Age-related impairments in nitric oxide-dependent cutaneous vasodilation and sweating during exercise: roles for oxidative stress and arginase?

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THESIS

Submitted to the
Faculty of Graduate and Postdoctoral Studies
In partial fulfillment of the requirements
For the degree of Master's of Science – Human Kinetics

Faculty of Heath Science, School of Human Kinetics
University of Ottawa, Canada

ACKNOWLEDGEMENTS

I wish to thank the following individuals for their guidance and support in the completion of this thesis. It would not have been possible without you.

To Dr. Glen Kenny – Thank you for allowing be to develop my research skills under your supervision. Your unrelenting commitment to your research and your students is unparalleled and I am truly grateful I have had the opportunity to work with you.

To my committee members Dr. François Haman and Dr. Éric Doucet; thank you for taking the time to provide positive and constructive feedback on my project. Without you, the completion of this thesis would not have been possible.

A big thank you to my colleagues at the Human and Environmental Physiology Research Unit. I have never before had the honour of working with such a talented and driven team of aspiring researchers.

A special thanks to Dr. Naoto Fujii whose guidance throughout my career as a young research has been pivotal.

A special thanks to Mr. Martin Poirier, Mr. Jeffrey Louie and Ms. Pegah Akbari for their amazing support and assistance throughout data collection and the writing of this thesis. I owe you both more than you know.

A very large thank you to all of the participants who volunteered for my project. Without your involvement in my gruelling studies, this thesis would not have been possible.

A special thank you to my incredible friends: Justin, Meagan, Emily, Dylan, Andrew, Jacob, Mateo, Warren and Maddie. I have never met a group of people quite like you and I know I will forever have your support in every movement I make.

Saving the biggest thank you for last, I am forever indebted to my parents. Your endless support and encouragement have helped develop me into the person I am today. It is because of you that I strive to make every day better than the one before it. You are a huge source of my ambition and success and I will hold that close to my heart forever.

ABSTRACT

This thesis sought to evaluate whether the impairments in nitric oxide (NO)-dependent cutaneous vasodilation and sweating observed in older adults during exercise in the heat stem from age-related increases in oxidative stress and/or arginase activity. Furthermore, we assessed whether changes in the sensitivity to NO at the level of the end-organ (i.e., cutaneous vasculature and sweat gland) also contribute. A total of 20 young (age, 23 ± 3 yrs) and 28 older (age, 63 ± 7 yrs) males completed one of two intermittent exercise protocols that consisted of two 30-min bouts of semi-recumbent cycling in the heat (35˚C) at a rate of metabolic heat production of 500 (protocol I; 11 young, 19 older) or 400 (protocol II; 9 young, 9 older) W. Each exercise bout was followed by a 20-min recovery period. During each protocol, local cutaneous vascular conductance (CVC; laser-Doppler flowmetry/mean arterial pressure) and sweat rate (SR, ventilated capsule) were continuously measured at four forearm skin sites. In protocol I, each forearm skin site was continuously perfused via intradermal microdialysis with either: 1) lactated Ringer's serving as a control (Control); 2) 10 mM N^G-nitro-$_L$-arginine methyl ester (L-NAME), a non-selective NO synthase inhibitor; 3) 10 mM ascorbate (Ascorbate), a non-selective antioxidant or 4) a combination of 10 mM ascorbate and 10 mM L-NAME (L-NAME + Ascorbate). In protocol II, the Ascorbate and L-NAME + Ascorbate skin sites were replaced with 5 mM N^{ω}-hydroxy-nor-Arginine + 5 mM S-(2-boronoethyl)-L-cysteine to inhibit arginase activity (Nor-NOHA+BEC) and 1 μM sodium nitroprusside, a nitric oxide donor (SNP). In the young adults during protocol I, CVC was reduced relative to Control at L-NAME (both $P<0.01$) and L-NAME + Ascorbate (both $P\leq0.03$) but similar to Control at Ascorbate (both $P\geq0.26$). In the older adults, CVC was reduced from Control during both exercise bouts at L-NAME (both $P\leq0.02$) but not Ascorbate (both $P\geq0.09$) or L-NAME + Ascorbate (both $P\geq0.15$). While L-NAME (both $P<0.04$) and L-NAME + Ascorbate (both $P<0.04$) attenuated SR in the younger adults during exercise (no effect of Ascorbate; both $P\geq0.36$), no differences between Control and any treatment site were observed in the older adults ($P=0.42$). However, correlational analysis revealed a moderate negative correlation between between VO_{2peak} and the change in SR from control at the Ascorbate site during both exercise bouts ($-0.55\leq r\leq-0.54$; both $P=0.02$) exercise. Furthermore, the change in SR from Control at L-NAME + Ascorbate was also found to be negatively correlated with VO_{2peak} in the second ($r=-0.54$; $P=0.02$) but not first ($r=-0.42$; $P=0.08$) exercise bout. In protocol II, CVC

was reduced from Control at L-NAME in the young and older adults during both (both $P<0.01$) and the first ($P=0.05$) exercise bout, respectively. Furthermore, SR was reduced from Control in the young (both $P\leq0.03$) but not older ($P=0.28$) adults at the L-NAME skin site. However, no influence of Nor-NOHA+BEC or SNP was observed in either age group for both CVC (all $P\geq0.38$) and SR ($P=0.28$). This thesis demonstrates that age-related increases in oxidative stress influence cutaneous vasodilation during exercise via mechanisms independent of NO. Furthermore, the current findings suggest an effect of oxidative stress on NO-independent SR in older adults but that secondary factors (i.e., aerobic fitness and/or physical activity level) may play a modulatory role. Finally, the results of this thesis demonstrate that, during exercise in the heat, neither elevated arginase activity nor changes in the sensitivity of the thermoregulatory end-organs to NO effect the CVC and SR responses in older adults.

Table of Contents

GLOSSARY

BEC – S-(2-boronoethyl)-L-cysteine

BH_4 – tetrahydrobiopterin

CVC – cutaneous vascular conductance

CVC_{max} – maximal cutaneous vascular conductance

L-NAME – N^G-nitro-$_L$-arginine methyl ester

Na^+/K^+-ATPase – sodium-potassium pump

NKCC – sodium-potassium-chloride cotransporter

NO – nitric oxide

Nor-NOHA – N^{ω}-hydroxy-nor-Arginine

NOS – nitric oxide synthase

PO/AH – pre-optic anterior hypothalamus

PU – perfusion units

ROS – reactive oxygen species

SNP – sodium nitroprusside

VO_{2peak} – peak rate of oxygen consumption

CHAPTER I

INTRODUCTION

1.1 Introduction

In humans, the maintenance of a stable body core temperature of ~37˚C is necessary for normal physiological function. The regulation of body temperature is under the control of the autonomic nervous system, which can elicit increases in cutaneous blood flow and sweating to facilitate the dissipation of heat from the skin to the environment. Accordingly, elevations in core and skin temperature induced by physical activity and/or elevated ambient temperatures elicit proportional increases in these heat loss responses (Gagge & Gonzalez, 1996). However, if the regulation of thermal homeostasis fails and core temperature is allowed to reach highly elevated levels, the risk of heat related illness and injury (i.e., heat exhaustion, heat stroke etc.) is greatly increased (Jay & Kenny, 2010; Kenny *et al.*, 2010; Kenney *et al.*, 2014). While healthy young adults are able to maintain body core temperature within a safe range under a variety of conditions, aging compromises the body's ability to dissipate heat (Larose *et al.*, 2013a, b, c) placing older adults at increased risk for catastrophic increases in core temperature (Kenny *et al.*, 2010). Indeed, the highest proportion of emergency room visits and deaths during extreme heat events occur in adults over 65 years of age (Ellis *et al.*, 1975; Semenza *et al.*, 1999).

In comparison to their younger counterparts, the capacity of older adults to increase cutaneous blood flow (Kenney, 1988; Holowatz *et al.*, 2003; Holowatz *et al.*, 2006a, b; Stanhewicz *et al.*, 2012; Smith *et al.*, 2013) and sweating (Inoue *et al.*, 1999; Inbar *et al.*, 2004; Larose *et al.*, 2013a, b, c; Smith *et al.*, 2013; Larose *et al.*, 2014) is attenuated during exercise and passively induced heat stress. In fact, age-related impairments in whole-body heat dissipation during exercise are evident in adults as young as 40 years of age (Larose *et al.*, 2013a). While the nature of the age-related reductions in whole-body heat loss has grown

substantially in recent years, a paucity of information exists regarding the underpinning physiological mechanisms.

A key role in the regulation of the heat loss responses has been identified for the biological signaling molecule nitric oxide (NO), which is produced via the conversion of L-arginine by the enzyme NO synthase. NO is required for full expression of the cutaneous vasodilatory response to whole-body passive heating (Kenney, 1988; Holowatz *et al.*, 2003; Holowatz *et al.*, 2006a, b; Wong & Minson, 2006; Stanhewicz *et al.*, 2012; Brunt *et al.*, 2013; Swift *et al.*, 2014) and exercise-induced heat stress (Welch *et al.*, 2009; Fujii *et al.*, 2014; McGinn *et al.*, 2014a, b; McNamara *et al.*, 2014; Meade *et al.*, 2015; Fujii *et al.*, 2016) as well as the sweating response to exercise. (Welch *et al.*, 2009; Fujii *et al.*, 2014; McGinn *et al.*, 2014b; Stapleton *et al.*, 2014a; Fujii *et al.*, 2016). However, in parallel with the aforementioned impairments in whole-body heat loss, NO-dependent to cutaneous vasodilation is attenuated in older adults during whole-body passive heating (Holowatz *et al.*, 2003; Holowatz *et al.*, 2006a, b) and exercise (Fujii *et al.*, 2016), whereas the contribution of NO to the sweating response during exercise is absent (Stapleton *et al.*, 2014a; Fujii *et al.*, 2015c; Fujii *et al.*, 2016).

Two mechanisms potentially underpinning the impairments in NO-dependent cutaneous vasodilation and sweating observed in older adults are elevations in oxidative stress and arginase activity. In regards to the former, aging is associated with an increase in the concentration of reactive oxygen species (ROS) in the skin as a result of an increase in the production of ROS as well as a concomitant decrease in the activity of ROS scavenging enzymes (Kohen, 1999; Lu *et al.*, 1999; Bouzid *et al.*, 2015a). It is well established that NO reacts readily with ROS such as superoxide to form peroxynitrite, leading to diminished NO bioavailability (Mortensen & Lykkesfeldt, 2014). In addition to elevations in basal ROS levels, aging is associated with

increases in activity of the enzyme arginase, which metabolizes L-arginine to form urea, in the skin (Berkowitz *et al.*, 2003; White *et al.*, 2006). Given that L-arginine is the precursor for both NO synthase and arginase as well as the fact that, in comparison to NO synthase, arginase preferentially binds L-Arginine, an age-related increase in arginase activity may result in the reciprocal inhibition of NO production via the sequestration of L-arginine (Berkowitz *et al.*, 2003; White *et al.*, 2006). Currently however, it is unknown if either of the aforementioned mechanisms contribute to the impairments in heat loss observed in older adults during exercise.

1.2 Rationale and Statement of the problem

There is evidence to suggest that both age-related increases in oxidative stress and arginase activity may impair heat loss during passively induced increases in core temperature (Holowatz *et al.*, 2006a, b). However, it is currently unclear whether either of these mechanisms contribute to the impairments in NO-dependent cutaneous vasodilation and sweating observed in older adults during exercise in the heat (Stapleton *et al.*, 2014a). Furthermore, it is unknown if the sensitivity of the end-organ (i.e., the cutaneous vasculature and sweat gland) to NO is altered in older relative to young adults. Therefore, pharmacological agents were delivered directly to the skin of young and older adults via intradermal microdialysis in order to 1) reduce the level of ROS (via a non-selective antioxidant), 2) inhibit the activity of arginase and 3) supply low-dose NO to analyze the resultant modulation, if any, of cutaneous vasodilation and sweating during both exercise and recovery in a hot environment.

1.3 Study objectives

The primary objective of this study was to assess the mechanisms underpinning the age-related impairments in the contribution of NO to the heat loss responses of cutaneous vasodilation and sweating during exercise in the heat. Specifically, this project aimed to:

1) Evaluate if local administration of the non-selective antioxidant ascorbate modulates the contribution of NO to the heat loss responses of cutaneous vasodilation and sweating.
2) Determine whether inhibition of arginase in the skin augments cutaneous vasodilation and sweating.
3) Assess the effect of low dose administration of sodium nitroprusside (a nitric oxide donor) on cutaneous vascular regulation and sweat rate.

1.4 Hypothesis

This project evaluated the hypothesis that local infusion of the antioxidant ascorbate in older adults via NO-dependent mechanisms would augment cutaneous blood flow and sweating via NO-dependent mechanisms. Further, the hypotheses that, in older adults, both inhibition of arginase activity in the skin as well as low dose administration sodium nitroprusside, a NO donor, would modulate cutaneous vasodilation and sweating, was also assessed.

1.5 Relevance of the study

To date, there has been extensive research on the influence of age on whole-body heat loss; however, a significant knowledge gap remains regarding the mechanisms underpinning these age-related impairments. First and foremost, this project provides important mechanistic insight regarding the age-related impairments in heat loss, which occur in adults as young as 40

years of age (Larose *et al.*, 2013a) and are greater in those who are physically inactive (Stapleton *et al.*, 2015), that may place older adults at a greater risk of heat related illness and injury in comparison to their younger counterparts (Kenny *et al.*, 2010). Second, the results gleaned from this study represent a first step in the development of practical strategies (e.g., ascorbate and/or L-arginine supplementation) aimed at protecting older individuals from the adverse effects of elevated core temperature experienced during severe and/or prolonged heat exposure.

1.6 Delimitations and limitations

This thesis evaluated the mechanisms underlying the age-related impairments in the relative contribution of NO to the heat loss responses during exercise. For this reason, regularly active (i.e., performing at least 30 minutes of structured physical activity a minimum of 2 times per week) older adults were recruited to ensure they could complete the experimental protocols. Therefore, the findings may not be representative of older adults with lower aerobic capacity. Furthermore, individuals with chronic pathophysiological conditions were excluded from this study. Consequently, the findings of this thesis may not apply to these individuals. Finally, this study was performed in males and is therefore not directly representative of the female population. However, the age-related impairments in heat loss have been observed in females (Larose *et al.*, 2013b).

