasodilation. Moreover, we show that the pattern of response was similar for both the young and older adults. However, we showed that older adults had an attenuated sweating responsiveness to the administration of pharmacological muscarinic receptor agonists (ACh and MCh) compared to the young adults. This impairment was observed during both the no-exercise resting (DOSE) and the postexercise resting recovery (Ex+DOSE) conditions. Conversely, our findings for age-related differences in CVC are less conclusive such that CVC was lower in the older compared to young adults with the use of ACh during the Ex+DOSE condition only. Together these findings suggest that peripheral factors do not modulate the postexercise suppression of heat loss responses of sweating and CVC in both young and older adults, despite the age-related impairments in sweat gland function.

It has been well documented that sweating and skin blood flow return to baseline levels in the first ~20 min following the cessation of dynamic exercise despite sustained elevations in core and muscle temperatures (Thoden *et al.*, 1994; Kenny *et al.*, 2006; Kenny *et al.*, 2009). Consistent with these observations, we observed a rapid reduction in sweating and CVC to baseline levels within 15 min of postexercise recovery (Figure 1) despite rectal temperature remaining significantly elevated above baseline resting values by ~0.6°C (Table

2). This postexercise disturbance in thermal homeostasis is thought to be the result of a nonthermal, centrally-mediated suppression of the thermoeffector responses of sweating and skin blood flow (Kenny & Jay, 2013). This notion is supported in part by the fact that the core temperature threshold for sweating and skin vasodilation, which is thought to be determined by central drive (Nadel *et al.*, 1974; Gisolfi & Wenger, 1984), is elevated following dynamic exercise (Jackson & Kenny, 2003; Kenny *et al.*, 2003). However, no changes in thermal sensitivity; an indicator of peripheral modulation, were observed (Nadel *et al.*, 1971; Nadel *et al.*, 1974; Jackson & Kenny, 2003). Nonetheless, there is no direct evidence indicating the impaired heat loss responses postexercise are entirely due to central mechanisms. That is, other mechanisms of peripheral origin, such as changes in the responsiveness of the effector organ (i.e., sweat glands and/or skin vessels) to pharmacological stimuli, may also be involved in the impaired heat loss responses postexercise. In the following section, we discuss how the present study findings provide important new information to address this knowledge gap.

Postexercise sweating and CVC in young adults

In the present study, we did not observe any difference in sweating with incremental doses of ACh or MCh between the DOSE and Ex+DOSE conditions in the young adults (Figure 1A and B). This finding indicates that the cholinergic sensitivity of the muscarinic receptors on the sweat gland is unaltered by a previous bout of exercise. Alternatively, studies have shown that AChE is involved in the regulation of sweating at low-to-moderate levels during passive heat stress (Shibasaki & Crandall, 2001; Kimura *et al.*, 2007). It is plausible, therefore, that the rapid postexercise suppression of sweating may be due to increased activity of the AChE enzyme. If this were true, we would expect to observe a

rightward shift in the dose response curve for ACh during the Ex+DOSE compared to the DOSE condition with minimal difference in the dose response curve between the DOSE and Ex+DOSE conditions for MCh. However, this was not the case in the present study (Figure 1A and B). Based on our observations, it appears that the postexercise suppression of the sweating response is independent of the modulation of AChE enzyme activity. We cannot however eliminate its involvement early in recovery (i.e., in the first 15 min) since the dose response protocol only commenced 15 min into postexercise recovery. Based on our results, we show that the postexercise attenuation of the sweating response is the result of a centrally mediated modulation as previously proposed (Journeay *et al.*, 2006; Shibasaki & Crandall, 2010; Kenny & Jay, 2013).

ACh-induced skin vasodilation is in part due to nitric oxide-dependent mechanisms (Kellogg, 2006; Medow *et al.*, 2008; Bruning *et al.*, 2012; Fujii *et al.*, 2013). Our results demonstrate no difference in the dose response curves for CVC between the DOSE and Ex+DOSE conditions using ACh in young adults (Figure 1C). Furthermore, our laboratory recently found that L-NAME, a nonselective nitric oxide inhibitor, reduced CVC relative to the control condition only during the first ~10 min into postexercise recovery (McGinn *et al.*, 2014). Together, these results imply that peripherally modulated mechanisms of skin vasodilation including changes in cholinergic sensitivity of the muscarinic receptor on the endothelium and nitric oxide-mediated pathways are not modified postexercise. In the present study, similar to using ACh, we did not observe any differences in skin vasodilation in the young adults with incremental doses of SNP between the DOSE and Ex+DOSE conditions (Figure 1D). Given that SNP is a nitric oxide donor that acts directly on the smooth muscle cell to cause relaxation and therefore vasodilation, we conclude that vascular smooth muscle function is also not altered postexercise. Consequently, as in the case of the

observed changes in postexercise sweating, we show that postexercise control of skin vasodilation in young adults is modulated by central factors.

Postexercise sweating and CVC in older adults

Similar to the young, the sweating response to administration of ACh and MCh in the older adults did not differ between the no-exercise resting (DOSE) and postexercise resting recovery (Ex-DOSE) conditions (Figure 2A and B). Thus, we show for the first time that despite an age-related attenuation in the sweating response (discussed below); as in the case of younger adults, the control of sweating in the postexercise recovery period in older adults is likely not mediated by mechanisms of peripheral origin. Likewise, the pattern of response in CVC was not different between the DOSE and Ex+DOSE conditions as assessed using the administration of incremental doses of ACh and SNP (Figure 2C and D). Thus, we show that the control of postexercise skin vasodilation is most likely due to central mechanisms; a response which parallels that observed in young adults.

Effects of aging on sweating and CVC

Numerous studies have examined age-related differences in thermoregulatory sweating during exercise and some have found reduced local/whole-body sweating and/or altered core temperature onset thresholds and thermosensitivity of the sweating response (Anderson & Kenney, 1987; Kenney & Anderson, 1988; Tankersley *et al.*, 1991; Inoue *et al.*, 1999a; Larose *et al.*, 2013a; Larose *et al.*, 2013c; Larose *et al.*, 2013d). In the present study, older adults likely had greater residual heat storage postexercise as indicated by a greater change in rectal temperature relative to baseline resting values at the end of the dose response protocol in the older (0.50 ± 0.28°C) compared to the young (0.23 ± 0.13°C) adults.

Despite this greater amount of heat, they were not able to produce more sweat during the dose response protocol. It has been postulated that the age-related impairments in sweating are due to differences in end organ function such as cholinergic sensitivity of the muscarinic receptors on sweat glands (Kenney & Fowler, 1988; Inoue *et al.*, 1999a). In the present study, we found that the dose-dependent sweating response to the administration of ACh and MCh was lower in older males relative to young males for the no-exercise resting conditions (DOSE) (Figure 3A and B). On the contrary, a recent study by Smith *et al.* (Smith *et al.*, 2013a) reported no age-related differences in the sweating response to administration of ACh from 1 x 10^{-7} to 1 x 10^{-1} log-molar performed during resting under non-heat stress conditions. While at first glance our results appear to contradict the findings by Smith *et al.* (2013), it is important to note that we only observed age-related decreases in sweating at the two highest doses of ACh (1 and 1.5 M). No significant difference in sweating between young and older males was observed at and below a concentration of ACh of 10^{-1} log-molar (Figure 3 and 4A). Taken together, it is plausible that a concentration of ACh >10^{-1} log-molar is required to clearly observe age-related reductions in end organ sweat gland function. On the other hand, age-related differences in sweating were observed with MCh even at a lower concentration (log-molar 1 x 10^{-4}). It is possible, therefore, that the effect of age on impairments in sweating can be masked or reduced by AChE enzyme activity. As such, MCh would be more suitable in the assessment of age-related differences in cholinergic sensitivity of muscarinic receptors on sweat glands.

Previous studies examining the effect of age on ACh-dependent skin vasodilation have yielded mixed conclusions (Sagawa *et al.*, 1988; Holowatz *et al.*, 2005; Bruning *et al.*, 2012; Smith *et al.*, 2013a). Some have found skin vasodilation to be impaired in older adults (Bruning *et al.*, 2012), while others have reported no age-related differences (Holowatz *et*

al., 2005; Smith *et al.*, 2013a). Consistent with previous reports, our findings were also inconclusive. While we did not observe attenuated skin vasodilation during the DOSE condition to ACh in the older compared to young adults (Figure 3C), we showed that endothelium function was impaired in the older adults during the Ex+DOSE condition (Figure 4C). Given that ACh-mediated skin vasodilation occurs via nitric oxide-dependent mechanisms as discussed above, the age-related reductions in skin vasodilation (Figure 4C) may reflect age-related decreases in nitric oxide-dependent skin vasodilation. Supporting this concept, it has been suggested that aging lowers nitric oxide-dependent skin vasodilation to ACh (Bruning *et al.*, 2012).

For the first time, our study assessed skin vasodilation in response to incremental doses of SNP in young and older adults. Our results show that the log EC_{50} for CVC was greater (i.e., closer to 0) in the older adults in comparison to the young adults during the DOSE condition (Figure 3D). This result suggests age-related decreases in smooth muscle sensitivity/responsiveness to nitric oxide (i.e., endothelium-independent vasodilation). This finding is consistent with one study demonstrating that expression of soluble guanylyl cyclase, the receptor for nitric oxide which causes smooth muscle relaxation, decreases with increasing age, which has been observed in the aortic ring of rats (Kloss *et al.*, 2000). In contrast, we did not observe a difference in the log EC_{50} for CVC during the Ex+DOSE condition. Furthermore, the CVC responses to incremental doses of SNP were not significantly different between the young and older adults during the DOSE or Ex+DOSE condition. It is unclear why the sensitivity to nitric oxide was impaired in the older adults during the DOSE condition only. Further studies are required to examine potential mechanisms.

Another interesting observation is that age-related reductions in skin vasodilation to ACh were not observed at the higher concentrations employed in this study (Figure 4C). This may indicate that there are other mechanisms compensating for the reduced nitric oxide-dependent mechanisms. One possibility is that endothelium-derived hyperpolarizing factors (EDHFs) are acting as a redundant mechanism as has been shown when nitric oxide-dependent vasodilation is reduced in humans (Luksha *et al.*, 2009) and rats (Goto *et al.*, 2012). EDHFs cause relaxation of smooth muscle cells and thus vasodilation in human skin by stimulating calcium-activated potassium (KCa) channels (Lorenzo & Minson, 2007; Brunt & Minson, 2012; Cracowski *et al.*, 2013). Taken together, it is plausible that age-related impairment of nitric oxide-dependent mechanisms may up-regulate the EDHF pathway(s). This may explain the lack of an age-related difference in CVC at the higher doses of ACh.

Some studies have reported that maximal CVC decreases with age (Martin *et al.*, 1995; Minson *et al.*, 2002; Hodges *et al.*, 2010), while others found no differences in maximal CVC induced by SNP between young and older adults (Bruning *et al.*, 2012; Smith *et al.*, 2013a). We did not observe a reduced absolute maximal CVC induced by 50 mM SNP in the older adults relative to their younger counterparts. The disparity in the pattern of response may be due to regional differences (Inoue & Shibasaki, 1996) where an age-related effect on the maximal skin vasodilatory capacity is not always evident at all areas of the skin, even when measured within the same body part (i.e., forearm).

Conclusions

The current study demonstrates that the postexercise suppression of heat loss responses is not mediated by factors of peripheral origin. This is evidenced by our

observation that no differences in sweating or CVC with incremental doses of pharmacological agonists were observed during the no-exercise resting condition (DOSE) or the postexercise resting recovery period (DOSE+Ex) in both young and older adults. Further, while we show marked impairment in sweat gland function in the older adults, the mechanisms underlying age-related changes in CVC were less conclusive.

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COMPETEING INTERESTS

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

Jill M. Stapleton and Glen P. Kenny contributed to the conception and design of the experiment, to the collection analysis and interpretation of data and drafting the article and revising it critically for important intellectual content. Naoto Fujii and Ryan McGinn and Katherine McDonald contributed to the collection, analysis, and interpretation of data and drafting the article and revising it critically for important intellectual content. We confirm that all authors approved the final version of the manuscript and all authors qualify for authorship.

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TABLES

Table 1. Participant characteristics for young and older adults.

Group	Age (years)	Height (m)	Body mass (kg)	Body surface area (m^2)	Body fat (%)	$\dot{V}O_2max$ ($mL \cdot kg^{-1} \cdot min^{-1}$)
Young	22 ± 3*	1.78 ± 0.10	81.7 ± 7.7	1.99 ± 0.11	14.3 ± 3.5*	46.3 ± 3.5*
Older	65 ± 3	1.75 ± 0.05	77.6 ± 12.9	1.94 ± 0.17	21.6 ± 6.7	34.3 ± 8.3

Values are mean ± standard deviation. $\dot{V}O_2max$, rate of maximum oxygen consumption. A significant difference ($P \leq 0.05$) between young and older adults is denoted by an asterisk (*).

Table 2. Mean arterial pressure and rectal temperatures for Ex+DOSE during baseline, end of exercise (End-Ex), following 15 minutes of recovery (Post-Ex) and during the plateau phase for each dose for young and older adults.

MAP	Baseline	End-Ex	Post-Ex	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6	Dose 7	Dose 8
Young	85 ±6	99 ±8	84 ±8	84 ±8	83 ±9	84 ±11	84 ±9	84 ±9	85 ±9	85 ±10	86 ±8
Older	88 ±8	103 ±12	81 ±8†	82 ±8	84 ±8	83 ±8	83 ±8	82 ±9	81 ±8	84 ±8	82 ±8
T_{re}											
Young	36.87 ±0.42	37.47 ±0.44†	37.44 ±0.45†	37.41 ±0.44†	37.35 ±0.43†	37.31 ±0.41†	37.21 ±0.42†	37.15 ±0.43§†	37.11 ±0.42†	37.09 ±0.42†	37.06 ±0.39†
Older	36.84 ±0.25	37.36 ±0.28†	37.45 ±0.13†	37.43 ±0.16†	37.41 ±0.21†	37.40 ±0.22†	37.35 ±0.25†	37.33 ±0.23†	37.30 ±0.25†	37.27 ±0.23†	37.27 ±0.22†

Values are mean ± standard deviation. MAP, mean arterial pressure (mmHg). T_{re}, rectal temperature (°C). A significant difference between young and older adults is denoted by an asterisk (*). A significant difference ($P \leq 0.05$) from baseline resting within age group is denoted by a dagger (†).

FIGURE LEGENDS

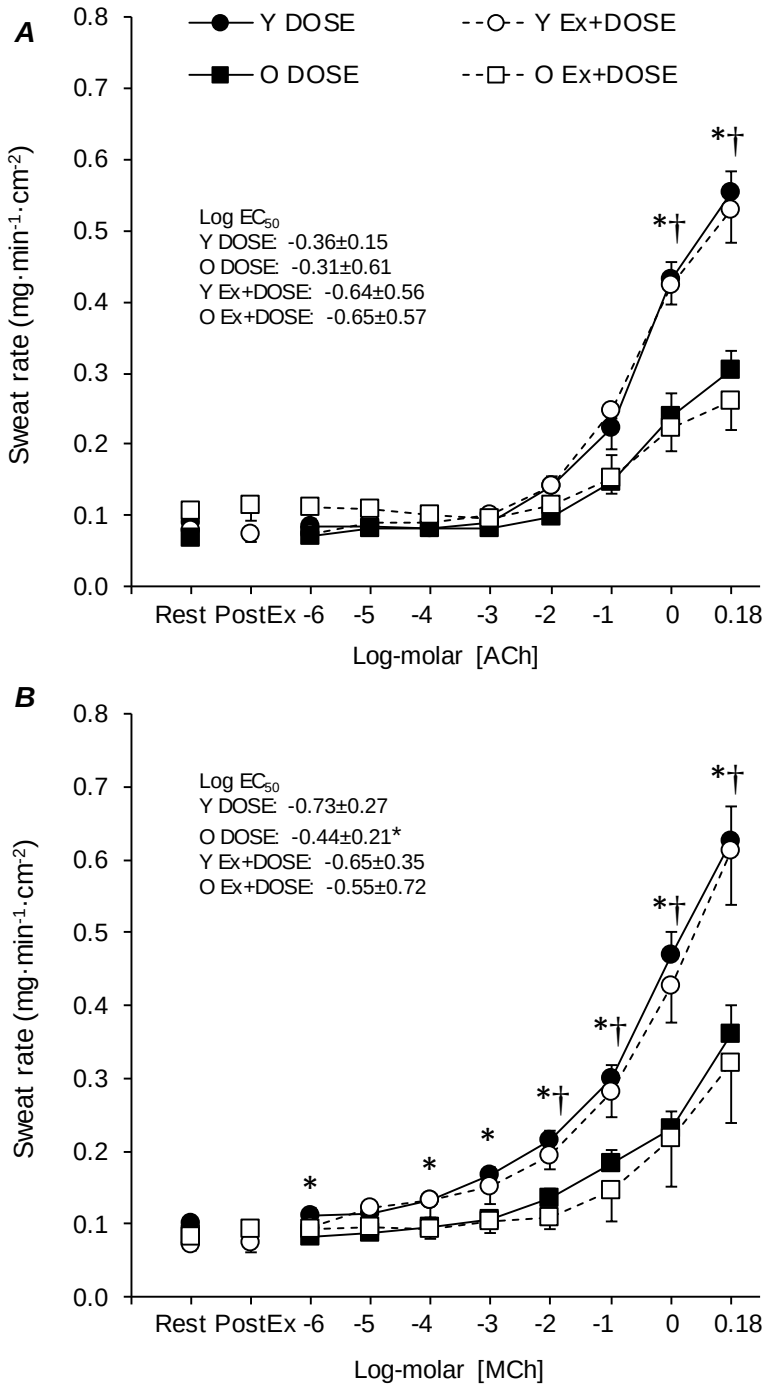

Figure 1. Mean ± standard error values for the DOSE (closed symbols) and Ex+DOSE (open symbols) condition for sweat rate to incremental doses of acetylcholine (ACh, Panel A) and methacholine (MCh, Panel B) in young (Y, circles) and older (O, squares) adults. Data are presented during baseline rest, 15-min postexercise (PostEx), and during the plateau phase of each dose. *, significant difference between young and older adults for the DOSE condition. †, significant difference between young and older adults for the Ex+DOSE condition ($P \leq 0.05$).

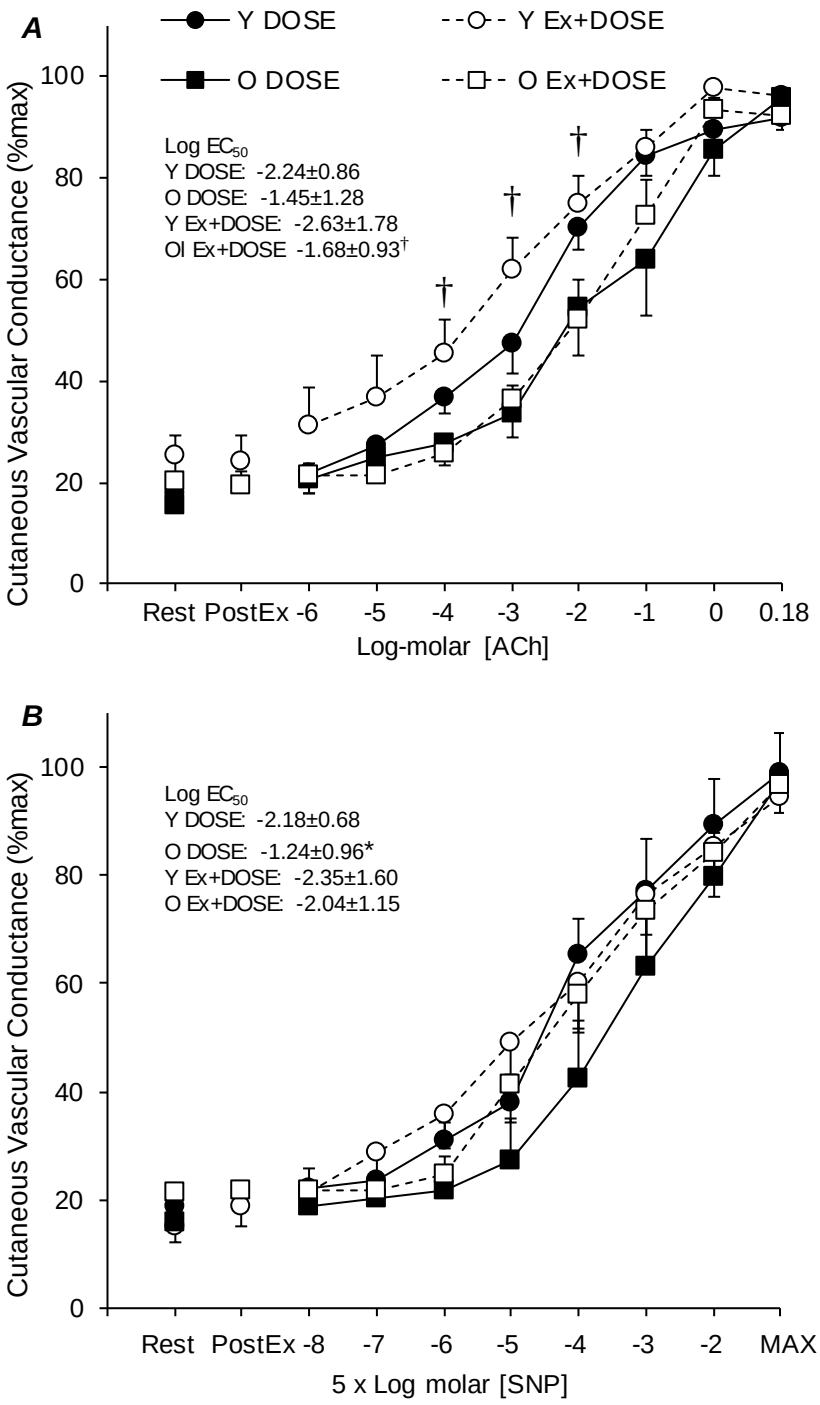

Figure 2. Mean ± standard error values for the DOSE (closed symbols) and Ex+DOSE (open symbols) conditions for cutaneous vascular conductance to incremental doses of acetylcholine (ACh, Panel A) and sodium nitroprusside (SNP, Panel B) in young (Y, circles) and older (O, squares) adults. Data are presented during baseline rest, 15-min postexercise (PostEx), and during the plateau phase of each dose. *, significant between young and older adults for the DOSE condition. †, significant between young and older adults for the Ex+DOSE condition ($P \leq 0.05$).

