The influence of ethnicity on local and whole-body heat loss responses during exercise in the heat: a comparison between young Canadian men of black-African and Caucasian descent

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Abstract

This thesis sought to evaluate whether the increased risk of heat-related illness observed in black-African descendants stems from impairements in local- and whole-body heat loss responses in this ethnic group. To evaluate this, in separate studies local- (study 1) and whole–body (study 2) heat loss responses were compared in young men (18-30 y) of black-African (n=21) and Caucasian (n=21) descent, matched for physical characteristics and fitness and born and raised in the same temperate environment. In study 1, we compared nitric oxide-dependent skin blood flow and sweating responses in young men of black-African (n=10) and Caucasian (n=10) descent during rest, exercise, and recovery in the heat. Both groups rested for 10-min, and then performed 50-min of moderate-intensity exercise at 200 W/m^2, followed by 30-min of recovery in hot-dry heat (35°C, 20% RH). Local cutaneous vascular conductance ($CVC_{\%max}$) and sweat rate (SR) were measured at two forearm skin sites treated with a) lactated-Ringer (Control), or b) 10 mM N^G-nitro-L-arginine methyl ester (L-NAME, NO synthase-inhibitor). L-NAME significantly reduced $CVC_{\%max}$ throughout rest, exercise, and recovery in both groups (both $p<0.001$). However, there were no significant main effects for the NO contribution to $CVC_{\%max}$ between groups (all $p>0.500$). L-NAME significantly reduced local SR in both groups (both $p<0.050$). The NO contribution to SR was similar between groups such that L-NAME reduced SR relative to control at 40 and 50 min into exercise (both $p<0.050$). In study 2, we assessed whole-body total heat loss (evaporative + dry heat exchange) in black-African (n=11) and Caucasian (n=11) men using direct calorimetry. Participants performed three, 30-min bouts of semi-recumbent cycling at fixed metabolic heat productions (and therefore matched heat loss requirements between groups) of 200

(light), 250 (moderate), and 300 W/m^2 (vigorous), each followed by 15-min recovery, in dry heat (40°C, ~13% relative humidity). Across all exercise bouts, dry (p=0.435) and evaporative (p=0.600) heat exchange did not differ significantly between groups. As such, total heat loss during light, moderate and vigorous exercise was similar between groups (p=0.777), averaging ((mean (SD)); 177 (10), 217 (13) and 244 (20) W/m^2 in men of black-African descent, and 172 (13), 212 (17) and 244 (17) W/m^2 in Caucasian men. Accordingly, body heat storage across all exercise bouts (summation of metabolic heat production and total heat loss) was also similar between the black-African (568 (142) kJ) and Caucasian groups (623 (124) kJ; p=0.356). This thesis demonstrates that ethnicity does not influence NO-dependent cutaneous vasodilation and sweating in healthy, young black-African descent and Caucasian men during exercise in the heat. Furthermore, we extend upon these observations by showing no differences in whole-body dry and evaporative heat exchange and therefore body heat storage.

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GLOSSARY

ACh: acetylcholine

AFD: black-African descendant

BEC: (S)-(2-boronoethyl)-L-cysteine-HCL

Nor-NOHA: Nω-hydroxynor-L-arginine

CAU: caucasian

CVC: cutaneous vascular conductance

CVC_{max}: maximal cutaneous vascular conductance

L-NAME: N^{G}-nitro-$_{L}$-arginine methyl ester

NO: nitric oxide

NOS: nitric oxide synthase

RH: relative humidity

SNP: sodium nitroprusside

VO_{2peak}: peak rate of oxygen consumption

CHAPTER 1

INTRODUCTION

Introduction

In healthy human beings, a stable body core temperature of approximately 37°C must be maintained to allow for optimal physiological function and survival, irrespective of environmental conditions. In a given environment, the regulation of body temperature is dictated by the ability to achieve a balance between the heat produced within the body, and the heat lost to the environment. This balance is known as thermoregulation, and is controlled by the autonomic nervous system via the activation of thermoeffector responses such as those that occur during exposure to cold (i.e., shivering, cutaneous vasoconstriction) and heat stress (i.e., cutaneous vasodilation, sweating) conditions (Shibasaki & Crandall, 2010).

Heat balance is disrupted when a human being is exposed to heat stress conditions, such as those associated with exposure to an elevated ambient temperature and/or exercise. Under such conditions, an increase in core temperature will occur until heat balance is re-established by an increase in heat dissipation mediated by an elevation in skin perfusion and sweating. During exposure to hot conditions, when air temperature exceeds skin temperature, this response is primarily dependent on the amount of sweat produced and subsequently evaporated. The increase in heat gain from the environment stimulates the sweating response which, combined with an increase in skin blood flow, allows for a greater rate of heat loss from the skin to the environment (Gagge & Gonzalez, 2010). Moreover, the increase in skin perfusion, and therefore skin temperature, reduces the gradient between air and skin temperature, reducing the rate of heat gain from the environment. Consequently, heat balance is re-established, and the core temperature returns to stable levels. Under elevated heat gain levels, such as conditions experienced

during exercise in a hot environment, the body's physiological capacity to dissipate heat may not be sufficient to balance the high rates of sweating required to achieve heat balance. Consequently, the body continues to store heat, resulting in a sustained increase in core temperature. If left unchecked, increases in body core temperature can place the individual at an increased risk of experiencing heat-related illness and injury (Jay & Kenny, 2010; Kenny *et al.*, 2010).

There are numerous individual factors, such as age, sex, fitness, acclimation status, and others, that can influence the body's physiological capacity to dissipate heat, which is mediated via changes in the regulation of skin blood flow and sweating. For example, females have a lower capacity to dissipate heat compared to males during exercise in the heat as evidenced by a lower response in evaporative heat loss (Gagnon & Kenny, 2012). Further, natural aging is also associated with marked reductions in whole-body heat dissipation, mediated primarily by a reduction in whole-body evaporative heat loss (Larose *et al.*, 2014; McGinn *et al.*, 2017). Other factors such as fitness (Lamarche *et al.*, 2018a; Lamarche *et al.*, 2018b), acclimation status (Poirier *et al.*, 2015; Poirier *et al.*, 2016) and the presence of chronic health conditions such as diabetes (Kenny *et al.*, 2013; Yardley *et al.*, 2013; Carter *et al.*, 2014; McGinn *et al.*, 2015) have also been shown to modulate the body's capacity to dissipate heat. Of importance, however, the influence of these intrinsic factors is heat load-dependent, such that differences are only evident above a certain level of heat load and therefore level of heat stress (Poirier *et al.*, 2015; Stapleton *et al.*, 2015b; Lamarche *et al.*, 2018a; Lamarche *et al.*, 2018b). For example, older adults may demonstrate a similar capacity to dissipate heat compared to their younger counterparts, and therefore a comparable core

temperature response, during a light exercise bout performed in a warm ambient condition. However, when a more intense exercise is performed, especially in the heat, age-related reductions in whole-body heat dissipation will lead to a more pronounced increase in the amount of heat stored and therefore result in a higher core temperature (Stapleton *et al.*, 2015b).

As outlined above, there are several factors that can alter the body's physiological capacity to dissipate heat which is linked directly to changes in the physiological heat loss responses of skin blood flow and sweating. Studies show that the regulation of these heat loss responses during an exercise-induced heat stress are mediated by an important key factor, the enzyme, nitric oxide (NO) synthase (NOS) (Welch *et al.*, 2009; Fujii *et al.*, 2014). Alterations in the relative contribution of this modulator can play a key role in mediating changes in the body's physiological capacity to dissipate heat. NOS, converts L-arginine to NO, a cell signaling protein produced during exercise and modulates the regulation of sweating and skin blood flow during an exercise-heat stress (Welch *et al.*, 2009; Stapleton *et al.*, 2014). In young males, NOS inhibition causes a reduction in both sweating (Amano *et al.*, 2017a) and cutaneous active vasodilation (Wong, 2013) during passive heating and exercise (Welch *et al.*, 2009; McGinn *et al.*, 2014). An impairment in the activity of NOS has also been linked to the observed age-related reductions in the heat loss response of skin blood flow. Specifically, age-related reductions in skin blood flow during both a passively-induced (Holowatz *et al.*, 2003; Holowatz *et al.*, 2006a, b) and an exercise-induced heat stress (Fujii *et al.*, 2015c; Fujii *et al.*, 2016a) are mediated via NOS-dependent mechanisms. In contrast, NOS-dependent sweating during exercise in the heat is diminished in older adults (Fujii *et al.*, 2015c).

Despite our growing knowledge of the influence of different individual factors (e.g., sex, ageing, fitness, and others) in the regulation of local and whole-body heat dissipation, there remains a paucity of information on the influence of ethnicity. Much of our understanding of the influence of individual factors on the physiological heat loss responses during an exercise-heat stress is based on the evaluation of individuals of various or similar ethnicities. Consequently, it remains unclear if individuals of different ethnicities may demonstrate divergent responses. This possibility exists given that epidemiological studies suggest that individuals with darker skin experience greater heat-related morbidity and mortality during heat stress (Taylor & McGwin, 2000; Knowlton *et al.*, 2009). For example, during the 1995 heatwave in Chicago, 739 excess deaths were recorded from death certificate data obtained from Illinois vital records files. It was discovered that, after accounting for socioeconomic factors, a disproportionate number of deaths were in individuals of black-African descent across all age groups (CDC, 2017). An earlier study by Taylor & McGwin (2000) showed that black-African descent males from the Southern United States had the highest death rates (Taylor & McGwin, 2000). Similarly, Knowlton and colleagues showed that the rate of emergency department visits were found to be highest among those of black-African descent as assessed during a 2006 heatwave in California (Knowlton *et al.*, 2009). Individuals of black-African descent are defined as any individual with dark skin of African origins (Statistics Canada, 2016, 2017). Taken together, these findings suggest that black-African descendants may have a reduced heat tolerance, albeit the underlying mechanisms for this response remain unclear.

Some insight regarding the effect of ethnicity on the physiological heat loss responses may be gleaned from the few studies that have examined thermoregulatory responses during local heating conditions (Hurr *et al.*, 2018; Kim *et al.*, 2018). Hurr and colleagues (2018), examined if elevated stress could be attributed to the reduced microvascular function present in black-African descent males/females relative to Caucasian males/females (n=12 per ethnic group) by inhibiting NOS via intradermal microdialysis. They observed a blunted NO-mediated skin blood flow response in the black-African descendants compared to their Caucasian counterparts (Hurr *et al.*, 2018). In another study, 9 black-African descent males/females matched to 9 Caucasian males/females were infused with Lactated Ringer's solution (control), 20 mM L-NAME (NOS inhibitor), 10 mM L-arginine (mimics the substrate L-arginine which enhances NO bioavailability), and a combination of 5.0 mM BEC and 5.0 mM nor-NOHA (arginase (enzyme that breaks down L-arginine) inhibitor) via intradermal microdialysis. They supplemented L-arginine and inhibited arginase to test if either of these methods would improve the lack of NO bioavailability present in black-African descendants which they hypothesized caused a decreased microvascular function in this group compared to Caucasians. They found that inhibition of NOS via L-NAME resulted in a blunted response in black-African descendants relative to Caucasians (Kim *et al.*, 2018). Together, these studies show that the NOS-mediated regulation of skin perfusion differs between black-African descendants and Caucasians.

Studies suggest that black-African descendants and Caucasians also differ in their capacity to sweat although findings have been equivocal. While some studies have reported that black-African descendants have reduced sweating responses relative to

their Caucasian counterparts (Kawahata & Adams, 1961; Marino *et al.*, 2004); others have shown that those responses are either the same in both groups (Baker, 1958) or lower in Caucasians relative to black-African descendants (Robinson *et al.*, 1941). For instance, Marino and colleagues investigated running performance and associated thermoregulatory responses of black-African descent and Caucasian runners in cool and warm conditions. On two separate occasions, two highly-trained groups (n=6 per group) of men consisting of black-African descendants and Caucasians performed moderate intensity treadmill running for 30 min at 70% of their peak treadmill running velocity followed by an 8 km self-paced performance run in a cool (15 °C) or warm (35 °C) humid (60% relative humidity) ambient condition. They demonstrated that during the 8 km self-paced run, the time of completion for the Caucasian group was marginally greater (3.22 minutes longer) in the warm conditions compared to their black-African descent counterparts, although the rate of increase in core temperatures did not differ between the two groups. However, they showed that the sweat rates for the black-African descendants were lower compared to their Caucasian counterparts in the warm conditions during the self-paced run (Marino *et al.*, 2004). Although this study shows that differences may exist between the two groups, the exercise design employed elicited between-group differences in metabolic heat productions, therefore, resulting in differences in the heat loss required to attain heat balance. Consequently, it remains unclear if differences in the body's capacity to dissipate heat exist between groups. Noteworthy, a field study conducted by Baker (1958) assessed heat dissipation in 8 men of black-African descent compared to 8 Caucasian males (matched for body surface area) in hot, dry conditions. Participants engaged in a walking exercise of 3 miles/hour, with no

incline, in hot-dry desert conditions and no differences in sweat rates were observed between groups, although black-African descendants demonstrated a greater final core temperature than the Caucasians. While these studies indicate that thermoregulatory responses may differ between groups, a controlled study employing a fixed heat load (and therefore thermal drive) is required to ascertain the extent to which ethnicity may alter the body's physiological capacity to dissipate heat.