CHAPTER II

REVIEW OF THE LITTERATURE

2.1 Basic Human Thermoregulation

Even small fluctuations in body temperature can pose a great challenge to homeostasis within the body; therefore, tight control of body temperature must be maintained to ensure normal physiological function (Taylor, 2006). However, factors such as environmental conditions (e.g., ambient temperature and humidity) and increases in metabolism (of which heat is the primary byproduct) due to physical activity can threaten the regulation of internal temperature. Body temperature regulation is thought to reside in the brain at the level of the pre-optic anterior hypothalamus (PO/AH), which receives and integrates afferent information from central (in the brain) and peripheral (in the skin) thermoreceptors and, if necessary, elicits specialized effector responses (i.e., increases in cutaneous blood flow and sweat production) in order to regulate core temperature (Boulant & Bignall, 1973; Hensel, 1981). This system operates via negative feedback to balance the rate of heat gained with the rate of heat loss to the environmental and ultimately controls core temperature within a narrow range (figure 1).

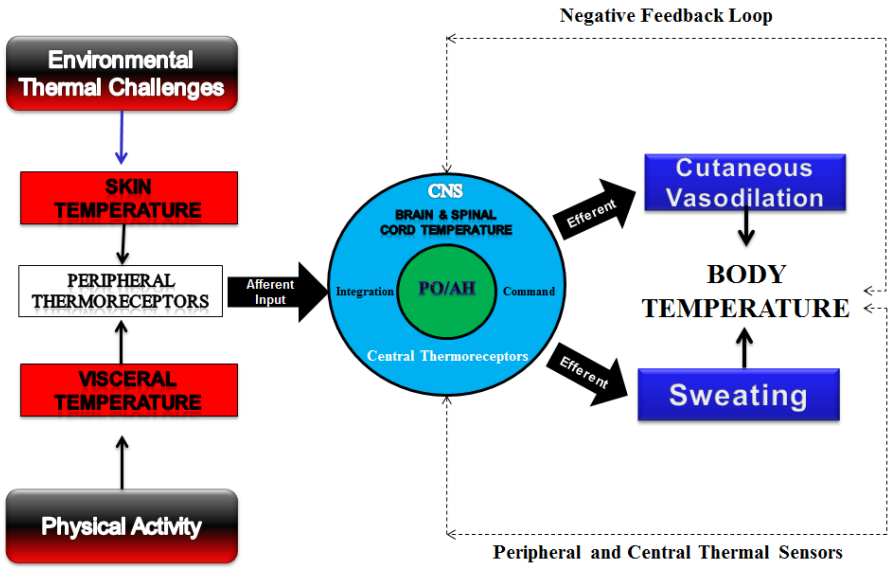

Figure 1. A schematic representation of the control of the heat loss responses (i.e., cutaneous vasodilation and sweating) in response to elevations in core and skin temperature during heat stress. Adapted from Nagashima *et al.* (2000).

During heat stress associated with exposure to a hot environment and/or exercise, the PO/AH receives information of elevated body core and/or skin temperatures and sends a signal via sympathetic nerves to the cutaneous vasculature and eccrine sweat glands in order to elicit cutaneous vasodilation and sweat production, respectively (Charkoudian, 2003; Johnson & Kellogg, 2010; Shibasaki & Crandall, 2010; Kenny & Jay, 2013; Johnson *et al.*, 2014). The former mechanism acts to increase the diameter of the cutaneous blood vessels and thereby the flow of blood through the shallow cutaneous circulation where its heat can be dissipated (Kenny & Jay, 2013; Johnson *et al.*, 2014). Fundamentally, this is achieved by an increase in skin temperature secondary to the elevation in cutaneous blood flow, which modulates the temperature gradient for dry heat exchange (i.e., heat loss via convection, conduction and radiation) between the skin the environment (Kenny & Jay, 2013; Johnson *et al.*, 2014).

While cutaneous vasodilation plays an important thermoregulatory role at rest and in situations of minor heat stress, sweating provides the body's greatest capacity for heat loss (Shibasaki & Crandall, 2010; Gagnon *et al.*, 2013; Kenny & Jay, 2013) and is the primary avenue of heat loss during exercise and/or exposure to a hot environment (Kenny & Jay, 2013). In fact, in situations in which environmental temperature exceeds that of the skin, sweating is the only mechanisms by which heat dissipation can occur (Kenny & Jay, 2013).

2.2 Human Heat Balance

The dynamic exchange of heat between the body and the environment can be modeled using the human heat balance equation (Gagge & Gonzalez, 1996; Kenny & Jay, 2013):

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

Where all terms are expressed in $W \cdot m^{-2}$ and,

S = rate of body heat storage

M = metabolic rate

W = rate of mechanical work

C = rate of convective and conductive heat loss from the skin

R = rate of radiative heat loss from the skin

E = rate of evaporative heat loss from the skin

As this equation illustrates, for the rate of body heat storage to be zero (i.e., body heat content and thereby body temperature is stable), the rate of metabolic heat production (a byproduct of metabolism) must match the rate of total heat loss to the environment. Heat loss is facilitated via dry (i.e., convection, conduction, and radiation) and evaporative mechanisms, with the former being dependent on the temperature gradient between the skin and the environment. Consequently, dry heat loss decreases as a function of the temperature gradient between the skin and the environment as ambient temperature approaches skin temperature (~34˚C). In fact, in conditions where the ambient temperature exceeds that of the skin, dry heat loss becomes negative such that a dry heat gain is experienced (Gagge & Gonzalez, 1996; Kenny & Jay, 2013). In contrast, evaporative heat loss (via sweating), which is dependent on the water vapour gradient between the skin and the environment, can only contribute to heat loss from the body and represents the primary avenue of heat loss during exercise and/or hot environments (Gagge & Gonzalez, 1996; Cain & McLellan, 1998; Gavin, 2003; Gagnon *et al.*, 2013; Kenny & Jay, 2013). During exercise and/or exposure to elevated ambient temperatures the body increases heat loss in an attempt to regain a state of heat balance such that heat load (i.e., the rate of heat gain via metabolism and the environment) is matched by total heat loss (Kenny & Jay, 2013). In fact,

the heat load experienced during exercise (also termed requirement for heat loss) is the major determinant of evaporative heat loss and therefore whole-body sweat rate, with the latter explaining ~95% of the variation in the former (Gagnon *et al.*, 2013).

2.3 Temperature Regulation during Heat Stress

Referring to the heat balance equation, heat stress (i.e., increases in body heat storage and temperature) can result from increases in metabolic heat production due to physical activity, exposure to hot and/or humid conditions (which impact dry heat exchange and evaporative heat loss, respectively) or a combination of these factors (Gagge & Gonzalez, 1996; Kenny & Jay, 2013). To prevent potentially catastrophic changes in body core temperature, the PO/AH elicits increases in cutaneous blood flow and sweating to elevate heat dissipation to the environment and ultimately maintain body core temperature within a physiological range (Taylor, 2006).

To quantify the nature of thermal stimulus integrated at the level of the PO/AH, researchers often use the measurable variable of mean body temperature, which is calculated based on simultaneous measurements of core temperature (typically esophageal and/or rectal) and the mean skin temperature across multiple skin sites (Lenhardt & Sessler, 2006). The effector response to a change in mean body temperature follows three distinct phases: the onset threshold, the thermosensitivity and the plateau (figure 2) (Charkoudian, 2003; Gagnon & Kenny, 2012). The onset threshold is the mean body temperature at which increases in cutaneous blood flow and sweating occur. Following the onset threshold, the thermosensitivity is the period in which increases in mean body temperature are paralleled by proportional increases in the heat loss responses. Finally, cutaneous vasodilation and sweating will fail to increase with further increases in mean body temperature. This phase is referred to as the plateau and may represent

either the maximal capacity of the heat loss responses or indicate that heat balance (i.e., metabolic and environmental heat gain is equivalent to heat dissipation) has occurred. Importantly, any factor of thermal or non-thermal origin that influences any or all of these phases will affect the rate of heat loss and thereby the rate of change in mean body temperature (Kenny & Jay, 2013). For example, following exercise there is a delay in the onset threshold (i.e., the heat loss responses are activated at a higher mean body temperature); however, the sensitivity of the response is increased, a phenomenon that has been termed the priming effect (Kenny & Journeay, 2010; Kenny & Jay, 2013).

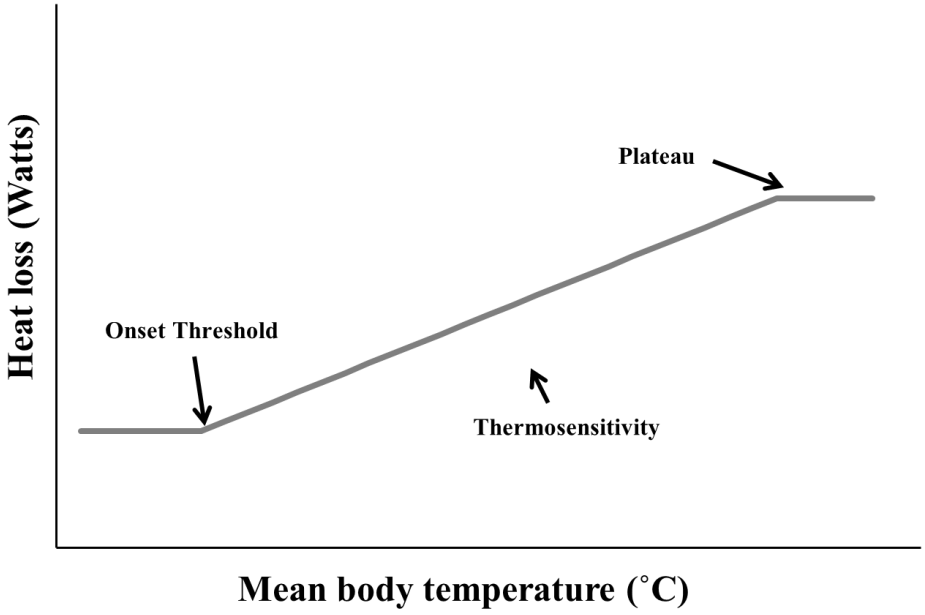

Figure 2. An illustration of the effector response to increases in mean body temperature during heat stress. The onset threshold refers to the mean body temperature threshold for the activation of the heat loss responses. Thereafter, heat loss increases proportionally to increases in mean body temperature – the thermosensitivity of the response. The plateau phase follows, which refers to the point at which increases in mean body temperature elicit no further increases in heat loss. This pattern of response is well established and has been demonstrated using local, whole-limb and whole-body measurements of heat loss. Adapted from Gagnon and Kenny (2012).

2.3.1 Cutaneous blood flow

Modulation of blood flow through the cutaneous circulation is an essential in the control of body temperature (Charkoudian, 2003; Johnson & Kellogg, 2010; Johnson *et al.*, 2014). To facilitate dry heat loss, cutaneous blood flow can increase from resting values of ~250 $mL \cdot min^{-1}$ to values of up to 8 $L \cdot min^{-1}$ in situations of severe heat stress (Rowell, 1974; Crandall & Gonzalez-Alonso, 2010). The cutaneous vasculature in humans is unique in the sense that it is under control of two populations of sympathetic nerves: an active vasoconstrictor system and an active vasodilator system (Johnson & Proppe, 1996). Sympathetic vasoconstriction is primarily mediated through presynaptic release of noradrenaline, and its withdrawal provides ~10-15% of the vasodilatory response to heat stress. In contrast, the activation of the active vasodilator system is thought to provide 85-90% of the cutaneous vascular response to heat stress; albeit, relatively little is known about the control of this system or the unknown vasodilator substances(s) that mediate its response (Charkoudian, 2003; Johnson & Kellogg, 2010; Johnson *et al.*, 2014). However, a prominent role has been established for NO, which accounts for ~30-45% of the vasodilatory response to whole-body passive heating (Kellogg *et al.*, 1998; Shastry *et al.*, 2000; McCord *et al.*, 2006) and has also been shown to be an important modulator of cutaneous blood flow during exercise (McCord *et al.*, 2006; Welch *et al.*, 2009; Fujii *et al.*, 2014; McGinn *et al.*, 2014a, b; Meade *et al.*, 2015; Fujii *et al.*, 2016).

2.3.2 Sweating

The primary avenue for heat dissipation during physical activity and/or environmental heat stress is the evaporation of sweat from the skin (Gagge & Gonzalez, 1996; Shibasaki & Crandall, 2010; Kenny & Jay, 2013). Increases in metabolic heat production and/or ambient

temperature place greater reliance on sweating (Shibasaki & Crandall, 2010; Gagnon *et al.*, 2013), which accounts to ≥80% of total heat loss during physical activity and/or exposure to high ambient temperatures compared to ~25% at rest (Cain & McLellan, 1998; Gavin, 2003). In fact, if ambient temperature exceeds that of the skin, evaporative heat loss is the only mechanisms through which heat dissipation can occur (Gagge & Gonzalez, 1996; Cain & McLellan, 1998; Gavin, 2003; Wendt *et al.*, 2007; Gagnon *et al.*, 2013; Kenny & Jay, 2013). Sweat production during heat stress stems from activation of the body's 2-4 million eccrine glands, which can support whole-body sweat rates as high as 2 $L \cdot hr^{-1}$ (American College of Sports Medicine *et al.*, 2007). The eccrine glands are activated via the binding of acetylcholine released from sympathetic cholinergic nerves to muscarinic receptors on the gland (Shibasaki & Crandall, 2010) (American College of Sports Medicine *et al.*, 2007). In contrast to cutaneous vascular regulation, relatively little is known about the biochemical pathways underpinning the sweating responses. It has however been demonstrated that NO is required for full expression of the sweat response during physical activity in young adults. Specifically, multiple studies report diminished local forearm sweat rate during exercise at a NO synthase-inhibited skin site relative to an untreated control site (Welch *et al.*, 2009; Gagnon & Kenny, 2011; Fujii *et al.*, 2014; McGinn *et al.*, 2014b; Stapleton *et al.*, 2014a). However, the involvement of NO is thought to be permissive such that NO itself cannot stimulate sweat production (Fujii *et al.*, 2014).

