The Contribution of Nitric Oxide to the Skin Blood Flow Response to Exercise in Boys and Men

Alexandra Woloschuk, BKin

Under the Supervision of Bareket Falk

Committee Members: Gary Hodges, Panagiota Klentrou

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Faculty of Applied Health Sciences

Brock University

St. Catharines, Ontario

Abstract

In response to heat stress, children sweat less than adults. However, little is known about their skin blood flow (SkBF) response. We investigated child-adult differences in SkBF during exercise (30 min at 60% VO_2max) and local heating (44°C) in 12 boys (9.7±1.2 y) and 12 men (22.2±2.0 y) using laser-Doppler flowmetry and L-NAME to inhibit nitric oxide (NO). The exercise-induced SkBF increase was greater in boys versus men (p=0.03). L-NAME blunted SkBF response during exercise in boys and men (p<0.01) (758±201 to 429±229 percent change from baseline *vs*. 541.6±167 to 352±109 percent change from baseline, respectively). Boys had a shorter time delay between the onset of exercise and onset of SkBF response compared with men (p<0.01) and L-NAME increased the time delay in boys and men (205±48 to 268±90 s *vs.* 309±71 to 376±116 s, respectively) (p=0.01). During local heating, SkBF increases were greater in boys versus men (p<0.01) and L-NAME blunted the SkBF response in boys and men (2594±939 to 1630±791 percent change from baseline *vs*. 1600±605 to 1046±345 percent change from baseline, respectively) (p<0.01). These data suggest that boys experience greater and faster increases in SkBF during exercise and local skin heating compared with men. NO influence on microvasculature and thermoregulatory function was not different between boys and men.

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Abbreviations/Key Terms

ANOVA	Analysis of Variance
bpm	Beats per minute
BSA	Body surface area
BSA/M	Body Surface-Area-to-Mass Ratio
% BF	Body Fat Percentage
CVP	Central venous pressure
CVC	Cutaneous vascular conductance
EDHF	Endothelium derived hyperpolarizing factor
FBF	Forearm blood flow
LDF	Laser Doppler flowmetry
L-NAME	Nω-nitro-L-arginine methyl ester
LSCI	Laser Speckle Contrast Imaging
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
PU	Perfusion Units
RPE	Rate of Perceived Exertion
SkBF	Skin blood flow
SRCE	Semi-recumbent cycle ergometer
$\bar{T}_{sk}$	Skin temperature
$\dot{V}O_2max$	Maximum volume of oxygen consumption
VOP	Venous Occlusion Plethysmography

1.0 Introduction

Differences in thermoregulatory responses to heat stress in children compared with adults may be attributed to different thermoregulatory strategies. During exercise, children rely on dry heat loss as the predominant method of dissipating heat by increasing skin blood flow (SkBF) (Falk & Dotan, 2008; Rowland, 2008). In contrast to children, adults rely more on sweating mechanisms to achieve evaporative cooling during exercise (Davies, 1981). Several geometric, metabolic, and cardiovascular factors have been explored and account for some of these age-related differences in temperature regulation. Although the SkBF response has been widely examined in adults, there are limited data on SkBF in children, especially during exercise. Investigating SkBF would contribute to the understanding of children's thermoregulation, which would elucidate the environmental conditions in which they may be more vulnerable to heat injuries. Such an understanding can improve recommendations to avoid heat injuries under stressful environmental conditions in both, a healthy population and those with chronic disease conditions.

2.0 Literature Review

2.1 Factors Affecting Child-Adult Differences in Heat Dissipation

2.1.1 Morphological

In order to dissipate heat during exercise, children predominantly rely more on dry heat loss by means of convection, conduction and radiation (Falk, 1998; Falk & Dotan, 2008) than on evaporative cooling, which is the predominant mechanism in adults. One explanation for this could be that children have a greater body surface-area-to-mass ratio (BSA/M) compared with adults (Bar-Or, 1980). With growth and development, BSA/M decreases with increasing organ, muscle, and bone size. In other words, children may have a greater opportunity to dissipate heat from their skin surface compared with adults. Not only are children smaller, they also have relatively less muscle mass compared with adults (as a proportion of body mass) and thus, may produce less heat (relative to their body size). The lower relative muscle mass and related lower heat production may result in a lower need to rely on sweating as a means to maintain thermal homeostasis. Indeed, Leites et al. (2016) compared boys to men working at the same relative workload and found similar thermoregulatory responses such as, heat production per unit body mass and rectal temperature. However, sweating rate was lower in boys compared with men, even when normalized to body surface area. It is important to note that SkBF was not measured.