CHAPTER 4: DISCUSSION AND CONCLUSIONS

4.1 Thesis discussion

The primary purpose of the current thesis was to examine the physiological capacity to dissipate heat in older adults during rest and exercise under various heat loads (metabolic and environmental). By accounting for factors such as body size, metabolic heat production and differences in aerobic fitness, the results from this thesis were able to delineate true age-related impairments in the body's physiological capacity to dissipate heat. In light of the fact that many studies examining heat loss responses of sweating and skin blood flow between young and older adults have been limited to local measures of sweating and skin blood flow and/or changes in core temperature, little was known about the consequence of age-related changes on whole-body heat loss. For most of the studies in this thesis, the Snellen whole-body direct calorimeter was employed to accurately measure whole-body heat dissipation (i.e., evaporative and dry heat exchange). Further, by assessing sweat rates and cutaneous vasodilation during rest and/or exercise with the use of pharmacological agonists and/or inhibitors, we were able to examine potential mechanisms involved in the impairment of heat loss in older adults. Gaining a better understanding of the mechanisms governing impaired heat loss responses will advance our understanding of why older adults suffer to a greater extent during heat stress when compared to their younger counterparts.

4.1.1 Physiological capacity to dissipate heat

There is a trend in our world climate today towards more frequent and extreme heat events, which are longer in duration. Even though Canada is not considered to be one of the hottest countries in the word, the temperate weather in addition to the large fluctuations in temperature throughout the year can make it difficult for individuals to become heat-

acclimatized. Therefore it is possible that people residing in these climates, such as Canada, may be more susceptible to heat-related illness during periods of above-normal temperatures. Given that heat waves are the cause of many preventable deaths around the world and within Canada, especially among older adults, the purpose of the first study was to examine whole-body heat loss and changes in body heat content in young (21 ± 3 years) and older adults (65 ± 5 years) during 2 hours of passive exposure to a hot-dry environment (36.5°C, 20% relative humidity) and a hot-humid condition (36.5°C, humidity 60% relative). We used conditions representative of the upper environmental extremes in Canada to assess the potential health risk to unacclimatized older adults living in temperate climates during heat waves. The results of the first study show that the cumulative change in body heat content after a 2 h exposure to a hot environment was significantly greater in older adults in both dry and humid conditions. Specifically, exposure to dry heat as well as humid heat posed a significantly greater thermal challenge for older adults as evidenced by greater changes in body heat content, suggesting that they may experience increased levels of thermal strain relative to their younger counterparts. Initiatives to increase awareness regarding the potential health risks associated with exposure to the heat, particularly when humidity is high, may be necessary to protect older individuals against heat illness and/or injury. The next question to consider was if these age-related impairments in whole-body heat loss and therefore greater increases in body heat storage were evident during exercise.

Previous studies reporting age-related detriments in whole-body heat loss and/or local sweating rate/onset/sensitivity, and/or greater increases in core and skin temperatures and greater body heat storage (Anderson & Kenney, 1987; Tankersley *et al.*, 1991; Kenney *et al.*, 1997; Inoue *et al.*, 1999a; Inbar *et al.*, 2004; Larose *et al.*, 2013a; Larose *et al.*, 2013d; Stapleton *et al.*, 2014) have been limited to a comparison based on a single heat load. As

such, it is not possible to determine from these studies at what level of heat stress age-related impairments in the body's physiological capacity to dissipate heat occur. Moreover, differences in heat load employed between studies have led to disparate findings such that some have reported no-age related differences in heat loss between young and middle-aged and/or older adults (Davies, 1979; Kenney, 1988; Pandolf *et al.*, 1988; Kenny *et al.*, 2010a; de Paula Viveiros *et al.*, 2012). Furthermore, many studies have not considered age-related changes in aerobic fitness. Given that as we age there is progressive decline in cardiorespiratory function leading to a ~7% reduction in VO_{2peak} per decade (Wilson & Tanaka, 2000) the influence of aerobic fitness in the context of aging is of paramount interest. While some studies have shown that maintaining a high level of VO_{2peak} through regular endurance-type exercise training may help attenuate the age-related impairments in thermoregulatory function (Buono *et al.*, 1991; Tankersley *et al.*, 1991; Inoue *et al.*, 1999a; Black *et al.*, 2008; Best *et al.*, 2012; de Paula Viveiros *et al.*, 2012), others have not (Larose *et al.*, 2013a; Larose *et al.*, 2013d). Furthermore, it is unknown how changes in aerobic fitness with increasing age affect the physiological maximal capacity for heat dissipation.

For the reasons outlined above, the second study was designed to examine age-related impairments in the body's physiological capacity to dissipate heat during exercise at progressively greater fixed rates of metabolic heat production in the heat (40°C and 15% RH). Additionally, the effects of aerobic fitness, in the context of aging, on whole-body heat loss in a group of trained and untrained middle-aged adults matched for VO_{2peak} with young and older adults respectively was examined. We showed that age-related impairments in whole-body evaporative heat loss in older relative to young males are evident at exercise-induced heat loads as low as 400 W. Moreover, middle-aged untrained males had a similar level of impairment compared to the older males. This impairment in the capacity to

dissipate heat was characterized by a reduced thermosensitivity of the evaporative heat loss response as well as a lower level of whole-body heat loss achieved during exercise. These findings are similar to Larose *et al.* (Larose *et al.*, 2013a) who showed that age-related impairments in the ability to dissipate heat occur as early as the age of 40. However, we showed that maintaining a high level of aerobic fitness (i.e., greater levels of VO_{2peak}) can help to reduce the apparent age-related impairments in the body's physiological capacity to dissipate heat in middle-aged adults. Recent studies have observed age-related impairments in whole-body heat loss responses despite being matched for aerobic fitness (Larose *et al.*, 2013a; Larose *et al.*, 2013c). Moreover, there was a correlation between age and whole-body sweat rate ($r = -0.39$, $P<0.001$) as well as body heat content ($r = 0.37$, $P<0.001$), where advancing age was associated with a reduction in whole-body sweating and therefore a greater change in body heat content. In contrast, VO_{2peak} did not significantly correlate with whole-body sweat rate ($r = 0.07$, $P = 0.516$) or body heat content ($r = -0.04$, $P = 0.696$). Thus, it is possible that a greater level of aerobic fitness may help attenuate the age-related impairments in the physiological capacity to dissipate heat in middle-aged adults, but as one continues to age, the effects of aerobic fitness are lessened or even lost. Future work is required to examine if this response is consistent ant various levels of heat loads and aerobic fitness.

Numerous studies, including study 2 of this thesis, have suggested that older males have an impaired ability to thermoregulate during exercise compared to younger males (Lind *et al.*, 1970; Smolander *et al.*, 1990; Tankersley *et al.*, 1991; Inbar *et al.*, 2004; Larose *et al.*, 2013a; Larose *et al.*, 2013d; Larose *et al.*, 2014). Moreover, sudomotor function is impaired in young females compared to young males (Gagnon *et al.*, 2008a; Ichinose-Kuwahara *et al.*, 2010; Gagnon & Kenny, 2011b, 2012b; Gagnon *et al.*, 2013a). However, there have been

very few studies that have examined the effects of increased age on the body's ability to dissipate heat during exercise in females (Drinkwater & Horvath, 1979; Anderson & Kenney, 1987; Kenney & Anderson, 1988; Larose *et al.*, 2013c). As such, from the few studies published examining the ability to dissipate heat in older compared to young females, no study has examined if there is a certain threshold in which age-related differences exist. Thus, the aim of the third study was, as in the case of the male study (study 2), to determine at what level of heat stress age-related impairments in the body's physiological capacity to dissipate heat occur in females. The main findings of study 3 show that whole-body heat loss was significantly lower in older females during exercise-induced heat loads of 250, 325 and 400 W compared to young females. Furthermore, as the level of heat load increased, so did the level of impairment in whole-body heat loss in the older adults indicating that the impaired heat loss is heat load dependent. As a result, the older females had a 1.7-fold greater cumulative change in body heat content compared to the young females at the end of the exercise-rest cycles. Additionally, the greater change in body heat content in the older females was the result of a lower level of whole-body heat loss during exercise only as no differences in heat loss were observed during recovery. Finally, despite the differences in whole-body heat loss, no differences were observed in local heat loss responses of sweating or skin blood flow. These age-related impairments are consistent with the very few studies that have examined the effects of aging on the body's ability to dissipate heat during exercise in females (Drinkwater & Horvath, 1979; Anderson & Kenney, 1987; Kenney & Anderson, 1988; Larose *et al.*, 2013c). However, we show for the first time that the magnitude of impairment at the level of the whole-body is hat load dependent.

Collectively, for the first time we were able to demonstrate that older males and females have greater impairments in their physiological capacity to dissipate heat compared

to their younger counterparts and that the level of impairment is heat dependent. For studies 2 and 3, we employed an incremental exercise model that required that the young, middle-aged (study 2 only) and older adults exercise at the same fixed absolute heat load and therefore requirement for heat loss. This experimental model was used to ensure that any differences in sweating (and therefore fluid loss) would be the result of age-related differences and not due to differences in the requirement for heat loss. Thus, for the first time we can determine what level of heat load older males and females may work or play safely without succumbing to a heat-related illness/injury and at what level of heat load the risk of suffering from heat stress become real. The results from these studies provide important insight into the specific heat load in which differences in the capacity to dissipate heat occur as a function of age and aerobic fitness. Currently, all work exposure limits are defined based on data extracted from studies conducted in young adults. Thus, by assessing how aging and level of aerobic fitness may alter the body's ability to dissipate as a function of increasing heat load, the data obtained from this study can be used to re-define work exposure limits based on the age and aerobic fitness level of the worker to reduce the risk of all workers from suffering a heat-related illness/injury. These findings also have important implications for health care providers, exercise specialists, health and safety managers and sporting event organizers (i.e., marathons, soccer tournaments, etc.) who can use our data to better understand the level of heat stress in which dangers associated with exercise in hot conditions occur and decide when it is safe to perform physical activity in the heat.

4.1.2 Mechanisms governing impaired heat loss responses in older adults

As was reported from the first three studies of this thesis, evaporation of sweat form the skin during passive and/or exercise in hot environments is the only avenue for heat loss.

Despite this, little is known about the underlying mechanisms regulating the impaired sweating response in older adults. Sweating during exercise in the heat is, in part, mediated through nitric oxide-dependent mechanisms in young adults. However, previous studies have shown aging reduces the nitric oxide bioavailability in the skin (Holowatz *et al.*, 2003; Holowatz *et al.*, 2006b, a; Stanhewicz *et al.*, 2012; Stanhewicz *et al.*, 2013). Yet, it remained unclear if aging reduces nitric oxide-dependent sweating during exercise in the heat. Thus, the fourth thesis study evaluated nitric oxide-dependent sweating in young and older adults as a way to determine if the impaired nitric oxide synthase system in older adults is responsible for some of the age-related impairments in the ability to dissipate heat. Successive exercise and recovery cycles were performed while measuring local sweat rate on two forearm skin sites which were continuously perfused via intradermal microdialysis with: 1) 0.9% saline as a control or 2) 10 mM N^G-nitro-L-arginine methyl ester (L-NAME), a non-selective nitric oxide synthase inhibitor. The findings revealed that local sweat rate is reduced at the L-NAME compared to the control condition at the end of all three 15 min exercise bouts in the young males. However, there was no effect of treatment condition on local sweat rate in the older males such that local sweat rate at the L-NAME treated and the control conditions were similar for all three exercise bouts. Furthermore, age-related impairments in sweating were observed at the control site at the end of the first exercise bout only. In contrast, local forearm sweat rate during each recovery period was not significantly different between the control and L-NAME treated conditions in both young and older adults, and the pattern of response was similar between groups. As such, nitric oxide-dependent sweating during short bouts of exercise in the heat is evident in young males, but not in older adults. These findings show that age-related impairment in sweating may be associated with age-related reductions in nitric oxide-mediated sweating. Understanding the

mechanisms associated with age-related impairment in sweating could aid in finding potential countermeasures and/or treatments to reduce risk of heat-related illness and/or injury. For example, as aspirin has been shown to elicit the release of nitric oxide from the vascular endothelium (Taubert *et al.*, 2004; Schroder, 2009), it is possible that a certain dosage of aspirin may help increase sweating in older adults, however this has yet to be examined. Another important consideration is whether the mechanisms of heat loss responses during postexercise recovery differ as a function of age.

To date, much of our limited understanding of the underlying mechanisms governing the control of heat loss responses following a bout of dynamic exercise is based on findings obtained in young adults (Kenny *et al.*, 2007; Kenny & Jay, 2013). In addition, it has yet to be determined whether or not peripheral factors, such as changes in sensitivity of the effector organ (i.e., sweat glands and/or skin vessels), contribute to the control of heat loss postexercise. Therefore, the aim of the fifth study was two-fold. The first objective was to examine the extent to which peripheral factors (i.e., sweat gland and skin vasodilatory function) contribute to the postexercise suppression of heat loss responses. Secondly, we examined whether there are differences in the mechanisms modulating postexercise heat loss as a function of age. The results revealed that the dose response relationships with incremental pharmacological agonists (acetylcholine, methacholine and sodium nitroprusside) were similar between the no-exercise resting condition and the postexercise resting recovery period for both sweating and skin vasodilation. Moreover, we observed that there was no difference in the pattern of response for sweating or skin vasodilation as a function of age. However, confirming the existence of age-related impairments in thermoregulatory function, we showed that older adults had an attenuated sweating response to the administration of pharmacological muscarinic receptor agonists (acetylcholine and

methacholine) compared to the young adults. This was evidenced by the older adults having a lower sweat rate compared to young adults at acetylcholine concentrations of 1 and 1.5 M and methacholine concentrations of 1×10^{-6} and 1×10^{-4} to 1.5 M. This impairment was observed during both the no-exercise resting and the postexercise resting recovery conditions. Conversely, our findings for age-related differences in skin vasodilation were less conclusive such that cutaneous vascular conductance was lower in the older compared to young adults with the use of acetylcholine during the postexercise resting recovery condition only. Together these findings suggest that peripheral factors do not modulate the postexercise suppression of heat loss responses of sweating and skin vasodilation in both young and older adults, despite the age-related impairments in sweat gland function.

4.1.3 Limitations

Participants recruited to volunteer in these series of studies were either untrained as determined by being physically active less than two times per week for the previous three months or highly endurance trained as determined by being physically active more than three times per week for the previous three months. Therefore, the results of this thesis cannot be applicable to obese or chronically ill populations. All female subjects were tested in their early to mid-follicular phase of the menstrual cycle or were post-menopausal during all experimental trials. Thus, further studies are required to examine the effect of sex hormones on the physiological capacity to dissipate heat.

One must consider that during the studies performed in the Snellen whole-body direct calorimeter (Study #1-3), the subject remained isolated in the calorimeter and access in and out of temperature chamber housing the calorimeter was restricted during the trial. As such, we were unable to take blood samples, measure body weight changes and/or monitor blood

pressure via brachial artery auscultation throughout the experimental trials. As a consequence, we were unable to determine the potential effects of age-related differences in hydration status and fluid distribution and/or mean arterial pressure changes on whole-body heat dissipation as a function of increases in passive and exercise-induced heat loads. Additional research is required to determine how changes in hydration status may influence this pattern of response as a function of increasing heat loads.

It is important to note that in the studies employing microdialysis (study #4 and study #5), local sweat rate was only measured at the forearm. Age-related decrements in sweating may not occur at an even rate across the body (Inoue *et al.*, 1991; Inoue & Shibasaki, 1996; Dufour & Candas, 2007; Smith *et al.*, 2013a) and have been found to vary between individuals of similar ages (Inoue *et al.*, 2004; Smith & Havenith, 2012; Smith *et al.*, 2013b). Recently, Smith *et al.* (Smith *et al.*, 2013a) observed reduced sweating during whole-body heating at rest in older compared to young adults at the arm, abdomen, thigh and lower back, with greatest impairment observed on the abdomen. Therefore, it is conceivable that a more pronounced age-related difference in the sweating response might have been observed if we had tested other areas of the body such as the abdomen.

4.2 Thesis conclusions

Collectively, the results from the five manuscripts presented in this thesis have addressed some key knowledge gaps in the literature pertaining to the effects of aging on whole-body and local heat loss during different levels of heat stress and/or pharmacological stimuli. Overall, the results show that older adults have a lower whole-body heat loss response during both passive heating and exercise above a certain heat load threshold. These age-related impairments in heat loss can be attributed to age-related reductions in nitric oxide-mediated

sweating, as well as to reductions of the responsiveness to cholinergic agents. Moreover, older adults, particularly those who are less aerobically fit, should use greater caution when exercising, especially in the heat. In conclusion, the findings suggest that true age-related impairments in the ability to dissipate heat are evident and should be considered when prescribing physical work to older adults, especially when work is performed in the heat.

CHAPTER 5:

CONTRIBUTION OF AUTHORS AND CO-AUTHORS

Thesis article #1:

Data collection was performed at the Human and Environmental Physiology Research Unit and the University of Ottawa. All authors contributed to the conception and design of the experiments. Data collection, analysis were performed by JM Stapleton. Interpretation of data was performed by JM Stapleton, J Larose and GP Kenny. All authors contributed to the drafting and critical revising of the manuscript and have approved the final version of the manuscript.

Thesis article #2:

Data collection was performed at the Human and Environmental Physiology Research Unit and the University of Ottawa. All authors contributed to the conception and design of the experiments. Data collection, analysis were performed by JM Stapleton and M Poirier. Interpretation of data was performed by all authors. All authors contributed to the drafting and critical revising of the manuscript and have approved the final version of the manuscript.

Thesis article #3:

Data collection was performed at the Human and Environmental Physiology Research Unit and the University of Ottawa. All authors contributed to the conception and design of the experiments. Data collection, analysis were performed by JM Stapleton. Interpretation of data was performed by all authors. All authors contributed to the drafting and critical revising of the manuscript and have approved the final version of the manuscript.

Thesis article #4:

Data collection was performed at the Human and Environmental Physiology Research Unit and the University of Ottawa. All authors contributed to the conception and design of the experiments. Data collection, analysis were performed by JM Stapleton and M Carter. Interpretation of data was performed by JM Stapleton, M Carter, N Fujii and GP Kenny. All authors contributed to the drafting and critical revising of the manuscript and have approved the final version of the manuscript.

Thesis article #5:

Data collection was performed at the Human and Environmental Physiology Research Unit and the University of Ottawa. All authors contributed to the conception and design of the experiments. Data collection, analysis were performed by JM Stapleton and K McDonald. Interpretation of data was performed by JM Stapleton, N Fujii, R McGinn and GP Kenny. All authors contributed to the drafting and critical revising of the manuscript and have approved the final version of the manuscript.

CHAPTER 6:

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CHAPTER 7:
APPENDICES

APPENDIX A

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ARTICLE

Do older adults experience greater thermal strain during heat waves?

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Abstract: Heat waves are the cause of many preventable deaths around the world, especially among older adults and in countries with more temperate climates. In the present study, we examined the effects of age on whole-body heat loss and heat storage during passive exposure to environmental conditions representative of the upper temperature extremes experienced in Canada. Direct and indirect calorimetry measured whole-body evaporative heat loss and dry heat exchange, as well as the change in body heat content. Twelve younger (21 ± 3 years) and 12 older (65 ± 5 years) adults with similar body weight (younger: 72.0 ± 4.4 kg; older: 80.1 ± 4.2 kg) and body surface area (younger: 1.8 ± 0.1 m^2; older: 2.0 ± 0.1 m^2) rested for 2 h in a hot–dry [36.5 °C, 20% relative humidity (RH)] or hot–humid (36.5 °C, 60% RH) environment. In both conditions, evaporative heat loss was not significantly different between groups (dry: p = 0.758; humid: p = 0.814). However, the rate of dry heat gain was significantly greater (by approx. 10 W) for older adults relative to younger adults during the hot–dry (p = 0.032) and hot–humid exposure (p = 0.019). Consequently, the cumulative change in body heat content after 2 h of rest was significantly greater in older adults in the hot–dry (older: 212 ± 25 kJ; younger: 131 ± 27 kJ, p = 0.018) as well as the hot–humid condition (older: 426 ± 37 kJ; younger: 317 ± 45 kJ, p = 0.037). These findings demonstrate that older individuals store more heat during short exposures to dry and humid heat, suggesting that they may experience increased levels of thermal strain in such conditions than people of younger age.

Key words: aging, passive heat stress, calorimetry, heat waves.