2.4 Aging

Aging is a progressive and complex process wherein changes at the molecular, cellular and organ level result in alterations in control of many of the bodies physiological functions (Chodzko-Zajko & Ringel, 1987; Gall & Parkhouse, 2004; Nelson *et al.*, 2010; Kenney *et al.*,

2014; Tseng *et al.*, 2014). As a result, older individuals are less able to appropriately respond to the internal and/or external stressors placed upon them. Examples of these changes in the context exercise- and/or environment-related stressors include reductions in aerobic (Nelson *et al.*, 2010) and muscular capacity (Gall & Parkhouse, 2004), alterations in cardiovascular regulation (i.e., decreased blood volume, cardiac muscle size and compliance) (Martin *et al.*, 2015) and the ability to cope with thermally challenging environments (Kenney *et al.*, 2014) as well as increases and decreases in fat and fat-free mass, respectively (Tseng *et al.*, 2014). While the above age-related changes are modifiable by interventions such as physical training (Yarasheski, 2003; Nelson *et al.*, 2010; Stapleton *et al.*, 2015), it should be noted that the eventual decline in the body's ability to respond to perturbations its internal and external environment is inevitable. Altogether, these changes associated with aging place older adults at a greater risk of adverse physiological events (e.g., cardiovascular complications, heat related illness) during physical exertion and/or exposure to hot environments (Kenney *et al.*, 2014).

2.4.1 Aging and Thermoregulation

In the context of thermoregulation, aging is associated with reduced tolerance to heat stress (Kenney *et al.*, 2014) as well as altered perception of thermal stimuli (Collins *et al.*, 1981; Chao *et al.*, 2007). Older adults are thought to be at a greater risk of heat-related illness and injury due to reductions in the physiological capacity to dissipate heat (Kenny *et al.*, 2010) as well as alterations in the cardiovascular system (which supports increases in cutaneous blood flow) (Kenney *et al.*, 2014). In line with this, recent studies report age-related reductions in whole-body heat loss (Kenny & Jay, 2013; Larose *et al.*, 2013a; Larose *et al.*, 2013b; Larose *et al.*, 2013c; Larose *et al.*, 2014) that occur in adults as young as 40 years of age (Larose *et al.*,

2013a), which likely result from a decreased capacity to increase both cutaneous blood flow (Kenney, 1988; Holowatz *et al.*, 2003; Holowatz *et al.*, 2006b; Holowatz & Kenney, 2007; Stanhewicz *et al.*, 2012; Smith *et al.*, 2013) and sweating (Inoue *et al.*, 1999; Inbar *et al.*, 2004; Larose *et al.*, 2013a; Larose *et al.*, 2013b; Larose *et al.*, 2013c; Smith *et al.*, 2013; Larose *et al.*, 2014) in comparison to their younger counterparts. Altogether, age-related reductions in thermoregulatory capacity result in greater increases in body heat storage and thereby body temperature during thermal challenge relative to young adults (Larose *et al.*, 2013a; Larose *et al.*, 2013b; Larose *et al.*, 2013c; Larose *et al.*, 2014), placing them at a greater risk of heat-related illness and injury relative to their younger counterparts (Kenny *et al.*, 2010; Kenney *et al.*, 2014).

2.4.1.1 Age and Nitric Oxide-dependent Heat Loss

In recent years, there has been great interest in the physiological mechanisms underpinning the aforementioned age-related impairments in the heat loss responses. These studies have clearly demonstrated that while NO-dependent vasodilation is an integral component to the cutaneous vasodilatory response to heat stress, attenuations in its involvement are present in older adults during both whole-body passive heating (Holowatz *et al.*, 2003; Holowatz *et al.*, 2006b, a) and exercise (Fujii *et al.*, 2016). Likewise, NO-dependent sweating, which has been clearly observed in younger individuals (Welch *et al.*, 2009; Fujii *et al.*, 2014; McGinn *et al.*, 2014b; Stapleton *et al.*, 2014a; Fujii *et al.*, 2016), is reportedly absent in their older counterparts (Stapleton *et al.*, 2014a; Fujii *et al.*, 2015c; Fujii *et al.*, 2016). The mechanisms underpinning the impaired contribution of NO to the heat loss responses in older adults during physical activity have yet to be elucidated. However, potential roles exist for

increases in oxidative stress and arginase activity in the skin, both of which are known to occur with aging (Kohen, 1999; Lu *et al.*, 1999; Berkowitz *et al.*, 2003; White *et al.*, 2006) (figure 3).

2.4.1.1.1 Oxidative Stress

In addition to the modulation of NO-dependent cutaneous vasodilation and sweating, aging is associated with an elevation in the production of ROS as well as a concomitant decrease in the activity of ROS scavenging enzymes in the skin (Kohen, 1999; Lu *et al.*, 1999; Bouzid *et al.*, 2015a). It is well established that NO reacts readily with ROS such as superoxide to form peroxynitrite, leading to diminished NO bioavailability (Mortensen & Lykkesfeldt, 2014). Additionally, elevated ROS levels can result in the uncoupling of NO synthase (which results in increased ROS production *per se*), which contributes to the ROS-mediated decreased in NO bioavailability (Mortensen & Lykkesfeldt, 2014). Thus, it is plausible that the impairments in NO-dependent cutaneous vasodilation and sweating in older adults stem from increased ROS in the skin (i.e., oxidative stress) (figure 3). In support of this mechanism, recent work demonstrates that, in parallel to reductions in NO-dependent vasodilation, local administration of ascorbate (a non-selective antioxidant) via intradermal microdialysis to the skin of older adults augments cutaneous vasodilation during whole-body passive heating (Holowatz *et al.*, 2006a). Furthermore, the effect of ascorbate on NO bioavailbility is mediated in part through the stabilization of an essential co-factor of NO synthase, tetrahydrobiopterin (BH_4), the bioavailability of which is also sensitive to the presence of ROS (Huang *et al.*, 2000; Mortensen & Lykkesfeldt, 2014). Indeed, direct administration of BH_4 to aged human skin has been shown to augment cutaneous vasodilation during whole-body passive heating via NO-dependent mechanisms (Stanhewicz *et al.*, 2012).

Apart from studies employing whole-body passive heating in older adults *in vivo*, indirect support for the influence of oxidative stress on the heat loss responses can be found in recent work from ours as well as other laboratories. Specifically, we recently demonstrated that local infusion of ascorbate augments NO-dependent cutaneous vasodilation during intense exercise in the heat (Meade *et al.*, 2015) wherein there is systemic increases in oxidative stress (Goto *et al.*, 2003; Seifi-Skishahr *et al.*, 2008; Sureda *et al.*, 2015); albeit, this work was performed only in young adults. Furthermore, in young adults local administration of angiotensin II, the effects of which are thought to be in-part mediated via an increased production of ROS, attenuates cutaneous vasodilation during local skin heating through ROS-related mechanisms (Stewart *et al.*, 2008). It should be noted however, that angiotensin II has also been shown to attenuate cutaneous vasodilation in younger adults during passive exposure to a hot environment and recovery from exercise in the heat; albeit, a role for oxidative stress in this response was not observed (Fujii *et al.*, 2015b). In regards to the sweating response, it has been shown that oxidative stress levels are associated with attenuations in the sweating responses induced by local administration of the cholinergic agonist acetylcholine (via electrophoresis) (Hoeldtke *et al.*, 2011). Furthermore, a ROS-mediated attenuation in local sweat rate with administration of angiotensin II in young adults has been observed during passive exposure and recovery from exercise in a hot environment (Fujii *et al.*, 2015b).

2.4.1.1.2 Arginase

Potentially underlying the age-related impairments in the contribution of NO to the heat loss responses are changes in the activity of the enzyme arginase, which is responsible for the conversion of L-arginine to urea in the final step of the urea cycle and upregulated in animal

models of aging (Berkowitz *et al.*, 2003; White *et al.*, 2006) (figure 3). Importantly, both NO synthase and arginase metabolize L-arginine and, in comparison to NO synthase, arginase preferentially binds this precursor. In this way, an age-related increase in arginase activity may reciprocally inhibit NO bioavailbility by sequestering L-arginine (Berkowitz *et al.*, 2003; White *et al.*, 2006) and therefore contribute to the impairment in NO-dependent cutaneous vasodilation and sweating observed in older adults. In support of this notion, local arginase inhibition via non-selective blockade in the skin (via microdialysis) of older adults has been shown to improve the local cutaneous vasodilatory response induced by whole-body passive heating to a level comparable to their younger counterparts (Holowatz *et al.*, 2006a, b; Stanhewicz *et al.*, 2012). Additionally, increased urea (the primary byproduct of arginase) content in eccrine sweat has been reported in older relative to younger adults, suggesting age-related upregulations in arginase activity at the level of the sweat gland (al-Tamer & Hadi, 1994).

2.5 Summary of key knowledge gaps

As summarized in figure 3, age-related increases in oxidative stress and arginase activity may impair NO-bioavailability and thereby cutaneous vasodilation and sweating in older adults during heat stress. However, despite evidence suggesting their involvement in the age-related impairments in cutaneous vasodilation during whole-body passive heating (Holowatz *et al.*, 2006b, a), it is currently unknown if these mechanisms influence the heat loss responses in older adults during exercise in the heat. Moreover, it is unclear whether the sensitivity of the thermoregulatory end-organs, namely the cutaneous vasculature and sweat gland, to NO is altered in older adults.

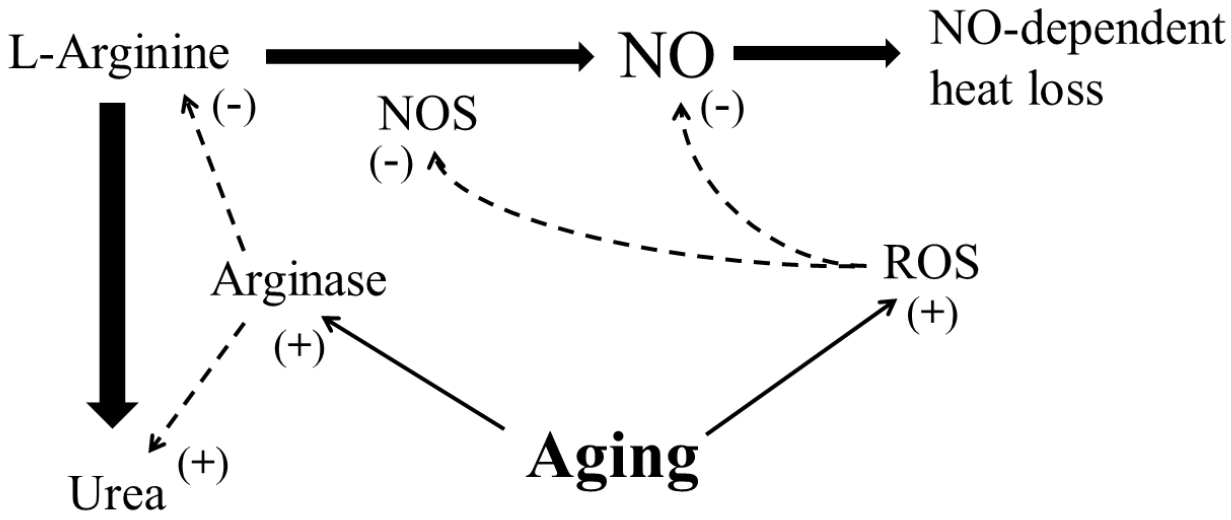

Figure 3. Summary of the potential mechanisms contributing to the impairments in NO-dependent heat loss (i.e., cutaneous vasodilation and sweating) observed in older adults. In this model, age-related increases in reactive oxygen species (ROS) in the skin reduce nitric oxide (NO) bioavailability directly through the reaction of ROS and NO, and indirectly through the modulation of NO synthase (NOS) activity. Furthermore, increases in the activity of the enzyme arginase, which requires the same precursor as NOS (i.e., L-Arginine), result in the reciprocal inhibition of NO production via the sequestration of L-arginine.

CHAPTER 3

METHODS

3.1 Participants

This study was in accordance with the *Declaration of Helsinki* and was approved by the University of Ottawa Health Sciences and Science Research Ethics Board. A total of 48 males (20 younger and 29 older) participated in the current study. Written and informed consent was obtained from all volunteers prior to their participation in the study. Participants were recruited via poster presentations, online discussion boards and word of mouth.

All participants who completed the current study were: 1) healthy (i.e., no history of cardiovascular, metabolic or respiratory disease and not currently taking medication related to these conditions); 2) an adult males aged between either 19 and 32 years (young) or 52 and 73 years (older) and 3) habitually active (i.e., performing at least 30 minutes of structured physical activity a minimum of 2 times per week). Each participant completed one screening and one of the two experimental protocols (see below). In protocol I, 11 young and 19 older males were included whereas 9 young and 9 older adults completed protocol II.

In the current thesis, males were chosen given the potential modulation in the control of body temperature associated with fluctuations in female sex hormone levels (i.e., estrogen and progesterone) (Charkoudian & Stachenfeld, 2014; Charkoudian & Stachenfeld, 2016) that occur throughout the menstrual cycle as well as during menopause. Furthermore, it has been demonstrated that in comparison to males, females exhibit a reduced capacity to dissipate heat independent of sex-related differences in body morphology and aerobic capacity (Gagnon *et al.*, 2008; Gagnon & Kenny, 2011, 2012).

3.2 Experimental Design

In each protocol, participants completed one preliminary and one experimental session. Prior to the start of each session, participants were instructed to refrain from alcohol, caffeine and/or strenuous physical activity for a minimum of 24 hours prior to each session. Furthermore, participants were instructed to drink 500 mL of water the night before as well as ~2 hours prior to each session to ensure adequate hydration and were also be asked to consume a small breakfast (no coffee or tea) approximately two hours before reporting to the laboratory on the day of each session.

3.2.1 Preliminary Session

During the screening session, body height, mass, density, as well as the peak rate of oxygen consumption (VO_{2peak}) were determined. Body height was measured with a physician 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 will be subsequently calculated from the measurements of body height and mass (Dubois & Dubois, 1916). Body density was measured using the hydrostatic weighing technique and used to calculate body fat percentage (Siri, 1956). VO_{2peak} was assessed by an automated indirect calorimetry system (MCD Medgraphics Ultima Series, Sun Tech Medical, MN, USA) during a progressive incremental cycling protocol performed on a semi-recumbent cycle ergometer (Corival, Lode B.V., Groningen, the Netherlands). Participants performed one minute of cycling at a starting workload of 100 W for the younger adults and 60 W for the older adults. Thereafter, the workload was increased by 20 W every minute until volitional fatigue and/or the participant could no longer maintain a

pedaling cadence >50 revolutions·min^{-1}. During the maximal incremental cycling protocol, the older participants were monitored via electrocardiogram by a trained technician.