Relative body composition differences found in children and adults also influence thermoregulatory responses to heat stress. Muscle contraction produces heat, which ultimately dissipates from the body (González-Alonso, Quistorff, Krustrup, Bangsbo, & Saltin, 2000). Thus, as stated above, more relative muscle mass in adults compared with children would require greater heat dissipation to occur at rest, as well as during exercise.

The amount of adiposity one has also affects thermoregulation, as adipose tissue acts as the body's natural insulator (Alexander et al., 2015). With maturation, females attain a greater percent of fat mass compared with males (and to girls) (Loomba-Albrecht & Styne, 2009). Prospective studies should indeed investigate the role of adiposity in thermoregulation between children and adults (females, in particular). However, since body composition is similar in boys and men, the role that adiposity plays in thermoregulation may not be as significant in explaining differences between child and adult males as it is when comparing females before and after puberty.

2.1.2 Sweating

The primary method of heat dissipation during heat stress in adults is sweat evaporation. Children, on the other hand, appear to rely more on dry heat loss. Kuno (1956) reported that the number of sweat glands is determined by approximately age 3. Thus, as a child grows and matures into an adult, fewer sweat glands are located on the skin surface within a given surface area. However, adults have larger sweat glands and greater sweat production per gland (Araki, Toda, Matsushita, & Tsujino, 1979; Wagner, Robinson, Tzankoff, & Marino, 1972), which consequently, may explain their greater reliance on evaporative cooling compared with children.

Consistently, during rest and exercise, children demonstrate a lower sweating rate compared with adults, regardless of the environmental conditions (Bar-Or, 1980; Falk, 1998; Inoue, Kuwahara, & Araki, 2004). Falk et al. (1991) showed increasing sweating rate in boys with increasing maturity, suggesting that larger differences would be expected between children and adults. Indeed, Rees and Shuster (1981) showed that sweating rate in boys, both absolute and after it was corrected for body surface area, was lower compared with

men. The authors speculated that men's higher sweating rate is androgen-induced during puberty. These findings support the notion that, when heat storage is similar, children have higher skin blood flow to dissipate heat compared with adults, who rely more on sweat evaporation.

2.1.3 Hormonal Differences

Hormonal differences between children and adults may also influence the thermoregulatory system. A greater relative muscle mass in men compared with boys is largely due to growth and the anabolic effects of testosterone. Testosterone responses to exercise or heat stress have been documented. However, very little is known about the direct effects of testosterone on heat dissipation in healthy individuals. In older men, one study found that exogenous testosterone had no effect on the cutaneous microvascular response to local heating (Sokolnicki, Khosla, & Charkoudian, 2007).

Hormone changes during puberty may partly explain the differences in the sweating response between children and adults. Most notably, Rees and Shuster (1981) reported no difference in sweating rate between girls and boys before puberty and that children and women had similar sweating rates relative to surface area, which was lower compared with men. The authors concluded that the secretory capacity per gland is higher in men and suggested that it could be due to endocrine factors, more specifically gonadal androgens, though they were not measured. Similarly, other studies have ascribed androgens as the cause for higher sweating rate in men (Araki et al., 1979; Inoue et al., 2004; Rowland, 2008).

Data on hormonal influences on SkBF in adult females are scarce. Sex hormones such as estrogen and progesterone in females impact thermoregulation (Charkoudian & Stachenfeld, 2014) and may influence cutaneous vasculature responses (Charkoudian &

Johnson, 2000). Thus, fluctuations in body temperature (Charkoudian & Johnson, 2000; Stephenson & Kolka, 1999) and changes in hormone levels throughout the menstrual cycle make studying women more complex and challenging.