Résumé : Les vagues de chaleur entraînent dans le monde entier de nombreux décès évitables, particulièrement chez les personnes âgées et dans les pays au climat plus tempéré. Dans cette étude, on examine les effets de l'âge sur la perte et le stockage de chaleur corporelle au cours d'une exposition passive à des conditions environnementales semblables aux températures extrêmes supérieures observées au Canada. On mesure par calorimétrie directe et indirecte la perte globale de chaleur corporelle par évaporation et d'échange de chaleur sèche ainsi que la variation du contenu corporel de chaleur. Douze adultes jeunes (21 ± 3 ans) et 12 adultes âgés (65 ± 5 ans) de masse corporelle (jeunes: 72,0 ± 4,4 kg; âgés: 80,1 ± 4,2 kg) et de surface corporelle (jeunes: 1,8 ± 0,1 m^2; âgés: 2,0 ± 0,1 m^2) similaires se reposent durant 2 h dans un environnement chaud et sec [36,5 °C, 20 % humidité relative (RH)] ou chaud et humide (36,5 °C, 60 % RH). Dans les deux conditions, on n'observe pas de différences significatives de perte de chaleur par évaporation dans les deux groupes (sec: p = 0,758; humide: p = 0,814). Toutefois, on observe un gain de chaleur sèche significativement plus grand (~10 W) chez les personnes âgées comparativement aux jeunes dans un environnement chaud et sec (p = 0,032) et chaud et humide (p = 0,019). Par conséquent, l'augmentation du contenu corporel de chaleur après 2 h de repos est significativement plus grande chez les adultes âgés dans un environnement chaud et sec (âgés: 212 ± 25 kJ; jeunes: 131 ± 27 kJ, p = 0,018) et dans un environnement chaud et humide (âgés: 426 ± 37 kJ; jeunes: 317 ± 45 kJ, p = 0,037). Ces observations révèlent que, comparativement à de jeunes personnes, les personnes âgées emmagasinent plus de chaleur au cours d'une brève exposition dans un environnement chaud (sec et humide) et peuvent vivre un plus grand stress thermique dans de telles conditions. [Traduit par la Rédaction]

Mots-clés : vieillissement, stress thermique passif, calorimétrie, vagues de chaleur.

Introduction

Changes in climate towards more frequent and extreme heat events, which are longer in duration, have posed a significant health risk around the world. In Europe, 70 000 people died during the 2003 heat wave as a result of prolonged exposure to extreme heat. Countries with generally mild summertime temperatures were especially affected (Fouillet et al. 2006; Poumadere et al. 2005; Rey et al. 2007; Robine et al. 2008). In France for example, approximately 15 000 deaths were attributed to the exceptional heat wave where temperatures exceeded 40 °C on 7 consecutive days (Fouillet et al. 2006; Poumadere et al. 2005; Rey et al. 2007). More recently, an estimated 55 000 people died in Russia during the summer 2010 heat wave (Osborn 2010), including approximately 11 000 deaths in Moscow (Barriopedro et al. 2011). In Canada, summers are

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generally mild, with mid-summertime temperatures averaging approximately 25 °C in many parts of the country (Government of Canada 2013*a*). Canadian summers also tend to be humid with relative humidity typically ranging between 50% and 70% (Government of Canada 2013*a*). The temperate weather in addition to the large fluctuations in temperature throughout the year can make it difficult for individuals to become heat acclimatized. Therefore it is possible that people residing in these climates may be more susceptible to heat-related illness during periods of above-normal temperatures as a result of low levels of heat adaptation compared with people living in warmer places year round.

The number of days with temperatures ≥30 °C and humidity index values exceeding 40 °C is expected to increase in Canada (Maxwell et al. 2006). Such extremes can have a considerable impact on the health of many people (Bustinza et al. 2013; Pengelly et al. 2007; Water, Air and Climate Bureau 2011), and older adults may be especially vulnerable to heat-related illness in these conditions (Frumkin et al. 2012; Kenny et al. 2010; Minson et al. 1998). A number of studies have demonstrated important age-related differences in local heat loss responses (e.g., sweat rate) and (or) core temperature during passive heat exposure of 60 to 240 min in duration (Crowe and Moore 1974; Dufour and Candas 2007; Fennell and Moore 1973; Miescher and Fortney 1989; Sagawa et al. 1988). In some cases, significant impairments in local heat loss or core temperature responses were evident after just 30 min of resting in the heat (Dufour and Candas 2007; Miescher and Fortney 1989). However, whether these differences in local indices of heat strain are meaningful from a whole-body perspective is currently unclear. Moreover, thermoregulatory responses as a function of age were observed under ambient air temperatures ranging between 40 and 45 °C. During extreme heat events, temperatures rarely rise above approximately 36–37 °C in Canada. For example, the hottest day of the year in Toronto, one of the largest Canadian metropolitan cities, was 36.7 °C during the 2012 North American heat wave (Government of Canada 2013*b*). This is considerably lower than temperatures recorded in southern parts of the United States where daily temperatures were as high as 42.8–45.0 °C during this particular heat wave (Burt 2012). Nevertheless, humidity index readings, which indicate the approximate temperature when heat and humidity are considered, have been recorded upwards of 45 °C during extreme heat events in Canada (Government of Canada 2013*a*).

In the present study, we used conditions representative of the upper temperature extremes in Canada to assess the potential public health risk to older adults living in mild climates during heat waves. We examined whole-body heat loss and changes in body heat content in younger (21 ± 3 years) and older adults (65 ± 5 years) during 2 h of passive exposure to a hot–dry condition (temperature: 36.5 °C; relative humidity: 20%; equivalent humidity index: 38 °C) and a hot–humid condition (temperature: 36.5 °C, relative humidity: 60%, equivalent humidity index: 51 °C). It was hypothesized that whole-body heat loss would be reduced in older adults compared with their younger counterparts thereby leading to a greater change in body heat content over 2 h of exposure in both heat stress conditions.

Materials and methods

Ethical approval

The experimental protocol was approved by the University of Ottawa Health Sciences and Science Research Ethics Board and The Health Canada and Public Health Agency of Canada Research Ethics Board in accordance with the Declaration of Helsinki. Volunteers provided written informed consent before participating in the study.

Participants

Twelve younger (21 ± 3 years, 4 females and 8 males) and 12 older (65 ± 5 years, 3 females and 9 males) adults who were not overly physically active (i.e., no more than 3 days per week of continuous exercise of ≤20 min in duration) participated in the study. Participants were nonsmokers and were free of known respiratory or cardiovascular disease. Younger and older participants had similar height (younger: 1.7 ± 0.1 m; older: 1.8 ± 0.1 m), weight (younger: 72.0 ± 4.4 kg; older: 80.1 ± 4.2 kg), body surface area (younger: 1.8 ± 0.1 m^2; older: 2.0 ± 0.1 m^2), and body mass index (younger: 24.7 ± 1.2 $kg \cdot m^{-2}$; older: 25.8 ± 1.1 $kg \cdot m^{-2}$).

Experimental design

Participants volunteered for 1 preliminary session and 2 experimental test sessions. During the preliminary session, measurements of height and body mass were obtained. Body surface area was subsequently calculated from the measurements of height and body mass (Du Bois and Du Bois 1989). We also inquired about physical activity patterns using a quantitative (3 month) and 7 day physical activity recall questionnaire proposed by Kohl et al. (1988).

The experimental sessions involved 2 tests that were performed on separate days with a minimum of 72 h between sessions. Participants were instructed to consume a light meal before their arrival and to avoid major thermal stimuli on their way to the laboratory. Participants were also asked to refrain from engaging in strenuous physical activity and consuming alcohol for 24 h as well as caffeine for up to 12 h before the testing sessions. To ensure euhydration, participants were instructed to drink 250 mL of water before bed, in the morning, and within 2 h of the experimental trial. No fluid was ingested for the duration of the experimental protocol. There were no differences between groups in hydration status, according to urine-specific gravity measurements, at the start of the hot–dry (younger: 1.017 ± 0.002; older: 1.012 ± 0.002) and hot–humid sessions (younger: 1.018 ± 0.003; older: 1.012 ± 0.002). The experimental sessions were performed in a whole-body direct air calorimeter, without prior laboratory acclimation sessions. Each session involved 120 min of passive heat exposure. During 1 experimental session, participants were seated in a whole-body calorimeter (a device for making very accurate measurements of the amount of heat emitted by the human body) regulated to 36.5 °C and 20% relative humidity (RH), whereas during the other session the environmental conditions were set to 36.5 °C and 60% RH. The order of the sessions was random; an equal amount of participants performed the hot–dry session first followed by the hot–humid session and vice versa. Participants wore a light pair of athletic shorts and sandals during the sessions, and female participants also wore a sports bra.

Measurements

A detailed explanation of how direct calorimetry measures whole-body heat loss and heat storage has been described in a previous publication (Kenny and Jay 2013). Also, a full technical description of the fundamental principles and performance characteristics of the Snellen calorimeter is available (Reardon et al. 2006). In summary, direct calorimetry measured whole-body evaporative loss and dry heat exchange (radiation, conduction, convection), yielding an accuracy of ± 2.3 W for the measurement of total heat loss. Indirect calorimetry was used to measure metabolic heat production. The change in body heat content (ΔH_b) was subsequently calculated by subtracting the total amount of heat production and heat loss over the 2 h sessions. The amount of evaporation required to achieve heat balance (E_{req}) was calculated from the sum of the rate of metabolic heat production and dry heat loss.

Local sweat production was measured using the ventilated capsule technique. A 3.8 cm^2 plastic capsule was attached to 3 skin sites (upper trapezius, forearm, and thigh) with an adhesive ring

and topical skin glue (Collodion HV, Mavidon Medical products, Lake Worth, Fla., USA). Compressed dry air was passed through the capsule at a rate of 1 L·min^{-1}. Water content of the effluent air was measured using high precision dew point mirrors (model 473, RH systems, Albuquerque, N.M., USA). Local sweat rate was calculated using the difference in water content between effluent and influent air multiplied by the flow rate and normalized for the skin surface area under the capsule.

Laser Doppler velocimetry was employed for measuring skin blood flow (PeriFlux System 5000, Main control unit; PF5010 LDPM). A laser Doppler probe (Perimed integrating probe 413, Järfälla, Sweden) was affixed to the forearm's surface in an area that did not seem overly vascular upon visual inspection and provided stable readings at rest. To measure maximal skin blood flow, the heater housing the laser Doppler probe was heated to 44 °C until maximal skin vasodilation was achieved (approx. 40 min) (Taylor et al. 1984). Cutaneous vascular conductance (CVC) was subsequently calculated as the ratio of skin blood flow perfusion units to mean arterial pressure and expressed as a percentage of maximum.

Visceral temperature was measured using a telemetric pill (VitalSense ingestible capsule thermometer is a Class II Medical Device according to 21 DFR 8982.1845; Mini Mitter Company Inc.) that moves freely and unobstructed through the digestive tract and is generally eliminated within 48 h of ingestion (McKenzie and Osgood 2004). The telemetric pill provides an estimate of internal body temperature.

Mean skin temperature was calculated as the weighted average of 4 skin temperature measurements: bicep (30%), chest (30%), quadriceps (20%), and back calf (20%) (Ramanathan 1964).

Systolic and diastolic blood pressures were determined using a Finometer (Finapres Medical Systems, Amsterdam, the Netherlands) from the beat-to-beat recording of the right middle finger arterial pressure waveform with the volume-clamp method (Penaz 1973) and physical criteria (Wesseling et al. 1995). Blood pressure measurements were used to calculate mean arterial pressure (diastolic blood pressure + 1/3 × pulse pressure).

Heart rate was monitored, recorded continuously, and stored using a Polar coded WearLink and transmitter, Polar RS400 interface, and Polar ProTrainer 5 software (Polar Electro Oy, Finland).

Urine samples and body mass were obtained prior to the start and immediately following the experimental sessions. Urine-specific gravity was determined using a handheld total solids refractometer (model TS400, Reichter Inc., Depew, N.Y., USA). Venous blood samples were also obtained prior to and immediately following each session, while participants remained seated upright, via a single venipuncture (Becton, Dickinson and Company [BD], Franklin Lakes, N.J., USA). The samples were transferred directly into serum with no additive and plasma K_2EDTA 5.4 mg BD Vacutainer® tubes (BD, Franklin Lakes, N.J., USA). The K_2EDTA blood was mixed by inversion and used to measure hematological parameters (Beckman Coulter, Miami, Fla., USA). Haemoglobin and hematocrit values were used to estimate the percent changes in plasma and blood volumes according to the method of Dill and Costill (1974).

Thermal discomfort was evaluated using the ASHRAE 7-point scale (TS; Scale "0 = Neutral" to "7 = Very, Very Hot") and was recorded every 10 min.

Statistical analysis

All dependent variables were compared between groups for each experimental condition (dry and humid) separately using a 2-way analysis of variance (ANOVA) with the repeated factor of time (levels: 30, 60, 90, and 120 min) and nonrepeated factor of age (levels: younger and older). Independent *t* tests were used for post hoc analysis when a main effect of group was observed. The values at 30, 60, 90, and 120 min were obtained by averaging the last minute of data collected during that time period. Independent *t* tests were also used to compare participant characteristics and the cumulative change in body heat content measured over the 120 min exposure. The level of significance for all analyses was set at $p \leq 0.05$. Analyses were performed using commercially available statistical software (GraphPad Prism 6.0, GraphPad Software, La Jolla, Calif., USA). All values are reported as mean ± standard error (SE). Note that visceral temperature was not measured in 1 of the younger participants in the hot–humid condition as well as in 2 older participants in both conditions; skin blood flow was only measured in ten younger and eight older participants.

Results

Younger versus older adults: hot–dry ambient condition

Calorimetry

Metabolic heat production, total heat loss, evaporative heat loss, E_{req}, and dry heat exchange during the hot–dry condition are presented in Fig. 1A and 1B. Metabolic heat production remained fairly consistent ($p = 0.219$), and there was no difference between groups ($p > 0.999$) during the dry heat exposure. Total heat loss, which combines evaporative heat loss and dry heat exchange, increased progressively ($p < 0.001$) for both groups ($p = 0.584$), mainly due to a progressive reduction in dry heat gain ($p < 0.001$). Moreover, the rate of dry heat gain significantly differed between groups ($p = 0.032$). At 60, 90, and 120 min, the rate of dry heat gain was 11 W ($p = 0.022$), 10 W ($p = 0.021$), and 12 W ($p = 0.008$), respectively, greater in older adults compared with younger adults. The difference in dry heat gain at 30 min fell just short of significance ($p = 0.056$). Evaporative heat loss on the other hand, remained constant throughout ($p = 0.291$) and was almost identical between groups ($p = 0.758$), averaging 104 ± 7 W in younger and 103 ± 7 W in older adults. E_{req}, calculated as the sum of metabolic heat production and dry heat exchange, remained stable during the dry heat exposure session ($p = 0.139$) and did not significantly differ between groups ($p = 0.328$). However, due to the greater rate of dry heat gain measured in older adults, E_{req} (and therefore the net heat load) was higher by an average of approximately 11 W in older adults. Following the 2 h exposure, the cumulative change in body heat content was significantly greater in the older age group relative to the younger group ($p = 0.018$) (Fig. 2B).

Local heat loss responses

Data from local sweat rates measured on the upper back, forearm, and thigh are presented in Table 1, whereas CVC data are presented in Table 2. Local sweat rates did not significantly increase during the session (all $p > 0.400$); however, sweat rate was significantly different between groups on the upper back ($p = 0.011$). Older adults had a greater sweat rate at 30 ($p = 0.035$), 60 ($p = 0.012$), 90 ($p = 0.005$), and 120 min ($p = 0.003$). Local sweating on the forearm ($p = 0.066$) and thigh ($p = 0.194$) were not different between groups. CVC remained the same throughout the session ($p = 0.182$). There was a difference between groups ($p = 0.019$) whereby CVC was higher in older adults at 60 ($p = 0.003$), 90 ($p = 0.010$), and 120 min ($p = 0.015$).

Core and skin temperatures

Visceral temperature during the hot–dry exposure is presented in Fig. 2A, whereas mean skin temperature data are presented in Table 1. Both visceral ($p < 0.001$) and mean skin ($p = 0.006$) temperatures increased over the 2 h but the differences between groups were not statistically significant ($p = 0.107$ and $p = 0.381$, respectively).

Cardiovascular responses and hydration status

Heart rate and mean arterial pressure data are presented in Table 2. Neither of these variables changed over the course of the exposure (heart rate: $p = 0.163$; mean arterial pressure: $p = 0.248$) as well, younger and older adults appeared to experience similar

Fig. 1. (A and C) Required amount of evaporative heat loss to achieve heat balance (E_{req}) (circles), evaporative heat loss (triangles) and dry heat exchange (squares). (B and D) Metabolic heat production (circles) and total heat loss (triangles). Data collected during passive exposure to a hot–dry (36.5 °C, 20% relative humidity) and hot–humid (36.5 °C, 60% relative humidity) condition for a duration of 2 h. Data for the younger group is presented in open symbols, whereas data for the older group is presented in dark filled symbols. * denotes a significant difference between groups. All values are presented as means ± SE.

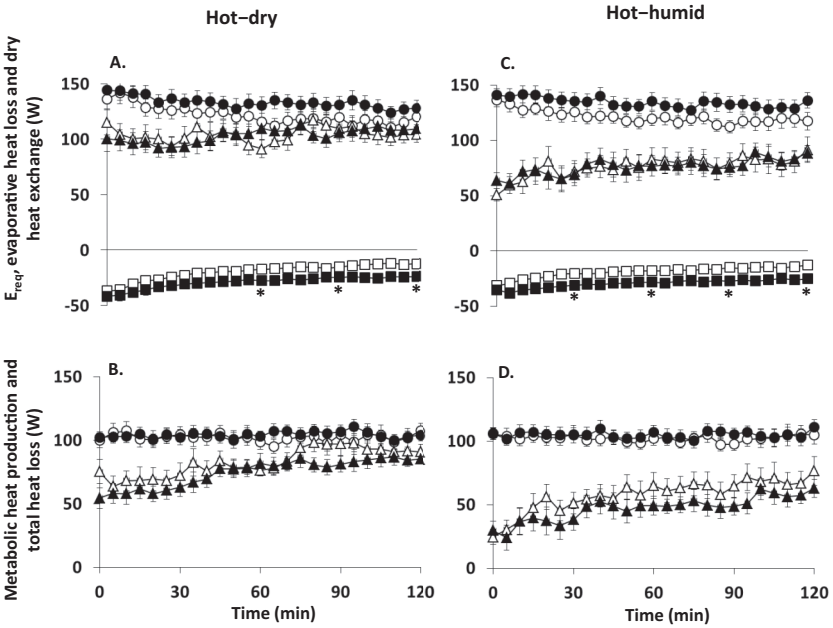
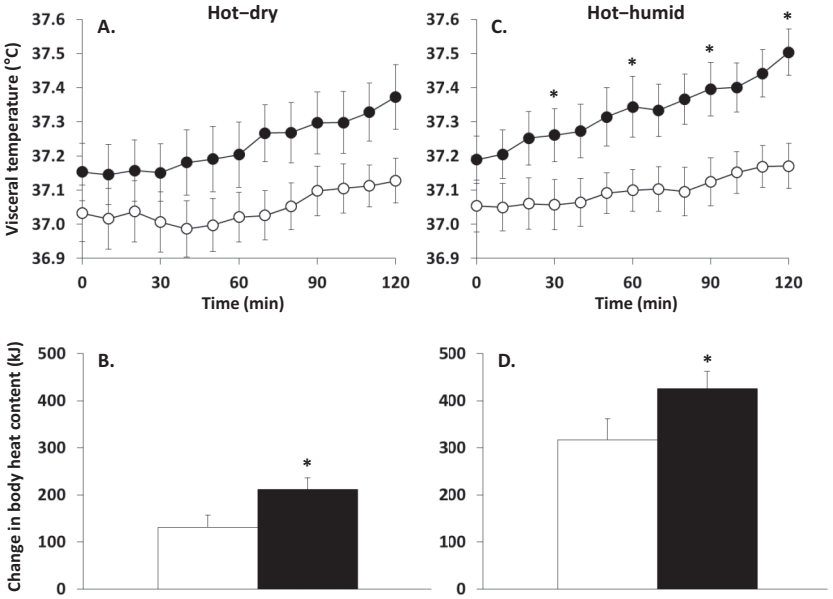


Fig. 2. (A and C) Visceral temperatures. (B and D) Cumulative change in body heat content. Data collected during passive exposure to a hot–dry (36.5 °C, 20% relative humidity) and hot—humid (36.5 °C, 60% relative humidity) condition for a duration of 2 h. Data for the younger group is presented in open symbols, whereas data for the older group is presented in dark filled symbols. * denotes a significant difference between groups. All values are presented as means ± SE.



levels of cardiovascular strain (heart rate: $p = 0.210$; mean arterial pressure: $p = 0.964$). The changes in blood volume (younger: −2.5 ± 0.5%; older: −3.9 ± 0.6%, $p = 0.077$), plasma volume (younger: −4.5 ± 0.8%; older: −6.6 ± 1.1%, $p = 0.101$), and urine-specific gravity (younger: 0.004 ± 0.001; older: 0.007 ± 0.001, $p = 0.219$) were not significantly different between groups. There was a trend for change in body weight to differ between groups ($p = 0.057$). Older adults had a slightly greater change in weight (−0.63 ± 0.05 kg) relative to younger adults (-0.51 ± 0.04 kg).