3.2.2 Experimental Sessions

Upon arrival to the laboratory on the day of the experimental session, participants provided a urine sample for the measurement of urine specific gravity (Reichert TS 400 total solids refractometer, Reichert Inc., Depew, NY, USA) and voided the remainder of their bladder prior to a baseline measurement of nude body mass. Participants were then rested in an upright-seated position for a 30-minute instrumentation period in a thermoneutral experimental room. During this time, four microdialysis fibres (Bioanalytical Systems Inc., West Lafayette, IN, USA) were inserted under aseptic conditions by advancing a 25-gaurge needle 20-25 mm through the dermal layer of the dorsal forearm skin. The microdialysis fibre was then passed through the lumen of the needle, which was subsequently withdrawn, leaving a 10 mm dialysis membrane within the dermal layer. Each fibre was secured to the skin using surgical tape and separated by ~2-4 cm, a distance sufficient to prevent 'cross-contamination' between treatment sites (Meade *et al.*, 2016). All microdialysis fibres were then perfused with lactated Ringer's solution at a rate of 4 μL·min^{-1} via a perfusion pump (model 400, CMA, Microdialysis, Solna, Sweden) for ~30 min. During this time, each skin site was instrumented for the measurement of local sweat rate and cutaneous blood flow.

Following the 30-min instrumentation period, participants entered a thermally controlled chamber regulated to 35˚C and 20% relative humidity wherein they performed one of two experimental protocols. In both protocols, participants remained seated on a semi-recumbent cycle ergometer located in the thermal chamber while the four microdialysis fibres were perfused

with pharmacological agents to evaluate the influence of age related increases in oxidative stress (protocol I; see section 3.2.2.1) or arginase activity as well as the effect of low-dose NO delivery (protocol II; see section 3.2.2.2) on cutaneous vasodilation and sweating. All agents employed (see sections 3.2.2.1, 3.2.2.2 and 3.4) were perfused in a counterbalanced manner (i.e., the location of the forearm [proximal-distal] perfused by each agent was not consistent between participants) at a rate of 4·μL min^{-1} via a microinfusion pump (Model 400, CMA Microdialysis, Solna, Sweden). Prior to the start of each protocol, each fibre was perfused with the respective pharmacological agents for ~60 min while final instrumentation of the participant was completed. It is important to note that a minimum of 90 min was allowed between fibre insertion and the start of the experimental protocol (i.e., 30 min prior to entering the thermal chamber and 60 min thereafter), which has been shown to be a sufficient time period for resolution of the local haemodynamic response induced by fibre insertion (Hodges *et al.*, 2009).

Following the 60-min perfusion period, 10 min of baseline data collection ensued. Thereafter, participants performed two successive 30-min bouts of semi-recumbent cycling with each exercise bout followed by a 20-min recovery period. Following the intermittent cycling protocol, each microdialysis fibre was perfused with 50 mM sodium nitroprusside (SNP, Hospira, Lake Forest, IL, USA) at a rate of 6 μL·min^{-1} to determine maximum cutaneous blood flow. Once a stable plateau in cutaneous blood flow was achieved (~20-30 min), a measurement of blood pressure was taken for the determination of maximal cutaneous vascular conductance (CVC_{max}). Following the observation of a stable plateau, the remaining instrumentation was removed and a final nude body mass measurement was collected. In both protocols, participants performed the intermittent cycling protocol as outlined above. However, to assess the influence of age-related increases in oxidative stress (protocol I) and arginase activity as well as low-dose

NO administration (protocol II) on the heat loss responses, different pharmacological agents were employed between protocols. Moreover, the intensity of exercise, defined as an absolute rate of metabolic heat production to ensure a similar drive for whole-body heat loss (Gagnon *et al.*, 2013; Kenny & Jay, 2013), also differed between the protocols.

3.2.2.1 Protocol I

In protocol I, the microdialysis fibres were assigned to receive 1) lactated Ringer's serving as a control (**Control**); 2) 10 mM N^G-nitro-$_L$-arginine methyl ester (**L-NAME;** Sigma-Aldrich), a non-selective NO synthase inhibitor; 3) 10 mM ascorbate (**Ascorbate**; Sigma-Aldrich), a non-selective antioxidant or 4) a combination of 10 mM ascorbate and 10 mM L-NAME (**L-NAME + Ascorbate**). These concentrations were chosen based on previous studies employing intradermal microdialysis in human skin for L-NAME (Holowatz *et al.*, 2003; Holowatz *et al.*, 2006b, a; Welch *et al.*, 2009; Fujii *et al.*, 2014; McGinn *et al.*, 2014a, b; Stapleton *et al.*, 2014a; Fujii *et al.*, 2015a, c; Meade *et al.*, 2015; Louie *et al.*, 2016) and ascorbate (Holowatz *et al.*, 2006a; Stewart *et al.*, 2008; Fujii *et al.*, 2015b; Meade *et al.*, 2015). By comparing the cutaneous vasodilatory and sweating responses during the separate and combined administration of ascorbate and inhibition of NO synthase to the untreated Control site in young and older adults, information regarding the influence of age-related increases in oxidative stress on NO-dependent heat loss was gleaned. Both exercise bouts were performed at a fixed rate of metabolic heat production of 500 W.

3.2.2.2 Protocol II

In protocol II, each microdialysis fibre received either 1) lactated Ringer's (**Control**); 2) 10 mM L-NAME (**L-NAME**), 3) 5 mM N^{ω}-hydroxy-nor-Arginine (Nor-NOHA; Sigma-Aldrich) + 5 mM S-(2-boronoethyl)-L-cysteine (BEC; Sigma-Aldrich) to inhibit arginase activity (**Nor-NOHA + BEC**); or 4) 1 μM SNP (Sigma-Aldrich), a nitric oxide donor (**SNP**). As in protocol I, these concentrations were chosen based on previous human studies employing intradermal microdialysis for Nor-NOHA and BEC (Holowatz *et al.*, 2006b, a; Stanhewicz *et al.*, 2012) as well as low-dose SNP (Houghton *et al.*, 2006). Assessing the influence of arginase inhibition and low dose SNP administration on the heat loss responses relative to the control site in the younger and older adults allowed for evaluation of the influence of arginase on the heat loss responses as well as provide information regarding how aging influences the sensitivity of the end-organ (i.e., cutaneous vasculature and sweat gland) to NO. In protocol II, both exercise bouts were performed at a fixed rate of metabolic heat production of 400 W.

3.3 Measurements

3.3.1 Core and skin temperature responses

A pediatric thermocouple probe of ~2 mm in diameter (Mon-a-therm, Mallinckrodt Medical, St. Louis, MO) inserted through the nose 40 cm past the entrance of the nostril was used for the continuous measurement of esophageal temperature. Mean skin temperature was calculated using 4 skin sites weighted to the regional proportions: 30% upper back, 30% chest, 20% thigh and 20% calf. All temperature data was collected using a data acquisition module at a sampling rate of 15 s and simultaneously displayed and recoded in spreadsheet format on a personal computer with LabVIEW software (version 7.0; National Instruments, TX, USA).

3.3.2 Local Heat Loss Responses

Cutaneous red blood cell flux (an index of cutaneous blood flow, expressed in perfusion units) was locally measured at a sampling rate of 32 Hz via laser-Doppler flowmetry (PeriFlux system 5000, Perimed, Stockholm, Sweden) using integrated laser-Doppler flowmetry probes with a 7-laser array (Model 413, Perimed) placed over the centre of each microdialysis fibre and housed within each sweat capsule (see below). Cutaneous vascular conductance (CVC) was evaluated as cutaneous red blood cell flux divided by mean arterial pressure (perfusion units·mmHg^{-1}) and presented as a percentage of CVC_{max} as determined during the maximal cutaneous vasodilation procedure.

Local sweat rate was measured using a specialized ventilated capsule placed directly over the center of each microdialysis membrane and affixed to the skin using double-sided adhesive rings and topical skin glue (Collodion HV, Mavidon Medical products, Lake Worth, FL, USA). The sweat capsules employed were specially designed to encompass the entire area of skin perfused by the microdialysis fibre, which we recently evaluated (Meade *et al.*, 2016). Dry compressed air was passed through each capsule at a rate of 0.2 L·min^{-1} while the water content of the effluent air was measured using capacitance hygrometry (Model HMT333, Vaisala, Helsinki, Finland). Long vinyl tubes were used for connections between the gas tanks and sweat and between the sweat capsule and hydrometer to allow for the internal gas temperature equilibrate with environmental temperature (~35˚C). Local sweat rate was calculated every 5 seconds using the difference in water content between the effluent and influent air multiplied by the flow rate and normalized for the skin surface are under the capsule (mg·min^{-1}·cm^{-2}).

3.3.3 Cardiovascular Responses

Arterial systolic and diastolic arterial pressures were monitored every 5 min with manual auscultation performed using a validated mercury column sphygmomanometer (Baumanometer Standby Model, WA Baum Co, Copiague, NY, USA). Mean arterial pressure was then calculated as diastolic pressure plus one-third pulse pressure (i.e., the difference between systolic and diastolic pressure).

A Polar coded transmitter was used to monitor heart rate. Data was recorded continuously and stored every 15 s using a Polar coded WearLink and transmitter, Polar RS400 interface, and Polar Trainer 5 software (Polar Electro, Oy, Kempele, Finland).

3.3.3 Metabolic heat production

Metabolic energy expenditure was measured with indirect calorimetry (Nishi, 1981). The oxygen and carbon dioxide concentrations of expired air was analyzed using electrochemical gas analyzers (AMETEK model S-3A/1 and CD3A, Applied electrochemistry, Pittsburg, PA, USA). A gas mixture of known concentration was used to calibrate the gas analyzer and a 3 L syringe was used to calibrate the turbine ventilometer approximately 30 min before the start of baseline data collection. Participants wore a full face mask (Model 7600 V2, Hans-Rudolf, Kansas City, MO, USA) attached to a 2-way T-shape non-rebreathing valve (Model 2700, Hans-Rudolf). Oxygen uptake and respiratory exchange ratio were obtained as 30 s averages and subsequently used to calculate metabolic rate. Metabolic heat production was then estimated as metabolic rate minus external work (Kenny & Jay, 2013).

3.3.4 Urine specific gravity

A handheld total solids refractometer (Model TS400, Reichter Inc., Depew, NY, USA) was used to evaluate urine specific gravity (an index of body fluid status) from the urine samples provided by the participant prior to the experimental protocol. Euhydration was defined as a urine specific gravity < 1.020, in line with the current guidelines regarding fluid replacement during physical activity (American College of Sports Medicine *et al.*, 2007).

3.4 Vasoactive and sudomotor agents

To assess the influence of aging on nitric oxide dependent cutaneous vasodilation and sweating, the following vasoactive and sudomotor agents were used in the employed experimental protocols (as described in Section 3.2.2.1 and 3.2.2.2).

1) Lactated Ringer's is a specialized solution that is isotonic to blood and was perfused at a Control site in each protocol to eliminate a potential confounding influence of perfusion via microdialysis on local cutaneous blood flow and sweating responses.
2) L-N^G-Nitroarginine methyl ester (L-NAME) is a non-selective NO synthase inhibitor. Blocking the enzymatic production of NO during heat stress diminishes the heat loss responses of cutaneous vasodilation and sweating to a greater extent in young relative to older adults (Fujii *et al.*, 2016).
3) Ascorbate is a non-selective antioxidant. Local infusion of ascorbate has been shown to augment the cutaneous vasodilatory response to whole-body passive heating in older adults (Holowatz *et al.*, 2006a).
4) N^{ω}-hydroxy-nor-Arginine (Nor-NOHA) is a non-selective arginase inhibitor. Blocking enzymatic activity of arginase has been shown to augment the cutaneous vasodilatory

response to whole-body passive heating in older adults (Holowatz *et al.*, 2006b, a; Stanhewicz *et al.*, 2012).

5) S-(2-boronoethyl)-L-cysteine (BEC) is a non-selective arginase inhibitor that has been previously used in conjunction with Nor-NOHA to ensure full blockade of arginase activity in human skin (Holowatz *et al.*, 2006b, a; Stanhewicz *et al.*, 2012).
6) Sodium Nitroprusside (SNP) is a nitric oxide donor which was delivered to the skin in small concentrations (i.e., 1 μM) in protocol II (Houghton *et al.*, 2006) and utilized in larger concentrations (i.e., 50 mM) at the end of each protocol during the maximal cutaneous vasodilation procedure (Holowatz *et al.*, 2003; Holowatz *et al.*, 2006b, a; Fujii *et al.*, 2014; McGinn *et al.*, 2014a, b; McNamara *et al.*, 2014; Fujii *et al.*, 2015a, b, c; Meade *et al.*, 2015; Fujii *et al.*, 2016).

3.5 Data analyses

Values for local forearm CVC and sweat rate at each treatment site as well as body core and skin temperatures and heart rate during the experimental protocol were presented as 5-min average of data recorded at the end of the following time periods: baseline, exercise 1 (25-30 min), recovery 1 (15-20 min), exercise 2 (25-30 min) and recovery 2 (15-20 min). Furthermore, blood pressure data was presented as an average of the two measurements taken over five minutes at each of the aforementioned time points. CVC_{max} was determined from averaged CVC data over a minimum of 2 min once a plateau was attained during the maximal cutaneous vasodilation procedure performed at the end of each protocol. Finally, the difference in sweat rate from control site at the L-NAME, Ascorbate, and L-NAME + Ascorbate sites in protocol I was determined during each exercise bout in the older adults (see statistical analysis section).