Catecholamines or stress hormones, such as epinephrine and norepinephrine, are released from the adrenal medulla to allow the body to adapt quickly in a stressful situation. Such situations of elevated stress can include sensation of danger, physical challenges, as well as increased physical activity. The term for the release of these hormones has been coined the "fight-or-flight" response due to several physiological changes that occur to facilitate survival, including cutaneous vasoconstriction. Increases in plasma catecholamines during exercise in the heat, while serving to enhance blood flow to working muscles, may increase the risk of hyperthermia in endurance-trained men by increasing vasoconstriction, thereby reducing cutaneous vascular conductance and dry heat dissipation (Mora-Rodríguez, González-Alonso, Below, & Coyle, 1996).

Differences in catecholamine levels during or after acute exercise in children or adolescents compared with adults are inconsistent. Three studies from the same laboratory have compared epinephrine and norepinephrine responses in male adolescents and adults and reported epinephrine increasing similarly in both groups (Pullinen, Mero, Huttunen, Pakarinen, & Komi, 2011; Pullinen, Mero, MacDonald, Pakarinen, & Komi, 1998) The epinephrine increase from pre-exercise was greater in adolescent boys (5±2.6 $nmo \cdot l^{-1}$) compared with men (2.5±2.5 $nmo \cdot l^{-1}$) (Pullinen, Mero, Huttunen, Pakarinen, & Komi, 2002). Norepinephrine increased more in men (32.7±13.2 $nmo \cdot l^{-1}$) compared with adolescent boys (15.7±7.8 $nmo \cdot l^{-1}$) (Pullinen et al., 1998). Rubin et al. (2014) compared boys with men and reported no differences in epinephrine response but a higher norepinephrine response in

men during exercise and a lower norepinephrine response in boys post-exercise. The exercise intervention (resistance training) was different in all four studies, which may explain the inconsistencies between the reported findings.

2.2 Factors affecting Skin Blood Flow

As mentioned above, the SkBF response to heat stress or exercise may change with increasing age. However, there are several additional factors (e.g., hydration, acclimation, fitness) which affect the SkBF response, as outlined below, which may differ between children and adults.

2.2.1 Age

Martin et al. (1995) showed that forearm SkBF is higher in children compared with adults while local heating was applied directly to the skin of the forearm for 60 min in resting conditions. Furthermore, Falk et al. (1992) showed that forearm SkBF was consistently higher in pre-pubertal boys during several bouts of exercise at $50\%\dot{V}O_{2max}$ in the heat, compared with mid-and late-pubertal boys, which suggests that SkBF decreases with maturity. Note that both studies used the venous occlusion plethysmography (VOP) method to examine SkBF.

The above findings are inconsistent with findings by Shibasaki et al. (1997) who reported no differences between boys and men in forearm SkBF during exercise at $40\%\ \dot{V}O_{2max}$ in the heat. There was however, higher SkBF in the back and chest in boys compared with men and the authors suggest it could be due to structural differences in cutaneous vasculature or that the vasculature in the trunk has a higher sensitivity to vasoactive peptides. Shibasaki et al. (1999) also reported no differences in forearm SkBF but a higher SkBF in the trunk in boys compared with men during resting conditions when

participants' legs were heated passively by a hot water bath. In these studies, SkBF was assessed using laser-Doppler flowmetry (LDF).

The findings by Shibasaki et al. (1999; 1997) in males are similar to findings by Drinkwater et al. (1977) who reported no significant differences in FBF using VOP between girls and women during exercise in the heat. The exercise protocol consisted of walking at 30% $\dot{V}O_{2max}$ on a treadmill for two-50 min sessions in various heat conditions. Despite the finding of similar skin blood flow in the two groups, it was suggested that the shift in blood volume to the periphery was the cause for girls to end exercise sooner (in the hotter sessions) compared with the women.