Thermal discomfort

Participants were asked to indicate their level of thermal discomfort throughout the exposure. Although perceived thermal discomfort did not increase or decrease during the session ($p = 0.245$), there was a significant difference between groups ($p = 0.051$). Older adults consistently rated their thermal sensation higher than that of younger adults and this was significantly different at 60 min (3.1 ± 0.4 vs. 2.2 ± 0.3, $p = 0.044$) and 120 min (3.1 ± 0.4 vs. 1.9 ± 0.3, $p = 0.008$).

Table 1. Local sweating rates and mean skin temperature during passive exposure to dry and humid heat.

	LSR (mg·min^{-1}·cm^{-2})							
	Arm		Back		Leg		T_{Sk} (°C)	
Min.	Younger	Older	Younger	Older	Younger	Older	Younger	Older
Hot–dry								
0	0.18±0.03	0.20±0.03	0.19±0.04	0.22±0.03	0.21±0.02	0.20±0.03	34.11±0.12	34.19±0.15
30	0.15±0.03	0.17±0.02	0.14±0.03	0.22±0.03*	0.18±0.02	0.17±0.02	34.47±0.13	34.36±0.18
60	0.14±0.03	0.21±0.02	0.14±0.03	0.24±0.03*	0.19±0.02	0.19±0.02	34.56±0.12	34.35±0.18
90	0.13±0.02	0.18±0.02	0.12±0.02	0.22±0.03*	0.21±0.02	0.15±0.01	34.57±0.11	34.40±0.19
120	0.11±0.02	0.20±0.03	0.12±0.02	0.23±0.03*	0.21±0.02	0.16±0.02	34.69±0.10	34.44±0.17
Hot–humid								
0	0.39±0.04	0.51±0.06	0.35±0.04	0.43±0.04*	0.48±0.04	0.39±0.03	34.41±0.10	34.28±0.07
30	0.33±0.03	0.43±0.04	0.30±0.04	0.41±0.05*	0.43±0.04	0.34±0.02	34.74±0.10	34.55±0.10
60	0.33±0.04	0.46±0.05	0.28±0.03	0.42±0.06*	0.42±0.03	0.31±0.02*	34.80±0.08	34.65±0.14
90	0.31±0.03	0.41±0.04	0.28±0.03	0.41±0.06*	0.39±0.03	0.30±0.02*	34.84±0.08	34.65±0.16
120	0.33±0.04	0.42±0.04	0.26±0.02	0.41±0.05*	0.40±0.03	0.31±0.02*	34.87±0.07	34.63±0.14

Note: Values are presented as mean ± SE. LSR, local sweat rate; T_{Sk}, mean skin temperature. *denotes a significant difference between younger and older adults.

Table 2. Cutaneous vascular conductance, heart rate, and mean arterial pressure responses during passive exposure to dry and humid heat.

	CVC (% of max)		HR (beats·min^{-1})		MAP (mmHg)	
	Younger	Older	Younger	Older	Younger	Older
Hot–dry						
0	18.4±1.4	21.0±1.9	78±4	77±3	85±2	95±3
30	19.0±1.8	23.7±1.8	80±4	78±3	94±3	95±5
60	17.6±1.3	24.5±1.6*	79±5	75±3	94±3	91±6
90	17.1±0.9	22.8±1.8*	84±5	74±2	94±2	97±6
120	17.2±1.0	22.6±1.9*	86±4	75±2	96±3	96±5
Hot–humid						
0	20.6±2.3	25.9±2.3	84±4	79±3	88±3	93±3
30	23.4±3.5	31.0±4.0	83±4	76±3	95±3	93±2
60	22.1±2.8	31.7±3.4*	86±4	81±4	95±3	93±2
90	21.6±3.0	33.8±3.7*	85±4	78±4	93±2	89±2
120	20.2±1.8	32.7±3.6*	86±5	80±3	94±2	92±2

Note: Values are presented as mean ± SE. CVC, cutaneous vascular conductance; HR, heart rate; MAP, mean arterial pressure. *denotes a significant difference between younger and older adults.

Younger versus older adults: hot–humid ambient condition

Calorimetry

We present the rates of metabolic heat production, total heat loss, evaporative heat loss, E_{req}, and dry heat exchange during the hot–humid experimental session in Fig. 1C and 1D. Similar to the hot–dry session, metabolic heat production remained constant ($p = 0.139$) and was not different between groups ($p = 0.535$). Total heat loss increased as the session progressed ($p < 0.001$). This was partly driven by a reduced rate of dry heat gain in both age groups ($p < 0.001$). Older adults had a significantly greater rate of dry heat gain compared with younger adults ($p = 0.019$). Significant differences between groups were observed at 30 (+11 W, $p = 0.011$), 60 (+10 W, $p = 0.032$), 90 (+12 W, $p = 0.032$), and 120 min (+12 W, $p = 0.021$). In contrast to the hot–dry session, evaporative heat loss increased significantly over the course of the humid heat exposure ($p < 0.001$), albeit this was similar between groups ($p = 0.814$). As a result of a greater rate of dry heat gain, the average E_{req} in older adults exceeded that of younger adults by approximately 13 W, although the difference between groups did not reach statistical significance ($p = 0.109$). The net amount of heat that was stored (and therefore the change in body heat content) over the 2 h hot–humid session was significantly greater in older adults relative to the younger adults ($p = 0.037$) (Fig. 2D).

Local heat loss responses

Local sweat rates on the upper back was significantly different between groups ($p = 0.025$); older adults had a greater sweat rate at 30 ($p = 0.016$), 60 ($p = 0.012$), 90 ($p = 0.016$), and 120 min ($p = 0.004$). Sweating on the forearm fell short of being significantly greater in older adults ($p = 0.056$). Finally, local sweating on the thigh was significantly greater in younger adults ($p = 0.010$). Significant differences occurred at 60 ($p = 0.030$), 90 ($p = 0.018$), and 120 min ($p = 0.031$). CVC remained significantly higher in older adults ($p = 0.042$) in the hot–humid condition compared with younger adults. CVC was significantly different between groups at 60 ($p = 0.031$), 90 (0.016), and 120 min ($p = 0.005$).

Core and skin temperatures

Both visceral ($p < 0.001$, Fig. 2C) and mean skin ($p = 0.004$) temperatures increased as the session progressed. Whereas mean skin temperature did not significantly differ between groups ($p = 0.213$), the increase in visceral temperature was significantly greater in older adults ($p = 0.019$). Differences were observed at 30 ($p = 0.040$), 60 ($p = 0.023$), 90 ($p = 0.011$), and 120 min ($p = 0.001$).

Cardiovascular responses and hydration status

Heart rate became significantly higher as the session progressed ($p = 0.010$), although this was similar in younger and older adults ($p = 0.242$). No differences in mean arterial pressure were measured over the course of the session ($p = 0.236$) and responses were similar between groups ($p = 0.390$). Younger and older adults had similar changes in blood volume (younger: –2.7 ± 0.5%; older: –2.5 ± 0.6%, $p = 0.441$), plasma volume (younger: –4.9 ± 0.9%; older: –3.9 ± 0.7%, $p = 0.464$) and urine-specific gravity (younger: 0.005 ± 0.002; older: 0.004 ± 0.002, $p = 0.442$). The difference in weight before and after the session fell short of statistical significance but tended to be more elevated in older adults (younger: –0.49 ± 0.02 kg, older: –0.59 ± 0.04 kg, $p = 0.057$).

Thermal discomfort

Thermal discomfort became increasingly greater over the course of the hot–humid exposure ($p = 0.002$) and older adults reported a higher thermal sensation ($p = 0.033$). This was significantly different at 30 (3.0 ± 0.2 vs. 2.1 ± 0.2, $p = 0.004$), 60 (3.2 ± 0.3 vs. 2.2 ± 0.2, $p = 0.007$), and 90 min (3.1 ± 0.3 vs. 2.3 ± 0.3, $p = 0.043$).

Discussion

In the present study, older adults had a greater change in body heat content compared with younger adults during short exposures to a hot–dry (36.5 °C, 20% RH) and hot–humid (36.5 °C, 60% RH) environment. This was in large part because heat load was more elevated in older adults during the exposures, owing to a greater rate of dry heat gain. This added heat load was not compensated for by a greater rate of evaporative heat loss, causing a greater rate of heat storage throughout the sessions that

resulted in a progressively greater change in body heat content among older adults.

A number of studies reported decrements in thermoregulatory function in older adults during passive exposure to extreme ambient conditions ranging from 40–90 °C with durations of 10 to 240 min (Armstrong and Kenney 1993; Drinkwater et al. 1982; Dufour and Candas 2007; Miescher and Fortney 1989; Sagawa et al. 1988; Shoenfeld et al. 1978). In the present study, we were concerned with investigating whole-body heat loss and heat storage under a lower environmental heat load as Canada does not typically experience such extreme temperatures, yet heat-related injuries and (or) deaths are still prevalent (Pengelly et al. 2007). The results from our study indicate that during exposure to a hot environment, with relatively low humidity, whole-body evaporative heat loss was similar between younger and older adults. Even so, older adults stored 38% more heat than their younger counterparts. It is important to note that older adults had a greater rate of dry heat gain (approx. 10 W), owing to the slightly lower mean skin temperature (and therefore greater air to skin temperature gradient). As shown in Fig. 1A, the amount of evaporative heat loss required to achieve balance (determined as the sum of metabolic heat load ± dry heat exchange) was actually more elevated in older adults compared with the younger group throughout the session. The average rate of evaporative heat loss required for older adults to achieve heat balance was approximately 133 W, whereas for young adults this was only approximately 122 W. Despite the slightly greater heat load, we did not observe a concomitant increase in the rate of whole-body evaporative heat loss in older adults. Evaporative heat loss accounted for approximately 85% of the evaporative heat loss required for heat balance in younger adults, but only approximately 77% in older adults. The net effect over the 2 h exposure was a greater cumulative increase in body heat content.

Canadian climates tend to be very humid in the summer. In fact, it is not uncommon for humidity indices (e.g., Humidex) to reach 45 °C with some extreme readings recorded as high as 51–53 °C during heat waves (Government of Canada 2013*a*). Such extreme conditions can severely impede thermoregulatory control by reducing the amount of heat that can be dissipated to the environment through evaporative heat loss; the primary physiological mechanism for cooling (McLellan et al. 1996; Taylor 2006). In the present study, evaporative heat loss was reduced to a similar extent (approx. 26%) in the hot–humid relative to the hot–dry condition in both younger and older adults. Consequently, heat storage increased by 59% in younger adults and 50% in older adults, compared with the amount of heat that was stored during the hot–dry condition. The change in body heat content remained higher in older adults (by 26%) compared with younger adults, again largely due to a greater rate of dry heat gain (approx. 10 W). Interestingly, local sweat rate was greater by approximately 55% and 49% (average from the three sites measured) for younger and older adults, respectively, in the humid condition compared with the dry environment, yet both groups stored more heat. Considering that the local sweat rate was somewhat more elevated in older adults (0.38 ± 0.04 vs. 0.31 ± 0.05 $mg \cdot min^{-1} \cdot cm^{-2}$) but that they still stored more heat would suggest that sweating efficiency was reduced to a greater degree in older individuals, thereby leading to increased thermal strain (Candas et al. 1979).

Core temperature measurements are commonly used to assess thermal stress among individuals in a variety of scenarios involving exposure to the heat. There is growing evidence from many population groups that core temperature measurements can severely underestimate whole-body heat storage as core temperature is only indicative of heat content within a specific region of the body (Kenny and Jay 2013). Our results indicate that core temperature increased progressively in younger (+0.09 ± 0.06 °C) and older adults (+0.22 ± 0.08 °C) during the hot–dry condition. In the hot–humid condition, the increase in visceral temperature was significantly more elevated in older adults (+0.32 ± 0.06 °C) than younger adults (+0.12 ± 0.04 °C). The change in core temperature, however, does not adequately reflect the much greater change in body heat content, and therefore the level of thermal strain experienced in each group. In fact, the small change in visceral temperature under both environmental conditions could lead to the misguided conclusion that the risk of developing a heat injury is relatively low. Conversely, the change in mean body temperature can more accurately be estimated using the change in body heat content measured by direct calorimetry with the equation $\Delta T_b = \Delta H_b/(b_m \times C_p)$ where ΔT_b is the change in mean body temperature, ΔH_b is the change in body heat content, b_m is body mass, and C_p is specific heat of the participant. Based on this equation, the estimated increase in mean body temperature would be approximately 0.52 °C and approximately 0.76 °C in younger and older adults, respectively, during the hot–dry exposure. For the hot–humid condition, the change in mean body temperature would actually be as high as approximately 1.34 °C (younger) and approximately 1.58 °C (older) after just 2 h. So although visceral temperature measurements may suggest minimal health risks in both groups under the conditions tested, it is important to keep in mind that mean whole-body temperature is much more elevated, with the greatest increase observed in older adults.

Perspectives

The main question behind the present study was whether or not exposure to extreme environmental conditions, which are representative of Canadian climates, poses a greater threat to the health of older adults relative to their younger counterparts. Increases in heat waves could be particularly concerning in places like Canada where summertime temperatures are generally mild, and temperatures fluctuate considerably throughout the year. As a result, individuals residing in such climates do not tend to be acclimatized to hot weather conditions and are likely more susceptible to the potential harmful effects of extreme heat compared with those living in countries and (or) cities where high summertime heat and humidity are common. In the present study, 2 h of exposure to a dry and humid heat stress resulted in greater body heat storage among older adults, suggesting a greater level of thermal strain compared with younger individuals. This is supported by the fact that older adults reported a higher level of thermal discomfort after only 60 and 30 min for the hot–dry and hot–humid environments, respectively. Nonetheless, there are a few things to consider when interpreting our study findings. First, the older adults in this study were healthy and not taking any medications. Thus, older adults with chronic medical conditions (e.g., diabetes, cardiovascular disease, respiratory disease, etc.) and (or) taking medication may be more vulnerable than the older adults who participated in the present study (Kenny and Jay 2013; Kenny et al. 2010). Moreover, the experimental sessions were conducted in a laboratory setting and did not factor in additional heat that could be gained through solar radiation. Also, the temperature selected to mimic the upper temperature extremes in Canada (i.e., 36.5 °C) may not be representative of extreme temperatures in large metropolitan cities, which could be even higher as a result of the "urban heat island effect" (Kovats and Hajat 2008; Lundgren et al. 2013). Finally, the exposure was purposely selected to be short. This was the first time that we used the direct calorimeter to assess whole-body heat loss and heat storage in a group of vulnerable older adults who had no prior experience in our laboratory. There are certain risks involved with doing these types of studies where participants are enclosed in a capsule-like device for an extended period of time. Given that previous studies had observed marked differences between younger and older adults after just 30 min (Dufour and Candas 2007; Miescher and Fortney 1989), we opted to only do a 2 h exposure. In reality, older adults may spend longer than 2 h exposed to high temperatures and (or) humidity during heat waves, hence our findings

underline a critical need to examine age-related changes in the heat stress responses during prolonged exposure (i.e., >4 h).

In summary, exposure to dry heat as well as humid heat posed a significantly greater thermal challenge for older adults as evidenced by greater changes in body heat content. Initiatives to increase awareness regarding the potential health risks associated with exposure to the heat, particularly when humidity is high, maybe necessary to protect older individuals against heat illness.

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APPENDIX B

Final published version of thesis article #4

Research Paper

Diminished nitric oxide-dependent sweating in older males during intermittent exercise in the heat

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New Findings

- **What is the central question of this study?**
  Sweating during exercise in the heat is, in part, mediated through nitric oxide-dependent mechanisms. It is unclear whether ageing reduces nitric oxide-dependent sweating during exercise in the heat.
- **What is the main finding and its importance?**
  Nitric oxide-dependent sweating during short bouts of exercise in the heat was observed in young men, but not in older adults. These findings show that age-related impairment in sweating may be associated with age-related reductions in nitric oxide-mediated sweating.

Nitric oxide (NO) is a signalling molecule that contributes to the control of many physiological pathways, including the heat-loss response of skin vasodilatation. Recently, NO has been implicated in the control of sweating during exercise in young adults. We tested the hypothesis that ageing reduces NO-dependent sweating during exercise in the heat. Ten young (23 ± 3 years old) and 10 older men (64 ± 5 years old), matched for body surface area, performed three successive 15 min bouts of exercise (Ex1, Ex2 and Ex3) at the same rate of metabolic heat production (300 W m^{-2}) in the heat (35°C, 20% relative humidity). Exercise periods were interspersed with 15 min recovery periods. Local sweat rate (ventilated capsule) was measured on two forearm skin sites, which were continuously perfused via intradermal microdialysis with 0.9% saline as control (CON) or 10 mM N^G-nitro-L-arginine methyl ester (L-NAME), a non-selective NO synthase inhibitor. Local sweat rate at the end of Ex1 was lower in the CON conditions in the older *versus* young men (0.69 ± 0.19 *versus* 0.90 ± 0.17 mg min^{-1} cm^{-2}, $P = 0.018$). In the young men, local sweat rate was reduced in the L-NAME-treated conditions compared with the CON conditions at the end of Ex1 (0.67 ± 0.14 *versus* 0.90 ± 0.17 mg min^{-1} cm^{-2}, $P = 0.004$), Ex2 (0.78 ± 0.20 *versus* 1.03 ± 0.20 mg min^{-1} cm^{-2}, $P = 0.013$) and Ex3 (0.78 ± 0.20 *versus* 1.03 ± 0.21 mg min^{-1} cm^{-2}, $P = 0.014$). In the older men, there was no main effect of treatment conditions on local sweat rate ($P = 0.537$) such that local sweat rates in the L-NAME-treated and CON conditions were similar (Ex1, 0.65 ± 0.20 *versus* 0.69 ± 0.19 mg min^{-1} cm^{-2}; Ex2, 0.80 ± 0.27 *versus* 0.91 ± 0.29 mg min^{-1} cm^{-2}; and Ex3, 0.84 ± 0.31 *versus* 0.94 ± 0.38 mg min^{-1} cm^{-2}). We conclude that ageing attenuates the influence of NO in the control of local forearm sweating observed in young adults during short 15 min bouts of exercise in the heat. This mechanism may, in part, explain the age-related impairments in sweating.

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Introduction

Evaporation of sweat promotes heat loss from the human body. Although the degree of evaporative heat loss from sweat production is affected by the ambient medium, temperature and water-vapour pressure, sweat evaporation is the main avenue for heat loss when ambient temperature exceeds mean skin temperature (~34°C) and the evaporative capacity of the environment does not exceed the required evaporation for heat balance (i.e. compensable conditions; Gisolfi & Wenger, 1984; Parsons, 2003). Compared with young adults, sweating has been shown to be reduced in older adults during exercise in the heat (Tankersley *et al.* 1991; Inoue *et al.* 1999; Larose *et al.* 2013*a*,*b*). Consequently, older individuals may be at an elevated risk of experiencing heat-related injuries during exercise in the heat. However, the underlying mechanism(s) for age-related impairments in sweating during exercise in the heat remains unclear.

Eccrine sweat glands possess both adrenergic and cholinergic receptors (Robertshaw, 1977; Schotzinger & Landis, 1988; Weihe *et al.* 2005). However, recent studies in humans have revealed that there is no contribution of adrenergic receptors to the sweating response (Buono *et al.* 2011; Machado-Moreira *et al.* 2012). For example, local β-adrenergic receptor blockade with propranolol did not affect the sweating response during exercise in the heat (Buono *et al.* 2011). Also, psychological sweating, which was originally thought to be mediated by non-cholinergic mechanisms (e.g. adrenergic mechanisms), was completely abolished using the systemic muscarinic receptor blockade atropine (Machado-Moreira *et al.* 2012). Furthermore, local and systemic muscarinic receptor blockade with atropine abolished thermal sweating (Kellogg *et al.* 1995; Machado-Moreira *et al.* 2012). It is therefore conceivable that ACh released from cholinergic nerves may be a key neurotransmitter in the control of sweating during heat stress. Additionally, a recent study by Welch *et al.* (2009) showed that sweating was lower in skin infused with N^G-nitro-L-arginine methyl ester [L-NAME; a non-selective nitric oxide synthase (NOS) inhibitor] through intradermal microdialysis, in comparison to a control skin site infused with 0.9% saline during continuous cycling exercise (~47% of maximum oxygen uptake) performed in a hot environment. This finding suggests that NO may also be an important modulator of the exercise-induced increases in sweating.

There is increasing evidence demonstrating that ageing reduces the NO bioavailability in the skin (Holowatz *et al.* 2003, 2006*a*,*b*; Stanhewicz *et al.* 2012, 2013), skeletal muscle (Nyberg *et al.* 2012) and serum (Toprakçi *et al.* 2000), as well as arterial and venous blood (Nyberg *et al.* 2012). It is therefore plausible that NO-dependent sweating during exercise in the heat may be compromised in older adults. Elucidating the mechanisms of age-related differences in sweating during exercise-induced heat stress, and specifically, assessing the potential role of NO in this response, would provide us with important new information about the regulation of sweating. This knowledge could be used to define potential countermeasures and/or treatments to improve heat dissipation in older adults engaged in exercise, thus mitigating the risk of heat-related injuries.