Physical characteristics can play an important role in mediating heat loss responses by altering heat exchange between the body and the surrounding environment and therefore body heat storage during exposure to heat stress. Relative to their Caucasian counterparts, black-African descendants tend to have a more compact stratum corneum (outermost layer of the skin) such that they have about twenty cell layers relative to the sixteen layers in their Caucasian counterparts (La Ruche & Cesarini, 1992). The lipid content of the epidermis of black-African descendants is also higher, resulting in greater cellular cohesion in the skin. Additionally, black skin is less hydrated than white skin (La Ruche & Cesarini, 1992). Such differences in skin structure may affect heat exchange between the skin and the environment because humans dissipate excessive heat mostly through the skin via heat transfer to the surrounding environment.

Human adaptation is marked by a progressive reduction in physiological strain after repeated exposure to stress (Taylor, 2006b) and plays an important role in determining an individual's physiological capacity to dissipate heat. The surrounding environment may confer phenotypic adaptations which are adaptations that arise from specific environmental stresses occurring within the individual's lifetime (Taylor, 2006b). It occurs when an organism modifies either its morphological configuration (such as sweat

gland size, fur thickness) or its physiological responses following repeated stress exposure (Taylor, 2006b). For example, it is well documented that individuals may increase their physiological capacity to sweat as a result of repeated exposure to thermal stress (heat acclimation) (Poirier *et al.*, 2016). While some investigators have ascribed ethnic differences between black-African descendants and Caucasians to genotypic (inherited) adaptations, others have attributed them to phenotypic adaptations or to some interaction of these two (Taylor, 2006b). In fact, studies have shown that individuals of black-African descent may possess physiological adaptations, such as improved sweating efficiency (Thomson, 1954; Yousef *et al.*, 1984), that may provide a thermoregulatory advantage in the heat (Robinson *et al.*, 1941; Baker, 1958; Wyndham *et al.*, 1964). Although these thermoregulatory adjustments may be attributed to genotypic (inherited) adaptations, (Baker, 1958; Scholander *et al.*, 1958; Roberts *et al.*, 1970), they may actually represent phenotypic adaptations as a result of the black-African descendants being residents of hotter climates than their Caucasian counterparts (Taylor, 2006b). However, there remains a paucity of information on the independent influence of these adaptations on different ethnic population groups; therefore, a study design isolating genotypic adaptations from phenotypic adaptations is required to assess heat exchange in black-African descendants and Caucasians.

1.2 Rationale and Statement of the Problem

To date, few studies have examined the influence of ethnicity on human thermoregulation to an exercise-heat stress. While it is thought that differences may exist in thermoregulatory function between black-African descendants and Caucasians, the

evidence has been equivocal. This is in large part due to the fact that studies conducted to date were not adequately designed to determine if differences in the body's physiological capacity to dissipate heat exist for a fixed heat load and if these differences (if any) are heat-load dependent (i.e., differences in heat dissipation only occurring above a certain level of heat stress). This is especially because previous studies have shown that individual factors associated with sex (Stapleton *et al.*, 2015b), ageing (Stapleton *et al.*, 2015a), fitness (Lamarche *et al.*, 2018a; Lamarche *et al.*, 2018b), acclimation (Poirier *et al.*, 2015), hydration (Meade *et al.*, 2019) and others alter the body's capacity to dissipate heat in a heat-load dependent manner. Additionally, previous investigators did not adequately design studies isolating genotypic adaptations from phenotypic adaptations in black-African descendants and Caucasians. To address this knowledge gap, my thesis was divided into two sub-studies evaluating local- and whole-body heat loss responses during an exercise-induced heat stress in Canadian of black-African descent relative to Caucasian Canadians matched for physical characteristics and fitness. In the first study, I assessed the relative contribution of NOS (a key modulator of heat loss responses) in the regulation of skin blood flow and sweating during an exercise-induced heat stress. To assess the influence of this enzyme, I blocked the activity of NOS via the infusion of a NOS inhibitor via intradermal microdialysis. In the second study, I compared whole-body evaporative and dry heat exchange in Caucasians and black-African descendants using a direct air calorimeter (a device which permits the very precise measurement of the amount of heat dissipated by the body). The participants performed an incremental exercise test which consisted of progressive increases in heat loads (and therefore thermal drive) in the heat. This evaluation allowed me to determine if ethnicity

modulated the body's physiological capacity to dissipate heat and if this response was heat-load dependent.

1.3 Study Objectives

The objectives of this study were to assess the NOS-dependent local heat loss responses of sweating and skin blood flow (study 1) as well as whole-body heat loss (study 2) responses in Canadians of black-African and Caucasian descent (matched for physical characteristics and fitness) during an exercise-induced heat stress.

This project aimed:

1) To examine the differences in the contribution of NOS, via the administration of the enzyme-specific inhibitor, L-NAME, in the regulation of skin blood flow and sweating in young black-African descent men as compared to their Caucasian counterparts during exercise in the heat.
2) To assess differences in whole-body heat loss responses (evaporative and dry heat loss) during exercise in the heat at increasing fixed rates of metabolic heat production equal to 200, 250 and 300 $W \bullet m^{-2}$ in young physically active males of black-African descent compared to their Caucasian counterparts. These rates of metabolic heat production were chosen to ensure a near compensable heat stress condition was achieved in the first bout, progressing to an uncompensable condition in the final exercise bout.

1.4 Research Hypothesis

In my first study, I evaluated the hypothesis that NO-dependent cutaneous vasodilation and sweating would be attenuated in young black-African descent men compared to Caucasians during exercise in hot, dry conditions. In my second study, I assessed the hypothesis that individuals of black-African descent would display elevations in evaporative heat loss and reductions in dry heat gain, which would enhance whole-body total heat loss relative to Caucasians. In addition, I evaluated the hypothesis that differences in the capacity to dissipate heat would be dependent on the level of heat stress (and therefore heat load).

1.5 Delimitations and Limitations

This study evaluated young adult males aged 18-30 years. Hence, the findings may not be representative of other age ranges or the female population. For this study, females were excluded given that it is well established that the mechanisms governing the regulation of heat loss responses differ between males and females (Fujii *et al.*, 2015a). In addition, the study required participants who were healthy; therefore, the conclusions coming from this study are not directly applicable to individuals who may suffer from chronic diseases, which may further alter thermoregulatory control.

1.6 Significance

There exists a significant knowledge gap regarding the influence of black-African descent ethnicity on heat loss responses. Therefore, this project is pivotal in examining the underlying thermoregulatory mechanisms of black-African descendants that may

place them at a greater risk of heat-related illness compared to their Caucasian counterparts. In addition, scientists have predicted that extremes in climate are increasing in both severity and frequency (Melillo *et al.*, 2014). The impact of ethnicity on thermal response to climate emergencies is thus critical as studies have shown that black-African descent males experience some of the highest death rates from heat-related illness across all age groups during exposure to extreme heat events (Taylor & McGwin, 2000; Knowlton *et al.*, 2009; CDC, 2017). Hence, increasing extremes in climate could lead to even higher death rates in this population group. Additionally, studying the underlying mechanisms of the influence of ethnicity on the regulation of heat loss will allow for a more profound understanding of the development of heat illness, especially in heat vulnerable individuals. This information will also be of vital importance in developing appropriate guidelines for protecting heat-vulnerable workers, which do not currently consider ethnicity. Additionally, the outcomes from the present thesis may have important implications for experimental design given investigators conducting studies on human thermoregulatory function often select participants from only one ethnic group to minimize the confounding influence of ethnicity (Stephens & Hoag, 1981; Glickman-Weiss *et al.*, 1997).

CHAPTER 2

REVIEW OF THE LITERATURE

2.1 Basic Human Thermoregulation

Small fluctuations in body temperature can be challenging to homeostasis. Given this challenge to homeostasis, human beings have developed the ability to use powerful autonomic responses to tightly regulate core body temperature at approximately 37ºC despite encountering diverse thermal environments. This regulation is done to allow for optimal physiological function and survival (Taylor, 2006a). Internal temperature can be affected by exposure to warm environmental conditions and physical activity. It has been well established that the pre-optic anterior hypothalamus (PO/AH) is the thermoregulatory centre of the body. This centre receives and integrates afferent input from the central and peripheral thermal receptors and adjusts various mechanisms (such as increases in sweat production) to maintain hypothalamic temperature (Carlisle, 1969; Boulant & Bignall, 1973; Hensel, 1981). This system operates via a negative feedback loop by adjusting effector responses to cold or warm stimuli to augment heat production (such as shivering) and to prevent or promote heat loss (through vasoconstriction and vasodilation/sweating). Increases and decreases in body temperature are sensed peripherally by cold and warm thermoreceptors located in the skin (in the epidermis). These fluctuations in heat are also detected centrally by thermoreceptors located in the brain and other deep body regions (in the hypothalamus, along the spinal cord, in the abdominal cavity, and in major blood vessels) (Carter *et al.*, 1999; Romanovsky, 2007). Elevations in core (central thermal input) and skin temperature (peripheral thermal input) are caused by the gain of heat from exposure to a hot environment and/or physical activity that activate the PO/AH via afferent input (shown in Figure 2). In turn, the activated PO/AH causes an increase in skin perfusion and sweat production which enhances heat

dissipation, thereby offsetting a greater increase in body core temperature (Armstrong *et al.*, 1993; Charkoudian, 2003; Johnson & Kellogg, 2010; Shibasaki & Crandall, 2010; Kenny & Jay, 2013; Johnson *et al.*, 2014). Cutaneous vasodilation increases the diameter of the cutaneous blood vessels, increasing the flow of blood through the cutaneous circulation where its heat can be dissipated to the surrounding environment (Kenny & Jay, 2013; Johnson *et al.*, 2014). Cutaneous vasodilation plays an important role at rest and in minor heat stress situations compared to sweating, which provides the body's primary avenue for heat loss, especially during exercise and exposure to a hot environment (Shibasaki & Crandall, 2010; Gagnon *et al.*, 2013; Kenny & Jay, 2013).

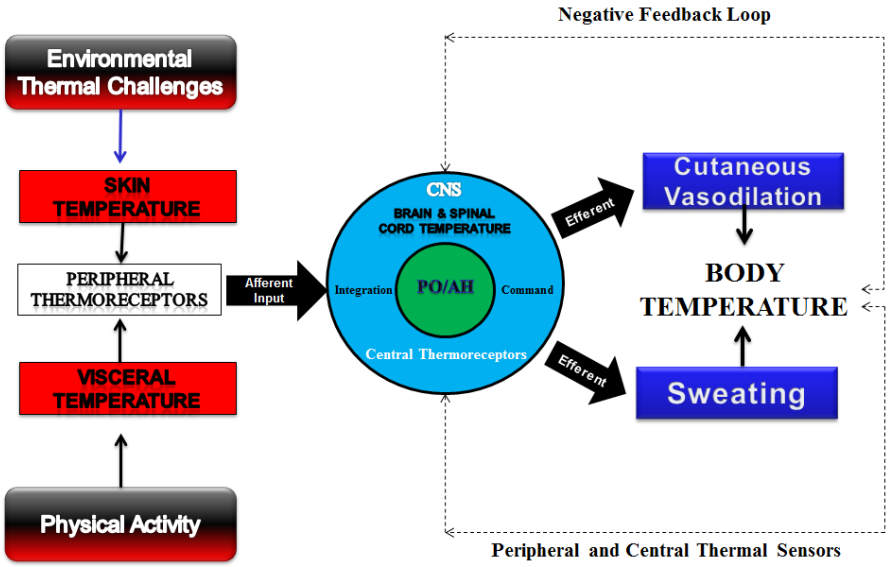

Figure 1. A representation of the control of heat loss responses of cutaneous vasodilation and sweating during heat stress in response to elevations in core and skin temperatures. From (Nagashima *et al.*, 2000).

2.2 Human Heat Balance

Healthy humans maintain core temperature at a near constant level (~37°C) regardless of environmental conditions. To maintain body core temperature, the thermoregulatory system adjusts physiological mechanisms to balance the rate at which heat is produced within the body with the rate at which heat is lost to the environment via dry heat exchange and evaporative heat loss. This relationship is defined by the following equations: $M - W = (K + C + R + E_{SK}) + (C_{RES} + E_{RES}) + S$, (all units, $W \bullet m^{-2}$);

Where,

M = rate of metabolic heat production

W = rate of mechanical work

K = rate of conductive heat loss

C = rate of convective heat loss from the skin

R = rate of radiative heat loss from the skin

E_{SK} = rate of evaporative heat loss from the skin

C_{RES} = rate of convective heat loss from pulmonary ventilation

E_{RES} = rate of evaporative heat loss from pulmonary ventilation

S = rate of body heat storage (Parsons, 2014).