Similarly, Rivera-Brown et al. (2006) also found no significant differences in SkBF between heat-acclimatized girls and women while completing a cycling protocol at 60% $\dot{V}O_{2max}$ in the heat. The VOP method was used to record SkBF at rest, 35 minutes into the session, and at fatigue. It was reported that the main reason girls and women stopped exercising was due to localized leg fatigue or discomfort from sitting on the bike and not because they were unable to overcome the heat stress. There were no significant differences between girls and women in terms of tolerance time (56.9±6.3 *vs.* 76.5±9.9 min, respectively), sweating rate (9.1±1.1 *vs.* 12.0±1.1 $ml \cdot m^{-2} \cdot min^{-1}$, respectively), increase in rectal temperature (0.9±0.1 *vs.* 1.1±0.1 °C, respectively), or heat storage (10.6±5.3 *vs.* 20.5±4.5 $W \cdot m^{-2}$, respectively).

2.2.2 Hydration Status and Blood Volume

Blood or plasma volume is dependent on hydration status. A decrease in plasma volume increases cardiovascular strain, resulting in higher HR and BP and lower stroke

volume and cardiac output at rest and the strain becomes exacerbated in the heat and/or with prolonged exercise (Sawka, Cheuvront, & Kenefick, 2015).

Heat stress from exercise leads to a cascade of events in the cardiovascular system. As expected, contracting skeletal muscles require increased perfusion in order to meet energy demands, which produce heat as a by-product. An increase in metabolic heat production results in thermoregulatory responses such as increasing sweating rate (and evaporative cooling) and increasing perfusion of the skin to allow dry heat transfer (Benzinger, Pratt, & Kitzinger, 1961) in order to achieve thermal balance. Cheatham et al. (2000) compared boys with men cycling for 40 minutes at a submaximal intensity and found a significantly greater decrease in plasma volume in men compared with boys. This study's findings support the notion that adults rely more on sweating rate and evaporative cooling (as suggested by the greater plasma volume lost) compared with children, who rely more on dry heat loss (as suggested by a lesser loss of plasma volume). Sweating rate and dry heat loss were not measured in this study.

Exercise in a warm environment causes further strain on the cardiovascular system and the circulatory response, which may compete with the thermoregulatory response (González-Alonso, Crandall, & Johnson, 2008). In warm conditions, the cardiovascular system must provide sufficient blood flow to the skeletal muscles to meet metabolic demands and sufficient blood flow to the skin to facilitate dry heat dissipation. That is, blood must continue to perfuse the skeletal muscles to continue performance and must reach the skin surface to remove heat generated during exercise. In adults, to dissipate heat during heat stress (i.e., during exercise and/or in warm environments), dry heat dissipation may be insufficient and sweating rate must increase. The latter may lead to hypohydration and

decreases in plasma volume, adding further strain to the cardiovascular system. In children, it could be speculated that during heat stress, the resultant cardiovascular strain may not be as prevalent as in adults since children sweat less. This would be an advantage for children as they would be able to maintain more of their plasma volume at a higher environmental heat or for a longer exercise period compared with adults.

Maintaining euhydration is important to sustain performance and prevent heat injury. Several studies have found that children are capable of staying hydrated when fluids are available, specifically when the beverages are flavoured (Wilk & Bar-Or, 1996; Wilk, Kriemler, Keller, & Bar-Or, 1998; Wilk, Rivera-Brown, & Bar-Or, 2007).

2.2.3 Heat Acclimatization

In adults, heat acclimatization predominantly occurs in the first 1 to 2 weeks of exposure to a hot environment (Tyler, Reeve, Hodges, & Cheung, 2016) and involves changes in several thermoregulatory responses required to meet the demands of the new environment (Périard, Travers, Racinais, & Sawka, 2016). Central and peripheral adaptations improve sweating and SkBF responses (Nadel, Mitchell, Saltin, & Stolwijk, 1971). Following 3 weeks of heat acclimation whereby participants cycled in 40°C, the threshold for sweating and the initiation of cutaneous vasodilation was evoked at a lower body temperature (Patterson, Stocks, & Taylor, 2004). Additionally, heat acclimatization improves cardiac output during exercise in the heat (Nielsen et al., 1993), which helps to alleviate the conflict between the cardiovascular and thermoregulatory system. The implication for this in regard to SkBF is that if body fluids are retained more efficiently, a greater amount of blood can be redirected to the periphery to expend heat.