The rate of metabolic heat production increases immediately upon the onset of exercise and is not initially offset by an increase in the rate of total heat loss [known as thermal inertia (Murgatroyd *et al.* 1993) or temporal dissociation (Webb & Annis, 1966)], thus giving rise to a pronounced increase in body heat storage during the early stages of exercise (Kenny & Jay, 2013). Hence, any delay associated with the activation of the sweating response, such as may occur with ageing, as recently demonstrated by Larose e*t al.* (2013*b*) where marked differences in whole-body evaporative heat loss were evident as early as ~10 min of exercise, would lead to more pronounced increases in body heat storage and therefore core temperature during exercise (Kenny & Jay, 2013). This would be exacerbated by the rapid attenuation of heat loss following cessation of exercise, which has been shown to be similar in both young and older adults despite the fact that older adults stored more heat during exercise (Larose *et al.* 2013*a*,*b*). While both young and older adults exhibited a greater evaporative heat loss with successive exercise bouts, the age-related impairments remained intact despite a greater increase in body heat content, and therefore thermal drive, with successive exercise bouts (Kenny *et al.* 2009; Larose *et al.* 2013*a*,*b*). Thus, the purpose of the present study was to test the following hypotheses: (i) NO-dependent sweating during exercise is reduced in older adults compared with their younger counterparts, and this response remains intact with successive exercise bouts; and (ii) the relative influence of NO may be less evident during recovery irrespective of the increase in core temperature observed with successive exercise bouts.

Methods

Ethical approval

This study was approved by the University of Ottawa Health Sciences and Science Research Ethics Board, in accordance with the Declaration of Helsinki. Written, informed consent was obtained from all participants prior to their involvement in the study.

Participants

Twenty males volunteered for the study and were divided into two groups of 10 young (23 ± 3 years old) and 10

older males (64 ± 5 years old). All participants were healthy, non-smoking, physically active males free from cardiovascular disease or diabetes. Young and older males were matched for physical activity behaviour (i.e. 3–4 days per week of continuous exercise of 30–60 min in duration). In addition, all experimentation occurred between the months of October and April to avoid the effects of natural acclimatization.

Experimental procedures

Each participant completed one preliminary and one experimental session. During the preliminary session, body height, mass and density, as well as maximal oxygen uptake ($\dot{V}_{O_2\,max}$) were determined. Body height was determined using a stadiometer (model 2391; Detecto, Webb City, MO, USA), while body mass was measured using a digital high-performance weighing terminal (model CBU150X; Mettler Toledo Inc., Mississauga, ON, Canada). Body surface area was subsequently calculated from the measurements of body height and mass (DuBois & DuBois, 1916). Body density was measured using the hydrostatic weighing technique, with a correction of 100 ml of trapped gas volume in the gastrointestinal tract (Adams & Beam, 2008), and used to calculate body fat percentage (Siri, 1956).

The $\dot{V}_{O_2\,max}$ was determined by indirect calorimetry (MOXUS system; Applied Electrochemistry, Pittsburgh, PA, USA) during a progressive incremental exercise protocol (Canadian Society for Exercise Physiology, 1986) performed on an upright constant-load cycle ergometer (Corival; Lode BV, Groningen, The Netherlands) to volitional fatigue. During the $\dot{V}_{O_2\,max}$ test, the older participants were monitored by electrocardiogram under supervision of a qualified technician.

During the experimental session, participants reported to the laboratory and changed into shorts and running shoes. The participants were asked to drink 500 ml of water the night before, as well as ∼2 h prior to the experimental session. They were also asked to refrain from alcohol, caffeine and exercise for 24 h prior to experimentation. Upon arrival at the laboratory, the participants provided a urine sample, and baseline body mass was measured. They subsequently rested quietly at an ambient room temperature of 24°C. During this time, two microdialysis fibres (MD 2000; Bioanalytical Systems, West Lafayette, IN, USA; 30 kDa cut-off, 10 mm membrane) were placed in the dermal space of the forearm under aseptic conditions. To place the fibre, a 25 gauge needle was first inserted into the dermal space of the dorsal aspect of the left forearm and then the needle exited 20–25 mm away from the point of entry. No anaesthesia was used during the insertion of the needle. The fibre was then inserted through the lumen of the needle. The needle was withdrawn, leaving the fibre in place. Each fibre was separated by at least 4.0 cm. After insertion, the fibres were perfused with a 0.9% saline solution at a rate of 2 μl min^{-1} via a micro infusion pump (CMA/400; CMA Microdialysis, Solna, Sweden).

Sixty minutes after the fibre placement (to allow for hyperaemia associated with the trauma of probe insertion to subside), the participant entered a thermal chamber regulated to an ambient air temperature of 35°C and a relative humidity of 20%. The participant rested for an additional 60 min baseline period in the upright seated posture while the rest of the instrumentation was placed. During the second hour, 10 mM L-NAME (Sigma Aldrich, Oakville, ON, USA), which has previously been used to inhibit NOS non-selectively in eccrine sweat glands (Lee & Mack, 2006; Welch *et al.* 2009), dissolved in saline solution was infused through one of the microdialysis fibres, while the second fibre continued to receive 0.9% saline as a control at a rate of 2 μl min^{-1}. The infusions continued for the remainder of the experimental protocol. Although it has been suggested that L-NAME has antimuscarinic effects, as shown in rat diaphragmatic microcirculation (Chang *et al.* 1997), a more recent study inferred minimal antimuscarinic effect of using 10 mM L-NAME on sweating in humans exercising in a hot environment (Welch *et al.* 2009). This confirmation is important because the sweating response during heat stress requires muscarinic receptor activation (Kellogg *et al.* 1995).

After the second 60 min baseline period, the participant was moved to an upright bicycle, where they rested for a 10 min baseline period followed by performing three 15 min bouts of cycling, each separated by 15 min of recovery. By design, each exercise was performed at a similar fixed rate of metabolic heat production of 300 ± 2 W m^{-2} (equivalent to 51.7 ± 7.4 and 69.6 ± 9.6% of $\dot{V}_{O_2\,max}$ for young and older males, respectively) to ensure a similar heat load between groups. Thus, any observed differences in the sweating response between groups would be attributed to the effects of ageing as opposed to differences in heat load and therefore thermal drive (Jay *et al.* 2011; Gagnon *et al.* 2013*b*). Following the intermittent exercise protocol, a postexercise urine sample was obtain and body mass was measured.

Measurements

The ventilated capsule technique was employed for the purpose of measuring local sweat rate. Local sweat rate was measured from a 3.8 cm^2 plastic capsule placed directly over the centre of the microdialysis membranes. The sweat capsules were attached to the forearm skin with adhesive rings and topical skin glue (Collodion HV; Mavidon Medical products, Lake Worth, FL, USA). Dry compressed air was passed through each capsule at a rate of 1.0 l min^{-1}. Water content of the effluent air was measured

using high-precision dew point mirrors (model 473; RH systems, Albuquerque, NM, USA). The gas tanks and tubes used for the measurement of sweat rate were placed inside the environmental chamber regulated at 35°C. Long 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 trial (i.e. 35°C). Local sweat rate was calculated every 1 s using the difference in water content between effluent and influent air multiplied by the flow rate and normalized for the skin surface area under the capsule (in milligrams per minute per square centimetre).

Oesophageal temperature (T_{oes}) was measured with a general purpose thermocouple temperature probe (Mallinckrodt Medical Inc., St Louis, MO, USA). The probe was inserted 40 cm past the entrance of the nostril while the participants sipped water through a straw. Rectal temperature (T_{re}) was measured using a general purpose thermocouple temperature probe (Mallinckrodt Medical Inc.) inserted to a minimum of 12 cm past the anal sphincter. Skin temperature was measured at four sites using thermocouples (Concept Engineering, Old Saybrook, CT, USA) attached to the skin with surgical tape. Mean skin temperature was calculated using the four skin temperatures weighted to the following regional proportions: upper trapezius, 30%; chest, 30%; quadriceps, 20%; and posterior calf, 20% (Ramanathan, 1964). Temperature data were collected using an HP Agilent data acquisition module (model 3497A; Agilent Technologies Canada Inc., Mississauga, ON, Canada) at a rate of one sample every 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).

Pre- and postexercise urine specific gravities were determined in duplicate using a hand-held total solids refractometer (model TS400; Reichter Inc., Depew, NY, USA).

Statistical analyses

The values for each exercise (Ex) and recovery period (R) were obtained by averaging the last minute of the exercise or recovery period. Values of T_{oes}, T_{re} and mean skin temperature were also presented as a change from baseline (ΔT_{oes}, ΔT_{re} and Δmean skin temperature). Dependent variables were analysed using a two-way ANOVA. The ANOVAs were performed with one factor of exercise time (three levels: Ex1, Ex2 and Ex3) or recovery time (three levels: R1, R2 and R3) and the second factor of treatment condition (two levels: CON and L-NAME) or age (two levels: young and older). When a significant main effect was observed, *post hoc* comparisons were carried out using Student's paired or unpaired *t* tests corrected for multiple comparisons using the Bonferroni procedure. Additionally, physical characteristics, baseline values for all variables, as well as local sweat rates as a function of increases in T_{oes} were analysed using a Student's unpaired *t* test to identify age-related differences.

We evaluated the age-related difference in sweat rate by comparing responses as follows: (i) at the end of exercise for each of the three exercise bouts; and (ii) as a function of an equivalent T_{oes} (i.e. thermal drive) as defined by the end-exercise response in young adults. Mean body temperature was calculated as follows: $0.8 \times T_{oes} + 0.2 \times$ mean skin temperature. The onset threshold and thermosensitivity of local sweat rate during each exercise period were determined from local sweat rate plotted against mean body temperature with a segmented regression analysis as described by Cheuvront *et al.* (2009) with aid of a computer algorithm (GraphPad Prism 6.0; GraphPad Software, La Jolla, CA, USA). The level of significance for all analyses was set at $P \leq 0.05$. Statistical analyses were performed using commercially available statistical software (GraphPad Prism 6.0). All values are reported as means ± SEM unless otherwise indicated as SD.

Results

Participant characteristics

Participant characteristics are presented in Table 1. There were no differences in height ($P = 0.348$), body mass ($P = 0.169$) and body surface area ($P = 0.225$) between groups. However, the younger males had a greater $\dot{V}_{O_2\,max}$, both as an absolute value ($P = 0.002$) and relative to body mass ($P = 0.010$). On the day of the experimental session, prior to Ex1 all participants were well hydrated according to urine specific gravity, a measure of hydration status, with similar values between groups (young, 1.014 ± 0.007 *versus* older, 1.016 ± 0.007, $P = 0.627$). In addition, the absolute change before and after exercise in urine specific gravity (young, 0.004 ± 0.005 *versus* older, 0.003 ± 0.006, $P = 0.820$) and weight (young, −1.13 ± 0.20 *versus* older, −1.12 ± 0.24 kg, $P = 0.892$) did not differ between groups.

Local sweat rate

Baseline rest. Baseline resting local sweat rate was similar between young and older males (Fig. 1*A* and *B*) and between treatment conditions (Fig. 2*A* and *B*; all $P > 0.05$).

Exercise. There was a main effect of age on local sweat rate during exercise in the CON conditions ($P = 0.038$) but not in the L-NAME-treated conditions ($P = 0.817$). Local sweat rate in the CON conditions was greater for the young compared with the older males at the end of

Table 1. Participant characteristics

Group	Age (years)	Height (cm)	Body mass (kg)	Body surface area (m^2)	Body fat (%)	$\dot{V}_{O_2\,max}$ ($l\ min^{-1}$)	$\dot{V}_{O_2\,max}$ ($ml\ kg^{-1}\ min^{-1}$)
Young	23 ± 3*	179.9 ± 6	81.6 ± 7.7	2.01 ± 0.11	17.6 ± 5.0	4.1 ± 0.8*	50.2 ± 9.1*
Older	64 ± 5	177.4 ± 5.3	75.7 ± 10.3	1.95 ± 0.12	19.5 ± 7.1	3.0 ± 0.6	39.5 ± 7.5

Abbreviation: $\dot{V}_{O_2\,max}$, maximal oxygen consumption. *Significant difference between young and older males ($P \leq 0.05$ for Student's unpaired *t* tests). All values are presented as means ± SD.

Ex1 ($P = 0.018$), but not at the end of Ex2 ($P = 0.291$) and Ex3 ($P = 0.519$; Fig. 1*A*). There was a main effect of treatment condition on local sweat rate in the young males ($P = 0.031$) but not in the older males ($P = 0.537$). In the young males L-NAME resulted in a lower local sweat rate compared with the CON conditions at the end of Ex1 ($P = 0.004$), Ex2 ($P = 0.013$) and Ex3 ($P = 0.014$; Fig. 2*A*). We found a main effect of time on local sweat rate in the CON and L-NAME-treated conditions (both $P < 0.001$). In the CON conditions, in both young and older males local sweat rate increased from the end of Ex1 to the end of Ex2 (both $P < 0.01$) but not from the end of Ex2 to the end of Ex3 (both $P > 0.05$; Fig. 1*A*). In the L-NAME-treated conditions, local sweat rate increased from the end of Ex1 to the end of Ex 2 in the older males ($P = 0.004$) but not in the young males ($P = 0.098$), and it did not increase from the end of Ex2 to the end of Ex 3 in both groups (both $P > 0.05$; Fig. 1*B*).

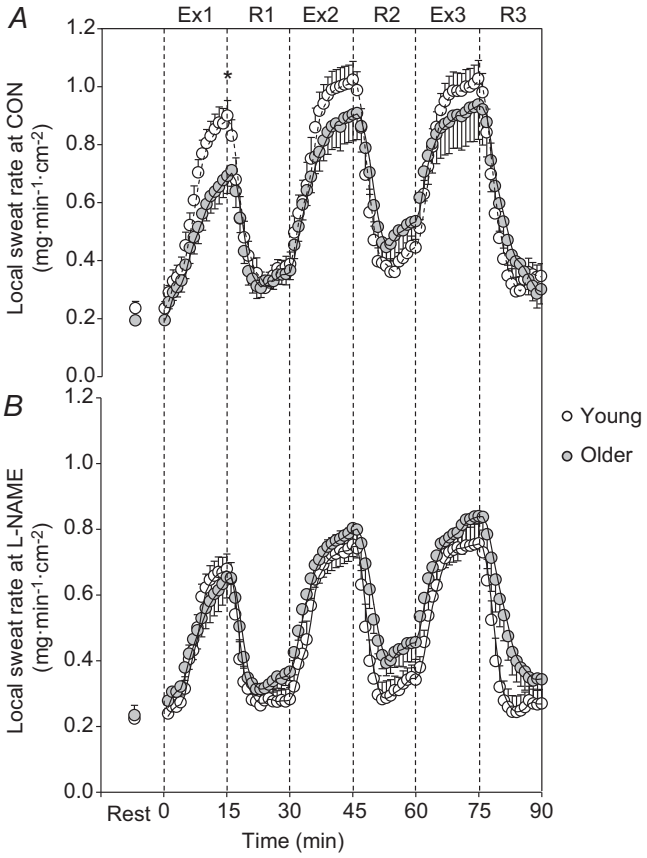

Figure 1. Local sweat rates in control (CON; *A*) and L-NAME-treated conditions (*B*) during exercise (Ex) and recovery (R) in the heat (35.0°C, 20% relative humidity)
Data from young males are presented in open circles, while data from older males are presented in grey circles. All values are presented as means ± SEM. *Significant difference between age groups ($P \leq 0.05$ for Student's unpaired *t* tests).

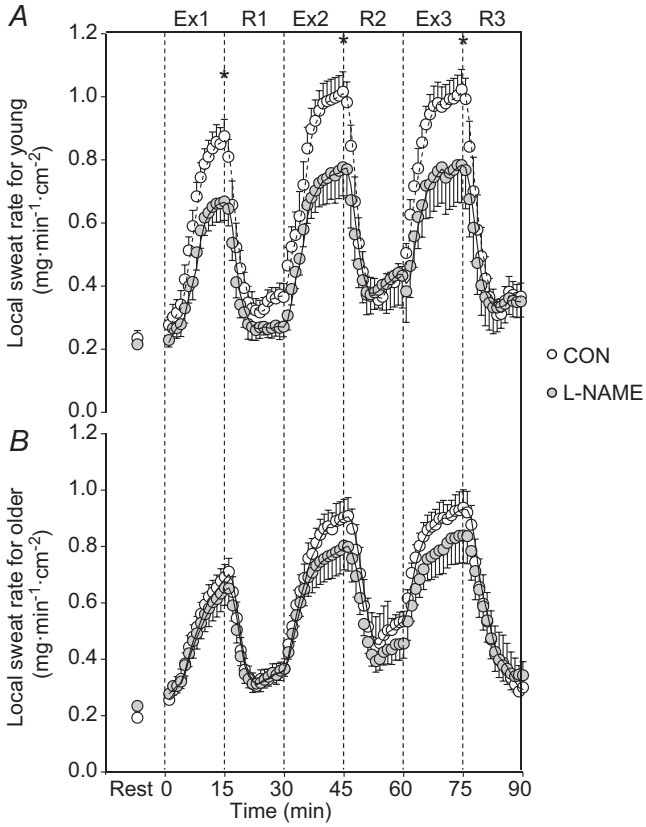

Figure 2. Local sweat rates for young (*A*) and older males (*B*) during exercise (Ex) and recovery (R) in the heat (35.0°C, 20% relative humidity)
Data from CON conditions are presented in open circles, while data from L-NAME-treated conditions are presented in grey circles. All values are presented as means ± SEM. *Significant difference between treatment conditions ($P \leq 0.05$ for Student's paired *t* tests).

When comparing the level of sweat rate for a similar increase in T_{oes} between groups with successive exercise (Fig. 3), the young group had a greater end-exercise sweat rate for a ΔT_{oes} of 0.54°C ($P = 0.004$) and 0.76°C ($P = 0.013$) as measured in Ex1 and Ex2, respectively, but not for a ΔT_{oes} of 0.81°C ($P = 0.188$) as measured in Ex3 in the CON conditions. There were no differences between groups in the L-NAME-treated conditions (all $P > 0.05$).

Recovery. During recovery, there was no main effect of age on local sweat rate in the CON ($P = 0.893$) or the L-NAME treatment conditions ($P = 0.227$). There was no main effect of treatment conditions on local sweat rate in the young ($P = 0.177$) or older males ($P = 0.856$). We found a main effect of time on local sweat rate in the CON conditions ($P = 0.006$) but not in the L-NAME-treated conditions ($P = 0.245$). However, in the CON conditions local sweat rate was not different between the end of R1 and R2 and between the end of R2 and R3 in both young and older males (all $P > 0.05$; Fig. 1*A*).

Core and skin temperatures

Baseline rest. Compared with the young males, the older males had a lower baseline T_{oes} at rest (young, 37.09 ± 0.22 *versus* older, 36.90 ± 0.17°C, $P = 0.047$). However, there were no between-group differences in baseline T_{re} (young, 37.17 ± 0.24 *versus* older, 37.07 ± 0.29°C, $P = 0.422$) and mean skin temperature (young, 34.94 ± 0.32 *versus* older, 34.77 ± 0.56°C, $P = 0.435$).

Exercise. There was no main effect of age on T_{oes} ($P = 0.817$), T_{re} ($P = 0.800$) and mean skin temperature ($P = 0.146$). There was a main effect of time on T_{oes}, T_{re} and mean skin temperatures (all $P < 0.001$). In both young and older males T_{oes}, T_{re} and mean skin temperature at the end of Ex2 were higher than those at the end of Ex1 (all $P < 0.05$; Table 2). In both young and older males, T_{oes} and mean skin temperature were not different between the end of Ex2 and the end of Ex3 (all $P > 0.05$); however, T_{re} increased from the end of Ex2 to the end of Ex3 ($P < 0.001$; Table 2). When core and mean skin temperatures were compared as a change from baseline, the results were similar. There was no main effect of age on ΔT_{oes} ($P = 0.067$), ΔT_{re} ($P = 0.273$) and Δmean skin temperature ($P = 0.217$). There was a main effect of time on ΔT_{oes}, ΔT_{re} and Δmean skin temperature (all $P < 0.001$). In both young and older males, ΔT_{oes}, ΔT_{re} and Δmean skin temperature at the end of Ex2 were higher than those at the end of Ex1 (all $P < 0.05$; Table 3). In

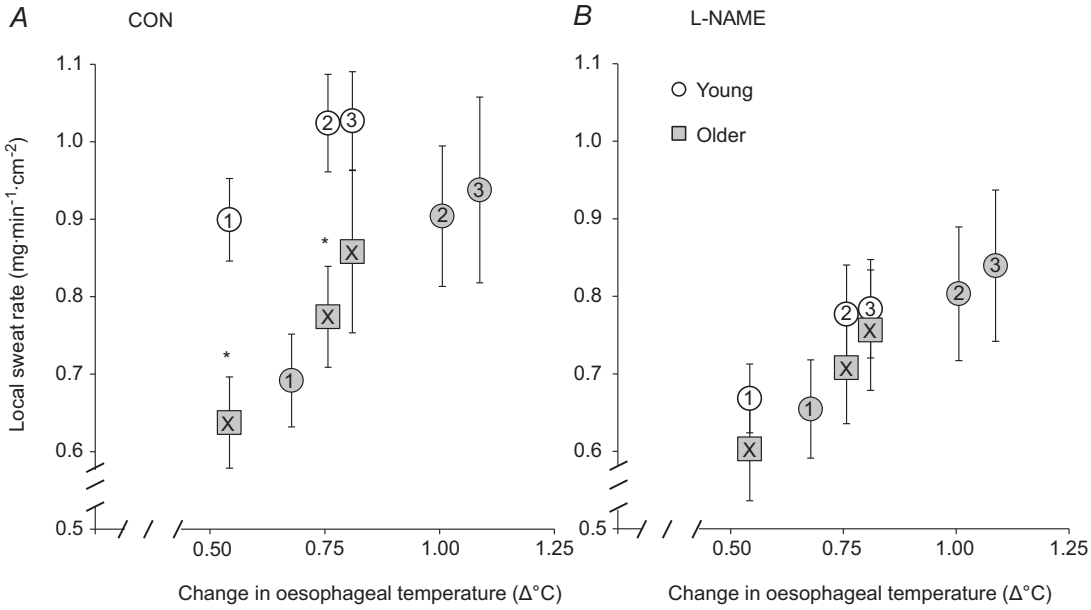

Figure 3. Local sweat rates at the end of each exercise bout for young (open circles) and older males (light grey circles) as a function of the successive exercise bouts in the CON (*A*) and L-NAME-treated conditions (*B*)
The squares with an 'X' represent the sweat rate achieved by the older males as a function of a similar increase in oesophageal temperature measured at the end of exercise for the young adults. The numbers represent the first (1), second (2) and third (3) exercise bouts, respectively. All values are presented as means ± SEM. *Significant difference between young and older males at the similar increase in oesophageal temperature ($P \leq 0.05$ for Student's unpaired *t* tests).