At room temperature, resting metabolic heat production is balanced primarily by dry heat loss via conduction/convection and radiation (terms described below) (Kenny & Jay, 2013). This balance is disturbed during exposure to a hot environment and/or changes in metabolic heat production due to physical activity. As ambient air temperature increases, the smaller temperature gradient between the skin surface and ambient air

reduces the capacity for dry heat exchange (Wendt *et al.*, 2007). When the environment is warmer than the skin, the body begins to gain heat via dry heat exchange, increasing the requirements for sweating and circulatory responses to achieve a given rate of heat dissipation. During physical activity, the increase in metabolic rate above resting levels augments the rate at which heat must be dissipated to the environment to prevent a dangerous rise in core temperature. The extent to which core temperature increases at steady state is largely independent of the ambient air temperature and is proportional to the metabolic rate (Keim *et al.*, 2002). With metabolic and/or environmental heat load, the increase in sweat rate and skin blood flow is proportional to the amount of evaporative heat loss required for heat balance. Under circumstances where impairments in sweating and skin blood flow (i.e., as occurs with aging, reduced fitness, others) lead to an insufficient rate of heat loss to offset an exercise-induced increase in metabolic heat production and/or environmental stress (i.e., elevated ambient temperature and/or humidity), core temperature continually rises. As it rises, the body continues to store heat, placing the individual at a greater risk of heat-related illness and injury. However, under normal circumstances, as core temperature rises, the heat is transferred to the skin surface via the blood and dissipated to the environment.

During these conditions, heat exchange occurs through convection, conduction, radiation, and evaporation (Wendt *et al.*, 2007). Convection is heat transfer that happens between a surface and a gas such as air or liquid such as water. It is dependent on the temperature gradient, the rate of movement and the relative heat storage capacity of the gas or liquid. Conduction occurs when heat is transferred when a surface comes into direct contact with another surface; it largely depends on the temperature difference

between the two surfaces. Radiation is the mechanism by which heat is lost or gained from the body to the environment by means of electromagnetic waves and depends on the thermal gradient between the body and the environment. Evaporation is the process of losing heat through the conversion of water to gas (Keim *et al.*, 2002).

2.3 Sweating

In human beings, the most important avenue of heat loss during environmental heat stress conditions and/or physical activity is the evaporation of sweat from the skin. Sweating accounts for ≥80% of total heat loss during exposure to high temperatures and/or physical activity, compared to 25% of heat loss during resting conditions (Cain & McLellan, 1998; Gavin, 2003). During exercise, internal and skin temperatures are the primary drivers of sweating (Nadel *et al.*, 1971a). However, once exercise has commenced, the rate of sweating changes without corresponding changes in internal or skin temperature (Van Beaumont & Bullard, 1963; Van Beaumont & Bullard, 1966).

Dynamic exercise triggers an increase in sweating (works in tandem with skin blood flow) to cause an exponential increase in the rate of whole-body heat loss, which occurs mainly through changes in evaporative heat loss. As exercise continues, the rate of increase in sweating (and skin blood flow) is reduced leading to a reduced rate of increase in the whole-body heat loss response. This reduced rate of increase in the whole-body heat loss response causes a decrease in the rate of body heat storage progressively until heat balance is achieved between the total rate of heat loss and the rate of metabolic heat production (Parsons, 2014).

There are two types of sweat glands: the apocrine gland and the eccrine gland. The apocrine glands are innervated by adrenergic nerves and are distributed in the armpits and pubic regions; they also secrete a viscous fluid, which has a distinct odor. The eccrine glands, which cover most of the body, are responsible for thermoregulatory sweating (Saga, 2002; Shibasaki *et al.*, 2006). These 2 to 4 million sweat glands are stimulated by cholinergic sympathetic nerves and secrete hypotonic sweat (water and electrolytes) (Saga, 2002). Eccrine sweat gland activation causes the sweat to be secreted onto the skin surface, therefore, promoting heat loss of the water content of sweat via evaporation (Wendt *et al.*, 2007).

Regional differences in local sweat rates have been reported in the chest and forearm during exercise. The sweating rate on the chest is greater than the forearm (Nadel *et al.*, 1971b). This difference in sweating rate has also been shown to be influenced by the workload. As exercise intensity increases, the local sweat rate is increased in both sites. At lower exercise intensities, moderate, and higher exercise intensities, the local sweat rate in the chest has been shown to be higher that the forearm at all three exercise intensities (Takano *et al.*, 1996; Kondo *et al.*, 1998).

In the context of black-African descent ethnicity, studies conducted have reported equivocal sweat responses in black-African descents compared to their Caucasian counterparts in exercise in the heat conditions (Thomson, 1954; Baker, 1958; Marino *et al.*, 2004). Although these studies have investigated differences in the sweating responses of these two groups, relatively little is known about the mechanisms underpinning these responses, and especially during exercise in the heat.

2.4 Skin Blood Flow

One of the central components of human thermoregulation is the ability of the body to adjust the cutaneous vasomotor tone, which regulates skin blood flow. This adjustment is essential to manage heat exchange during exposure to heat stress (exposure to a hot environment and/or performing physical activity). In non-glabrous skin, the control of skin blood flow is regulated by the two branches of the sympathetic nervous system: a noradrenergic active vasoconstrictor system and a cholinergic active vasodilator system (Charkoudian, 2003; Kellogg, 2006). The regulation of non-glabrous skin is in contrast to glabrous skin (palms, soles, and lips), which are innervated only by sympathetic vasoconstrictor nerves (Johnson & Proppe, 1996). The sympathetic vasoconstrictor system is mediated primarily through the presynaptic release of the neurotransmitter, norepinephrine, and an unknown vasoconstricting co-transmitter (Stephens *et al.*, 2001). Although a specific vasoconstricting co-transmitter has not been specified, some such as neuropeptide Y (NPY) have been postulated (Stephens *et al.*, 2004). During heat stress, withdrawal of the vasoconstrictor activity provides approximately 10 to 20 percent of the vasodilatory response (Rowell, 1974; Pergola *et al.*, 1994). In contrast, the sympathetic vasodilator system is accountable for approximately 80 to 95 percent of the total increase in skin blood flow (Johnson & Proppe, 1996); albeit, little is known about the control of this system or the neurotransmitter that mediates its response (Charkoudian, 2003; Johnson & Kellogg, 2010). Skin blood flow can increase from 250 $ml \cdot min^{-1}$ during resting normothermic conditions to values of up to 6 to 8 $L \cdot min^{-1}$ which occurs during maximal heat stress conditions (Rowell, 1974). During exercise and/or exposure to a hot environment, vasomotor reflexes lead to cutaneous vasodilation, which leads to an

increase in skin blood flow. These reflexes redirect the blood (returning from the active muscles and core tissues) to the skin, hence, increasing convective/ conductive and/or radiative transfer of heat from the core to the periphery and allowing heat to be dissipated to the surrounding environment (Hardy, 1961; Hammel, 1968). These large increases often require an increase in cardiac output and redistribution of blood flow from inactive tissues, such as the splanchnic region (which demonstrate vasoconstriction) (Kenney & Johnson, 1992). These adjustments are sufficient to match the demand for increased skin blood flow so that oxygen supply to organs such as the heart is not compromised (Johnson & Proppe, 1996). In the context of black-African descent ethnicity, numerous studies have investigated the skin blood flow responses of black-African descendants compared to their Caucasian counterparts after exposure to different modulators, such as NOS, cyclooxygenase, acetylcholine and others (Maley *et al.*, 2014; Maley *et al.*, 2015, 2017a; Kim *et al.*, 2018); however, none of these studies have evaluated this response during exercise in the heat.

2.5 Thermoregulation from a Calorimetric Perspective

Dynamic exercise causes a rise in metabolic heat production, resulting in an increase in body heat storage, and a corresponding elevation in muscle tissue and core temperature (Parsons, 2014). During the early stages of exercise, there is an immediate and rapid rise in the rate of metabolic heat production which causes a rapid increase in the body heat storage due to the slow rise in the rate of total heat loss (Murgatroyd *et al.*, 1993; Webb, 1995). The time taken for the rate of total heat loss to balance the rate of metabolic heat production is dependent upon the temperature, humidity of the

surrounding environment, and the intensity of the exercise (Kenny *et al.*, 2008). As exercise continues, the reflex physiological mechanisms of skin blood flow and sweating are triggered, causing an increase in the rate of whole-body heat loss (Benzinger, 1969). This increase leads to a progressive decrease in the rate of body heat storage until the rate of total heat loss matches the rate of metabolic heat production, leading to heat balance such that the rate of body heat storage will be zero and core temperature will achieve steady-state (Parsons, 2014).

In the context of ethnicity, various studies have investigated the differences in heat loss responses in black-African descendants versus Caucasians. Most of these studies have employed a single continuous exercise-induced heat load (Thomson, 1954; Marino *et al.*, 2004) during whole-body heating. Therefore, it could not be determined if ethnic-related differences existed at lower exercise intensities, and if these differences would be exacerbated at higher heat loads. Determining if these differences exist at different heat loads is critical as ethnic differences may not be present in lower exercise intensities; however, they may exist at higher intensities. In addition, none of these studies have utilized a direct air calorimeter. In the literature, there is a lack of information employing an exercise model consisting of intermittent successive bouts of exercise with progressive increases in heat load to examine the threshold at which ethnic-related differences exist. Therefore, the effect of ethnicity on whole-body heat loss as a function of increases in exercise-induced heat load remains to be explored.

2.6 Ethnicity and Whole-body Heat Loss Responses

Individual factors, such as age, sex, and fitness have been shown to have an influence on the body's ability to lose heat during heat stress conditions (Poirier *et al.*, 2015; Stapleton *et al.*, 2015b; Lamarche *et al.*, 2018a; Lamarche *et al.*, 2018b). Ethnicity is associated with heat-related morbidity and mortality in some studies (Taylor & McGwin, 2000; Kaiser *et al.*, 2007) but not in others (Green *et al.*, 2010). For example, recent epidemiological studies have shown that black-African descendants have an increased vulnerability to heat-related morbidity and mortality across all age groups (Kaiser *et al.*, 2007; Gosling *et al.*, 2009; CDC, 2017).

In the context of thermoregulation, some studies demonstrate that differences in heat loss responses between different ethnicities exist. For example, Duncan and Horvath conducted a study in which young sedentary adult males of Malaysian, Indian and Chinese (n=29) origin (they all resided in tropical Malaysia) were subjected to a standard heat stress test. The subjects, matched for fitness, performed a 2-hour treadmill walk at 50% of their VO_{2max} during exposure to an ambient temperature of approximately 34.9 °C dry bulb and approximately 32.1 °C wet bulb. They concluded that the Malaysian ethnic population group exhibited a superior thermoregulatory ability based on their low core temperatures and low sweat rate responses compared to the other two ethnicities (Duncan & Horvath, 1988).

In the context of black-African descent ethnicity, studies have shown equivocal responses. These studies have focused on the evaluation of both local sweat rates and whole-body sweat rates in young black-African descendants and Caucasian males. Specifically for whole-body sweating, some investigators have reported that black-African

descendants have reduced sweating responses relative to their Caucasian counterparts (Riggs & Sargent, 1964; Wyndham *et al.*, 1964; Marino *et al.*, 2004); others have shown that those responses are either unchanged (Baker, 1958) or lower in Caucasians relative to black-African descendants (Robinson *et al.*, 1941). For example, Thomson (1954) conducted a study investigating the heat loss responses of black-African descendants compared to their Caucasian counterparts (n=47). To explore potential differences in sweating, Thomson subjected his participants to exercise in a controlled thermal environment of 100 °F dry-bulb. He found significantly higher skin temperatures, lower sweat rates and lower terminal rectal temperatures in black-African descendants compared to Caucasians. Therefore, the authors concluded that black-African descendants had superior heat dissipation. He also believed that since black-African descendants had lower rectal temperatures, the lower sweat rates were utilized to preserve fluid and electrolytes. This difference in dissipation can also be attributed to other factors, such as the structure of the skin. Black skin has more cell layers, and is less hydrated compared to white skin (La Ruche & Cesarini, 1992). This difference in skin structure may also affect heat exchange between the skin and the environment. For local sweat rates, investigators have focused on evaluating if sweat gland distribution and the total number of sweat glands differ between the two groups. These investigators have shown no significant differences in sweat gland distribution (Glaser, 1934; Thomson, 1954); however, while some investigators have shown, on average, a higher total number of sweat glands in black-African descendants (Glaser, 1934); others have shown a lower total number of sweat glands in black-African descendants relative to Caucasians (Kawahata & Adams, 1961) and others have found them to be similar (Thomson, 1954).

For example: Kawahata and Adams (1961) sought to investigate if a difference existed in the number of sweat glands in black-African descent (n=9; ages: 19-36) and Caucasian (n=10; ages: 16-26) men, with similar body surface areas. The participants sat in a heated chamber (41-42 °C) for roughly 30 to 50 minutes to obtain maximal sweating. They used the counting method (counted the number of sweat glands under 10X magnification) to obtain the total number of sweat glands on 20 different skin regions of the body. They found that the total number of sweat glands were significantly greater in Caucasians compared to black-African descendants. This shows that a difference may exist between these two groups. Although significant knowledge has been acquired concerning the difference in sweat responses of different ethnicities, further research is still required to extend our understanding of these heat loss responses in these two ethnic groups.