All of the above adaptations were documented in adults. In children, acclimation occurs to a similar extent as adults, although at a slower rate (Inbar, Bar-Or, Dotan, & Gutin, 1981). However, the effect of acclimatization on SkBF in children has not been examined. In comparing the SkBF response between children and adults, it is nevertheless prudent to ensure that all participants are similarly acclimated/acclimatized.

2.2.4 Fitness Level

Individuals with a higher fitness level are characterized by higher VO_{2max}, lower resting heart rate, and a greater efficiency at utilizing metabolites during exercise, compared with those of lower fitness level. Similar to heat acclimatization, thermoregulatory responses are also more efficient in those with higher fitness. Morrison et al. (2006) showed that a lower fitness level in men was associated with lower tolerance to passive hyperthermia. Not surprisingly, Mora-Rodriguez et al. (2010) reported higher heat production, a larger rise in rectal temperature, higher SkBF (at the forearm), higher sweating rate, and lower mean skin temperature (taken at the leg, thigh, arm, and chest) in trained endurance athletes versus untrained men and women. Therefore, with higher fitness, more heat is produced (most likely due to larger amounts of muscle mass, or higher absolute work load), and similar to heat acclimatization, increases in sweating and SkBF are necessary to dissipate the excess heat.

All the above studies are documented in adults. In children, there are very few studies on the effects of heat acclimatization. It appears that children acclimatize similarly to adults, but at a slower rate. However, unlike the case in adults, in children both heat acclimatization and increased fitness (training) enhance heat dissipation to a similar extent (Inbar et al., 1981).

2.3 The Role of Nitric Oxide

2.3.1 Nitric Oxide, Nitric Oxide Synthase & L-NAME

Endogenous nitric oxide (NO) is a vasodilator located in endothelial and skeletal muscle cells (Casey, Walker, Ranadive, Taylor, & Joyner, 2013). Nitric oxide synthase (NOS) continuously produces NO to maintain a steady balance in blood pressure (Kopincová, Púzserová, & Bernátová, 2012). At a microvascular level, mechanisms of cutaneous vasodilation via NOS continue to be explored.

With local skin heating up to a maximum of 44°C, two independent pathways affecting SkBF are evident: 1) initially, there is a rapid increase in SkBF caused by activation of warm-sensitive local sensory nerves and then 2) a plateau occurs due to the local release of NO (Kellogg, Liu, Kosiba, & O'Donnell, 1999). Kellogg et al. (1999) also showed that NOS inhibition attenuated the vasodilation response to higher skin temperature (41°C). In a later study, Kellogg et al. (2008) demonstrated that specifically, endothelial NOS increased production of NO during an increase in local skin temperature to 41.5°C. NO production accounts for 60% of the increase in SkBF (Kellogg et al., 1999; Minson, Berry, & Joyner, 2001). However, the other 40% is mostly unaccounted for. After inhibiting the effects of NOS and endothelial-derived hyperpolarizing factors (EDHF), which are a class of vasodilators found in vascular beds, Brunt and Minson (2012) were able to demonstrate that under local heating, vasodilation was further reduced, suggesting that part of the unknown 40% is due to EDHF.

Cutaneous microvascular reactivity decreases with age in adults (Tew, Saxton, & Hodges, 2012) but the mechanism is unclear. DeSouza et al. (2002) found a decrease in NO bioavailability with aging (DeSouza et al., 2002), which hinders cutaneous microvascular

dilation and thus, heat dissipation. Studies of the responses to local heating in young compared with older adults indicate that older adults have attenuated NO-mediated cutaneous vasodilation (Hodges et al., 2017; Minson, Holowatz, Wong, Kenney, & Wilkins, 2002), and that there is a decline in resting FBF and peak FBF associated with ageing (Hodges et al., 2010). No such studies are available in youth.