Table 2. Core and mean skin temperature responses at the end of successive exercise and recovery periods

	T_{oes} (°C)		T_{re} (°C)		Mean skin temperature (°C)	
Time	Young	Older	Young	Older	Young	Older
Ex1	37.66 ± 0.37	37.61 ± 0.23	37.51 ± 0.27	37.37 ± 0.22	35.61 ± 0.32	35.42 ± 0.55
Ex2	37.87 ± 0.38	37.93 ± 0.23	37.89 ± 0.30	37.84 ± 0.29	35.75 ± 0.36	35.52 ± 0.51
Ex3	37.92 ± 0.38	38.05 ± 0.30	38.05 ± 0.33	38.20 ± 0.28	35.76 ± 0.40	35.36 ± 0.54
R1	37.38 ± 0.26	37.35 ± 0.17	37.60 ± 0.29	37.56 ± 0.29	35.29 ± 0.42	35.08 ± 0.46
R2	37.50 ± 0.29	37.48 ± 0.22	37.80 ± 0.33	38.01 ± 0.33	35.30 ± 0.43	35.05 ± 0.47
R3	37.49 ± 0.38	37.39 ± 0.29	37.88 ± 0.31	38.16 ± 0.35	34.99 ± 0.53	34.64 ± 0.58

Abbreviations: Ex, exercise; R, recovery; T_{oes}, oesophageal temperature; and T_{re}, rectal temperature. No significant differences were observed between age groups. All values are presented as means ± SD.

Table 3. Relative changes in core and mean skin temperature responses at the end of successive exercise and recovery periods

	ΔT_{oes} (°C)		ΔT_{re} (°C)		ΔMean skin temperature (°C)	
Time	Young	Older	Young	Older	Young	Older
Ex1	0.57 ± 0.25	0.71 ± 0.24	0.35 ± 0.08	0.31 ± 0.09	0.67 ± 0.19	0.61 ± 0.16
Ex2	0.78 ± 0.29	1.03 ± 0.25	0.72 ± 0.16	0.77 ± 0.14	0.82 ± 0.27	0.69 ± 0.22
Ex3	0.84 ± 0.31	1.12 ± 0.38	0.89 ± 0.17	1.07 ± 0.18	0.82 ± 0.32	0.62 ± 0.27
R1	0.29 ± 0.09	0.46 ± 0.12	0.43 ± 0.11	0.49 ± 0.12	0.35 ± 0.31	0.35 ± 0.31
R2	0.41 ± 0.14	0.58 ± 0.20	0.63 ± 0.16	0.91 ± 0.17	0.36 ± 0.33	0.36 ± 0.33
R3	0.40 ± 0.29	0.49 ± 0.31	0.71 ± 0.16	1.07 ± 0.20	0.20 ± 0.39	0.37 ± 0.09

Abbreviations: Ex, exercise; R, recovery; ΔT_{oes}, change in oesophageal temperature; and ΔT_{re}, change in rectal temperature. No significant differences were observed between age groups. All values are presented as means ± SD.

both young and older males, ΔT_{oes} and and Δmean skin temperature were not different between the end of Ex2 and the end of Ex3 (all $P > 0.05$); however, ΔT_{re} increased from the end of Ex2 to the end of Ex3 ($P < 0.001$; Table 3).

Recovery. There was no main effect of age on T_{oes} ($P = 0.674$), T_{re} ($P = 0.245$) and mean skin temperature ($P = 0.416$). We found a main effect of time on T_{re} and mean skin temperature (both $P < 0.001$) but not on T_{oes} ($P > 0.05$). In both young and older males, T_{re} increased from the end of R1 to the end of R2 ($P < 0.001$) but not from the end of R2 to the end of R3 ($P < 0.05$; Table 2). In the young and older males, mean skin temperature did not change from the end of R1 to the end of R2 ($P < 0.05$; Table 2). Mean skin temperature decreased from the end of R2 to the end of R3 in the older males ($P < 0.05$). No differences were measured in the young males (both $P > 0.05$; Table 2). Likewise, there was no main effect of age on ΔT_{oes} ($P = 0.062$) and Δmean skin temperature ($P = 0.980$). There was, however, a main effect of age on ΔT_{re} ($P = 0.040$). The older males had a greater ΔT_{re} at the end of R2 ($P = 0.002$) and R3 ($P = 0.001$) compared with the younger males. We found a main effect of time on ΔT_{re} and Δmean skin temperature (both $P < 0.001$) but not on ΔT_{oes} ($P > 0.05$). In both young and older males, ΔT_{re} increased from the end of R1 to the end of R2 ($P < 0.001$) but not from the end of R2 to the end of R3 ($P < 0.05$; Table 3). In the young and older males, Δmean skin temperature did not change from the end of R1 to the end of R2 ($P < 0.05$; Table 3). ΔMean skin temperature decreased from the end of R2 to the end of R3 in the older males ($P < 0.05$). No differences were measured in the young males ($P > 0.05$; Table 3).

Mean body temperature onset threshold and sensitivity for local sweat rate

There was no main effect of age or treatment conditions on the mean body temperature onset threshold or on the thermosensitivity for local sweat rate (all $P > 0.05$; Table 4). Therefore, the onset threshold for local sweat rate was not different between young and older adults in the CON conditions and in the L-NAME-treated conditions. Also, no differences in thermosensitivity between young and older adults for local sweat rate were found in the CON conditions and in the L-NAME-treated conditions. There was a main effect of time on the mean body temperature onset threshold for local sweat rate for both the CON and the L-NAME-treated conditions (both $P < 0.001$) but not for thermosensitivity in the both conditions (both $P > 0.05$). Irrespective of group or treatment conditions, mean body temperature at the onset threshold for local

Table 4. Absolute and relative mean body temperature onset thresholds and thermosensitivities for each exercise period

	Absolute onset threshold for sweat rate (°C)		Relative onset threshold for sweat rate (°C)		Thermosensitivity for sweat rate (mg min^{-1} cm^{-2} °C^{-1})	
Time	Young	Older	Young	Older	Young	Older
CON						
Ex1	36.79 ± 0.24	36.58 ± 0.27	0.11 ± 0.13	0.07 ± 0.14	0.63 ± 0.83	0.90 ± 0.58
Ex2	36.99 ± 0.25	36.92 ± 0.19	0.30 ± 0.13	0.41 ± 0.16	1.58 ± 0.91	1.01 ± 0.43
Ex3	37.08 ± 0.26	36.99 ± 0.31	0.40 ± 0.15	0.48 ± 0.30	1.90 ± 1.42	0.76 ± 0.47
L-NAME						
Ex1	36.81 ± 0.30	36.55 ± 0.27	0.12 ± 0.18	0.06 ± 0.16	1.28 ± 0.69	1.00 ± 0.82
Ex2	37.03 ± 0.26	36.90 ± 0.24	0.35 ± 0.11	0.39 ± 0.15	1.24 ± 0.64	0.86 ± 0.49
Ex3	37.11 ± 0.24	37.03 ± 0.28	0.43 ± 0.14	0.51 ± 0.25	1.42 ± 0.85	0.75 ± 0.50

Abbreviations: CON, control; Ex, exercise; and L-NAME, N^G-nitro-L-arginine methyl ester. No significant differences were observed between age groups. All values are presented as means ± SD.

sweat rate increased from Ex1 to Ex2 (all $P < 0.05$) but not from Ex2 to Ex3 (all $P > 0.05$). When mean body temperature threshold was expressed as a change from baseline, there was still no main effect of age or treatment conditions (all $P > 0.05$; Table 4).

Discussion

The present study is the first to examine age-related changes in the role of NO in local forearm sweat rate during intermittent exercise in the heat. Older males had a lower local sweat rate at the end of the first exercise bout compared with the young males but thereafter local sweat rate was similar between groups for the subsequent two exercise–recovery cycles. We showed that while NOS inhibition with L-NAME reduced sweating at the end of each exercise bout relative to the control conditions in the young males, no effect of L-NAME was evident in the older males. Additionally, local sweat rate at the end of each recovery period was not affected by L-NAME compared with the control conditions in either the young or older males. We show that ageing diminishes NO-dependent sweating during short bouts of exercise in the heat.

Age-related reduction in sweating

By design, we used a similar fixed rate of metabolic heat production for the young and older adults (Jay *et al.* 2011; Gagnon *et al.* 2013*b*). In the present study, local sweat rate measured in the CON conditions at the end of the first exercise bout was lower in the older males compared with the young males (Fig. 1*A*). However, given that end-exercise core temperature differed between groups, it was not possible to define clearly the extent to which differences in thermal drive may have influenced the sweating response. When we compared the sweat rates based on an equivalent core temperature response, we showed that older adults demonstrated a reduced capacity to dissipate heat as evidenced by a markedly lower sweat rate. Therefore, our results show that true age-related impairments in sweating are evident and remain intact with successive exercise bouts.

In contrast to exercise, there were no differences in sweat rate measured during the recovery period between the young and older adults. In fact, we show that the young and older adults demonstrate a rapid reduction in sweating followed by a plateau at near-baseline resting levels, which is maintained for the duration of the recovery period. These findings are consistent with recent reports demonstrating that whole-body evaporative heat loss is attenuated to a similar extent in young and older adults despite the fact that older adults store more heat at the end of exercise (Larose *et al.* 2013*a*,*b*). As our study findings demonstrate, the pattern of the postexercise sweating response cannot be attributed to age-related differences in NO-dependent influences on sweating. It could be argued, however, that the control of sweating postexercise in both young and older adults may be mediated by factors of non-thermal origin. This mechanism is discussed in greater detail in subsequent sections.

Nitric oxide-dependent sweating in young males

Our finding of a diminished local sweat rate in the L-NAME-treated conditions in comparison to the CON conditions in the young males (Fig. 2*A*) demonstrates that increases in NO augment sweat production during exercise. A similar result was also shown in a previous study of young males during 30 min of continuous moderate-intensity exercise (~47% peak oxygen uptake; Welch *et al.* 2009). Additionally, it is important to note that the attenuation in local sweat rate measured in the L-NAME-treated conditions relative to the CON conditions was not limited to the first exercise bout. In

fact, we observed a similar attenuation during the second and third exercise bouts despite the greater increase in core temperature and therefore thermal drive.

While this study was not specifically designed to assess the underlying mechanisms for the NO-dependent influences on sweating, some insight may be gleaned from previous studies. First, K^+ and Cl^- channels are involved in sweat secretion (Sato, 1993). Nitric oxide activates Cl^- channels, as demonstrated in human lung epithelial cells (Kamosinska *et al.* 1997). Thus, NO may activate Cl^- channels in human sweat glands, contributing to the sweating response in young adults. Secondly, calcium-activated potassium (KCa) channels are found in equine sweat gland epithelial cells (Huang *et al.* 1999), and NO activates KCa channels in the aortic smooth muscle of rabbits (Bolotina *et al.* 1994). Therefore, it is possible that a similar response may occur in human sweat glands, contributing to the sweat response in young adults. Taken together, one or more of these mechanisms may explain the role of NO in sweating in young adults. Alternatively, it is equally possible that none of these mechanisms may explain the observed pattern of response. Further studies are required to examine these potential mechanisms.

In the present study, L-NAME did not reduce local sweat rate at the end of each recovery period compared with the CON conditions in our younger subjects, suggesting that unlike the situation during exercise, there is no role for NO in the sweat response during recovery. Alternatively, it is possible that the role of NO is less evident at low sweat rates similar to those levels measured during recovery (i.e. ∼0.2–0.4 mg min^{-1} cm^{-2} for our younger males). In keeping with our findings, Kellogg *et al.* (1998) reported no effect of NOS inhibition on local sweat rates below 0.40 mg min^{-1} cm^{-2} during a passive heat stress in young males. Likewise, we showed no role for NO in sweating at the beginning of exercise when local sweat rates were below ∼0.4 mg min^{-1} cm^{-2}; however, a role of NO in sweating was observed at the end of each exercise bout when local sweat rate exceeded ∼0.9 mg min^{-1} cm^{-2} in the young males (Fig. 2*A*). Also, Welch *et al.* (2009) reported no role of NO in sweating in young males during the beginning of continuous exercise in the heat when local sweat rate was below 1.3 mg min^{-1} cm^{-2}, but thereafter they observed a clear contribution of NO to sweating. As such, it is possible that NO-dependent sweating in young adults occurs only above a certain sweat rate. Alternatively, it is plausible that other factors of non-thermal origin may have exerted a more pronounced effect in the control of sweating in the postexercise period. Numerous studies have reported that whole-body evaporative heat loss (i.e. sweating) and skin blood flow are rapidly attenuated following dynamic exercise despite sustained elevations in core and muscle temperature in young adults (Thoden *et al.* 1994; Kenny *et al.* 2006, 2009). This has been ascribed to a non-thermal baroreflex-mediated response associated with postexercise hypotension (Kenny & Jay, 2013). While we cannot confirm that the lack of NO-dependent sweating is a result of a greater non-thermal-mediated suppression of sweating, the pattern of response observed in this study is consistent with previous reports of non-thermal modulation of sweating.

Diminished NO-dependent sweating in older males

We found that local sweat rate at the end of each exercise bout remained similar between the CON and L-NAME-treated conditions in the older adults (Fig. 2*B*), and the pattern of response remained intact with successive exercise bouts. These findings suggest that the bioavailability of NO is reduced in aged skin, possibly diminishing NO-dependent sweating. Currently, the precise mechanism by which ageing impairs NO-dependent sweating remains unclear. However, studies examining skin blood flow during whole-body heat stress at rest show that the bioavailability of NO is reduced in aged skin (Holowatz *et al.* 2003, 2006*a*,*b*; Stanhewicz *et al.* 2012, 2013). This is thought to be due to age-related differences in oxidative stress (Holowatz *et al.* 2006*a*) and/or arginase activity (Holowatz *et al.* 2006*b*). Further studies are required to assess these mechanisms.

We show that sweat rate in the L-NAME-treated conditions was significantly increased from the end of Ex1 to the end of Ex2 ($P = 0.004$). This was equivalent to a ∼19% increase in sweating (Fig. 1*B*). In view of the fact that sweat rate was comparable between the CON and L-NAME-treated conditions, this suggests that increases in sweat rate may be mediated through NO-independent mechanisms in older adults. An *in vitro* study showed that prostaglandin E_1, which is produced by cyclo-oxygenase (COX)-1 and -2, can directly increase sweat rate (Sato, 1977). Furthermore, an age-dependent COX-2 upregulation was reported in the mesenteric artery of rats (Ramos-Alves *et al.* 2012), thereby modulating the production of prostaglandin E_1, which ultimately increases sweating. Although speculative, upregulation of COX-2 may also occur in the eccrine sweat glands of older adults, increasing prostaglandin E_1 and therefore sweat rate.

Similar to the response measured in young adults, there were no differences in sweat rate in the CON or L-NAME-treated conditions during recovery in the older adults. Moreover, the level of sweating achieved during recovery was similar between young and older adults despite the marked differences in end-exercise sweating. As discussed in the previous subsection, non-thermal factors have been shown to have a pronounced influence on heat dissipation following cessation of exercise. In light of the similar sweating responses observed between young and older adults following cessation of exercise,

it is plausible that modulation of the sweating response may be influenced by non-thermal factors. To date, little is known about the relative contribution of thermal and non-thermal factors in the regulation of postexercise heat loss in older adults. However, given the similar postexercise sweating response measured in both the young and the older adults, it is conceivable that, as reported in the case of young adults (Kenny & Gagnon, 2010), the attenuation in sweating may be linked to a baroreceptor-mediated suppression of heat dissipation in older adults.

Limitations

It is important to note that in the present study local sweat rate was measured only at the forearm. Age-related decrements in sweating may not occur at an even rate across the body (Inoue *et al.* 1991; Inoue & Shibasaki, 1996; Dufour & Candas, 2007; Smith *et al.* 2013*a*) and have been found to vary between individuals of similar ages (Inoue *et al.* 2004; Smith & Havenith, 2012; Smith *et al.* 2013*b*). Recently, Smith *et al.* (2013*a*) observed reduced sweating during whole-body heating at rest in older compared with young adults at the arm, abdomen, thigh and lower back, with greatest impairment observed on the abdomen. Therefore, it is conceivable that a more pronounced age-related difference in the sweating response might have been observed in other areas, such as the abdomen. It is possible that L-NAME did not diffuse to all of the skin area covered by the sweat capsule (3.8 cm^2). However, we show that the relative reduction in sweat rate during exercise measured in the L-NAME-treated conditions (~30%) in the present study was similar to the findings reported by Welch *et al.* (2009; ~25%), who used a smaller sized sweat capsule (0.567 cm^2). Taken together, these finding suggest that the use of sweat capsules ranging from 0.567 to 3.8 cm^2 do not influence the effectiveness of L-NAME in the regulation of sweating. Additionally, in the present study we examined age-related differences in the sweating response in males only. Recent studies have found sex-related differences in the sweat response (Gagnon *et al.* 2008, 2013*a*). As such, our findings cannot be generalized to young or older females.

Conclusions

We show that inhibition of NOS with L-NAME significantly attenuates local forearm sweat rate relative to the control conditions during each of the 15 min exercise bouts despite progressively greater increases in thermal drive in young adults. In contrast, there was no significant difference in sweating between the CON and L-NAME-treated conditions in the older adults. Local forearm sweat rate during each recovery period was not significantly different between the CON and L-NAME-treated conditions in both young and older adults, and the pattern of response was similar between groups. In summary, NO-dependent sweating during intermittent exercise is impaired in older adults.

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Additional Information

Competing interests

None declared.

Author contributions

J.M.S. and G.P.K. contributed to the conception and design of the experiment, to the collection, analysis and interpretation of data and to drafting the article and revising it critically for important intellectual content. N.F. and M.C. contributed to the collection, analysis and interpretation of data and to drafting the article and revising it critically for important intellectual content. All authors approved the final version of the manuscript and all authors qualify for authorship.

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APPENDIX C

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ORIGINAL RESEARCH

Age-related differences in postsynaptic increases in sweating and skin blood flow postexercise

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dose response, exercise, heat loss, nonthermal factors, skin perfusion, sweat rate.

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Abstract

The influence of peripheral factors on the control of heat loss responses (i.e., sweating and skin blood flow) in the postexercise period remains unknown in young and older adults. Therefore, in eight young (22 ± 3 years) and eight older (65 ± 3 years) males, we examined dose-dependent responses to the administration of acetylcholine (ACh) and methacholine (MCh) for sweating (ventilated capsule), as well as to ACh and sodium nitroprusside (SNP) for cutaneous vascular conductance (CVC, laser-Doppler flowmetry, % of max). In order to assess if peripheral factors are involved in the modulation of thermoeffector activity postexercise, pharmacological agonists were perfused via intradermal microdialysis on two separate days: (1) at rest (**DOSE**) and (2) following a 30-min bout of exercise (**Ex+DOSE**). No differences in sweat rate between the DOSE and Ex+DOSE conditions at either ACh or MCh were observed for the young (ACh: $P = 0.992$ and MCh: $P = 0.710$) or older (ACh: $P = 0.775$ and MCh: $P = 0.738$) adults. Similarly, CVC was not different between the DOSE and Ex+DOSE conditions for the young (ACh: $P = 0.123$ and SNP: $P = 0.893$) or older (ACh: $P = 0.113$ and SNP: $P = 0.068$) adults. Older adults had a lower sweating response for both the DOSE (ACh: $P = 0.049$ and MCh: $P = 0.006$) and Ex+DOSE (ACh: $P = 0.050$ and MCh: $P = 0.029$) conditions compared to their younger counterparts. These findings suggest that peripheral factors do not modulate postexercise sweating and skin blood flow in both young and older adults. Additionally, sweat gland function is impaired in older adults, albeit the impairments were not exacerbated during postexercise recovery.

Introduction

Thermoregulatory control of sweating and skin blood flow during postexercise recovery is altered such that at the cessation of dynamic exercise, there is a rapid decrease in sweating and skin blood flow despite a significant residual heat load (Kenny and Jay 2013). As a result, the rate of whole-body heat loss is reduced and consequently is paralleled by a prolonged elevation in core and muscle temperatures above preexercise baseline levels for 60–90 min (Kenny et al. 2006, 2007; Kenny and Gagnon 2010). It has been suggested that centrally mediated factors of nonthermal origin (i.e., baroreceptor loading status) can modulate the control of heat loss following exercise in young adults (Carter et al. 2002; Jackson and Kenny 2003; Wilson et al. 2004; Journeay et al. 2006; Kenny et al. 2006, 2008; Jay et al. 2007). However, it has yet to be determined whether or not peripheral factors, such as sensitivity of the effector organ (i.e., sweat glands and/or skin vessels), contribute to the control of heat loss postexercise.