3.6 Statistical Analysis

To assess the effect of each pharmacological treatment, local CVC and sweat rate were analyzed using a two-way repeated-measures analysis of variance within each protocol with the factors of time period (five levels: baseline, exercise 1, recovery 1, exercise 2 and recovery 2) and of treatment site (four levels: protocol I: Control, L-NAME, Ascorbate and L-NAME + Ascorbate; protocol II: Control, L-NAME, Nor-NOHA + BEC and SNP). To compare physiological responses between age groups, a separate two-way mixed model analysis of variance was performed with the factors of time (five levels) and age (two levels: young and older) to evaluate CVC and sweating at the Control site as well as the body (esophageal and mean skin) temperature and cardiovascular (mean arterial pressure and heart rate) variables. Local forearm absolute maximal CVC (expressed in perfusion units (PU)·mm Hg^{-1}) attained during SNP infusion was analyzed for each protocol with a two-way mixed-model analysis of variance with the factors of treatment site (four levels) and age (two levels). When a significant main effect was observed, *post hoc* multiple comparisons were carried out using Student's paired t-tests and corrected for multiple comparisons to maintain a fixed chance of Type I error of 5% using the Holm-Bonferroni procedure. Furthermore, Student's independent t-test were used in each protocol to compare participant physical characteristics as well as pre-trial urine specific gravity and the percent change in body mass during the trial between the young and older adults. The level of significance for all analyses was set at $P \leq 0.05$. All values were reported as mean ± 95 % confidence interval (calculated as 1.96 · standard error of the mean).

During data analysis in protocol I, a highly variable influence of ascorbate administration on the local sweating response in older adults was observed. For this reason, data for additional

participants were collected (i.e., n=19) and the influence of each treatment on local sweat rate (i.e., the change in sweat rate from Control at the L-NAME, Ascorbate, and L-NAME + Ascorbate sites) was compared to participant VO_{2peak} as well as the absolute local sweat rate achieved at the Control site during both exercise bouts via Pearson product moment correlations. Likewise, a Pearson Product moment correlation was used to assess the relationship between VO_{2peak} and local sweat rate at Control.

CHAPTER IV

RESULTS

4.1. Protocol I

4.1.1. Participant characteristics

The characteristics (mean ± standard deviation) of the young and older participants in protocol I are presented in table 1. In comparison to their younger counterparts, age was greater in the older adults ($P<0.01$) whereas no differences between age groups were noted for body height ($P=0.89$), weight ($P=0.78$) or surface area ($P=0.84$). Furthermore, VO_{2peak} ($P<0.01$) and body fat percentage ($P<0.01$) were lower and greater, respectively, in the older relative to younger adults.

Table 1. Participant characteristics for protocol I

	Young	**Older**
Age (yrs)	24 ± 4	62 ± 7*
Body height (m)	1.75 ± 0.07	1.75 ± 0.05
Body weight (kg)	78.9 ± 10.1	77.7 ± 10.6
Body surface area (m^2)	1.94 ± 0.14	1.93 ± 0.14
VO_{2peak} ($mLO_2 \cdot min^{-1} \cdot kg^{-1}$)	45.3 ± 6.6	36.7 ± 6.8*
Body fat %	14.9 ± 4.6	27.3 ± 7.3*

Presented values are mean ± standard deviation. Young adults n=11, older adults n=19. VO_{2peak}, rate of peak oxygen consumption as determined during a maximal incremental cycling protocol; Body fat % assessed using the hydrostatic weighing technique. *, $P \leq 0.05$ vs. Young.

4.1.2. Hydration status, body temperature and cardiovascular variables

Pre-trial measures of urine specific gravity indicated that, in line with current guidelines (i.e., urine specific gravity <1.020), both the young (1.010 ± 0.004) and older (1.012 ± 0.007) participants were adequately hydrated prior to the start of the experimental protocol (American College of Sports Medicine *et al.*, 2007). Furthermore, no differences in pre-trial urine specific gravity ($P=0.24$) or the change in body weight during the experimental protocol (young, 1.84 ± 0.21%; older, 1.79 ± 0.32%) were observed between age groups ($P=0.40$).

In the young adults, esophageal temperature (table 2) was elevated from baseline values during both exercise bouts (both $P<0.01$) and recovery periods (both $P<0.01$; main effect of time, $P<0.01$). Furthermore, esophageal temperature was greater at the end of the second exercise bout and recovery in comparison to the first exercise ($P<0.01$) and recovery ($P<0.01$), respectively. In parallel, esophageal temperature was increased from baseline values throughout the experimental protocol in the older adults (all $P<0.01$). Moreover, esophageal temperature was elevated in the second relative to first exercise bout ($P<0.01$) and recovery period ($P<0.01$). No differences in esophageal temperature were observed between the young and older adults (main effect of age group, $P=0.57$). In both the young and older adults, mean skin temperature (table 2) was elevated from baseline values at the end of both exercise bouts (all $P\leq0.02$; main effect of time, $P<0.01$). However, no differences were observed between age groups (main effect of age group, $P=0.62$).

Mean arterial pressure (table 2) was elevated from baseline values during both exercise bouts (both $P\leq0.02$) and reduced from baseline in the first ($P=0.02$) but not second ($P=0.29$) recovery in the young adults (main effect of time, $P<0.01$). Similarly, in the older adults, mean arterial pressure was elevated from baseline during both exercise bouts (both $P<0.01$). Mean arterial pressure was attenuated compared to baseline in the second recovery ($P<0.01$) but not the first ($P=0.07$) recovery. Throughout the incremental exercise protocol, mean arterial pressure was elevated in the older relative to younger adults (all $P\leq0.02$) with the exception of the second recovery ($P=0.39$; main effect of age group, $P=0.02$). Heart rate (table 2) was elevated from baseline values throughout the incremental exercise protocol in both the young and older adults (all $P<0.01$; main effect of time, $P<0.01$). Furthermore, heart rate was increased in the second relative to first exercise (both $P<0.01$) and recovery (both $P<0.01$). However, no differences in heart rate were observed between the young and older adults (main effect of age group, $P=0.62$).

Table 2. Body temperatures and cardiovascular responses during resting and intermittent exercise in protocol I.

	Baseline	Exercise 1	Recovery 1	Exercise 2	Recovery 2
Esophageal temperature, ˚C					
Young	37.02 ± 0.11	37.61 ± 0.19*	37.33 ± 0.14*	37.82 ± 0.19*‡	37.44 ± 0.16*‡
Older	36.99 ± 0.18	37.78 ± 0.18*	37.32 ± 0.13*	38.00 ± 0.19*‡	37.41 ± 0.13*‡
Mean skin temperature, ˚C					
Young	35.01 ± 0.29	35.56 ± 0.38*	35.11 ± 0.31	35.59 ± 0.39*	35.16 ± 0.36
Older	34.95 ± 0.33	35.43 ± 0.30*	35.02 ± 0.34	35.51 ± 0.48*	35.08 ± 0.37
Mean arterial pressure, mmHg					
Young	90 ± 4	97 ± 5*	85 ± 5*	95 ± 5*	88 ± 7
Older	96 ± 5†	107 ± 6*†	93 ± 5†	104 ± 6*†	91 ± 4*
Heart rate, beats·min^{-1}					
Young	71 ± 6	130 ± 11*	85 ± 7*	135 ± 12*‡	88 ± 7*‡
Older	69 ± 6	123 ± 13*	83 ± 10*	130 ± 14*‡	89 ± 11*‡

Presented values are mean ± 95% confidence interval. Young (n=11) and older (n=19) adults performed an intermittent exercise protocol consisting of two exercise bouts each followed by a recovery period. Both exercise bouts were performed at a fixed rate of heat production of 500 W. Esophageal and mean skin temperatures as well as heart rate responses represent an average of the final 5 min of the corresponding time period. Mean arterial pressure values represents an average of two measurements taken over the final 10 minutes of the corresponding time period. *, significant difference vs. Baseline; ‡, significant difference between Exercise 1 vs Exercise 2 or Recovery 1 vs Recovery 2; †, significant difference vs. young; P≤0.05.

4.1.3. Local forearm cutaneous vascular conductance response

4.1.3.1. Young adults

Local forearm CVC (figure 4) was elevated from baseline values during both exercise bouts (all $P \leq 0.03$) but similar to baseline during both recovery periods (all $P \geq 0.17$) in the young adults at all treatment sites (main effect of time, $P < 0.01$). Throughout baseline as well as both exercise bouts and recovery periods, CVC was attenuated at the L-NAME (all $P < 0.01$) and L-NAME + Ascorbate skin (all $P \leq 0.03$) sites (main effect of treatment site, $P < 0.01$). In contrast, no differences in CVC were noted between Control and Ascorbate (all $P \geq 0.26$).

4.1.3.2. Older adults

In the older adults, forearm CVC (figure 4) was elevated from baseline values throughout both exercise bouts and recovery periods at all treatment sites (all $P \leq 0.03$; main effect of time, $P < 0.01$). At baseline, CVC was reduced compared to Control at the L-NAME ($P \leq 0.01$) and L-NAME + Ascorbate ($P = 0.01$) skin sites but elevated at the Ascorbate site ($P = 0.04$; main effect of treatment site, $P < 0.01$). During both exercise bouts, CVC remained reduced from Control at L-NAME (both $P \leq 0.02$), whereas no differences were observed between Control and either Ascorbate (both $P \geq 0.09$) or L-NAME + Ascorbate (both $P \geq 0.15$). Finally, during bother recovery periods, CVC was reduced from Control at the L-NAME (both $P \leq 0.02$) and L-NAME + Ascorbate (both $P \leq 0.01$) skin sites; however, no differences were observed at the Ascorbate site (both $P \geq 0.10$).

4.1.4.3. Comparison between age groups

No differences in local forearm CVC were observed between age groups at the Control site (main effect of condition, P=0.15). Furthermore, when CVC_{max}, obtained during perfusion of 50 mM SNP at the end of the trial, was assessed, no differences were observed between the young and older adults at the Control (young, 1.73 ± 0.10 PU·mmHg^{-1}; older, 1.80 ± 0.12 PU·mmHg^{-1}), L-NAME (young, 1.88 ± 0.16 PU·mmHg^{-1}; older, 1.79 ± 0.10 PU·mmHg^{-1}), Ascorbate (young, 1.68 ± 0.14 PU·mmHg^{-1}; older, 1.66 ± 0.09 PU·mmHg^{-1}) or L-NAME + Ascorbate (young, 1.60 ± 0.15 PU·mmHg^{-1}; older, 1.67 ± 0.13 PU·mmHg^{-1}) skin sites (main effect of age group, P=0.97). Likewise, similar CVC_{max} responses were observed between treatment sites within each age group (main effect of treatment site, P=0.63)

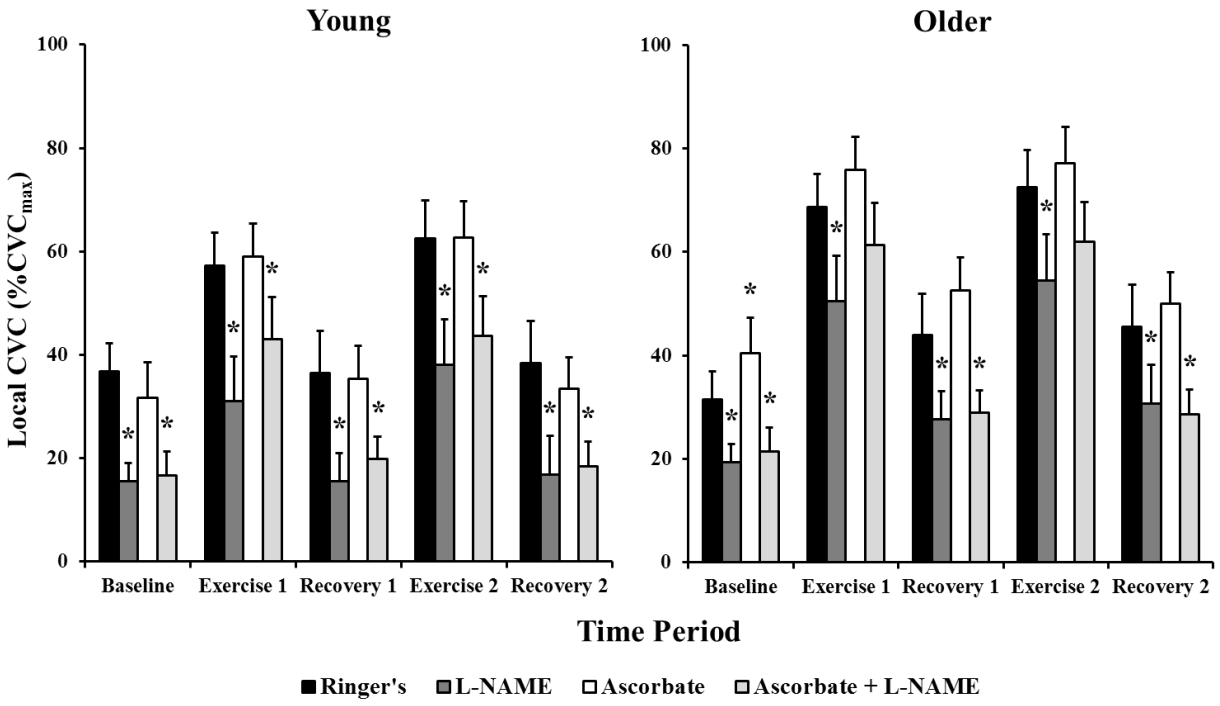

Figure 4. Local forearm cutaneous vascular conductance (CVC; $\%CVC_{max}$) during the two successive exercise bouts performed at a fixed rate of metabolic heat production of 500 W for the young (n=11; panel A) and older (n=19; panel B) adults in protocol I. Throughout the intermittent exercise protocol, four skin sites were continuously perfused via intradermal microdialysis with 1) lactated Ringer's (black bars) to act as a control; 2) 10 mM N^G-nitro-$_L$-arginine methyl ester (L-NAME, dark grey bars) to inhibit nitric oxide synthase activity; 3) 10mM ascorbate (Ascorbate, white bars), an antioxidant, or; 4) a combination of 10 mM L-NAME + 10 mM Ascorbate (L-NAME + Ascorbate, light grey bars). Values are mean ± 95% confidence interval. Each data point represents an average of the last 5 min of each time period. *, significant difference vs. Control; P≤0.05.