The benefits of exercise training have been explored as a way to combat the issue of declining vascular reactivity with age. After 48 weeks of mild or moderate endurance training, post-menopausal females improved cutaneous microvascular reactivity (Tew, George, Cable, & Hodges, 2012) and cutaneous dilation function in response to local heating (Hodges et al., 2010). Training seems to modify the endothelium, resulting in an increase in NO bioavailability (Tew et al., 2012). Following just one bout of exercise, SkBF was reported to improve in response to local heating, suggesting positive adaptations to the NOS mechanism (McNamara, Keen, Simmons, Alexander, & Wong, 2014).

Predominantly, studies have used N^{ω}-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NOS, to study the cutaneous microvasculature dilation response (Kopincová et al., 2012). L-NAME is an L-arginine analogue that uses substitution to inhibit NOS and ultimately, NO production. L-NAME is effective at blocking cutaneous vascular conductance (CVC = perfusion units (PU)·$mmHg^{-1}$) during exercise (Welch, Foote, Hansen, & Mack, 2009) and the effects of NO during local heating (Casey et al., 2013; Dreyfuss et al., 2013). In all the above studies, adult participants were investigated. To my knowledge, no study has used L-NAME to investigate microvasculature and thermoregulatory function in healthy children.

2.4 Methods of Investigating Skin Blood Flow

2.4.1 Venous Occlusion Plethysmography

Venous Occlusion Plethysmography (VOP) is a technique used extensively to measure SkBF in children and adults in various pharmacological and physiological capacities (Falk et al., 1992; Hodges et al., 2010; Johnson, Minson, & Kellogg, 2014; Wilkinson & Webb, 2001). VOP is conducted by placing a cuff on a proximal part of a limb (such as the forearm, calf), which is then inflated to a pressure between the venous pressure and the arterial diastolic pressure in order to prevent venous return from the limb. The rate of change in limb volume, either in circumference (Whitney, 1953) or displacement (Greenfield, 1960), is then used to calculate SkBF in milliliters (mL) of blood per minute per 100 mL tissue (Johnson et al., 2014).

A major limitation of VOP is that it can only measure blood flow to an extremity and it assumes that any changes in limb circumference or volume reflect changes in blood flow to the skin only. Therefore, the muscles in the extremity being measured must be inactive. That is, VOP cannot measure perfusion to the skin separately from muscle blood flow. Saumet et al. (1988) demonstrated that SkBF, when measured by LDF, is not influenced by skeletal muscle blood flow, but this cannot be demonstrated using VOP. In addition, since the use of VOP assumes that the muscles in the limb in which the measurement is made (usually the forearm) are inactive, the measurement is generally not performed during exercise but rather, before and after exercise or between bouts of exercise (Wilkinson & Webb, 2001).

2.4.2 Laser-Doppler Flowmetry

LDF is the most commonly used method to assess SkBF (Iredahl, Löfberg, Sjöberg, Farnebo, & Tesselaar, 2015). LDF is a non-invasive technique that uses a single-point sensor recording, which is placed on the skin surface (Oberg, 1990). The most common skin surface

location is the volar forearm (Iredahl et al., 2015). LDF measures SkBF via the Doppler shift, which uses single-frequency laser light that reflects off moving red blood cells and records the frequency distribution of the light (photons) (Oberg, 1990).

Although LDF has a high temporal resolution, which allows for the continuous measurement of SkBF, it has several limitations. Since LDF is limited to a single skin site, it reflects the SkBF at that site only. Spatial variability cannot be measured unless the probe is moved (Kvandal et al., 2006). Multiple probes can be employed to address this issue. However, they are very costly and would still be insufficient at providing estimated measurements for an entire limb surface as there is no way to eliminate gaps between probes. Further, the LDF is unable to measure absolute flow, such as in $ml \cdot min^{-1}$. Instead, arbitrary perfusion units (PU) are often used (Cracowski, Minson, Salvat-Melis, & Halliwill, 2006). In order to obtain a meaningful value from PU, the recorded values are often expressed as a percentage of maximal SkBF, which is usually determined by local heating.