Peripheral factors modulating heat loss postexercise can be assessed by examining changes in sweat production and skin vasodilation to increasing doses of pharmacological agonists. For example, exogenously administering incremental doses of acetylcholine (**ACh**) and methacholine (**MCh**) can be employed to examine sweat gland function (Kenney and Fowler 1988; Inoue et al. 1999; Lee and Mack 2006; Kimura et al. 2007; Gagnon et al. 2013; Smith et al. 2013; Metzler-Wilson et al. 2014). Furthermore, differences in sweat rate observed between ACh (hydrolyzed by acetylcholinesterase, **AChE**) and MCh (resistant to AChE) can allude to whether or not the response is mediated by AChE enzyme activity (Kimura et al. 2007). Likewise, perfusion of endothelium-dependent (ACh) and/or endothelium-independent (sodium nitroprusside, **SNP**) agonists in an incremental manner can be utilized to examine skin vascular function (Lee and Mack 2006; Medow et al. 2008; Bruning et al. 2012; Gagnon et al. 2013; Smith et al. 2013). To the best of our knowledge, no study has examined if peripheral mechanisms contribute to the disturbance of postexercise heat loss responses of sweating and skin blood flow.

To date, much of our limited understanding of the underlying mechanisms governing the control of postexercise heat loss responses is based on findings obtained in young adults. Human aging is associated with attenuated sweating and skin vasodilation during exercise (Anderson and Kenney 1987; Kenney and Anderson 1988; Tankersley et al. 1991; Inoue et al. 1999; Larose et al. 2013a,b,c). However, the extent to which these age-related impairments in the thermoeffector activity may influence heat dissipation during the postexercise recovery period remains unclear. Some insight may be gleaned from a recent study by Larose et al. (2013c) who examined local and whole-body heat loss responses in young and older adults during intermittent exercise in the heat (Larose et al. 2013c). They found that despite greater heat storage during each of the four 15-min exercise bouts in the older adults, the magnitude of the postexercise suppression in whole-body evaporative heat loss, as measured by direct calorimetry, was similar between the young and older males (Larose et al. 2013c). A similar pattern was measured for the local responses of sweating and skin blood flow. These findings suggest the likely possibility that the underlying factors affecting postexercise heat dissipation may be of similar origin for young and older adults.

Previous studies have compared sweat rates and skin vasodilation between young and older adults at rest with the use of pharmacological stimulation and have yielded conflicting results. While some studies have reported an attenuated sweating response in older adults as assessed by a subcutaneous injection of 5 mmol/L of MCh (Kenney 1988; Inoue et al. 1999), others observed no differences using intradermal microdialysis to administer increasing doses (1×10^{-7} to 0.1 mol/L) of ACh (Smith et al. 2013). Furthermore, Bruning et al. (2012) reported an attenuated ACh-induced skin vasodilation in middle-aged (53 ± 1 years) compared with younger (23 ± 1 years) adults at the highest concentration employed (0.1 mol/L), while infusing ACh via intradermal microdialysis in a dose–response manner (Bruning et al. 2012). Others, however, have not observed any differences in skin blood flow between young and older adults receiving doses of ACh from 1×10^{-7} to 0.1 mol/L (Holowatz et al. 2005; Smith et al. 2013). To date, no study has evaluated if age-related differences in sweating or skin vasodilation exist at higher doses of ACh or MCh (>0.1 mol/L). It remains to be determined if older adults have an attenuated responsiveness to the administration of pharmacological agonists (>0.1 mol/L) compared to their younger counterparts and whether or not the same pattern of response exists postexercise.

Thus, the purpose of this study was twofold: to examine (1) the extent to which peripheral factors (i.e., sweat gland and skin vasodilatory function) contribute to the postexercise suppression of heat loss responses; and (2) whether there are differences in the mechanisms modulating postexercise heat loss as a function of age. We hypothesized that: (1) peripheral factors would not modulate the postexercise suppression of heat loss as determined by local measurements of sweating and skin blood flow and, (2) the mechanisms for postexercise suppression of heat loss would not differ as a function of age, but older adults would have an attenuated responsiveness

to the administration of the pharmacological agonists compared to their younger counterparts.

Methods

Ethical approval

This study was approved by the University of Ottawa Health Sciences and Science Research Ethics Boards, in accordance with the Declaration of Helsinki. Written, informed consent was obtained from all the participants prior to their involvement in the study.

Participants

Sixteen males volunteered for the study and were divided into two groups of eight young (18–25 years) and eight older (61–70 years) adults. All participants were healthy, nonsmoking, physically active males free from cardiovascular disease and diabetes. Physical characteristics of the participants are presented in Table 1.

Experimental procedures

Each participant completed one preliminary and two experimental sessions. The experimental sessions were performed in a random order and on separate days with a minimum of 72 h and maximum of 2 weeks between sessions. During the preliminary session, body height, mass, and density, as well as maximum oxygen uptake (VO_2max) were determined. Body height was determined using a stadiometer (Detecto, model 2391, Webb City, MO), whereas body mass was measured using a digital high-performance weighing terminal (model CBU150X; Mettler Toledo Inc., Mississauga, ON, Canada). Body surface area was subsequently calculated from the measurements of body height and mass (DuBois and DuBois 1916). Body density was measured using the hydrostatic weighing technique, and body fat percentage was calculated using the Siri equation (Siri 1956). VO_2max was measured during a progressive cycle ergometer protocol which consisted of a 2-min warm-up at 40 W followed by 20 W increments every minute until the participant could no longer maintain a pedaling cadence of at least 60 rpm. Continuous electrocardiographic monitoring was used for the older males during the maximal exercise test under the supervision of a qualified technician.

Participants performed the experimental sessions at the same time of day and were asked to drink 500 mL of water the night prior to, as well as the morning of the experimental session. They were also asked to refrain from alcohol, caffeine, and exercise 24 h prior to experimentation. Upon arrival at the laboratory, the participants provided a urine sample and a baseline body mass was measured. They subsequently rested quietly in an upright semirecumbent posture on a bed in a room set to an ambient temperature of 24°C and 20% relative humidity. During this time, three microdialysis fibers (MD 2000; Bioanalytical Systems, West Lafayette, IN) were placed in the dermal space of the forearm under aseptic conditions. To place the fibers, a 25-gauge needle was inserted into the dermal space of the lateral mid-anterior aspect of the left forearm and then exited the skin 20–25 mm away from the point of entry. The microdialysis fiber was inserted through the lumen of the needle. The needle was subsequently withdrawn, leaving the semipermeable membrane (30 KDa cutoff, 10 mm membrane) in place under the skin. After insertion, the fibers were perfused with lactated Ringer's solution at a rate of 2 μL/min via a perfusion pump (CMA/400; CMA Microdialysis, Solna, Sweden).

For one of the experimental conditions (**DOSE**), participants remained resting in an upright semirecumbent posture on the bed in a nonheat stress environment (i.e., ambient temperature of 24°C and 20% relative humidity) for 60–90 min after the fiber placement (to allow for hyperemia associated with fiber insertion trauma to subside; Anderson et al. 1994). Baseline resting data were obtained for 10 min following the hyperemia response. Subsequently, increasing doses of **MCh** (*site 1*) and **ACh** (*site 2*) were infused in a dose-dependent manner at two mid-anterior forearm skin sites to assess the sweating response. The ACh infusion at site 2 as well as infusion

Table 1. Participant characteristics for young and older adults.

Group	Age (years)	Height (m)	Body mass (kg)	Body surface area (m^2)	Body fat (%)	$\dot{V}O_2$ max (mL/kg/min)
Young	22 ± 3*	1.78 ± 0.10	81.7 ± 7.7	1.99 ± 0.11	14.3 ± 3.5*	46.3 ± 3.5*
Older	65 ± 3	1.75 ± 0.05	77.6 ± 12.9	1.94 ± 0.17	21.6 ± 6.7	34.3 ± 8.3

Values are mean ± standard deviation. $\dot{V}O_2$ max, rate of maximum oxygen consumption. A significant difference ($P \leq 0.05$) between young and older adults is denoted by an asterisk (*).

of **SNP** (*site 3*) was used in a dose-dependent manner to assess skin vasodilation (all pharmacological agonists were from Sigma Aldrich, Oakville, ON, Canada). All pharmacological agonists were infused in 10-fold increments, from 1×10^{-6} to 1 mol/L for MCh and ACh, and from 5×10^{-6} to 5×10^{-2} mol/L for SNP (Gagnon et al. 2013). Each dose was initially primed through the microdialysis membrane at an infusion rate of 100 μL/min for ~1 min, thereafter, each dose was infused for 8 min at a rate of 2 μL/min. This amount of time ensured a plateau in sweat rate or skin blood flow was reached at each concentration of the agonists. A higher dose of ACh and MCh (1.5 mol/L) was infused at the end for an additional 25 min, while the maximum dose of SNP (5×10^{-2} mol/L) continued to be infused to ensure a steady-state maximal response to the highest concentration employed.

For the second experimental condition (**Ex+DOSE**), after fiber placement, the participants entered a thermal chamber regulated to an ambient air temperature of 30°C and 20% relative humidity. The participants rested for 60–90 min on a semirecumbent cycle ergometer (Corival; Lode B.V., Groningen, Netherlands) while the remainder of the instrumentation was placed. Once the instrumentation was placed and the hyperemia response had subsided, baseline resting data were obtained for 10 min after which the participants performed a 30-min exercise bout. To ensure that both groups received a similar heat load, they exercised at the same constant rate of metabolic heat production of ~250 W (equivalent to 46.3 ± 4.7% VO_2max for the young and 55.7 ± 6.9% VO_2max for the older males). Following the exercise bout, participants rested for 15 min to allow local sweat rate and skin blood flow to return to baseline resting values (Kenny et al. 2007). At this point, the dose–response relationships for sweating and skin vasodilation were assessed in the same manner as for the DOSE experimental session.

Measurements

The ventilated capsule technique was employed for the purpose of measuring local sweat rate. Sweat rate was measured from 3.8-cm^2 plastic capsules attached to the skin with adhesive rings and topical skin glue (Collodion HV; Mavidon Medical products, Lake Worth, FL). The sweat capsules were placed directly over the fiber membrane of each agonist sites (i.e., skin sites 1 and 2). The sweat capsule at the ACh site (*site 2*) also housed the laser-Doppler flow probe (see details below), allowing for the simultaneous measurement of local sweat rate and skin blood flow. Compressed dry air was passed through each capsule at a rate of 0.5 L/min. Long 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 trial. Water content of the effluent air was measured using high precision dew point mirrors (model 473; RH systems, Albuquerque, NM) or capacitance hygrometers (Vaisala, Woburn, WA). Both instruments offer precise, quality measures of changes in humidity at the skin (RH systems: dew point accuracy = ±0.2°C and Viasala: absolute humidity accuracy = ~1.08 g/m^3). Local sweat rate was calculated using the difference in water content between effluent and influent air multiplied by the flow rate and normalized for the skin surface area under the capsule. Local skin blood flow was estimated at 32 Hz using laser-Doppler velocimetry (PeriFlux System 5000; Perimed AB, Stockholm, Sweden). A laser-Doppler probe (integrating probe 413; Perimed AB) was placed directly over the microdialysis membrane at the ACh (*site 2*) and SNP (*site 3*) sites. Cutaneous vascular conductance (CVC) was subsequently calculated as the ratio of skin blood flow perfusion units to mean arterial pressure and expressed as a percentage of maximum.

Systolic and diastolic blood pressures were determined manually using brachial auscultation at the end of each 8-min infusion during the DOSE condition. Mean arterial pressure was then calculated as diastolic blood pressure + 1/3 × pulse pressure (difference between systolic and diastolic pressure). Additionally, mean arterial pressure was measured continuously using a Finometer (Finapres Medical Systems, Amsterdam, the Netherlands) from the beat-to-beat recording of the left middle finger arterial pressure waveform with the volume-clamp method (Penaz 1973) and physiocal criteria (Wesseling et al. 1995) during the Ex+DOSE condition. The left middle finger was supported at heart level for calibration and for the duration of the experimental protocol. Blood pressures were verified during the Ex+DOSE condition by manual brachial auscultation.

Rectal temperature was measured during the Ex+DOSE condition using a general purpose thermocouple temperature probe (Mallinckrodt Medical Inc., St-Louis, MO) inserted to a minimum of 12 cm past the anal sphincter. Rectal temperature data were collected using a HP Agilent data acquisition module (model 3497A; Agilent Technologies Canada Inc., Mississauga, ON, Canada) at a rate of one sample every 15 sec and simultaneously displayed and recorded in spreadsheet format on a personal computer with LabVIEW software (Version 7.0; National Instruments, Austin, TX).

A preexperimental test of urine sample was obtained to ensure all participants were in a euhydrated state. Urine-specific gravity was determined in duplicate using a handheld total solids refractometer (model TS400; Reichter Inc., Depew, NY).

Data analysis

To determine the concentration of the agonist causing 50% of the maximal response (EC_{50}), dose–response curves were created by plotting local sweat rate and CVC as a function of the log concentration of the agonist and fitted using a nonlinear regression analysis with a Hill slope of 1 (GraphPad Prism 6.0; GraphPad Software, La Jolla, CA; Davis et al. 2007; Kimura et al. 2007). The log EC_{50} is an indicator of sensitivity of the end organ to the agonist, where a negative log EC_{50} closer to 0 indicates a lower sensitivity. Baseline resting data for both the DOSE and Ex+DOSE conditions were obtained by averaging the final 5 min of the 10-min baseline resting period. Postexercise data were obtained for the Ex+DOSE condition only by averaging the final min of the 15-min postexercise recovery period. Sweat rate and CVC averages were obtained during the final min of each 8-min dose. The dose–response curves were compared within age groups for the DOSE and Ex+DOSE conditions to assess whether peripheral factors influence heat loss responses postexercise. Additionally, the dose–response curves were compared between age groups for the DOSE and Ex+DOSE conditions separately to assess the effect of age on end-organ function and on the mechanism of the postexercise suppression of heat loss responses.

Statistical analysis

Sweating, CVC, mean arterial pressure, and rectal temperature (Ex+DOSE only) data were analyzed using a two-way repeated measures analyses of variance protocol using the repeated factor of agonist concentration (eight levels: 10-fold increments from 1×10^{-6} to 1 and 1.5 mol/L for MCh and ACh and from 5×10^{-6} to 5×10^{-2} mol/L for SNP) and the nonrepeated factor of test condition (DOSE vs. Ex+DOSE) and age (two levels: young and older), separately. When a significant main effect was observed for test condition (DOSE vs. Ex+DOSE), post hoc comparisons were carried out using Student's paired two-tailed *t*-tests. Likewise, when a significant main effect was observed for age (young vs. older), post hoc comparisons were carried out using Student's unpaired two-tailed *t*-tests. Additionally, physical characteristics as well as baseline resting, end exercise (Ex+DOSE only) and 15-min postexercise (Ex+DOSE only) values, the log EC_{50} and absolute maximal CVC values were analyzed using Student's unpaired two-tailed *t*-tests. Within group comparisons (i.e., baseline resting vs. 15-min postexercise values) were analyzed using Student's paired two-tailed *t*-tests. The level of significance for all analyses was set at $P \leq 0.05$. Analyses were performed using commercially available statistical software (GraphPad Prism 6.0; GraphPad Software, La Jolla, CA). All values are reported as mean ± standard deviation unless otherwise indicated as standard error.

Results

Participant characteristics

Participant characteristics are presented in Table 1. There were no differences in height ($P = 0.460$), body mass ($P = 0.459$), and body surface area ($P = 0.534$) between groups. However, the younger males had a greater maximum oxygen consumption relative to body mass ($P < 0.001$). On the day of both experimental sessions, baseline urine-specific gravity did not significantly differ between groups (DOSE: young = 1.026 ± 0.006 vs. older = 1.019 ± 0.005, $P = 0.229$ and Ex+DOSE: young = 1.017 ± 0.007 vs. older = 1.017 ± 0.004, $P = 0.967$). During the Ex+DOSE condition, young and older adults exercised at a fixed rate of heat production which was kept similar between the young (257 ± 12 W/m^2) and older (240 ± 18 W/m^2, $P = 0.114$) adults.

Postsynaptic sweating during no-exercise resting (DOSE) and postexercise recovery (Ex+DOSE)

Sweating responses at baseline rest, 15-min postexercise (Ex+DOSE only) and to incremental doses of ACh and MCh for the young and older adults are presented in Figure 1A and B, respectively. Baseline resting sweat rate was similar between the DOSE and Ex+DOSE conditions in both young and older adults for the ACh (young: $P = 0.614$, older: $P = 0.105$) and MCh (young: $P = 0.123$, older: $P = 0.666$) skin sites. For the Ex+DOSE condition, there were no differences in sweat rates between baseline rest and 15-min postexercise in either young or older adults for both the ACh (young: $P = 0.453$, older: $P = 0.301$) and MCh (young: $P = 0.348$, older: $P = 0.152$) sites. Likewise, there was no main effect of the experimental condition such that sweating responses were similar between DOSE and Ex+DOSE in young and older adults during the incremental doses of both ACh (young: $P = 0.992$, older: $P = 0.775$) and MCh (young: $P = 0.710$, older: $P = 0.738$).

Age-related effects on postsynaptic sweating

No-exercise resting condition (DOSE)

There were no significant differences in baseline resting sweat rate between young and older males at both the

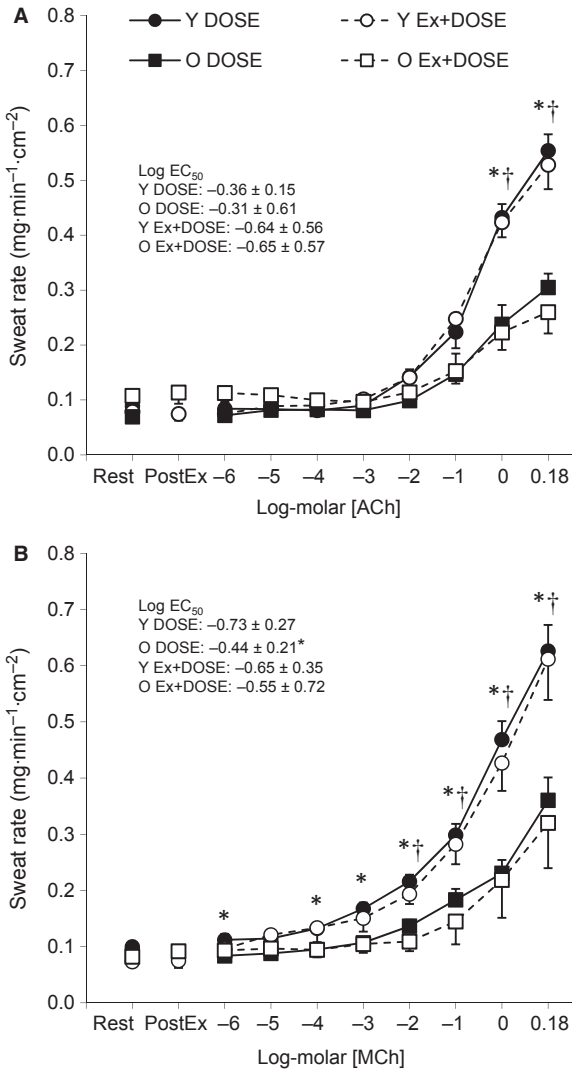

Figure 1. Mean ± standard error values for the DOSE (closed symbols) and Ex+DOSE (open symbols) condition for sweat rate to incremental doses of acetylcholine (ACh, Panel A) and methacholine (MCh, Panel B) in young (Y, circles) and older (O, squares) adults. Data are presented during baseline rest, 15-min postexercise (PostEx), and during the plateau phase of each dose. *Significant difference between young and older adults for the DOSE condition. †Significant difference between young and older adults for the Ex+DOSE condition ($P \leq 0.05$).

ACh ($P = 0.230$) and MCh ($P = 0.276$) skin sites. Sweating increased as a function of increasing concentrations for both ACh and MCh (both $P \leq 0.001$). Furthermore, there was a main effect of age on local sweat rate for ACh ($P = 0.049$) and MCh ($P = 0.006$). For ACh, sweat rate did not differ between age groups at the lower concentrations (i.e., 1×10^{-6}–1×10^{-1} mol/L), but was greater in the young compared to the older males at the two higher concentrations (1 and 1.5 mol/L) employed (both $P < 0.05$). For MCh, sweat rate was greater in the young compared to the older males at 1×10^{-6} and 1×10^{-4} to 1.5 mol/L (all $P < 0.05$). The log EC_{50} did not differ between age groups for ACh ($P = 0.814$), but was lower (i.e., further away from 0) for the young compared to the older adults for MCh ($P = 0.035$).

Postexercise resting recovery condition (Ex+DOSE)

There were no significant differences in baseline resting sweat rate between the young and older adults at both the ACh ($P = 0.351$) and MCh sites ($P = 0.934$). Likewise, there were no differences observed between groups in sweat rate 15-min postexercise for ACh ($P = 0.121$) or MCh ($P = 0.246$). Sweating increased as a function of increasing concentrations of both ACh and MCh (both $P < 0.001$). There was a main effect of age on local sweat rate for ACh ($P = 0.05$) and MCh ($P = 0.029$). For ACh, sweat rate did not differ between age groups at the lower concentrations (i.e., 1×10^{-6} to 1×10^{-1} mol/L), but was greater in young compared to older males at the two highest concentrations (1 and 1.5 mol/L) employed (both $P < 0.05$). For MCh, sweat rate was greater in young compared to older males at 1×10^{-2} to 1.5 mol/L (all $P < 0.05$). However, the log EC_{50} did not differ between age groups for ACh ($P = 0.483$) or MCh ($P = 0.362$).