2.6 Ethnicity and Nitric Oxide-Dependent Heat Loss

It is well established that, during exercise in the heat, NOS contributes to the cutaneous vasodilation and sweating responses in young males. A number of studies have investigated the underlying physiological mechanisms of these heat loss responses in relation to NO (Holowatz *et al.*, 2005; Lee & Mack, 2006; Welch *et al.*, 2009; Fujii *et al.*, 2014; Stapleton *et al.*, 2014; Louie *et al.*, 2016). These studies show that the inhibition of NOS impairs sweat production as well as cutaneous vasodilation during exercise in the heat (Welch *et al.*, 2009; Fujii *et al.*, 2014; Stapleton *et al.*, 2014; Fujii *et al.*, 2015b; Fujii *et al.*, 2015c; Fujii *et al.*, 2016a; Louie *et al.*, 2016). Although NO-dependent sweating and skin blood flow have been clearly observed in younger males (Welch *et al.*, 2009;

Fujii *et al.*, 2014; McGinn *et al.*, 2014; Stapleton *et al.*, 2014; Fujii *et al.*, 2015b), it is unclear if these responses are influenced by ethnicity.

To the best of our knowledge, only two studies have assessed the role of ethnicity on heat loss responses during NOS inhibition, and these were limited to local skin heating only. While the mechanisms that occur during local skin heating can produce different results from whole-body heating, some insights may be gleaned from these studies. For example, Hurr and colleagues (2018) investigated the hypothesis that black-African descendants have a reduced microvascular function relative to Caucasians due to elevated oxidative stress. Hence, they infused 10 mM Tempol (compound that mimics the superoxide dismutase enzyme which breaks down superoxides leading to elevated oxidative stress), and 10 mM Ascorbic acid (an antioxidant) to test if this can restore the blunted skin blood flow response in black-African descendants via intradermal microdialysis on two skin sites. Simultaneously, they also infused Ringer's solution (control solution) on a third skin site on the forearm of 24 young adults (6 males and 6 females of black-African descent, 6 males and 6 females of Caucasian descent). This infusion was followed by the administration of 10 mM L-NAME to assess the contribution of NO to skin blood flow at all three skin sites during local heating on the forearm at 39°C. They discovered that there was a blunted NO skin blood flow response at the control and ascorbic acid sites, but an augmented tempol NO contribution in the black-African descendants compared to their Caucasian counterparts. They concluded that tempol administration improves the blunted skin blood flow response to local heating in black-African descendants relative to their Caucasian counterparts. Therefore, these results indicate that the elevated free radical production mediates the impaired ability to

effectively utilize nitric oxide production during local heating (Hurr *et al.*, 2018). In another study, Kim and colleagues (2018) investigated if there were any racial differences in the microvascular function of black-African descendants relative to Caucasians and if this difference was attributable to a lack of NO bioavailability. Therefore, they tested if L-arginine (substrate which is converted into NO) supplementation or inhibition of arginase (enzyme which breaks down L-arginine) would improve the lack of NO bioavailability. Hence, they infused Lactated Ringer's solution (control), 20 mM L-NAME (nitric oxide synthase inhibitor), 10 mM L-arginine (mimics the substrate L-arginine which was used to enhance NO bioavailability), and a combination of 5.0 mM BEC and 5.0 mM nor-NOHA (arginase inhibitor which inhibits the breakdown of L-arginine) via intradermal microdialysis in black-African descendants compared to their matched Caucasian counterparts (n=9 per group). They discovered that the supplementation of L-arginine improved the blunted vasodilatory response in black-African descendants but not Caucasians after local heating. On the other hand, the inhibition of arginase had no effect on the cutaneous microvascular function of either group. In addition, the magnitude of reduction in skin blood flow after NOS inhibition was greater in Caucasians relative to control, therefore, indicating a reduced NO-mediated vasodilation in the black-African descendants (Kim *et al.*, 2018).

While these studies have investigated the contribution of NOS in black-African descendants during local skin heating, little is known about the contribution of NOS during exercise in the heat in the context of ethnicity. Therefore, the mechanisms underpinning the contribution of NOS to the heat loss responses of sweating and skin blood flow in black-African descendants during exercise in the heat have yet to be elucidated.

CHAPTER 3

METHODS AND RESULTS OF THESIS

Article I:

The effects of nitric oxide synthase inhibition on cutaneous vasodilation and sweating in second-generation young men of black-African and Caucasian descent during exercise in the heat

The effects of nitric oxide synthase inhibition on cutaneous vasodilation and sweating in second-generation young men of black- African and Caucasian descent during exercise in the heat

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NEW FINDINGS (99/100 words)

What is the central question of this study?

- Nitric oxide modulates cutaneous vasodilation and sweating during an exercise-heat stress in young men. However, it remains uncertain whether these effects are reduced in black-African descendants who commonly demonstrate reduced nitric oxide bioavailability. Therefore, we assessed whether black-African descendants display reduced nitric oxide-dependent cutaneous vasodilation and sweating compared to Caucasians under these conditions.

What is the main finding and its importance?

- Nitric oxide-dependent cutaneous vasodilation and sweating were similar between groups, indicating that reduced nitric oxide bioavailability in black-African descendants does not attenuate these heat loss responses during an exercise-heat stress.

Abstract (250/250 words)

Men of black-African descent are at an increased risk of heat related illness relative to their Caucasian counterparts. This may, in part, be due to reduced cutaneous nitric oxide (NO) bioavailability in this population, which may alter local cutaneous vasodilation and sweating. To evaluate this, we compared these heat loss responses in young men (18-30 y) of black-African (n=10) and Caucasian (n=10) descent during rest, exercise, and recovery in the heat. Participants were matched for physical characteristics and fitness, and they were all born and raised in the same temperate environment (i.e. Canada; second-generation and higher). Both groups rested for 10-min, and then performed 50-min of moderate-intensity exercise at 200 W/m^2, followed by 30-min of recovery in hot-dry heat (35°C, 20% RH). Local cutaneous vascular conductance ($CVC_{\%max}$) and sweat rate (SR) were measured at two forearm skin sites treated with a) lactated-Ringer (Control), or b) 10 mM N^G-nitro-L-arginine methyl ester (L-NAME, NO synthase-inhibitor). L-NAME significantly reduced $CVC_{\%max}$ throughout rest, exercise, and recovery in both groups (both $p<0.001$). However, there were no significant main effects for the NO contribution to $CVC_{\%max}$ between groups (all $p>0.500$). L-NAME significantly reduced local SR in both groups (both $p<0.050$). The NO contribution to SR was similar between groups such that L-NAME reduced SR relative to control at 40 and 50 min into exercise (both $p<0.050$). We demonstrate that ethnicity per se does not influence NO-dependent cutaneous vasodilation and sweating in healthy young black-African descent and Caucasian men during exercise in dry heat.

INTRODUCTION

Heat-related mortality is higher among men of black-African descent compared to Caucasians (white Europeans) (CDC, 2017). While the reasons behind this are multifactorial, it may in part be attributed to a reduced capacity for heat dissipation via attenuated vasodilator and sweating responses at the cutaneous end-organ level (i.e. cutaneous vasculature and eccrine sweat glands). Indeed, black-African descendants demonstrate reduced forearm (Ozkor *et al.*, 2014) and local cutaneous (Maley *et al.*, 2017a) vasodilator responsiveness relative to Caucasians. Further, sweat gland activation during passive heat exposure is lower in black-African descent men than in Caucasians (Kawahata & Adams, 1961), albeit relative increases in sweat output in response to phamacological stimulation have also been reported for men of black-African descent (Gibson & Shelley, 1948).

In young men, nitric oxide (NO) has been established as an important modulator of cutaneous vasodilation and sweating during an exercise-induced heat stress (Welch *et al.*, 2009; McNamara *et al.*, 2014; Stapleton *et al.*, 2014; Fujii *et al.*, 2016b). However, previous work was not designed to examine potential ethnic differences in the contribution of NO to these heat loss responses. Importantly, *in vitro* evidence indicates that NO bioavailability may be reduced in individuals of black-African descent relative to Caucasians, which has been linked to excess superoxide production in this population (Kalinowski *et al.*, 2004; Mason *et al.*, 2005). In support of this, NO-dependent cutaneous vasodilation is also attenuated in young men of black-African descent, which can be counteracted with local delivery of the superoxide dismutase mimetic Tempol (Hurr *et al.*, 2018) or with L-arginine supplementation (Kim *et al.*, 2018). While other factors

associated with reduced NO bioavailability, such as advanced age, are known to attenuate cutaneous vasodilation and sweating during exercise (Fujii *et al.*, 2016b), it remains uncertain whether this translates into local impairments of these heat loss responses in young men of black-African descent.

Therefore, we assessed NO-dependent cutaneous vasodilator and sweating responses in young black-African descent and Caucasian men during and following an exercise-heat stress. We hypothesized that NO-dependent cutaneous vasodilation and sweating would be attenuated in young African descent men compared to Caucasians under these conditions.

METHODS

Ethical Approval

This study was approved by the University of Ottawa Health Sciences and Science Research Ethics Board in accordance with the guidelines set forth by the *Declaration of Helsinki.* All volunteers provided verbal and written informed consent prior to participation.

Participants

Twenty healthy, non-smoking young black-African descent (n=10) and Caucasian (n=10) men matched for peak oxygen uptake ($\dot{V}O_{2peak}$) and physical characteristics (i.e. body mass and surface area) participated (Table 2). Ethnicity was based on self-classification, whereby participants reported their ethnicity and that of their parents. Participants were eligible if they were second or higher generation Canadians (i.e., born and raised in Canada) as defined by Statistics Canada (Dobson *et al.*, 2018) and if both parents were of the same ethnicity.

Experimental Design

All participants completed one screening and one experimental session, separated by ≥48 h. For all sessions, all participants were instructed to consume ~250-500 ml of water ~2 h before the study and were advised to abstain from over-the-counter medications for 48 h, from strenuous exercise, alcohol and caffeine for 12 h, and from food for 2 h prior to and throughout the experiment.

During the screening session, body mass and height were measured using a digital weight scale platform (Model CBU150X, Mettler Toledo Inc., OH, USA) and an eye-level physician stadiometer (Model 2391, Detecto Scale Company, Webb City, MO, USA), respectively. $\dot{V}O_{2peak}$ was assessed using an automated indirect calorimetry system (Medgraphic Ultima; Medical Graphic, St. Paul, MN, USA) via a progressive incremental cycling exercise protocol on a semi-recumbent cycling ergometer (Corival; Lode B.V., Groningen, the Netherlands). The protocol began with 1 minute of cycling at a workload of 80 W, which was then increased by 20 W/min until volitional fatigue. The participants maintained a consistent pedaling rate between 60-100 revolutions·min^{-1}.

Upon arrival for the experimental session, participants voided their bladders and after confirming euhydration [urine specific gravity: <1.025; (Kenefick & Cheuvront, 2012)], changed into athletic shorts, then measured the pre-trial body mass on a weighing terminal (Model CBU150X, Mettler Toledo Inc., OH, USA). Participants then sat in a semi-recumbent position on a medical bed in a non-heat stress environment (25°C) while two microdialysis fibres (30 kDa cutoff, 10 mm membrane) (MD2000; Bioanalytical Systems, West Lafayette, IN, USA) were inserted into the dermal layer of skin on the left dorsal forearm under aseptic conditions. Each skin site was initially wiped with an alcohol swab.

At each forearm site, a 25-gauge needle was inserted in the dermal layer of the skin (~2.5 cm in length). The microdialysis fibre was subsequently threaded through the lumen of the needle, after which the needle was removed from the skin, leaving the fibre embedded under the skin. Each fibre was secured with surgical tape and separated by at least 4 cm. The process was repeated for the other skin site. After fibre insertions, participants were then transferred to an adjacent thermal chamber regulated at 35°C and 20% relative humidity (Can-Trol Environmental Systems, Markham, ON, Canada) where they remained resting on a semi-recumbent cycler ergometer for a minimum of 75 min. At the start of the resting period in the heat, perfusion of pharmacological agents was initiated at the two microdialysis sites at a rate of 4 μl·min^{-1} with a microinfusion pump (model 400; CMA Microdialysis, Solna, Sweden). The sites were perfused with a) lactated Ringer (CTRL, Baxter, Deerfield, IL, USA) or b) 10 mM *N*^{G}-nitro-L-arginine methyl ester (L-NAME, Sigma-Aldrich, St. Louis, MO, USA), as previously described (Fujii *et al.*, 2014). Drug infusion continued for at least 75 min before baseline resting measurements commenced to ensure that vasodilation due to fibre insertion trauma had subsided (Anderson *et al.*, 1994).

Participants rested for 10 min, after which they performed 50-min of cycling at a fixed rate of metabolic heat production of 200 W·m^{-2} equivalent to 47(7) (AFD) and 44(5) (CAU) %$\dot{V}O_{2peak}$, followed by 30-min of recovery. Following recovery, 50 mM sodium nitroprusside (SNP, Sigma-Aldrich) was infused for 20-25 min at both sites at a rate of 6 μL/min until maximum cutaneous perfusion was achieved for at least 2 min. Afterwards, the participant's post body mass was measured.

Measurements

Local cutaneous blood flow [expressed in perfusion units (PU)], which is an index of cutaneous blood flow] and sweat rate (SR, expressed in $mg \cdot min^{-1} \cdot cm^{-2}$) were measured simultaneously at both skin sites using laser-Doppler flowmetry (PeriFlux System 5000, Perimed, Stockholm, Sweden) and custom designed ventilated capsules, as previously described (Meade *et al.*, 2016). Cutaneous vascular conductance (CVC) was calculated as perfusion units divided by mean arterial pressure and expressed as a percentage of maximum during SNP ($CVC_{\%max}$).