2.4.3 Laser Speckle Contrast Imaging

Laser speckle contrast imaging (LSCI) is a novel approach to measuring SkBF that allows for a high spatial (Davis, Kazmi, & Dunn, 2014) and temporal resolution, using a high frame rate camera (Iredahl et al., 2015). The LSCI assesses continuous skin perfusion over a large measurement area (Roustit, Millet, Blaise, Dufournet, & Cracowski, 2010), in contrast to the LDF's single- or multi-point probe location(s). Using a contrast analysis, the LSCI uses changes in speckle pattern (Draijer, Hondebrink, van Leeuwen, & Steenbergen, 2009) – bright and dark areas of interference seen when laser light rebounds off a moving object, such as a red blood cell – to provide an index of blood flow (Boas & Dunn, 2010), expressed in perfusion units (PU).

In recent years, several studies have compared the LDF to the LSCI in measuring responses to various provocations in the skin. Examining post-occlusive reactive hyperemia (PORH), Iredahl et al. (2015) found lower inter-subject variability using LSCI compared with LDF. Puissant et al. (2013) reported similar results when examining variability using a neurotransmitter vasodilator. Studies with LSCI have also reported better inter-day reproducibility compared with LDF when measuring PORH in adults (Roustit et al., 2010; Tew, Klonizakis, Crank, Briers, & Hodges, 2011). Better inter-site variability was also reported in the LSCI compared with LDF when examining local heating (Millet, Roustit, Blaise, & Cracowski, 2011). All of these studies were conducted in adults.

The LSCI has previously been validated (against LDF) in adults, under resting conditions, while conducting post-occlusive reactive hyperemia tests and with various pharmaceutical interventions (Cracowski, Gaillard-Bigot, Cracowski, Roustit, & Millet, 2011; Puissant et al., 2013; Roustit et al., 2010; Roustit & Cracowski, 2012; Tew et al., 2011). However, it is not clear whether the technique can be used during exercise, given the potential of invariably more movement. LSCI has not been validated in children undergoing any type of heat stress. Its extent of use in children has been limited to studies associated with wound healing (Lindahl, Tesselaar, & Sjöberg, 2013; Mirdell, Iredahl, Sjöberg, Farnebo, & Tesselaar, 2016).

2.2.4 Iontophoresis

Diffusing ions into the skin via an electrical current, known as Iontophoresis, is a technique that has recently been used in combination with LDF to explore cutaneous vascular responses (Johnson et al., 2014). Local iontophoresis of various substances such as acetylcholine (most prevalently used in microvasculature research) have been used to

examine endothelial function and explore nitric oxide (NO) mechanisms involved in regulating blood flow and blood pressure (Pfeiffer, Leopold, Schmidt, Brunner, & Mayer, 1996). Another common substance used in local iontophoresis is L-NAME, an L-arginine analogue, which is most commonly used to inhibit NO Synthase (NOS). NOS is responsible for the production of NO, facilitating vasodilation. That is, an increase in NOS promotes dilation and ultimately affects vascular reactivity (Kopincová et al., 2012).

Iontophoresis is used in children and adults in various capacities. For example, pilocarpine iontophoresis stimulates sweat, often used for diagnosing cystic fibrosis (LeGrys, 1996) and iontophoresis is involved in the treatment for hyperhidrosis (Dahl & Glent-Madsen, 1989) by decreasing sodium concentration and sweat volume (Walling & Swick, 2011). However, L-NAME Iontophoresis has not been used in children to examine the SkBF response.

3.0 Statement of Purpose

The effect of age on the SkBF response to heat stress is unclear and previous reports are inconsistent. A single study by Shibasaki et al. (1997) has looked at SkBF differences between boys and men during exercise in the heat using LDF. No differences in forearm SkBF were found. However higher SkBF in the trunk in boys was reported. In contrast, Falk et al (1992) using VOP, found higher forearm SkBF in early- compared with late-pubescent boys, suggesting that even greater differences between children and adults would be expected. The two studies that examined females during exercise in the heat found no differences in forearm SKBF, using VOP (Drinkwater et al., 1977; Rivera-Brown et al., 2006).