Postsynaptic skin vasodilation during no-exercise resting (DOSE) and postexercise recovery (Ex+DOSE)

Cutaneous vascular conductance responses at baseline rest, 15-min postexercise (Ex+DOSE only) and to incremental doses of ACh and SNP for the young and older adults are presented in Figure 2A and B, respectively. Baseline resting values for CVC were similar between young and older adults for the DOSE and Ex+DOSE conditions for both ACh (young: $P = 0.308$, older: $P = 0.113$) and SNP (young: $P = 0.949$, older: $P = 0.068$) skin sites. For the Ex+DOSE condition, CVC returned to preexercise baseline levels such that there was no difference between baseline rest and 15-min postexercise levels in young and older adults for both the ACh (young: $P = 0.826$, older: $P = 0.853$) and SNP (young: $P = 0.187$, older: $P = 0.883$) sites. Additionally, CVC responses were not different in either young or older adults at the ACh (young: $P = 0.123$, older: $P = 0.832$) or SNP (young: $P = 0.893$, older: $P = 0.360$) sites between the DOSE and Ex+DOSE conditions during the infusion of the incremental doses.

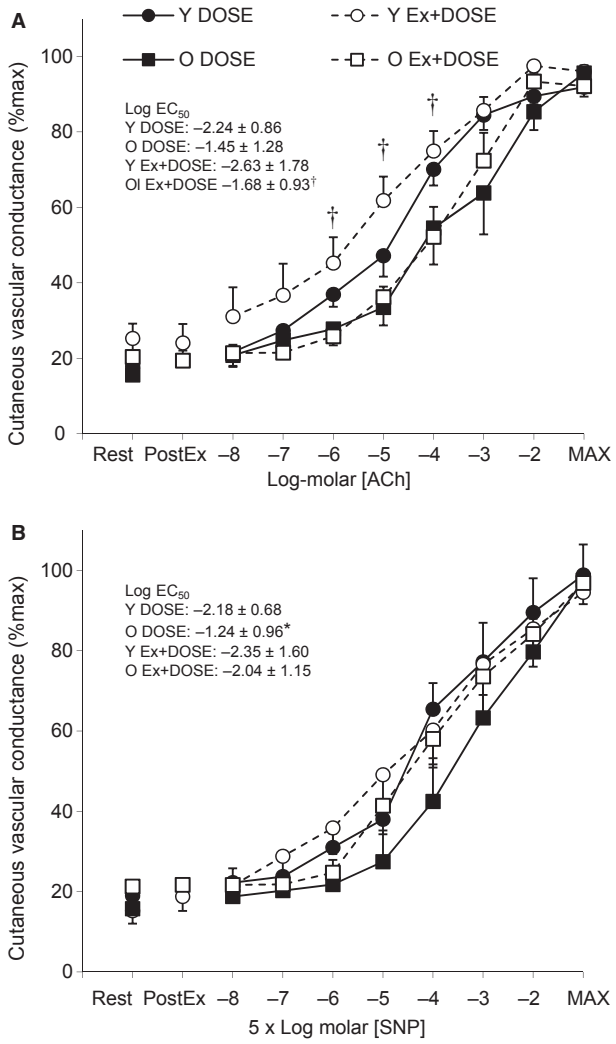

Figure 2. Mean ± standard error values for the DOSE (closed symbols) and Ex+DOSE (open symbols) conditions for cutaneous vascular conductance to incremental doses of acetylcholine (ACh, Panel A) and sodium nitroprusside (SNP, Panel B) in young (Y, circles) and older (O, squares) adults. Data are presented during baseline rest, 15-min postexercise (PostEx), and during the plateau phase of each dose. *Significant between young and older adults for the DOSE condition. †Significant between young and older adults for the Ex+DOSE condition ($P \leq 0.05$).

Age-related effects on postsynaptic skin vasodilation

No-exercise resting condition (DOSE)

There were no significant differences in baseline resting CVC between young and older males at both the ACh ($P = 0.864$) and SNP ($P = 0.507$) skin sites. CVC increased as a function of increasing concentrations of both ACh and SNP (both $P \leq 0.001$), but there was no main effect of age on CVC for ACh ($P = 0.127$) or SNP ($P = 0.131$). In contrast, the log EC_{50} was lower for the young compared to the older adults for SNP ($P = 0.041$), but not for ACh ($P = 0.087$). Additionally, maximal absolute CVC values did not differ between young (ACh: 1.65 ± 0.46 and SNP: 1.78 ± 0.48 perfusion units/mmHg) and older (ACh: 1.78 ± 0.83 and SNP: 1.97 ± 0.62 perfusion units/mmHg) adults (both $P > 0.10$). Mean arterial pressure did not change throughout the protocol ($P = 0.120$) and there was no main effect of age ($P = 0.873$) between young (average: 87 ± 10 mmHg) and older (average: 87 ± 8 mmHg) adults.

Postexercise resting recovery condition (Ex+DOSE)

There were no significant differences in preexercise baseline resting CVC between the young and older males at both the ACh ($P = 0.401$) or SNP ($P = 0.191$) skin sites. Likewise, there were no differences between groups observed in CVC 15-min postexercise at the ACh ($P = 0.425$) or SNP ($P = 0.530$) sites. CVC increased as a function of increasing concentrations of both ACh and SNP (both $P \leq 0.001$). There was a main effect of age on CVC for ACh ($P = 0.014$). CVC was greater in the young at 1×10^{-4} to 1×10^{-2} mol/L compared to the older males (all $P < 0.05$). In contrast, there was no main effect of age on CVC for SNP ($P = 0.573$). Consequently, the log EC_{50} was lower for the young compared to older adults for ACh ($P = 0.044$), but was not different between groups for SNP ($P = 0.665$). Additionally, maximal absolute CVC values did not differ between young (ACh: 1.91 ± 0.53 and SNP: 1.37 ± 0.70 perfusion units/mmHg) and older (ACh: 1.63 ± 0.85 and SNP: 1.23 ± 0.44 perfusion units/mmHg) adults (both $P > 0.10$).

Age-related effects on rectal temperature and mean arterial pressure responses

There were no differences between age groups in baseline resting ($P = 0.835$), end-exercise ($P = 0.572$), or 15-min postexercise ($P = 0.933$) rectal temperatures (Table 2). Additionally, rectal temperature decreased as a function of time from end of exercise to end of the dose–response protocol ($P < 0.001$), but was not different between age groups ($P = 0.354$, Table 2). However, the older adults had a greater change in rectal temperature from preexercise baseline resting values at the end of the dose–response protocol (0.50 ± 0.28°C) compared to the young adults (0.23 ± 0.13°C, $P = 0.041$). There were no

Table 2. Mean arterial pressure and rectal temperature responses for Ex+DOSE during baseline, end of exercise (End-Ex), following 15 min of recovery (Post-Ex), and during the plateau phase for each dose for young and older adults.

	Baseline	End-Ex	Post-Ex	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6	Dose 7	Dose 8
MAP											
Young	85 ± 6	99 ± 8	84 ± 8	84 ± 8	83 ± 9	84 ± 11	84 ± 9	84 ± 9	85 ± 9	85 ± 10	86 ± 8
Older	88 ± 8	103 ± 12	81 ± 8†	82 ± 8	84 ± 8	83 ± 8	83 ± 8	82 ± 9	81 ± 8	84 ± 8	82 ± 8
T_{re}											
Young	36.87 ± 0.42	37.47 ± 0.44†	37.44 ± 0.45†	37.41 ± 0.44†	37.35 ± 0.43†	37.31 ± 0.41†	37.21 ± 0.42†	37.15 ± 0.43†	37.11 ± 0.42†	37.09 ± 0.42†	37.06 ± 0.39†
Older	36.84 ± 0.25	37.36 ± 0.28†	37.45 ± 0.13†	37.43 ± 0.16†	37.41 ± 0.21†	37.40 ± 0.22†	37.35 ± 0.25†	37.33 ± 0.23†	37.30 ± 0.25†	37.27 ± 0.23†	37.27 ± 0.22†

Values are mean ± standard deviation.
MAP, mean arterial pressure (mmHg); T_{re}, rectal temperature (°C).
A significant difference ($P \leq 0.05$) from baseline resting within age group is denoted by a dagger (†).

age group differences at baseline resting ($P = 0.336$), end exercise ($P = 0.253$), or 15-min postexercise in mean arterial pressure ($P = 0.551$, Table 2). However, the older adults had a significantly lower mean arterial pressure at 15-min postexercise compared to preexercise baseline resting values (Table 2, $P = 0.049$). No differences were observed in the young adults ($P = 0.369$). Mean arterial pressure did not differ over time during the agonist infusion protocol ($P > 0.10$) and was not different between age groups ($P = 0.217$, Table 2).

Discussion

A key finding of this study was our observation that the dose–response relationships with incremental pharmacological agonists (ACh, MCh and SNP) were similar between the no-exercise resting condition (DOSE) and the postexercise resting recovery period (Ex+DOSE) for both sweating and skin vasodilation. Moreover, we show that the pattern of response was similar for both the young and older adults. However, we showed that older adults had an attenuated sweating responsiveness to the administration of pharmacological muscarinic receptor agonists (ACh and MCh) compared to the young adults. This impairment was observed during both the no-exercise resting (DOSE) and the postexercise resting recovery (Ex+DOSE) conditions. Conversely, our findings for age-related differences in CVC are less conclusive such that CVC was lower in the older compared to young adults with the use of ACh during the Ex+DOSE condition only. Together these findings suggest that peripheral factors do not modulate the postexercise suppression of heat loss responses of sweating and CVC in both young and older adults, despite the age-related impairments in sweat gland function.

It has been well documented that sweating and skin blood flow return to baseline levels in the first ~20 min following the cessation of dynamic exercise despite sustained elevations in core and muscle temperatures (Thoden et al. 1994; Kenny et al. 2006, 2009). Consistent with these observations, we observed a rapid reduction in sweating and CVC to baseline levels within 15 min of postexercise recovery (Figures 1 and 2) despite rectal temperature remaining significantly elevated above baseline resting values by ~0.6°C (Table 2). This postexercise disturbance in thermal homeostasis is thought to be the result of a nonthermal, centrally mediated suppression of the thermoeffector responses of sweating, and skin blood flow (Kenny and Jay 2013). This notion is supported in part by the fact that the core temperature threshold for sweating and skin vasodilation, which is thought to be determined by central drive (Nadel et al. 1974; Gisolfi and Wenger 1984), is elevated following dynamic exercise

(Jackson and Kenny 2003; Kenny et al. 2003). However, no changes in thermal sensitivity, an indicator of peripheral modulation, were observed (Nadel et al. 1971, 1974; Jackson and Kenny 2003). Nonetheless, there is no direct evidence indicating the impaired heat loss responses postexercise are entirely due to central mechanisms. That is, other mechanisms of peripheral origin, such as changes in the responsiveness of the effector organ (i.e., sweat glands and/or skin vessels) to pharmacological stimuli, may also be involved in the impaired heat loss responses postexercise. In the following section, we discuss how this study findings provide important new information to address this knowledge gap.

Postexercise sweating and CVC in young adults

In this study, we did not observe any difference in sweating with incremental doses of ACh or MCh between the DOSE and Ex+DOSE conditions in the young adults (Fig. 1A and B). This finding indicates that the cholinergic sensitivity of the muscarinic receptors on the sweat gland is unaltered by a previous bout of exercise. Alternatively, studies have shown that AChE is involved in the regulation of sweating at low-to-moderate levels during passive heat stress (Shibasaki and Crandall 2001). It is plausible, therefore, that the rapid postexercise suppression of sweating may be due to increased activity of the AChE enzyme. If this were true, we would expect to observe a rightward shift in the dose–response curve for ACh during the Ex+DOSE compared to the DOSE condition with minimal difference in the dose–response curve between the DOSE and Ex+DOSE conditions for MCh. However, this was not the case in this study (Fig. 1A and B). Based on our observations, it appears that the postexercise suppression of the sweating response is independent of the modulation of AChE enzyme activity. We cannot, however, eliminate its involvement early in recovery (i.e., in the first 15 min) since the dose–response protocol only commenced 15 min into postexercise recovery. Based on our results, we show that the postexercise attenuation of the sweating response is the result of a centrally mediated modulation as previously proposed (Journeay et al. 2006; Shibasaki and Crandall 2010; Kenny and Jay 2013).

ACh-induced skin vasodilation is in part due to nitric oxide-dependent mechanisms (Kellogg 2005; Medow et al. 2008; Bruning et al. 2012; Fujii et al. 2013). Our results demonstrate no difference in the dose–response curves for CVC between the DOSE and Ex+DOSE conditions using ACh in young adults (Fig. 2A). Furthermore, our laboratory recently found that L-NAME, a nonselective nitric oxide inhibitor, reduced CVC relative to the control condition only during the first ~10 min into postexercise recovery (McGinn et al. 2014). Together, these results imply that peripherally modulated mechanisms of skin vasodilation including changes in cholinergic sensitivity of the muscarinic receptor on the endothelium and nitric oxide-mediated pathways are not modified postexercise. In this study, similar to using ACh, we did not observe any differences in skin vasodilation in the young adults with incremental doses of SNP between the DOSE and Ex+DOSE conditions (Fig. 2B). Given that SNP is a nitric oxide donor that acts directly on the smooth muscle cell to cause relaxation and therefore vasodilation, we conclude that vascular smooth muscle function is also not altered postexercise. Consequently, as in the case of the observed changes in postexercise sweating, we show that postexercise control of skin vasodilation in young adults is modulated by central factors.

Postexercise sweating and CVC in older adults

Similar to the young, the sweating response to administration of ACh and MCh in the older adults did not differ between the no-exercise resting (DOSE) and postexercise resting recovery (Ex-DOSE) conditions (Fig. 1A and B). Thus, we show for the first time that despite an age-related attenuation in the sweating response (discussed below); as in the case of younger adults, the control of sweating in the postexercise recovery period in older adults is likely not mediated by mechanisms of peripheral origin. Likewise, the pattern of response in CVC was not different between the DOSE and Ex+DOSE conditions as assessed using the administration of incremental doses of ACh and SNP (Fig. 2A and B). Thus, we show that the control of postexercise skin vasodilation is most likely due to central mechanisms; a response which parallels that observed in young adults.

Effects of aging on sweating and CVC

Numerous studies have examined age-related differences in thermoregulatory sweating during exercise and some have found reduced local/whole-body sweating and/or altered core temperature onset thresholds and thermosensitivity of the sweating response (Anderson and Kenney 1987; Kenney and Anderson 1988; Tankersley et al. 1991; Inoue et al. 1999; Larose et al. 2013a,b,c). In this study, older adults likely had greater residual heat storage postexercise as indicated by a greater change in rectal temperature relative to baseline resting values at the end of the dose–response protocol in the older (0.50 ± 0.28°C)

compared to the young (0.23 ± 0.13°C) adults. Despite this greater amount of heat, they were not able to produce more sweat during the dose–response protocol. It has been postulated that the age-related impairments in sweating are due to differences in end-organ function such as cholinergic sensitivity of the muscarinic receptors on sweat glands (Kenney and Fowler 1988; Inoue et al. 1999). In this study, we found that the dose-dependent sweating response to the administration of ACh and MCh was lower in older males relative to young males for the no-exercise resting conditions (DOSE; Fig. 1A and B). On the contrary, a recent study by Smith et al. (2013) reported no age-related differences in the sweating response to administration of ACh from 1×10^{-7} to 1×10^{-1} log-molar performed during resting under non-heat stress conditions. While at first glance our results appear to contradict the findings by Smith et al. (2013), it is important to note that we only observed age-related decreases in sweating at the two highest doses of ACh (1 and 1.5 mol/L). No significant difference in sweating between young and older males was observed at and below a concentration of ACh of 10^{-1} log-molar (Fig. 1A). Taken together, it is plausible that a concentration of ACh $> 10^{-1}$ log-molar is required to clearly observe age-related reductions in end-organ sweat gland function. On the other hand, age-related differences in sweating were observed with MCh even at a lower concentration (log-molar 1×10^{-4}). It is possible, therefore, that the effect of age on impairments in sweating can be masked or reduced by AChE enzyme activity. As such, MCh would be more suitable in the assessment of age-related differences in cholinergic sensitivity of muscarinic receptors on sweat glands.

Previous studies examining the effect of age on ACh-dependent skin vasodilation have yielded mixed conclusions (Holowatz et al. 2005; Bruning et al. 2012; Smith et al. 2013). One study found skin vasodilation to be impaired in older adults (Bruning et al. 2012), whereas others have reported no age-related differences (Holowatz et al. 2005; Smith et al. 2013). Consistent with previous reports, our findings were also inconclusive. While we did not observe attenuated skin vasodilation during the DOSE condition to ACh in the older compared to young adults (Fig. 2A), we showed that endothelium function was impaired in the older adults during the Ex+DOSE condition (Fig. 2A). Given that ACh-mediated skin vasodilation occurs via nitric oxide-dependent mechanisms as discussed above, the age-related reductions in skin vasodilation (Fig. 2A) may reflect age-related decreases in nitric oxide-dependent skin vasodilation. Supporting this concept, it has been suggested that aging lowers nitric oxide-dependent skin vasodilation to ACh (Bruning et al. 2012).

For the first time, our study assessed skin vasodilation in response to incremental doses of SNP in young and older adults. Our results show that the log EC_{50} for CVC was greater (i.e., closer to 0) in the older adults in comparison to the young adults during the DOSE condition (Fig. 2B). This result suggests age-related decreases in smooth muscle sensitivity/responsiveness to nitric oxide (i.e., endothelium-independent vasodilation). This finding is consistent with one study demonstrating that expression of soluble guanylyl cyclase, the receptor for nitric oxide which causes smooth muscle relaxation, decreases with increasing age, which has been observed in the aortic ring of rats (Kloss et al. 2000). In contrast, we did not observe a difference in the log EC_{50} for CVC during the Ex+DOSE condition. Furthermore, the CVC responses to incremental doses of SNP were not significantly different between the young and older adults during the DOSE or Ex+DOSE condition. It remains unclear why the sensitivity to nitric oxide was impaired in the older adults during the DOSE condition only. Further studies are required to examine potential mechanisms.

Another interesting observation is that age-related reductions in skin vasodilation to ACh were not observed at the higher concentrations employed in this study (Fig. 2A). This may indicate that there are other mechanisms compensating for the reduced nitric oxide-dependent mechanisms. One possibility is that endothelium-derived hyperpolarizing factors (EDHFs) are acting as a redundant mechanism as has been shown when nitric oxide-dependent vasodilation is reduced in humans (Luksha et al. 2009) and rats (Goto et al. 2012). EDHFs cause relaxation of smooth muscle cells and thus vasodilation in human skin by stimulating calcium-activated potassium (KCa) channels (Lorenzo and Minson 2007; Brunt and Minson 2012; Cracowski et al. 2013). Taken together, it is plausible that age-related impairment of nitric oxide-dependent mechanisms may upregulate the EDHF pathway(s). This may explain the lack of an age-related difference in CVC at the higher doses of ACh.

Some studies have reported that maximal CVC decreases with age (Martin et al. 1995; Minson et al. 2002; Hodges et al. 2010), while others found no differences in maximal CVC induced by SNP between young and older adults (Bruning et al. 2012; Smith et al. 2013). We did not observe a reduced absolute maximal CVC induced by 50 mmol/L SNP in the older adults relative to their younger counterparts. The disparity in the pattern of response may be due to regional differences (Inoue and Shibasaki 1996), where an age-related effect on the maximal skin vasodilatory capacity is not always evident at all areas of the skin, even when measured within the same body part (i.e., forearm).

Conclusions

This study demonstrates that the postexercise suppression of heat loss responses is not mediated by the factors of peripheral origin. This is evidenced by our observation that no differences in sweating or CVC with incremental doses of pharmacological agonists were observed during the no-exercise resting condition (DOSE) or the postexercise resting recovery period (DOSE+Ex) in both young and older adults. Furthermore, while we show marked impairment in sweat gland function in the older adults, the mechanisms underlying age-related changes in CVC were less conclusive.

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Conflict of Interest

The authors declare that they have no competing interests.

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APPENDIX D

Ethics approval notice for thesis research projects

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
Pierre	Boulay	Health Sciences / Ed. Physique et sportive	Co-investigator
Naoto	Fujii	Health Sciences / Human Kinetics	Co-investigator
Ronald	Sigal	Medicine / Medicine	Co-investigator
Martin	Poirier	Health Sciences / Human Kinetics	Research Assistant
Jill	Stapleton	Health Sciences / Human Kinetics	Research Assistant
Joanie	Larose	Health Sciences / Human Kinetics	Project Coordinator

File Number: H12-11-04

Type of Project: Professor

Title: Body Heat Storage during Work in the Heat in Type 2 Diabetes Mellitus/Heat Stress in Older Adults and Individuals with Type 2 Diabetes

Renewal Date (mm/dd/yyyy)	Expiry Date (mm/dd/yyyy)	Approval Type
02/27/2014	02/26/2015	Ia

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

Special Conditions / Comments:
N/A

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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.research.uottawa.ca/ethics/forms.html.

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.research.uottawa.ca/ethics/forms.html.

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

Signature:

Mélanie Rioux
Ethics Coordinator
For Gilles Morier, Acting Director of the Office of Research Ethics and Integrity

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