Blood pressure (Baumonometer Standby Model; WA Baum, Copiague, NY, USA), heart rate (Polar Electro, Kempele, Finland), aural canal temperature (an index of body core temperature) (Braun Thermoscan Pro 6000; Welch Allyn, Skaneateles Falls, NY, USA), skin temperatures (Concept Engineering, Old Saybrook, CT, USA) and metabolic energy expenditure (AMETEK model S-3A/1 and CD3A, Applied Electrochemistry, Pittsburgh, PA, USA) were all measured as previously described (Fujii *et al.*, 2018).

Data analysis

Baseline resting values were obtained by averaging measurements performed over the 10 min. Values at the start of exercise (time 0) were obtained during the last 5 min before exercise commenced. Local forearm SR and CVC as well as core body and mean skin temperature data acquired during the exercise and recovery periods were obtained by averaging measurements made over the last 5 min of each 10 min interval. Maximal CVC was defined as the highest consecutive 2-minute interval during SNP infusion.

Statistical analysis

SR and $CVC_{\%max}$ were analyzed using a two-way repeated-measures analysis of variance (ANOVA) with factors of time (10 levels: 10 min throughout baseline, and every 10 min during the 50-min exercise and 30-min recovery periods) and treatment site (two levels: CTRL, L-NAME) separately performed in each group (AFD, CAU). Group differences in SR and $CVC_{\%max}$ at the control sites were analyzed using a two-way mixed-model ANOVA with the factors of time and group. Group differences in NO-dependent SR and $CVC_{\%max}$ were evaluated by examining the absolute differences between L-NAME and control sites across time using a two-way mixed-model ANOVA with the factors of time and group. Body temperatures (i.e., core body and skin temperature) and cardiovascular (mean arterial pressure, heart rate) variables were analyzed using a two-way mixed-model ANOVA with the factors of time (three levels: baseline, end-exercise and end-recovery) and group (2 levels: AFD, CAU). When a significant main effect or interaction was detected, multiple comparisons tests were performed using the Bonferroni method. Physical characteristics and hydration status were compared between-groups using unpaired, two-tailed *t* tests. Alpha was set at $p<0.05$ for all statistical comparisons. Values are reported as mean (95% CI) or mean (SD), where appropriate. Statistical analyses were conducted using GraphPad Prism v.8.2 (GraphPad, CA, USA).

RESULTS

Cutaneous vascular conductance

For $CVC_{\%max}$, the treatment site by time interactions were not significantly different for either group (p=0.647, AFD; p=0.751, CAU). However, both demonstrated significant main effects for time (p<0.001, both groups) and treatment site (p=0.001, AFD; p=0.007, CAU) such that L-NAME significantly reduced $CVC_{\%max}$ relative to control throughout the protocol (Figure 1). At the control sites, $CVC_{\%max}$ was similar between groups (p=0.947 for group by time interaction, p=0.916 for main effect of group, and p<0.001 for main effect of time). The NO-dependent effects on $CVC_{\%max}$ were not significantly different between groups (p=0.993 for the group by time interaction, p=0.794 for main effect of group, and p=0.477 for main effect of time).

For maximum absolute CVC for AFD (CTRL: 1.72 (0.65) $PU \cdot mmHg^{-1}$; L-NAME: 1.27 (0.67) $PU \cdot mmHg^{-1}$) and CAU (CTRL: 1.81 (0.28) $PU \cdot mmHg^{-1}$; L-NAME: 1.80 (0.66) $PU \cdot mmHg^{-1}$) there were no significant main effects for treatment site, group or the treatment site by group interaction (all p>0.05).

Sweat rate

For SR there was a significant treatment site by time interaction (p=0.049) and a significant main effect of time (p<0.001) but not treatment site (p=0.073) for black-African descent men. For Caucasians, the treatment site by time interaction was not statistically significant (p=0.202), however, there were significant main effects for time (p<0.001) and treatment site (p=0.044) (Figure 1). At the control sites, SR was similar between groups (p=0.254 for group by time interaction, p=0.228 for main effect of group, and p<0.001 for

main effect of time). The NO-dependent effects on SR were not significantly different between groups ($p=0.535$ for the group by time interaction, $p=0.496$ for main effect of group, and $p=0.028$ for main effect of time). However, L-NAME significantly reduced SR relative to control at 40 and 50 min into exercise (both $p<0.05$).

Body temperatures and cardiovascular responses

For core body and skin temperatures, heart rates, and mean arterial pressures there were no significant effects of group or the group by time interaction between black-African descendants and Caucasians ($p>0.05$). For all variables there were significant main effects for time ($p<0.05$) such that all responses were elevated from baseline at end-exercise and end-recovery as well as at end-exercise compared to end-recovery (all $p<0.05$) (Table 1).

Hydration status and body weight loss

All participants were euhydrated, as confirmed by baseline urine specific gravity of 1.016 (0.01) (AFD) and 1.016 (0.01) (CAU). Following the experiment, body mass was reduced by 1.1 (0.16) kg (AFD) and 1.2 (0.13) kg (CAU), respectively.

DISCUSSION

This is the first to compare NO-dependent cutaneous vasodilation and sweating responses in young black-African descent and Caucasian men during rest, exercise, and recovery in dry-heat. Consistent with previous work, we observed NO-dependent attenuations in cutaneous vasodilation and sweating responses in both groups. However,

in contrast to our hypothesis, these effects were not attenuated in black-African descent men relative to Caucasians.

Consistent with previous work in young men of various ethnicities (Welch *et al.*, 2009; McNamara *et al.*, 2014; Stapleton *et al.*, 2014; Fujii *et al.*, 2015c) , we showed that nitric oxide synthase (NOS) inhibition attenuated cutaneous vasodilation throughout rest, exercise, and recovery in the heat. Here we extend upon prior work by showing that NOS inhibition attenuated cutaneous vasodilation to a similar extent in black-African descent and Caucasian men, indicating that ethnicity does not influence NO-dependent cutaneous vasodilation under the conditions tested. Our current findings contrast with previously identified attenuations in NOS-dependent cutaneous vasodilation during local skin heating in young black-African descent compared to Caucasian men (Hurr *et al.*, 2018; Kim *et al.*, 2018). This discrepancy may be due to higher levels of cutaneous perfusion achieved during these local heating studies (~80-90 $CVC_{\%max}$) compared to the current protocol (~55-60 $CVC_{\%max}$ at end-exercise). As such, under exercise conditions eliciting higher levels of cutaneous perfusion, NO-dependent cutaneous vasodilation may be attenuated in young black-African descent men relative to Caucasians, however, this remains to be confirmed.

Consistent with previous work in young men (Welch *et al.*, 2009; McNamara *et al.*, 2014; Stapleton *et al.*, 2014; Fujii *et al.*, 2015c), we showed that NOS inhibition attenuated sweating and this effect was most evident at end-exercise when local sweat rates were at their maximum. Here, we extend on these prior findings by showing that NOS-dependent sweating is similar between black-African descent and Caucasian men under these conditions. Additionally, local forearm sweat rates at the untreated control sites

were comparable between groups, which demonstrates that the overall capacity for sweating may not be different between black-African descent and Caucasian men under these conditions. NOS-dependent sweating during exercise is more evident in individuals who produce higher sweat rates for a given heat load, regardless of aerobic fitness (Amano *et al.*, 2017b). As such, the similar sweating capacities observed between groups should have resulted in a comparable NOS contribution to this response assuming NO bioavailability is not compromised, which is consistent with our current observations. This, along with similar NOS-dependent cutaneous vasodilator responses between groups, indicate that NO bioavailability was not compromised in the black-African descent men under the current conditions.

This is the first study designed to compare NO-dependent cutaneous vasodilation and sweating in young black-African descent and Caucasian men during exercise in dry heat. As previously mentioned, one potential mechanism contributing to altered cutaneous vasodilation and sweating responses in young black-African descendants may be decreased NO bioavailability which is often ascribed to inherited (genotypic) adaptations (Mata-Greenwood & Chen, 2008). However, when examining ethnic groups who may reside in different climates (i.e. temperature vs. tropical), phenotypic adaptations resulting from long-term residence in a hot environment (i.e. heat acclimatization) may influence such comparisons, obscuring inherited (genetic) adaptations (Taylor, 2006b). Therefore, in the present study, we selected males born and raised in the same temperate climate in Canada (i.e. second generation or higher) to minimize the effects of phenotypic adaptations resulting from environmental influences on NO-dependent cutaneous vasodilation and sweating.

In addition to isolating the inherited (i.e. genetic) effects of ethnicity, the groups were also matched for physical characteristics, and we employed a design eliciting a similar fixed rate of metabolic heat production between groups, and therefore, the same heat loss required to attain heat balance. This approach minimized the influence of possible variables that could influence heat exchange, and therefore, allowed for isolating the influence of ethnicity on NO-dependent cutaneous vasodilation and sweating between groups. Using this novel approach, we demonstrated that ethnicity *per se* does not influence NO-dependent cutaneous vasodilation or sweating in healthy young men during rest, moderate-intensity exercise, and recovery in dry-heat. That said, differences in cutaneous vasodilator responsiveness between black-African descent and Caucasian men have been observed under other experimental conditions (Maley *et al.*, 2017a; Hurr *et al.*, 2018; Kim *et al.*, 2018). This indicates that ethnicity should remain an important consideration when designing mechanistic studies to evaluate local thermoeffector control depending on the modulator being investigated and the experimental conditions being utilized. Further, while this study provides novel insights into the effects of ethnicity on NO-dependent cutaneous vasodilation and sweating, the results are limited to healthy young black-African descent and Caucasian men. Therefore, future studies are necessary to evaluate these effects in other ethnic groups and in women and older adults who may demonstrate more pronounced reductions in these heat loss responses via reduced NO bioavailability compared to young men.

CONCLUSION

We show that inherited (i.e. genetic) adaptations associated with ethnicity do not influence nitric oxide-dependent cutaneous vasodilation and sweating during rest, exercise, and recovery in the heat. Our findings provide valuable insights into the mechanisms regulating cutaneous vasodilation and sweating in young black-African descent men who may have a reduced capacity to dissipate heat during exercise, resulting in a greater risk of heat-related injury relative to Caucasians.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s). The results of the current study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

AUTHOR CONTRIBUTIONS

C.M.M., G.W.M, N.F., and G.P.K. conceptualized and designed the research. C.M.M., M.D.S. and G.W.M contributed to data collection. C.M.M. and G.W.M. analyzed the data. G.W.M. prepared figures. C.M.M. and G.W.M drafted the manuscript. All authors interpreted the results, edited the manuscript and approved the final version.

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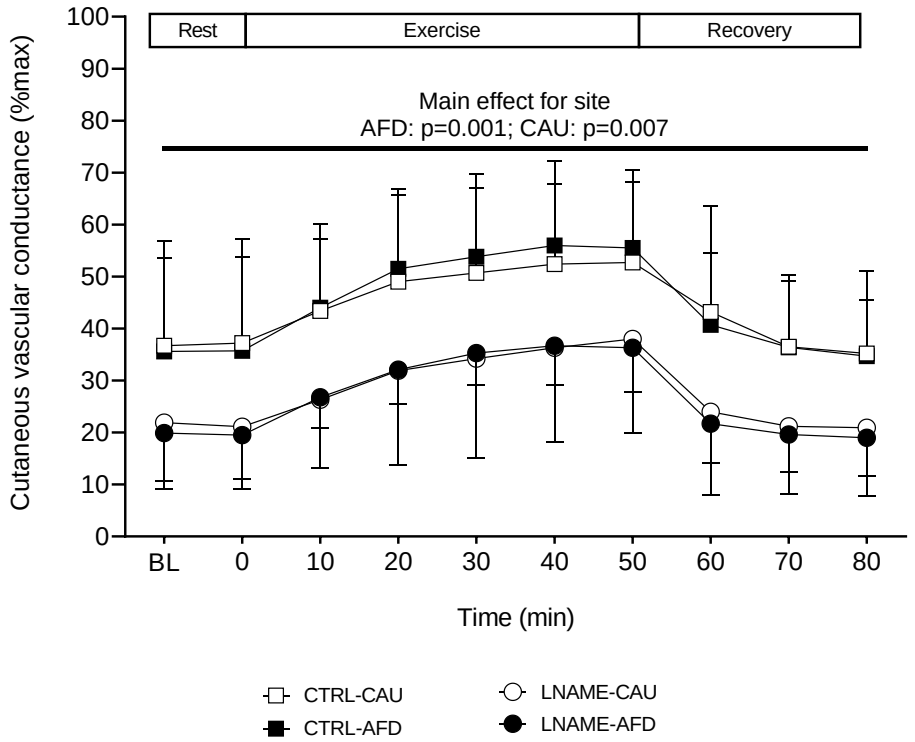
FIGURE CAPTION AND TABLES

Figure 1. Time course changes (n=10 in each group) in local forearm cutaneous vascular conductance (A) and forearm sweat rate (B) during rest, exercise at 200 W/m^2 (equivalent to moderate intensity exercise) heat load), and recovery in black-African descent (AFD, black symbols) and Caucasian (CAU, white symbols) men. Two skin sites on the dorsal forearm were continuously treated with either 1) lactated Ringer solution (CTRL, squares) or 2) 10 mM N^G-nitro-L-arginine methyl ester (L-NAME, circles). Values are presented as mean (SD). Each value during exercise and recovery represents the average of the last 5 min of each 10 min interval. *Significantly different from control for both groups at $p<0.05$.