4.1.4. Local forearm sweat response

4.1.4.1. Young adults

In the young adults, local sweat rate (figure 5) was elevated from baseline values at all treatment sites during each exercise and recovery periods (all $P<0.01$; interaction of time and treatment site, $P=0.05$). While no differences in local sweat rate were observed between treatment sites at rest (all $P\geq0.06$), local sweat rate was attenuated at the L-NAME (both $P\leq0.04$) and L-NAME + Ascorbate (both $P\leq0.04$) sites in comparison to Control during both exercise bouts. In contrast, during exercise no differences in local sweat rate between Control and Ascorbate were observed (both $P\geq0.36$). With the exception of L-NAME + Ascorbate during the first bout ($P=0.01$), no differences in local sweat rate were observed between Control and any treatment site during either recovery period (all $P\geq0.12$).

4.1.4.2. Older adults

Local sweat rate in the older adults (figure 5) was increased from baseline values at the end of both exercise bouts and recovery periods (all $P<0.01$; main effect of time, $P<0.01$). However, in contrast to the younger adults, no differences were observed between treatment sites throughout the experimental protocol (main effect of treatment site, $P=0.42$).

4.1.4.3. Comparison between age groups

When a comparison was made between local sweat rate measured at the Control site between older and younger adults, no differences were noted (main effect of age group, $P=0.16$).

4.1.3.2.1. Correlational analysis

No association was found between VO_{2peak} and the influence of L-NAME infusion on local sweat rate during either exercise bout (exercise 1, r=-0.128; exercise 2, r=-0.194; both P≥0.43). In contrast, a negative correlation was observed between VO_{2peak} and the change in sweat rate at the Ascorbate site during both the first (r=-0.55; P=0.02) and second (r=-0.54; P=0.02) exercise. Furthermore, the influence of combined L-NAME + Ascorbate infusion on local sweat rate was found to be negatively correlated in the second (r=-0.54; P=0.02) but not first (r=-0.42; P=0.08) exercise bout. When a comparison was made between the change in local sweat rate induced at each treatment site and the level of sweating achieved at Control, a negative correlation was observed for L-NAME in the first (r=-0.54; P=0.02) exercise bout but not the second (r=-0.40; P=0.09). During both exercise bouts, a negative correlation also existed between sweat rate at the control and the change in sweat rate induced at both the Ascorbate (exercise 1, r=-0.67; exercise 2, r=-0.67; both P>0.01) and L-NAME + Ascorbate (exercise 1, r=-0.54; exercise 2, r=-0.58; both P≥0.02) skin sites. Finally, a correlation was observed between VO_{2peak} and local sweat rate at the Control site during both the first (r=0.45; P=0.05) and second (r=0.47; P=0.04) exercise bouts.

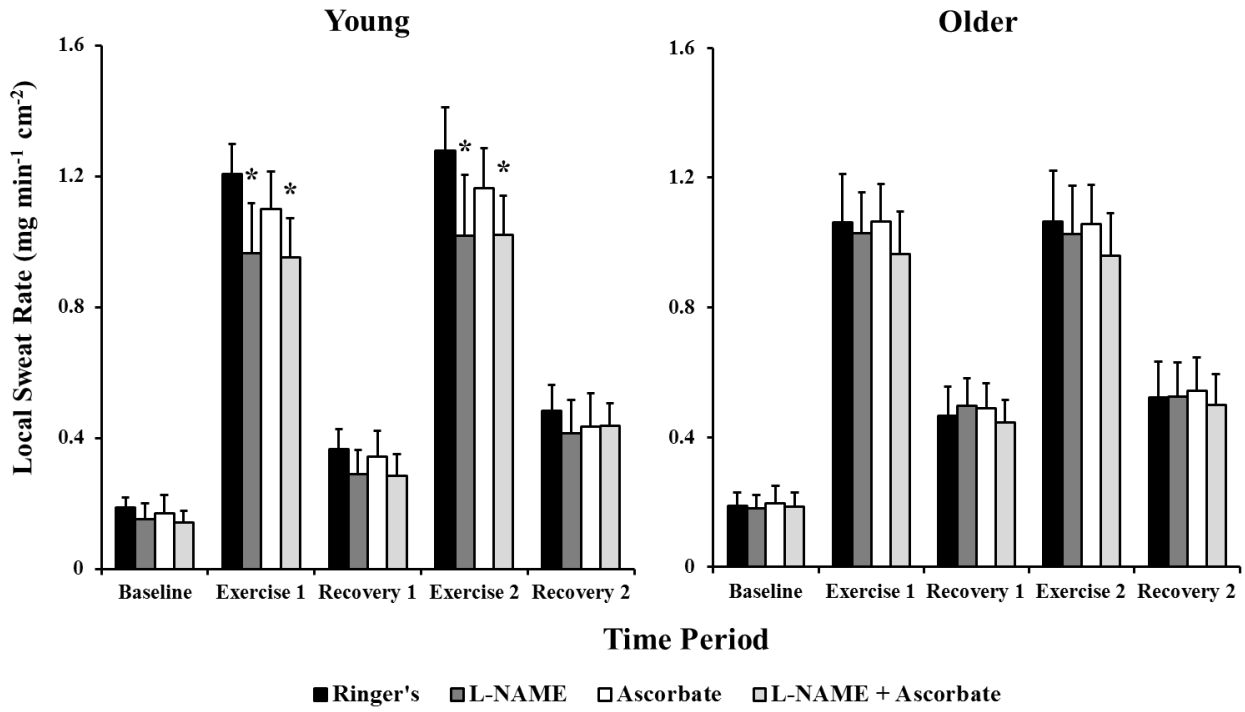

Figure 5. Local forearm sweat rate ($mg \cdot min^{-1} \cdot cm^{-2}$) during the two successive exercise bouts performed at a fixed rate of metabolic heat production of 500 W for the young (n=11; panel A) and older (n=19; panel B) adults in protocol I. Throughout the intermittent exercise protocol, four skin sites were continuously perfused via intradermal microdialysis with 1) lactated Ringer's (black bars) to act as a control; 2) 10 mM N^G-nitro-$_L$-arginine methyl ester (L-NAME, dark grey bars) to inhibit nitric oxide synthase activity; 3) 10mM ascorbate (Ascorbate, white bars), an antioxidant, or; 4) a combination of 10 mM L-NAME + 10 mM Ascorbate (L-NAME + Ascorbate, light grey bars). Values are mean ± 95% confidence interval. Each data point represents an average of the last 5 min of each time period. *, significant difference vs. Control; $P \leq 0.05$.

4.2. Protocol II

4.2.1. Participant physical characteristics

The characteristics (mean ± standard deviation) of the young and older participants in protocol II are presented in table 3. Age was greater in the older adults (P<0.01) whereas no differences between age groups were noted for body height (P=0.69), weight (P=0.30) or surface area (P=0.40) between age groups. Furthermore, VO_{2peak} (P<0.01) and body fat percentage (P<0.01) were lower and greater, respectively, in the older relative to younger adults.

Table 3. Participant characteristics for protocol II

	Young	**Older**
Age (yrs)	23 ± 3	66 ± 6
Body height (m)	1.73 ± 0.06	172 ± 0.09
Body weight (kg)	75.6 ± 5.8	71.4 ± 10.3
Body surface area (m^2)	1.90 ± 0.09	1.84 ± 0.18
VO_{2peak} ($mLO_2 \cdot min^{-1} \cdot kg^{-1}$)	45.9 ± 7.1	32.1 ± 2.7*
Body fat %	13.6 ± 4.2	25.2 ± 4.5*

Presented values are mean ± standard deviation. Young adults n=9, older adults n=9. VO_{2peak}, rate of peak oxygen consumption as determined during a maximal incremental cycling protocol; Body fat % assessed using the hydrostatic weighing technique. *, significant difference vs. Young; P≤0.05.

4.2.2. Body temperature, cardiovascular and hydration status variables

Urine specific gravity was similar between age groups (young, 1.013 ± 0.005; older, 1.016; P=0.41) and indicated that both groups were adequately hydrated (i.e., urine specific gravity <1.020) (American College of Sports Medicine *et al.*, 2007). Furthermore, the change in body weight over the experimental protocol was not different between the young (-1.64 ± 0.28%) and older (-1.50 ± 0.28%) adults (P=0.49).

In the young adults, esophageal temperature (table 4) was elevated from baseline values during both exercise bouts (both $P<0.01$) and the first ($P<0.01$) but not second recovery ($P=0.08$; main effect of time, $P<0.01$). Furthermore, esophageal temperature was elevated in the second relative to first exercise bout ($P=0.02$). However, no differences in esophageal temperature were observed between recovery bouts ($P=0.62$). In the older adults, esophageal temperature was elevated from baseline values throughout both exercise bouts (both $P<0.01$) and recovery periods (both $P <0.01$). Moreover, esophageal temperature in the older adults was elevated in the second exercise bout in comparison to the first ($P=0.04$) but not in the second relative to first recovery period ($P=0.44$). No differences in esophageal temperature were noted between age groups (main effect of age group, $P=0.61$). In the young adults, skin temperature (table 4) was elevated from baseline during both exercise bouts (both $P\leq0.03$), but not during either recovery period (both $P\geq0.10$; main effect of time, $P<0.01$). Further, no differences in mean skin temperature were observed between exercise bouts ($P=0.71$) or recovery periods ($P=0.74$). In the older adults, mean skin temperature was increased in relation to baseline values during both exercise bouts ($P<0.01$) and recovery periods (both $P<0.01$) as well as in the second exercise bout in comparison to the first ($P<0.01$). In contrast, mean skin temperature was similar between recoveries ($P=0.31$). No differences in mean skin temperature were observed between the young and older adults (main effect of condition, $P=0.61$).

Mean arterial pressure (table 4) remained similar to baseline in the young adults throughout the experimental protocol and did not differ between each exercise bout and recovery period (all $P>0.01$; interaction of time and age group, $P=0.03$). In the older adults mean arterial pressure was elevated from baseline values during both exercise bouts (both $P\leq0.02$) and reduced during the second ($P=0.02$) but not first ($P=0.26$) recovery period. Furthermore, while mean

arterial pressure in the older adults was similar between exercise bouts (P=0.07), the response in the second recovery was reduced relative to that in the first (P=0.05). When a comparison between age groups was made, it was found that a greater mean arterial pressure response occurred in the older relative to younger adults in the first exercise bout (P=0.03), but at no other time point throughout the protocol (all P≥0.10). Heart rate response (table 4), was elevated from baseline values in the younger adults during both exercise bouts (both P<0.01) but similar to baseline during both recovery periods (both P≥0.11; main effect of time, P<0.01). Furthermore, no differences in heart rate were noted between exercise bouts (P=0.09) or recovery periods (P=0.39). In the older adults, heart rate was elevated from baseline values throughout the experimental protocol (all P≤0.01). Moreover, heart rate increased from the first to second exercise bout (P<0.01) and recovery period (P<0.01). However, no differences were observed between age groups (main effect of age group, P=0.95).

Table 4. Body temperatures and cardiovascular responses during resting and intermittent exercise in protocol II.

	Baseline	Exercise 1	Recovery 1	Exercise 2	Recovery 2
Esophageal temperature, ˚C					
Young	37.12 ± 0.22	37.55 ± 0.22*	37.34 ± 0.19*	37.67 ± 0.22*‡	37.37 ± 0.18
Older	37.07 ± 0.11	37.70 ± 0.21*	37.36 ± 0.12*	37.86 ± 0.20*‡	37.42 ± 0.19*
Mean skin temperature, ˚C					
Young	34.97 ± 0.25	35.54 ± 0.25*	35.20 ± 0.30	35.55 ± 0.27*	35.18 ± 0.18
Older	34.83 ± 0.27	35.30 ± 0.24	35.19 ± 0.19	35.38 ± 0.38	35.30 ± 0.19
Mean arterial pressure, mmHg					
Young	89 ± 3	92 ± 4	87 ± 2	91 ± 3	86 ± 3
Older	92 ± 5	101 ± 7*	98 ± 6	98 ± 6*	86 ± 2*
Heart rate, beats·min^{-1}					
Young	76 ± 9	112± 10*	81 ± 7	114 ± 10*	82 ± 0.7
Older	69 ± 10	113 ± 15*	82 ± 11*	120 ± 15*‡	84 ± 12*‡

Presented values are mean ± 95% confidence interval. Young (n=9) and older (n=9) adults performed an intermittent exercise protocol consisting of two exercise bouts each followed by a recovery period. Both exercise bouts were performed at a fixed rate of heat production of 400 W Esophageal and mean skin temperatures as well as heart rate responses represent an average of the final 5 min of the corresponding time period. Mean arterial pressure values represents an average of two measurements taken over the final 10 minutes of the corresponding time period. *, significant difference vs. Baseline; ‡, significant difference in Exercise 1 vs Exercise 2 or Recovery 1 vs Recovery 2; $P \leq 0.05$.

4.2.3. Local forearm cutaneous vascular conductance response

4.2.3.1. Young adults

Relative to baseline, local forearm CVC (figure 6) was elevated during the both exercise bouts at all treatment sites (all $P \leq 0.04$) with the exception of the L-NAME site during the first exercise bout ($P=0.08$; main effect of time, $P<0.01$). In contrast, CVC was similar to baseline values at all treatment sites during both recovery periods (all $P \geq 0.10$) except that it was elevated at the Nor-NOHA + BEC site during the first recovery ($P<0.01$). When a comparison was made between treatment sites, it was found that throughout the experimental protocol CVC was reduced relative to Control at the L-NAME skin site (all $P<0.01$) but similar to Control at the Nor-NOHA + BEC (all $P \geq 0.43$) and SNP (all $P \geq 0.18$) sites (main effect of treatment site, $P<0.01$).

4.2.3.2. Older adults

In the older adults, local forearm CVC (figure 6) was elevated in comparison to baseline at the Control site during both exercise bouts (both $P<0.01$) but neither recovery period (both $P \geq 0.08$; main effect of time, $P<0.01$). At the L-NAME site, CVC remained similar to control throughout the experimental protocol (all $P \geq 0.12$) whereas, in parallel to the Control site, CVC was elevated at the Nor-NOHA + BEC and SNP skin sites during the exercise bouts (all $P \leq 0.02$) but not recoveries (all $P \geq 0.15$; main effect of treatment site, $P<0.01$). At the L-NAME site, CVC was reduced in comparison to Control during baseline ($P<0.01$) and the first ($P=0.02$) but not second ($P=0.17$) exercise bout, as well as during both recovery periods. However, no differences in CVC from Control were observed at Nor-NOHA + BEC (all $P \geq 0.06$) or SNP (all $P \geq 0.10$) throughout the experimental protocol.