Rest
Exercise
Recovery
Main effect for site
AFD: p=0.001; CAU: p=0.007
Cutaneous vascular conductance (%max)
100
90
80
70
60
50
40
30
20
10
0
BL
0
10
20
30
40
50
60
70
80
Time (min)
CTRL-CAU
CTRL-AFD
LNAME-CAU
LNAME-AFD

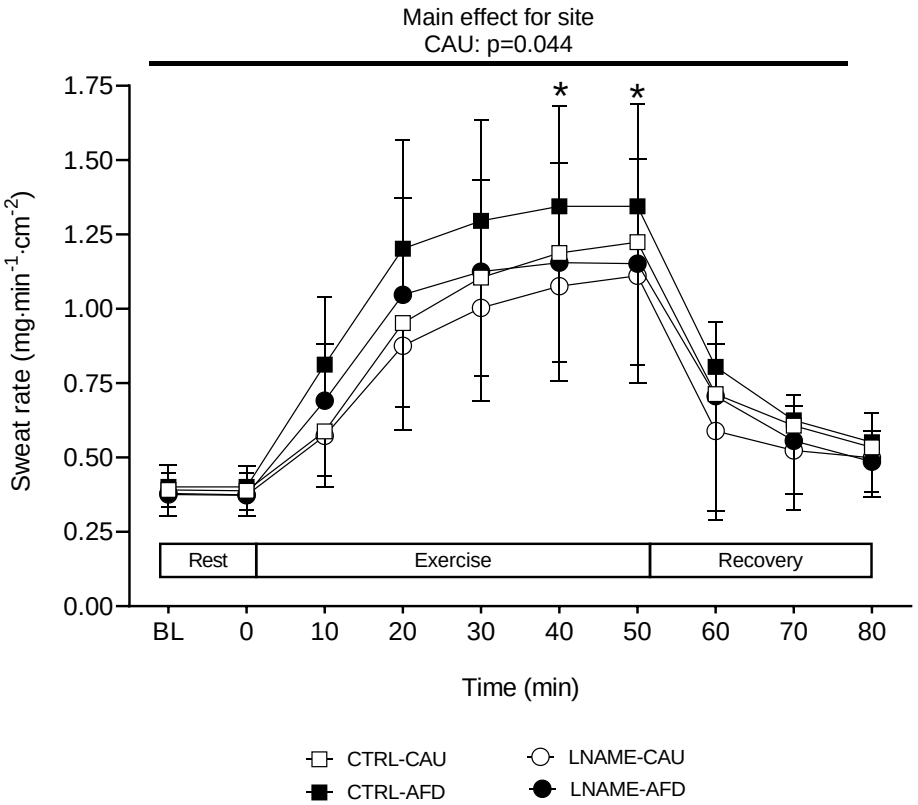

Main effect for site
CAU: p=0.044
*
*
Sweat rate (mg·min-1·cm-2)
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Rest
Exercise
Recovery
BL
0
10
20
30
40
50
60
70
80
Time (min)
CTRL-CAU
CTRL-AFD
LNAME-CAU
LNAME-AFD

Table 1. Thermal and cardiovascular responses

		Rest	End-Exercise	End-Recovery
Core temperature (°C)				
	AFD	37.1 (0.3)	37.8 (0.3)	37.4 (0.3)
	CAU	37.1 (0.3)	37.7 (0.3)	37.4 (0.3)
Mean skin temperature (°C)				
	AFD	34.4 (0.3)	35.2 (0.4)	34.5 (0.5)
	CAU	34.2 (0.5)	35.3 (0.4)	34.6 (0.5)
Heart rate (beats·min^{-1})				
	AFD	77 (11)	135 (17)	88 (16)
	CAU	76 (10)	130 (19)	90 (13)
Mean arterial pressure (mmHg)				
	AFD	95 (6)	106 (11)	95 (5)
	CAU	93 (4)	102 (12)	93 (5)

Values are presented as mean (SD), n=10 subjects in each group. All values represent the average over the final 5 min of each time interval. AFD, black-African descendant; CAU, Caucasian.

Table 2. Participant characteristics for black-African descent and Caucasian groups

	Black-African descent	Caucasian
Age (yr)	22(3)	23(4)
Height (m)	1.75(0.05)	1.77(0.07)
Body mass (kg)	78.21 (12.46)	78.86(8.10)
Body surface area (m^2)	1.94(0.16)	1.96(0.12)
Body fat (%)	13.477.91)	19.34(4.72)
Peak oxygen consumption ($mL \cdot kg^{-1} \cdot min^{-1}$)	39.29(7.55)	40.81(3.78)

Values are presented as mean (SD), n=10 participants in each group.

Article II:
Whole-body heat exchange in second generation black-African and Caucasian men during exercise eliciting matched heat-loss requirements in dry heat

Whole-body heat exchange in second generation black-African and Caucasian men during exercise eliciting matched heat-loss requirements in dry heat

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Running title: Ethnicity and whole-body heat dissipation

Key words: ethnicity, exercise, heat stress

Exposition word count: 3378/4000

Number of references: 24

Subject area: Environmental and exercise physiology

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NEW FINDINGS (100/100 words)

What is the central question of this study?

- Black-African descendants are thought, by some, to have inherited physiological adaptations conducive to survival in hot climates. We therefore assessed whether Canadians of black-African descent display enhanced whole-body total heat loss (evaporative + dry heat exchange) compared to Caucasian-Canadians during exercise eliciting matched heat-loss requirements in dry heat.

What is the main finding and its importance?

- Neither whole-body total heat loss nor body heat storage differed significantly between groups, irrespective of the exercise-intensity. Our findings indicate that genotypic adaptations associated with ethnicity do not appreciably modify whole-body heat exchange during exercise-heat stress.

ABSTRACT (Word count: 250/250)

Ethnicity has long been thought to modulate thermoregulatory function; however, an evaluation of whole-body heat exchange in second-generation (born and raised in the same climate) men of black-African descent and Caucasian men (white European descendants) during exercise eliciting matched heat-loss requirements remained unavailable. We therefore used direct calorimetry to assess whole-body total heat loss (evaporative + dry heat exchange) in young (18-30 years), second-generation black-African (*n*=11) and Caucasian (*n*=11) men. Participants performed three, 30-min bouts of semi-recumbent cycling at fixed metabolic heat productions (and therefore matched heat loss requirements between groups) of 200 (light), 250 (moderate), and 300 W/m^2 (vigorous), each followed by 15-min recovery, in dry heat (40°C, ~13% relative humidity). Across all exercise bouts, dry (*p*=0.435) and evaporative (*p*=0.600) heat exchange did not differ significantly between groups. As such, total heat loss during light, moderate and vigorous exercise was similar between groups (*p*=0.777), averaging ((mean (SD)); 177 (10), 217 (13) and 244 (20) W/m^2 in men of black-African descent, and 172 (13), 212 (17) and 244 (17) W/m^2 in Caucasian men. Accordingly, body heat storage across all exercise bouts (summation of metabolic heat production and total heat loss) was also similar between the black-African (568 (142) kJ) and Caucasian groups (623 (124) kJ; *p*=0.356). We demonstrate that, when assessed in young, second-generation black-African and Caucasian men during exercise eliciting matched heat-loss requirements in dry heat, ethnicity did not significantly modulate whole-body dry and evaporative heat exchange or the resulting changes in total heat loss and body heat storage.

INTRODUCTION

It has long been thought that, compared to Caucasians (white European descendants) originating from more temperate climates, individuals of black-African descent possess physiological adaptations that may provide a thermoregulatory advantage during work in the heat (Robinson *et al.*, 1941; Baker, 1958; Wyndham *et al.*, 1964). These include increases in skin temperature that buffer dry heat gain by minimizing the thermal-gradient between the skin and environment (Thomson, 1954), as well as increases in sweat secretion in response to pharmacological stimulation (Gibson & Shelley, 1948) and improved sweating efficiency (*i.e*., reduced wasteful sweating in humid environments) (Thomson, 1954; Yousef *et al.*, 1984), which optimize evaporative heat loss. While such thermoregulatory adjustments are often ascribed to inherited (genotypic) adaptations that are conducive to survival in extreme environments (Baker, 1958; Scholander *et al.*, 1958; Roberts *et al.*, 1970), they may simply represent phenotypic adaptations (*i.e*., heat acclimatization) associated with the black-African descendants studied being residents of hotter climates than their Caucasian counterparts (Taylor, 2006b).

Given that genotypic adaptations occurring in individuals of black-African descent would be conveyed across generations, it has been suggested that a more appropriate design to isolate these effects from any phenotypic adaptation would be to assess heat exchange in black-African descendants and Caucasians who both have long-term residence in the same climate (Taylor, 2006b). However, previous comparisons of thermoregulatory function in black-African descendants and Caucasians during exercise-heat stress satisfying this important design consideration are sparse (Robinson *et al.*,

1941; Yousef *et al.*, 1984; Marino *et al.*, 2004), and have been directed toward assessing heat tolerance in a field setting (Robinson *et al.*, 1941; Yousef *et al.*, 1984) or exercise performance (Marino *et al.*, 2004). To that end, those investigators were often unable to standardize the environmental conditions (Robinson *et al.*, 1941; Yousef *et al.*, 1984) and incorporated only indirect estimates of heat exchange (*e.g.*, body mass change measured prior to and following the experiment) (Robinson *et al.*, 1941; Yousef *et al.*, 1984; Marino *et al.*, 2004), which provide limited information on the effects of ethnicity on time-dependent changes on both whole-body dry and evaporative heat exchange. Further, in the most recent of those studies (Marino *et al.*, 2004), the exercise performed elicited between-group differences in metabolic heat production, and thus, the heat loss required to attain heat balance, making it impossible to draw valid comparisons of heat exchange between ethnic groups. Therefore, despite extensive research on this topic, no study in our view has been adequately designed to assess whether genotypic adaptations (if any) occurring in individuals of black-African descent modulate whole-body heat exchange during exercise-heat stress.

We therefore used direct calorimetry to measure whole-body dry and evaporative heat exchange in second-generation Canadians of black-African descent and Caucasian ethnicity during exercise eliciting matched heat-loss requirements in dry heat. By studying men born and raised in a primarily temperate climate (Canada), we were able to minimize potential between-group differences in phenotypic adaptation. Further, we employed an experimental protocol involving three successive bouts of cycling consisting of increasing, fixed rates of metabolic heat production of 200, 250, and 300 W/m^2, to ensure the area-specific requirement for heat loss was matched between groups. We hypothesized that

individuals of black-African descent would display elevations in evaporative heat loss and reductions in dry heat gain that would enhance whole-body total heat loss (evaporative + dry heat exchange) relative to Caucasians.

METHODS

Ethical approval

The experimental protocol was approved by the University of Ottawa Health Sciences and Science Research Ethics Board and conforms to the latest revision of the *Declaration of Helsinki*, except for registration in a database. Written informed consent was obtained from all participants.

Participants

Twenty-two young (18-30 years), black-African (*n*=11) and Caucasian (*n*=11) men, with similar peak oxygen consumption ($\dot{V}O_{2peak}$) and physical characteristics participated (Table 1). Ethnicity was based on self-classification. All participants reported their ethnicity and their parent's ethnicity and were enrolled only if both parents were of black-African descent or Caucasian, respectively. All participants belonged to either the second or higher generations (*i.e*., born and raised in Canada) as defined by Statistics Canada (Dobson *et al.*, 2018). Black-African participants were descendants from the black ethnic groups of Africa, while Caucasian participants included white-European descendants. Participants were non-smokers, were not taking any medication and did not report a history of cardiovascular, respiratory, or metabolic disease.

Experimental procedures

All participants completed one preliminary session and one experimental trial on separate days (separated by >48h). During the preliminary session, body height, mass and density, as well as $\dot{V}O_{2peak}$ were determined. Body surface area was derived from measures of height (model 2391, Detecto, Webb City, MO, USA) and body mass (IND560, Mettler Toledo Inc., Mississauga, ON, Canada) (Du Bois & Du Bois, 1916). Body density was measured using the hydrostatic weighing technique and used to estimate body fat percentage (Siri, 1956). $\dot{V}O_{2peak}$ was assessed by indirect calorimetry (MCD Medgraphics Ultima Series, MGC Diagnostics, Saint Paul, MN, USA) during an incremental exercise test (CSEP, 1986) on a semi-recumbent cycle ergometer (Corival, Lode B.V., Groningen, Netherlands) in thermoneutral conditions (~23°C).

For the experimental session, all participants were instructed to arrive at the laboratory adequately rested and hydrated as well as abstained from exercise, caffeine and alcohol 24h prior. After confirming euhydration (urine specific gravity <1.025) (Kenefick & Cheuvront, 2012), participants changed into athletic shorts and shoes, and were instrumented in a temperate room (~25ºC). Participants then entered a direct air calorimeter regulated to an air temperature of 40°C and relative humidity of ~13% to complete 15-min seated rest (rest) followed by three, 30-min bouts of cycling at metabolic heat productions of 200, 250, 300 W/m^2, each followed by 15-min recovery. These work rates were equivalent to ~30, 45 and 60% of each individual's pre-determined $\dot{V}O_{2peak}$, and were therefore classified as light, moderate and vigorous exercise, respectively.

Measurements

The Snellen direct air calorimeter was used to quantify whole-body evaporative and dry heat exchange (Kenny *et al.*, 2017). Briefly, the calorimeter inflow and outflow values of absolute humidity and air temperature were measured at 8 second intervals using high precision dew point hygrometry (RH Systems model 373H, Albuquerque, NM, USA), and resistance temperature detectors (Black Stack model 110V 50/60H2, Fluke Corporation, WA, USA). Air mass flow through the calorimeter was measured by differential thermometry over a known heat source placed in the effluent air stream. Evaporative heat loss was calculated using the calorimeter outflow – inflow difference in absolute humidity, multiplied by the air mass flow ($kg \cdot s^{-1}$) and the latent heat of vaporization of sweat (2426 $J \cdot g^{-1}$). Dry heat loss was calculated from the calorimeter outflow – inflow difference in air temperature, multiplied by the air mass flow and specific heat capacity of air (1005 $J \cdot kg^{-1} \cdot °C^{-1}$), with negative values denoting heat gain from the environment (*i.e*., when air temperature exceeds that of the skin). Oxygen consumption, carbon dioxide production and minute ventilation were derived continuously from measures of expired gases and air flows (Moxus modular metabolic system, AEI Technologies, Bastrop, TX, USA), and used to approximate metabolic rate. External work rate was controlled to maintain metabolic heat production (metabolic rate – external work) during exercise (Kenny *et al.*, 2017). To account for respiratory heat exchange, expired air was recycled back into the calorimeter.

Rectal temperature was measured using a thermocouple probe (Mon-a-therm General Purpose Temperature Probe, Mallinckrodt Medical, St. Louis, MO, USA) inserted ~12 cm past the anal sphincter. Skin temperature was measured at the bicep, chest,

thigh, and calf using T-type thermocouples (Concept Engineering, Old Saybrook, CT, USA), and used to derive mean skin temperature as the weighted average of the four sites: biceps 30%, chest 30%, thigh 20%, and calf 20% (Ramanathan, 1964). All temperatures were collected continuously using an HP Agilent data acquisition module (model 3497A; Agilent Technologies Canada, Mississauga, ON, Canada). Heart rate was recorded continuously using a Polar M400 monitor (Polar Electro, Oy, Finland).

Data and statistical analysis

The cumulative change in body heat content (kJ) was calculated as the temporal summation of metabolic heat production and total heat loss (evaporative + dry heat loss) across all exercise bouts. Metabolic heat production, total heat loss, dry and evaporative heat exchange, rectal and mean skin temperatures, and heart rate were expressed as minute averages, with an average of the final 5-mins of each rest and exercise period used for statistical analyses. These data were analyzed using a mixed, two-way ANOVA with the non-repeated factor of group (black-African descent, Caucasian) and the repeated factor of time (rest, light, moderate, vigorous). *Post hoc* comparisons were performed using paired (time) or unpaired (group), two-tailed *t*-tests when a main effect or interaction was observed, with the *p* value being adjusted using the Holm-Bonferroni procedure. Physical characteristics were compared between-groups using unpaired, two-tailed *t* tests. Alpha was set at 0.050 for all statistical comparisons. Based on the effect size (Cohen's *d* =1.89) associated with previously reported ethnicity-related differences in whole-body sweat rate of 6.8 (1.1) ml/min during exercise in the heat (Marino *et al.*, 2004), a minimum of six subjects per group were required to detect between-group differences of this effect size with at least 80% statistical power (Faul *et al.*, 2007).

Therefore, with the current sample (n=11 per group) these analyses were adequately powered (>80%). Data are reported as mean (SD). Statistical analyses were completed using Prism 8 (GraphPad, CA, USA).

RESULTS

Metabolic heat production, evaporative heat exchange, and total heat loss increased over time (*i.e.*, increasing exercise intensity), while dry heat loss increased over time (main effect of time: all p<0.001) (Figure 1). However, those data were similar between-groups (main effect of group: all p≥0.385) (Figure 1). As such, the cumulative change in body heat content (mean (SD)) across all three exercise periods was also similar between the individuals of black-African descent (568 (142) kJ) and Caucasians (623 (124) kJ) (p=0.353). Rectal and mean skin temperatures also increased over time (main effect of time: both p<0.001) (Figure 2), however no between-group differences were observed (main effect of group: p=0.358 and p=0.892, respectively). A group-by-time interaction was observed for heart rate (p=0.023), although *post-hoc* comparisons revealed that heart rate for the individuals of black-African descent at baseline and during light, moderate and vigorous exercise (78 (13), 130 (17), 148 (17) and 170 (18) beats/min, respectively) did not differ significantly from the Caucasians (80 (15), 122 (21), 140 (23) and 165 (21) beats/min, respectively) (all p≥0.805).

DISCUSSION

The present study was designed to provide what is thought to be the first assessment of whole-body dry and evaporative heat exchange in second-generation

black-African descendants and Caucasians during exercise eliciting matched heat-loss requirements in dry heat. Contrary to our working hypothesis, ethnicity did not significantly modify dry or evaporative heat exchange, or the resulting changes in total heat loss, irrespective of the metabolic heat production employed. This was paralleled by similar changes in body heat storage, body core and mean skin temperatures, as well as cardiovascular strain throughout exercise between groups. Therefore, although genotypic adaptations associated with ethnicity have long been thought to enhance heat exchange among individuals of black-African descent, we demonstrate that such effects are of minimal importance during exercise in hot, dry conditions.

Since the early work of Robinson and colleagues (Robinson *et al.*, 1941), who reported that Caucasians (laboratory staff) were less successful in regulating body temperature than individuals of black-African descent (cotton workers), it has been thought, by some, that black-African descendants may have inherited physiological adaptations that convey a thermoregulatory advantage during work in the heat (Baker, 1958; Roberts *et al.*, 1970). Others, however, have suggested those effects may simply represent phenotypic adaptations (*i.e.*, heat acclimatization) associated with the black-African descendants studied residing in hotter climates than their Caucasian counterparts (Taylor, 2006b). To minimize any phenotypic effects, we assessed heat exchange in black-African descendants and Caucasians who were both born and raised in the same primarily temperate climate (Canada). Further, to perform a detailed assessment of heat exchange during exercise eliciting a matched requirement for heat loss between ethnic groups, we used direct calorimetry to quantify time-dependent changes in whole-body dry and evaporative heat exchange during exercise at increasing, fixed rates of metabolic

heat production. With this unique approach, we could determine the effects of any physiological (genotypic) adaptation conveyed across generations to individuals of black African descent on whole-body heat exchange.

Compared to their Caucasian counterparts, individuals of black-African descent have been reported to display increases in skin temperature (Thomson, 1954) that can buffer dry heat gain in hot environments, coupled with increases in sweat secretion (Gibson & Shelley, 1948) and improved fluid conservation (Thomson, 1954; Yousef *et al.*, 1984), which act to optimize evaporation. We therefore assumed that, should these thermoregulatory adjustments be owed to inherited physiological (genotypic) adaptations, individuals of black-African descent would display enhanced whole-body total heat loss relative to Caucasians residing in the same climate (Canada). In contrast, whole-body total heat loss (Figure 1) and the resulting changes in body heat storage and body core temperature (Figure 2) were similar between groups throughout exercise, indicating that any genotypic adaptation associated with ethnicity does not appreciably modify whole-body heat exchange during exercise in dry heat. While larger confirmatory studies over varying environmental conditions are required, especially given the known effects of ethnicity on skin blood flow (Maley *et al.*, 2017b, a) and mean skin temperature (Newman, 1969; Farnell *et al.*, 2008) in cooler conditions, and since genotypic thermoregulatory adaptations (*e.g*. greater sweating efficiency) may be more pronounced in humid environments (Baker, 1958; Yousef *et al.*, 1984), our findings suggest that ethnicity-related differences in heat exchange proposed in previous studies represent phenotypic, rather than genotypic adaptations. Although this suggestion is not novel (Taylor, 2006b), we provide perhaps the most definitive supporting evidence to date.

The outcomes from the present study may have important implications for experimental design. For a number of years, investigators conducting studies on human thermoregulatory function have often selected participants from only one ethnic group to minimize the confounding influence of ethnicity (Stephens & Hoag, 1981; Glickman-Weiss *et al.*, 1997). This may remain an important consideration for more mechanistic research directed at evaluating thermoeffector control, especially since there is evidence of ethnicity-related differences in the control of cutaneous blood flow (Maley *et al.*, 2017b, a). However, given the observed similarities in whole-body heat exchange, body heat storage and cardiovascular strain between individuals of black-African descent and their Caucasian counterparts in the present study, this approach may be unwarranted for applied research, provided both groups have resided in the same climate for a prolonged duration (*i.e.*, to minimize any phenotypic effects).

CONCLUSION

Genotypic adaptations associated with ethnicity have long been thought to enhance heat exchange among individuals of black-African descent relative to Caucasians. However, we show that whole-body heat exchange is generally similar between young men of black African descent and Caucasians born and raised in a temperate climate during light-to-vigorous exercise in dry heat. Although there is a need for larger confirmatory studies across differing environmental conditions, our findings suggest that any inherited (genotypic) adaptations associated with ethnicity do not appreciably modulate whole-body heat exchange during exercise-heat stress.

DISCLOSURES

None to declare. The results of the study are presented clearly, honestly and without fabrication, falsification or inappropriate data manipulation

AUTHOR CONTRIBUTIONS

C.M.M., S.R.N., and G.P.K. concept and design of research; C.M.M., S.R.N., A.W.D., and S.S. performed experiments. C.M.M. analyzed data. S.R.N. prepared figures and performed statistical analyses. C.M.M., S.R.N., and G.P.K. drafted the manuscript; all authors interpreted results of experiments as well as edited, revised and approved final version of manuscript.

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FIGURE CAPTION AND TABLES

Figure 1. Metabolic heat production (A), dry heat loss (B), evaporative heat loss (C), and total heat loss (D) for individuals of black-African descent (black-African descent) (*n*=11) and Caucasians (Caucasian) (*n*=11) during 15-min seated rest followed by three, 30-min bouts of cycling at metabolic heat productions of 200 (light), 250 (moderate), 300 W/m^2 (vigorous) in dry heat (40˚C, ~13% relative humidity). Data are individual responses (transparent lines and symbols) with means and standard deviations (solid lines and symbols). Results (*p* values) from the mixed, two-way ANOVA used to compare groups (black-African descent, Caucasian) over time (rest, light, moderate, vigorous) are presented in the bottom right of each panel. No significant between-group differences were observed (all $p>0.050$).

Figure 2. Body core (rectal) temperature (A) and mean skin temperature (B) for individuals of black African descent (black-African descent) (*n*=11) and Caucasians (Caucasian) (*n*=11) during 15-min seated rest followed by three, 30-min bouts of cycling at metabolic heat productions of 200 (light), 250 (moderate), 300 W/m^2 (vigorous) in dry heat (40˚C, 13% relative humidity). Data are individual responses (transparent lines and symbols) with means and standard deviations (solid lines and symbols). Results (*p* values) from the mixed, two-way ANOVA used to compare groups (black-African descent, Caucasian) over time (rest, light, moderate, vigorous) are presented in the bottom right of each panel. No significant between-group differences were observed (all $p>0.050$).

Table 1. Physical characteristics of the black-African descent (black-African descent; *n*=11) and Caucasian (Caucasian; *n*=11) groups.

Group		Age (yr)	Height (cm)	Body mass (kg)	A_D (m^2)	$\dot{V}O_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	Body fat (%)
Black-African descent	Mean (SD)	21 (3)	176 (5)	78.3 (15.6)	1.94 (0.18)	41.0 (7.2)	14.2 (7.5)
	min-max	18-28	168-185	58.2-109.9	1.66-2.33	34.3-56.7	5.9-28.5
Caucasian	Mean (SD)	24 (5)	175 (7)	76.6 (8.0)	1.92 (0.11)	43.3 (4.8)	15.5 (5.3)
	min-max	18-30	161-183	62.9-89.5	1.75-2.10	38.0-54.1	8.7-23.5

Notes: A_D, body surface area; $\dot{V}O_{2peak}$, peak oxygen consumption. Due to technical difficulties in one preliminary session, body fat could not be determined in one individual of black-African descent.