4.2.3.3. Comparison between age groups

No differences in CVC were observed between the young and older adults during the intermittent exercise protocol (main effect of age group, P=0.71). Furthermore, CVC_{max} was similar between age groups at the Control (young, 1.85 ± 0.15 PU·mmHg^{-1}; older, 1.86 ± 0.19 PU·mmHg^{-1}), L-NAME (young, 1.89 ± 0.16 PU·mmHg^{-1}; older, 1.79 ± 0.15 PU·mmHg^{-1}), Nor-NOHA + BEC (young, 1.69 ± 0.13 PU·mmHg^{-1}; older, 1.98 ± 0.15 PU·mmHg^{-1}) and SNP (young, 1.84 ± 0.19 PU·mmHg^{-1}; older, 1.48 ± 0.16 PU·mmHg^{-1}) skin sites (main effect of age group, P=0.85). Likewise, no differences in CVC_{max} were noted between treatment sites within each age group (main effect of treatment site, P=0.71).

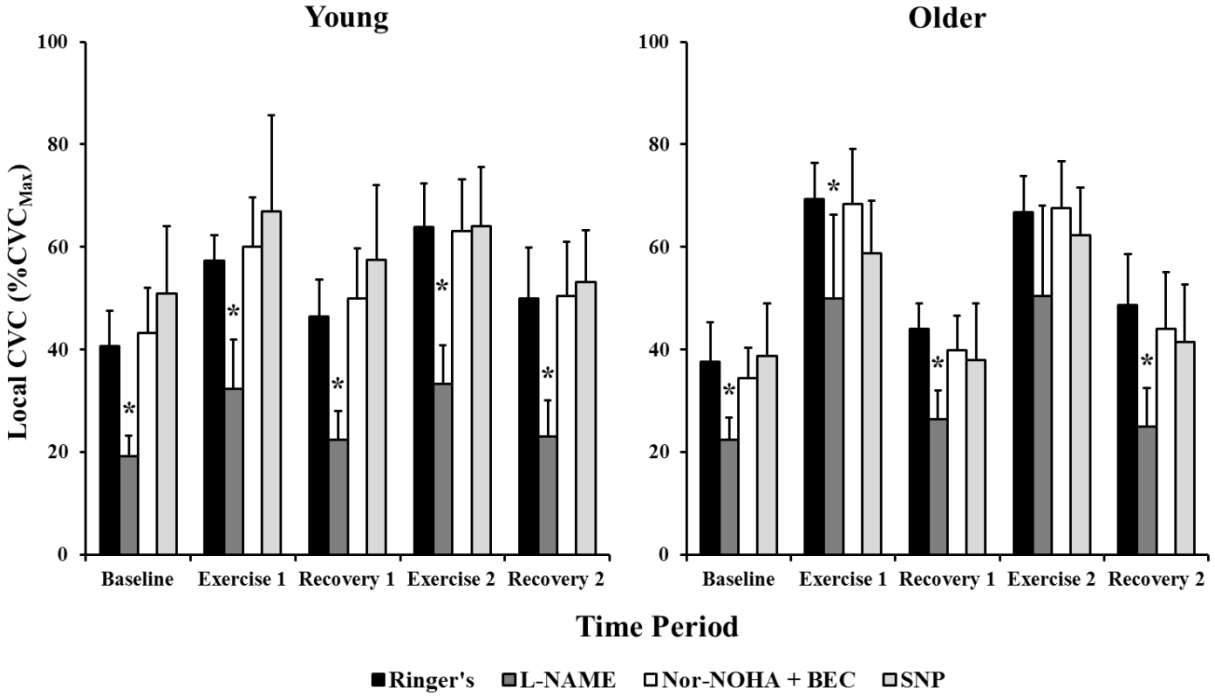

Figure 6. Local cutaneous vascular conductance (CVC; %CVC_{max}) during the two successive exercise bouts performed at a fixed rate of metabolic heat production of 400 W for the young (n=9; left panel) and older (n=9; right panel) adults in protocol II. Throughout the intermittent exercise protocol, four skin sites were continuously perfused via intradermal microdialysis with 1) lactated Ringer's (black bars) to act as a control; 2) 10 mM N^G-nitro-$_L$-arginine methyl ester (L-NAME, dark grey bars) to inhibit nitric oxide synthase activity; 3) 5 mM N^{ω}-hydroxy-nor-Arginine + 5 mM S-(2-boronoethyl)-L-cysteine (Nor-NOHA + BEC, white bars), to inhibit arginase activity, or; 4) 1μM sodium nitroprusside (SNP, light grey bars), a nitric oxide donor. Values are mean ± 95% confidence interval. Each data point represents an average of the last 5 min of each time period.

4.2.4. Local forearm sweat response

4.2.4.1. Young adults

Local sweat rate (figure 7) at all treatment sites was elevated relative to baseline values during both exercise bouts and recovery periods in the younger adults (all $P \leq 0.01$; interaction of time and condition, $P=0.03$). No differences in local sweat rate were observed between treatment sites at baseline (all $P \geq 0.18$). During both exercise bouts, sweat rate was attenuated at the L-NAME (both $P \leq 0.03$) but not Nor-NOHA + BEC (both $P \geq 0.57$) or SNP (both $P \geq 0.38$) skin sites, relative to Control. In parallel to baseline, no differences in local sweat rate were observed between Control and any treatment site during either recovery period (all $P \geq 0.09$).

4.2.4.2. Older adults

In comparison to Baseline, local sweat rate (figure 7) in the older adults was elevated at all treatment sites during both exercise bouts and recovery periods (all $P \leq 0.01$; main effect of time, $P<0.01$). However, no differences in the sweating response were observed between treatment sites (main effect of treatment site, $P=0.28$).

4.2.4.3. Comparison between age groups

No differences in local sweat rate were observed between the young and older participants throughout the experimental protocol (main effect of age group, $P=0.76$).

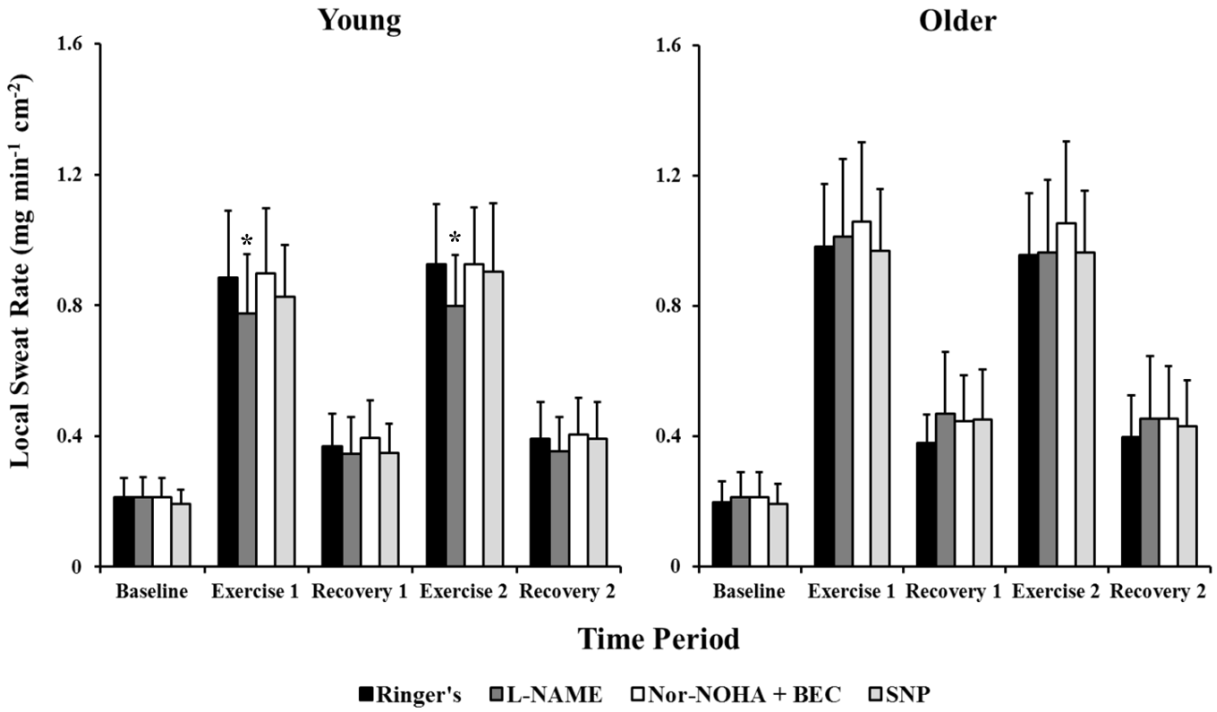

Figure 7. Local forearm sweat rate ($mg \cdot min^{-1} \cdot cm^{-2}$) during the two successive exercise bouts performed at a fixed rate of metabolic heat production of 400 W for the young (n=9; left panel) and older (n=9; right panel) adults in protocol II. Throughout the intermittent exercise protocol, four skin sites were continuously perfused via intradermal microdialysis with 1) lactated Ringer's (black bars) to act as a control; 2) 10 mM N^{G}-nitro-$_{L}$-arginine methyl ester (L-NAME, dark grey bars) to inhibit nitric oxide synthase activity; 3) 5 mM N^{ω}-hydroxy-nor-Arginine + 5 mM S-(2-boronoethyl)-L-cysteine (Nor-NOHA + BEC, white bars), to inhibit arginase activity, or; 4) 1μM sodium nitroprusside (SNP, light grey bars), a nitric oxide donor. Values are mean ± 95% confidence interval. Each data point represents an average of the last 5 min of each time period.

CHAPTER V

DISCUSSION

This thesis sought to determine the influence of age-related increases in oxidative stress and arginase activity as well as changes in the sensitivity of the thermoregulatory end-organs (i.e., the cutaneous vasculature and the sweat gland) to NO on the regulation of cutaneous vasodilation and sweating during exercise in the heat. The findings of protocol I indicate that during passive exposure to a hot environment, oxidative stress impairs NO-dependent cutaneous vasodilation. During exercise, oxidative stress also influences cutaneous vascular regulation, but only when NO production is inhibited. However, the influence of oxidative stress on NO-dependent vasodilation, as observed during baseline, is absent during postexercise recovery. Furthermore, the current thesis suggests that oxidative stress may also influence the sweating response to exercise but that factors such as aerobic fitness and/or sudomotor function play a modulatory role. Finally, in protocol II, it was demonstrated that neither arginase inhibition nor low dose SNP infusion influences the cutaneous vasodilatory and sweating responses in young and older adults during moderate intensity intermittent exercise in the heat.

5.1. Oxidative Stress

During thermal challenge, older adults display reductions in whole-body heat loss (Kenny & Jay, 2013; Larose *et al.*, 2013a; Larose *et al.*, 2013b; Larose *et al.*, 2013c; Larose *et al.*, 2014) in comparison to their younger counterparts as a result of attenuations in the cutaneous blood flow (Kenney, 1988; Holowatz *et al.*, 2003; Holowatz *et al.*, 2006b; Holowatz & Kenney, 2007; Stanhewicz *et al.*, 2012; Smith *et al.*, 2013) and sweating (Inoue *et al.*, 1999; Inbar *et al.*, 2004; Larose *et al.*, 2013a; Larose *et al.*, 2013b; Larose *et al.*, 2013c; Smith *et al.*, 2013; Larose *et al.*, 2014) responses to whole-body passive and exercise induced heat stress. Moreover, aging is associated with impairments in the contribution of NO to cutaneous vasodilation (Fujii *et al.*,

2016) and sweating (Stapleton *et al.*, 2014a; Fujii *et al.*, 2016) during exercise as well as elevations in oxidative stress in the skin due to both an increase in the production of ROS as well as a decrease in the number of ROS scavenging enzymes (Kohen, 1999; Lu *et al.*, 1999; Bouzid *et al.*, 2015a). Given that ROS such as superoxide react readily with NO, thereby decreasing its bioavailability (Mortensen & Lykkesfeldt, 2014), we tested the hypothesis that oxidative stress contributes to the age-related reductions in NO-dependent cutaneous vasodilation and sweating.

Evidence for an influence of oxidative stress on NO-dependent cutaneous vasodilation can be observed during baseline in protocol I wherein local infusion of ascorbate (a non-selective antioxidant) resulted in elevated cutaneous blood flow in older adults but this response was abolished when ascorbate was administered and NO production was concomitantly inhibited (via local infusion of the non-selective NO synthase inhibitor L-NAME). There are several mechanisms by which oxidative stress may influence NO-dependent cutaneous vasodilation. In addition to the direct effect of ROS on NO bioavailability, oxidative stress also results in the uncoupling of NO synthase as well as destabilization of BH_4, an essential co-factor for NO production (Mortensen & Lykkesfeldt, 2014). Regardless of the exact mechanism(s), the current findings are in line with previous work showing that local infusion of ascorbate (Holowatz *et al.*, 2006a) and BH_4 (Stanhewicz *et al.*, 2012) augments the cutaneous vasodilatory response to whole-body passive heating in older adults, with the latter response occurring via NO-dependent mechanisms (the contribution of NO to the former was not assessed).

In contrast to the findings for baseline, independent infusion of ascorbate did not influence cutaneous vasodilation during either exercise bout. When NO synthase was inhibited during administration of ascorbate however, cutaneous vasodilation was augmented in the older adults. This is evidenced by the fact that while independent infusion of L-NAME attenuated

cutaneous vasodilation during exercise, the cutaneous vasodilatory response remained similar to Control during simultaneous infusion of L-NAME and ascorbate. It may be that the large contribution of NO to cutaneous vasodilation masks other vasodilatory mechanisms that are ROS sensitive. For instance, ROS can influence the activity of various ion channels that are known to modulate cutaneous vascular tone such as the sodium-potassium pump (Na^+/K^+-ATPase), sodium-potassium-chloride cotransporter (NKCC), and K^+ channel (Elliott & Schilling, 1992; Elliott & Koliwad, 1995; Liu & Gutterman, 2002). In support of this notion, we recently demonstrated an effect of Na^+/K^+-ATPase activity on NO-independent vasodilation, but again, only when NO production was inhibited (Louie *et al.*, 2016). However, the exact mechanisms underpinning the current and previous (Louie *et al.*, 2016) findings remain to be elucidated.