Figure 1

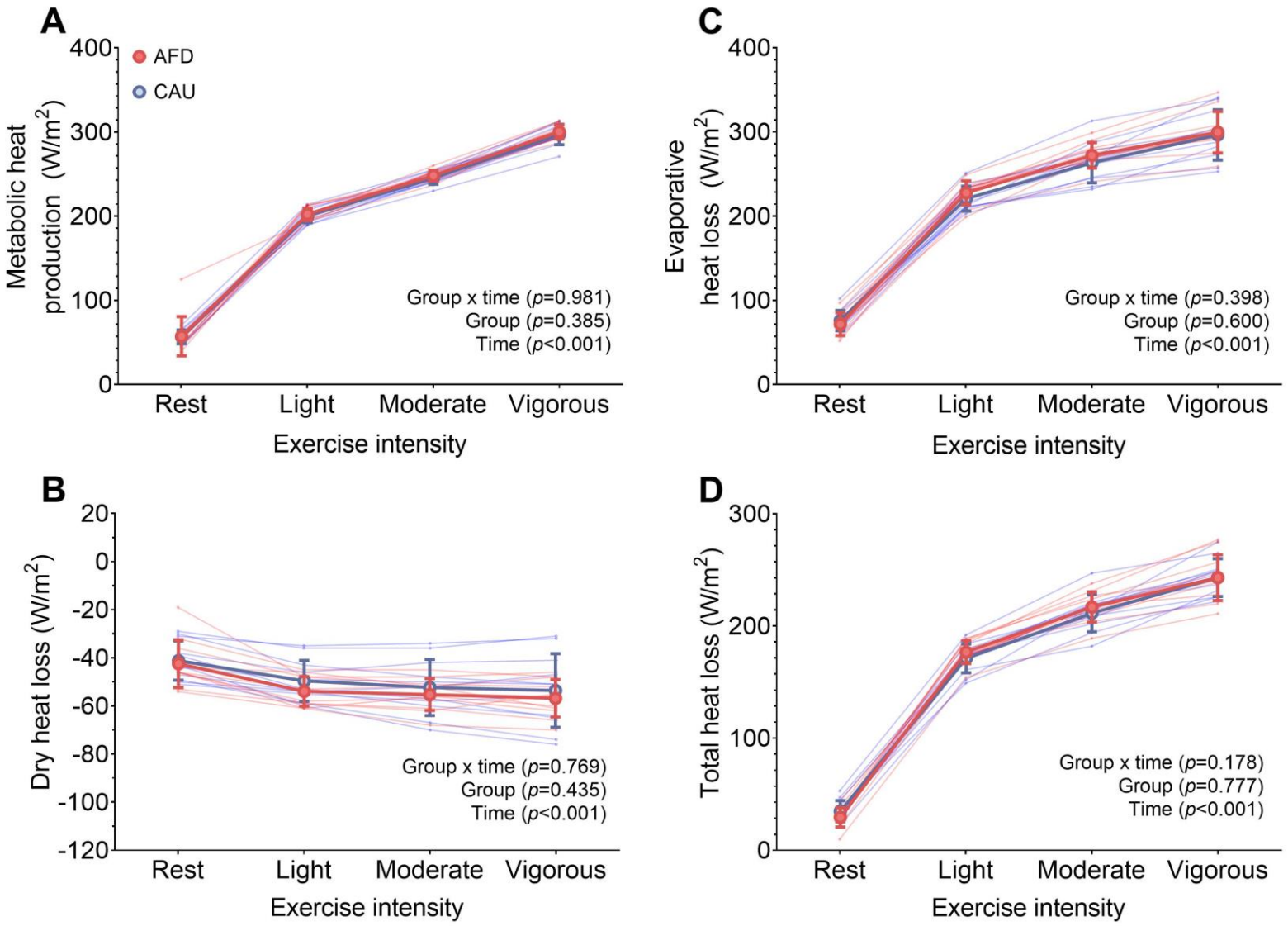
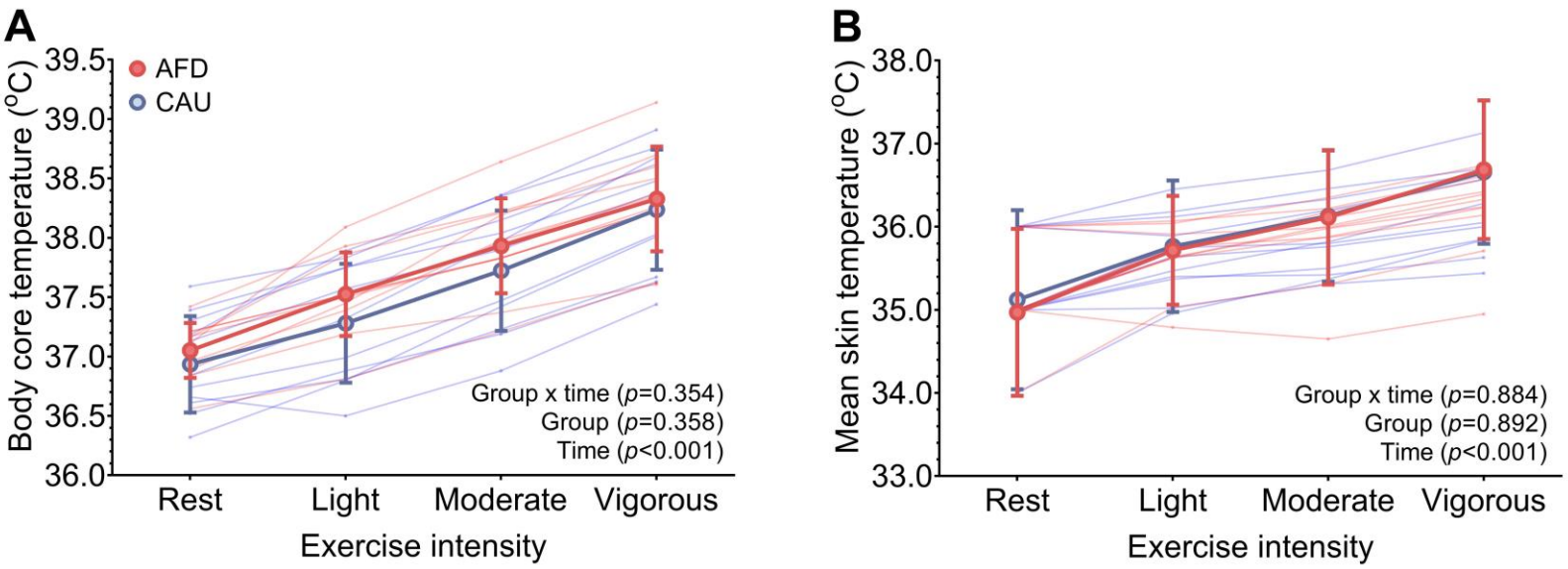

A
AFD
CAU
Metabolic heat production (W/m2)
400
300
200
100
0
Group x time (p=0.981)
Group (p=0.385)
Time (p<0.001)
Rest
Light
Moderate
Vigorous
Exercise intensity
C
Evaporative heat loss (W/m2)
400
300
200
100
0
Group x time (p=0.398)
Group (p=0.600)
Time (p<0.001)
Rest
Light
Moderate
Vigorous
Exercise intensity
B
Dry heat loss (W/m2)
20
0
-20
-40
-60
-80
-100
-120
Group x time (p=0.769)
Group (p=0.435)
Time (p<0.001)
Rest
Light
Moderate
Vigorous
Exercise intensity
D
Total heat loss (W/m2)
300
200
100
0
Group x time (p=0.178)
Group (p=0.777)
Time (p<0.001)
Rest
Light
Moderate
Vigorous
Exercise intensity

Figure 2


A
AFD
CAU
Body core temperature (°C)
39.5
39.0
38.5
38.0
37.5
37.0
36.5
36.0
Group x time (p=0.354)
Group (p=0.358)
Time (p<0.001)
Rest
Light
Moderate
Vigorous
Exercise intensity
B
Mean skin temperature (°C)
38.0
37.0
36.0
35.0
34.0
33.0
Group x time (p=0.884)
Group (p=0.892)
Time (p<0.001)
Rest
Light
Moderate
Vigorous
Exercise intensity

CHAPTER 4

GENERAL CONCLUSIONS OF THE THESIS

The current thesis investigated whether ethnicity influenced the relative contributions of nitric oxide to cutaneous vasodilation and sweating and if differences in whole-body heat exchange led to differences in body heat storage during exercise in the heat in young men of black-African and Caucasian descent. These men were born and raised in the same temperate environment (Canada) and matched for physical characteristics and fitness.

In the first study, we assessed the relative contribution of nitric oxide to cutaneous vasodilation and sweating between these two groups during exercise in dry heat. Prior reports showed attenuations in cutaneous vasodilation after NOS inhibition in young males of various ethnicities during exercise in the heat (Welch *et al.*, 2009; McNamara *et al.*, 2014; Stapleton *et al.*, 2014; Fujii *et al.*, 2015c). The current results support the previous literature by demonstrating that a nitric oxide-dependent attenuation in cutaneous vasodilation was present in young men. Further, it extends on these prior findings by demonstrating that ethnicity does not modulate NOS-dependent cutaneous vasodilation specifically during moderate intensity exercise in the heat. Additionally, these findings are in contrast to previous work conducted during local skin heating conditions (Hurr *et al.*, 2018; Kim *et al.*, 2018) showing attenuations in NOS-dependent cutaneous vasodilation in young men of black-African descent compared to Caucasian men. This discrepancy may be due to differences in the regulation of skin blood flow during local heating conditions compared to exercise conditions. Local heating of the skin causes a direct and substantial vasodilation in the area being warmed. This resulted in higher vasodilation levels (~80-90 $CVC_{\%max}$) in the previous studies. On the other hand, exercise in the heat raises internal temperature and leads to a rapid increase in cutaneous

vasodilation. As exercise continues, and internal temperature approaches 38°C, maximum levels of cutaneous vasodilation are reached, which were (~55-60 $CVC_{\%max}$) in the current study.

We also showed that both groups displayed similar attenuations in NOS-dependent sweating during exercise in the heat and this was consistent with previous work in young men of various ethnicities (Welch *et al.*, 2009; McNamara *et al.*, 2014; Stapleton *et al.*, 2014; Fujii *et al.*, 2015c). The current thesis findings extend upon these observations by showing that ethnicity does not modulate NOS-dependent sweating in young black-African and Caucasian descent men. However, whether this response is unique to these two population groups is unclear. To the best of our knowledge, no other studies have evaluated NOS-dependent sweating during an exercise heat stress in other ethnic groups and therefore, further research is warranted for confirmation.

In the second study, we evaluated whether the relative contribution of dry and evaporative heat loss and therefore whole-body heat exchange differed between the two groups. This was particularly important given prior observations of differences in local cutaneous vasodilation (Hurr *et al.*, 2018; Kim *et al.*, 2018) (which may impact sweating) between black-African and Caucasian-descent men. Differences in cutaneous perfusion and sweating can result in alterations in dry and evaporative heat loss respectively. Despite prior reports indicating altered thermoeffector activity between these two groups, we showed that whole-body heat exchange did not differ between black-African and Caucasian descent men and that this response remained intact regardless of the level of heat stress as defined by increases in exercise intensity.

At the whole-body level, prior reports have demonstrated equivocal findings on heat loss responses between black-African and Caucasian descent men (Riggs & Sargent, 1964; Marino *et al.*, 2004). While some studies have reported that black-African descendants have reduced sweating responses relative to their Caucasian counterparts (Riggs & Sargent, 1964; Wyndham *et al.*, 1964; Marino *et al.*, 2004); others have shown that those responses are either unchanged (Baker, 1958) or higher in black-African descendants (Robinson *et al.*, 1941) compared to Caucasians. The discrepancy in the findings may be due to various factors confounding the independent influence of ethnicity. This includes unmatched physical characteristics, which elicited between-group differences in metabolic heat production, and thus, the heat loss stimulus required to attain heat balance. To overcome these shortcomings, we selected groups with similar physical characteristics and employed a design eliciting similar rates of fixed metabolic heat production and therefore heat load to assess the influence of ethnicity on whole-body heat exchange. Additionally, previous studies examining these two ethnic groups, compared individuals who were born and raised in different climatic regions, which resulted in the introduction of phenotypic adaptations (Thomson, 1954; Baker, 1958). For example: Thomson (1954) conducted a study investigating the heat loss responses of black-African descendants compared to their Caucasian counterparts (n=47) during exercise in the heat. Both groups were tested in Nigeria, West Africa; however, while the black-African descendants were from Nigeria (tropical climate), the Caucasian group were originally from Europe (temperate climate). Therefore, due to the selection of ethnic groups from different climatic regions, Thomson's study resulted in the introduction of phenotypic adaptations. In the current thesis, we select men born and raised in Canada

(temperate climate) to minimize the effect of phenotypic adaptations and to allow for the isolation of genotypic adaptations.

It is beyond any doubt that rising global temperature is the biggest health threat of the century with enormous consequences for humanity. At the national level, a staggering report prepared by Environment and Climate Change Canada (Ministry of Environment and Climate Change) indicates that Canada is warming at a rate twice as fast as the rest of the world, with annual average temperatures increasing by 1.7°C since 1948 (versus a 0.8°C increase globally), with some regions exhibiting unparalleled increases of 2.3°C (Bush & Lemmen, 2019). It is a wakeup call for all Canadians, especially given new research predicts that periods of extreme heat in Canada will be more frequent, intensified, and likely to be five times more deadly. Our study findings demonstrate that while black-African descendants have an elevated risk of a heat-related injury, those of Canadian descent (second generation) do not demonstrate a reduced heat tolerance as evidenced by a similar capacity to dissipate heat during an exercise-heat stress. Consequently, these findings indicate that heat management solutions and strategies can provide an equal level of protection to both young black-African and Caucasian men. However, further work is required to assess if this response remains intact in older adults given that as noted above, the risk of heat-related morbidity and mortality is elevated in older individuals especially in those with chronic health conditions with black-African descendants being at a greater risk relative to their Caucasian counterparts (Whitman *et al.*, 1997; CDC, 2017).

In summary, we showed that ethnicity does not influence local NOS-dependent cutaneous vasodilation and sweating nor does it influence whole-body dry and

evaporative heat exchange or the resulting changes in body heat storage during exercise in the heat in young men of black-African and Caucasian descent, who were born and raised in the same temperate environment and matched for physical characteristics and fitness.

CHAPTER 5

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