During postexercise recovery, cutaneous vasodilation returned to baseline values; however, in contrast to baseline, no influence of oxidative stress on NO-dependent cutaneous vasodilation was observed in the older adults. These findings suggest that the mechanisms underpinning cutaneous vasodilation differ between pre- and postexercise. One possible explanation may be that while exercise (Goto *et al.*, 2003), especially when performed in a warm environment (Sureda *et al.*, 2015), has been shown to increase levels of ROS, it is also associated with a postexercise increase in ROS scavenging enzymes. In combination with previous work demonstrating that aging is also associated with elevations in endogenous antioxidant activity (Pansarasa *et al.*, 1999; Inal *et al.*, 2001) it is possible that the lack of a ROS-mediated reduction in NO-dependent in cutaneous vasodilation in older adults during recovery, may be the result of an increase in the activity of endogenous antioxidants. Support for this notion also exists in a recent study from our laboratory wherein we observed that following high intensity exercise, which is associated with marked elevations in oxidative stress (Goto *et al.*, 2003), the influence

of ROS on NO-dependent cutaneous vasodilation was quickly abolished in young adults (Meade *et al.*, 2015). Altogether, the influence of age-related increases in oxidative stress appears to differ depending on the type of heat stress (i.e., passive exposure to a hot environment or exercise-induced) as well as factors such as the performance of exercise *per se.*

In line with previous work (Boulant & Bignall, 1973; Welch *et al.*, 2009; Fujii *et al.*, 2014; McGinn *et al.*, 2014b; Stapleton *et al.*, 2014a; Fujii *et al.*, 2016), NO was observed to contribute to the sweating response to exercise in younger adults whereas, when expressed as the group mean, no influence of NO synthase inhibition on the local sweating response was observed in their older counterparts. However, correlational analysis revealed that the influence of oxidative stress on sweating in older adults may be modulated by secondary factors – namely, aerobic capacity and/or thermal effector activity. Specifically, a moderate negative correlation was observed between the influence of ascorbate infusion on local sweat rate (i.e., the difference in sweat rate between Control and the ascorbate treated skin site) and VO_{2peak} (exercise 1, r=-0.55; exercise 2, r=-0.54) as well as the magnitude of the sweating response observed at the Control site (exercise 1, r=-0.67; exercise 2, r=-0.67). As a potential mechanism, exercise training has been shown to decrease markers of oxidative stress as well as increase endogenous antioxidant levels in older adults during both resting and exercise conditions (Gomez-Cabrera *et al.*, 2008; Traustadottir *et al.*, 2012; Rowinski *et al.*, 2013; Bouzid *et al.*, 2015a; Bouzid *et al.*, 2015b). Furthermore, recent work has demonstrated that while sedentary middle-aged (age, ~49 years) adults (VO_{2peak}, ~37 $ml \cdot min^{-1} \cdot kg^{-1}$) displayed reduced levels of whole-body sweating in relation to their younger counterparts (VO_{2peak} ~50 $ml \cdot min^{-1} \cdot kg^{-1}$), this age-related impairment was abolished in endurance-trained individuals (VO_{2peak}, ~ 51 $ml \cdot min^{-1} \cdot kg^{-1}$) (Stapleton *et al.*, 2015). Of note, the influence of aerobic capacity on whole-body heat loss is also in line with the

current findings of a moderate correlation between VO_{2peak} and sweat rate at Control during both exercise bouts (exercise 1, r=0.45; exercise 2, r=0.47). These results in combination with the current findings suggest that differences in oxidative stress secondary to differences in aerobic fitness and/or habitual activity levels may modulate sweat rate during exercise in older individuals.

It is important to note that the influence of ascorbate on sweat rate during exercise appears to be independent of NO. Specifically, the moderate negative correlation between fitness and the influence of ascorbate on sweating was preserved when L-NAME was co-infused during the second exercise bout (r=-0.54). Similarly, the influence of combined L-NAME and ascorbate perfusion was negatively correlated to the sweat rate achieved at Control during both exercise bouts (exercise 1, r=-0.54; exercise 2, r=-0.58). The reasons underpinning the apparent NO-independent influence of oxidative stress on the sweating response to exercise cannot be determined from the current data. However, the associated mechanisms may be related to the influence of ROS on ion-channels crucial to fluid movement in the eccrine sweat gland, and therefore sweat production and expulsion. As mentioned in the discussion of an NO-independent influence of ROS on CVC, oxidative stress has been shown to influence the function of various membrane transport proteins including the Na^+/K^+-ATPase, NKCC, and K^+ channel (Elliott & Schilling, 1992; Elliott & Koliwad, 1995; Liu & Gutterman, 2002). In line with this notion, using a protocol similar to the current thesis we recently demonstrated that the Na^+/K^+-ATPase is an important contributor to exercise-induced increases in sweat rate via mechanisms independent of NO (Louie *et al.*, 2016). Clearly, future research is warranted to determine the separate and combined influence of factors such as age, fitness and habitual activity level on the precise mechanism underpinning regulation of sweating during exercise.

5.2. Arginase

In addition to elevations in oxidative stress, we hypothesized that age-related increases in the activity of the arginase enzyme (Kohen, 1999; Lu *et al.*, 1999; Berkowitz *et al.*, 2003; White *et al.*, 2006), which more readily reacts with L-arginine (precursor for NO production) in comparison to NO synthase, contribute to the impairments in heat loss seen in older adults (Stapleton *et al.*, 2014a; Fujii *et al.*, 2016). However, the results from protocol II do not support this notion as inhibition of arginase did not influence the mechanisms underlying the heat loss responses in either the young or older adults, despite reductions in NO-dependent cutaneous vasodilation and sweating observed in the older adults during both exercise bouts. This is in contrast to previous findings demonstrating that arginase inhibition augmented the cutaneous vasodilatory response to whole-body passive heating in older adults to a level similar to their younger counterparts (Holowatz *et al.*, 2006a, b).

It is important to note that the mechanisms underpinning the regulation of cutaneous vasodilation, as well as sweating, differ between passively- and exercise-induced heat stress. For example, in the previous studies assessing the influence of age-related changes in arginase activity on the cutaneous vasodilatory response to whole-body passive heating, local skin temperature at each treatment site was maintained at ~33˚C (Holowatz *et al.*, 2006a, b). In contrast, passive exposure to a hot environment (35˚C) in the current study resulted in relatively greater skin temperatures such that mean skin temperature reached values of ~34.9-35.5˚C throughout the incremental exercise bouts in protocol II. Given that *in vitro* studies demonstrate that the activity of enzymes such as NO synthase are temperature sensitive (Venturini *et al.*, 1999), differences in local skin temperature may contribute to the disparate responses observed between the current thesis and previous work (Holowatz *et al.*, 2006a, b). Future research should

be directed at evaluating the influence of factors such as local skin temperature on the mechanisms underpinning the heat loss responses.

5.3. End-organ sensitivity to exogenous nitric oxide

The current thesis demonstrated that low-dose infusion of sodium nitroprusside did not influence cutaneous vasodilation or sweating in the older adults. At first glance it would appear that to sensitivity of the end organ to NO may be altered with aging. However, it is important to note that no influence of SNP was noted in the younger adults. Throughout the experimental protocol, the contribution of NO to cutaneous vasodilation (~26%CVC_{Max}) remained relatively unchanged in the younger adults, which may suggest that it is exposure to a hot environment and not exercise *per se* that modulates the contribution of NO to cutaneous vasodilation. In other words, the increase in skin temperature during the initial exposure period prior to baseline data collection (~60 min) was sufficient to elevate endogenous nitric oxide production to its functional maximum. However, no influence of SNP was observed on cutaneous vasodilation in the older adults despite the fact that attenuations in its contribution were noted in the second exercise bout. Similarly, despite the lack of NO-dependent sweating, low dose SNP infusion did not influence the sweating response during either exercise bout. Together, these findings may indicate that the changes in the contribution of NO to the heat loss responses be secondary to alterations in signaling pathways downstream of NO. For example, we recently demonstrated that cyclooxygenase is an important modulator of sweat rate during exercise and likely contributes to the sweating response in an interactive manner with NO (Fujii *et al.*, 2014). Furthermore, unpublished data from our laboratory indicate that in younger adults, purinergic receptor activation also contributes to exercise induced increases in sweating via NO-dependent

mechanisms (Akbari *et al.,* article under review for publication in the *American Journal of Physiology*). Given that neither cyclooxygenase (Fujii *et al.*, 2015c) nor purinergic receptor (Akbari *et al.,*) inhibition in older adults has been shown to influence the sweating response to exercise, the age-related impairment in NO-dependent heat loss observed during exercise may be in part-explained by changes pathways downstream of NO, in addition to the aforementioned influence of oxidative stress as observed in protocol I.

5.4. Considerations

The primary consideration of this thesis is that the respective influences of oxidative stress and arginase activity on the heat loss responses in older adults were studied separately. It is possible that if both factors contribute to the age-related impairments in NO-dependent heat loss that removing the influence of only oxidative stress (protocol I) or arginase (protocol II) may have a reduced effect on cutaneous vasodilation and sweating relative to a situation in which both pathways were inhibited in concert. However, previous studies demonstrate augmentation of the cutaneous vasodilatory response to whole-body passive heating in older adults with independent infusion of ascorbate (Holowatz *et al.*, 2006a) or arginase inhibition (Holowatz *et al.*, 2006a, b). Currently, it is unclear as to why these findings differ from the current thesis wherein we demonstrate no influence independent ascorbate infusion or arginase inhibition on cutaneous vasodilation or sweating. As discussed it appears that the influence of these pathways may differ based on the type of heat stress (i.e., whole-body passive heating, passive exposure to a hot environment or exercise-induced heat stress) as well as additional factors including aerobic fitness and/or physical activity level.

Interestingly, in both protocols similar levels of CVC and sweating as well as body temperature responses were observed between the young and older adults. While age-related impairments in whole-body heat loss (Kenny & Jay, 2013; Larose *et al.*, 2013a; Larose *et al.*, 2013b; Larose *et al.*, 2013c; Larose *et al.*, 2014) are clearly observed in adults as young as 40-years of age (Larose *et al.*, 2013a), this is not always reflected by local heat loss response measurements (Larose *et al.*, 2014; Stapleton *et al.*, 2015). As a potential explanation, it has been shown that the magnitude of the age-related attenuation in cutaneous vasodilation and sweating may be dependent on the site of measurement (Smith *et al.*, 2013). A more pronounced separation may have been seen if a different area of skin was utilized such as the chest or back which typically exhibit greater sudomotor activity than the forearm (Kenny & Jay, 2013); however, in the current thesis the forearm was chosen as the measurement site to prevent movement artifact as well as to reduce discomfort during fibre insertion. Furthermore, this site was also chosen to facilitate comparison between previous studies, given that the majority of the work aimed at evaluating the mechanisms underpinning the heat loss responses employed forearm skin sites (Kellogg *et al.*, 1998; Holowatz *et al.*, 2003; Holowatz *et al.*, 2006a, b; Wong & Minson, 2006; Holowatz & Kenney, 2007; Stewart *et al.*, 2008; Hodges *et al.*, 2009; Welch *et al.*, 2009; Stanhewicz *et al.*, 2012; Brunt *et al.*, 2013; Fujii *et al.*, 2014; McGinn *et al.*, 2014a, b; McNamara *et al.*, 2014; Stapleton *et al.*, 2014a; Swift *et al.*, 2014; Fujii *et al.*, 2015a, b, c; Meade *et al.*, 2015; Fujii *et al.*, 2016; Meade *et al.*, 2016).

It is important to note that the older group adults recruited for the current thesis exhibited greater aerobic capacities and physical activity levels than may be expected in the general population. This factor may also contribute to the similar heat loss observed between groups, given the previously mentioned improvements in whole-body (Stapleton *et al.*, 2015) as well as

local (Okazaki *et al.*, 2002) heat loss responses associated with regular physical activity. Additionally, the elevated fitness level of the older adults likely contributes to the similar body (i.e., core and skin) temperature responses observed between groups. Specifically, age-related impairments in body heat regulation are dependent on the combined exercise and environmental heat load, with older adults reaching their capacity for whole-body heat dissipation at lower heat loads in relation to their younger counterparts (Stapleton *et al.*, 2015). Given the influence of endurance training on whole-body heat dissipation discussed above (Stapleton *et al.*, 2015), the heat loads employed in the current thesis may not have exceeded the heat loss capacity of the individuals recruited. That being said, it is also important to note that many studies demonstrate reductions in whole-body heat loss in older relative to young adults yet similar changes in body core temperature during exercise (Larose *et al.*, 2013a; Larose *et al.*, 2013c; Larose *et al.*, 2014; Stapleton *et al.*, 2014b), a response likely owing a heterogeneous distribution of heat within the body's tissues (i.e., core, skin, muscle) (Taylor *et al.*, 2014). Regardless, the current thesis clearly demonstrates age-related modulation of the mechanisms underpinning the heat loss responses; however, further study into how these changes influence whole-body heat dissipation in older individuals of varying levels of aerobic fitness and/or physical activity is warranted.

CHAPTER VI

CONCLUSION

6.1 Conclusion

In conclusion, we demonstrate that oxidative stress influences cutaneous vasodilatory response to passive exposure to a hot environment in older adults via NO-dependent mechanisms while, in contrast, oxidative stress influences cutaneous vasodilation during exercise via pathways independent of NO. However, no influence of oxidative stress on cutaneous vascular regulation in older adults was seen during postexercise recovery. Furthermore, the findings of this thesis suggest that the local sweating response to exercise may be sensitive to age-related increases in oxidative stress but that secondary factors such as aerobic fitness may play a modulatory role. Finally, we show that neither increases in arginase activity nor changes in the sensitivity of the thermoregulatory end-organ to NO effect the cutaneous vasodilatory and sweating in older adults during moderate intensity intermittent exercise in the heat.

6.2 Acknowledgements

This research was supported by the Natural Sciences and Engineering Research Council of Canada [Discovery Grant (RGPIN-06313-2014) and Discovery Grants Program - Accelerator Supplements (RGPAS-462252-2014); all funds held by G.P. Kenny]. Dr. Glen P. Kenny was supported by a University of Ottawa Research Chair Award. Mr. Robert D. Meade was supported by a Natural Sciences and Engineering Research Council Alexander Graham Bell Graduate Scholarship.

CHAPTER VII

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