To my knowledge, no study has examined the effect of age (children vs. young adults) on SkBF during exercise in thermoneutral conditions. Studying thermoregulatory effects under these conditions would be important because during exercise in thermoneutral (and cool) conditions, SkBF plays a more important role in heat dissipation than in extreme heat (where ambient temperature is above skin temperature), and this may be particularly evident in children.

Additionally, while there are several recent studies examining the mechanism of the microvascular response to various stimuli, using iontophoresis, there are no such studies in children. Such studies can help elucidate the explanatory mechanisms explaining the apparent differences between children and adults in regard to heat dissipation.

This study aims to divulge potential age-related differences in SkBF responses to systemic (exercise) and local heat. Additionally, L-NAME iontophoresis will be used to elucidate mechanisms explaining potential age-related differences in SkBF responses. As mentioned earlier, L-NAME is a NOS inhibitor, which is the enzyme needed to produce NO.

Since NO is the predominant vasodilator in the endothelium (accounting for 60% of increases in SkBF), blunting its effect would decrease SkBF. Intuitively, if children are more reliant on SkBF to dissipate heat during exercise compared with adults, a greater impact (a decreased SkBF response) would then be evident in children. Thus, if age-related differences in the SkBF response are observed, the use of L-NAME may provide insight into the mechanism responsible for such differences. Conducting this study would provide additional literature about children's thermoregulatory 'strategies' and the mechanisms involved in cutaneous vasculature in comparison to adults.

Based on the literature, it was hypothesized that SkBF responses to exercise and to local heating would be greater in the boys than in the men as a means to dissipate heat. It was also hypothesized that L-NAME would blunt the SkBF response and that this effect would be greater in boys compared with men.

4.0 Methods

4.1 Participants

Twelve male adults (age 22.2±1.99 y) and twelve boys (9.8±1.14 y) volunteered for the study. Inclusion criteria required participants to be healthy, non-smokers, non-acclimatized (to heat), not taking any medication, with no lower leg injuries within the past 6 months, with a blood pressure within the normal range and without any contraindications reported on the medical screening questionnaire (Appendix A).

4.2 Procedure

Participants made two visits to the laboratory, approximately 1 h and 2.5 h in duration, respectively. Written informed consent was provided by adult participants and parents of child participants. Child participants provided written informed assent. All participants completed a medical screening questionnaire (Appendix A) prior to engaging in any exercise. All testing took place during the months of January to April.

4.2.1 Visit 1

Participants completed the Godin Leisure-Time Exercise Questionnaire (Godin & Shephard, 1985) (Appendix B) to determine any within- or between-group differences in physical activity. Anthropometric measurements were recorded. Body composition was measured as described by Slaughter et al. (1988). Pubertal status (Tanner, 1962) (Appendix C) was self-reported.

Participants were fitted on a semi-recumbent cycle ergometer (SRCE) and completed a 2-5-minute warm-up, followed by an exercise test consisting of three phases: a submaximal phase, a progressive exercise phase to volitional exhaustion ($\dot{V}O_2max$), and a 'supramaximal' phase (verification of $\dot{V}O_2max$). Throughout the test, oxygen consumption, heart rate (HR)

(bpm), power (W) and rate of perceived exertion (RPE) (Borg, 1973) were monitored and recorded by the same investigator.

4.2.2 Visit 2

At the start of the session, the left, dorsal forearm was inspected for an optimal location to place an iontophoresis probe and LDF probes. A site that had an absence of any superficial veins, scars, cuts, etc. was chosen. Hair was trimmed, if necessary, and the skin was cleaned with an alcohol swab. To inhibit NOS, A 100 μA current of a 2% L-NAME solution was locally iontophoresed (Perimed, Sweden) to the dermis from a 1.4 cm^2 surface for 10 min. Following the iontophoresis, the treated area was marked with a permanent marker. A 40-minute rest period followed, to allow the local SkBF to return to baseline (Johnson et al., 2014). This protocol was based on previous work (Hodges et al., 2017; Iredahl, Tesselaar, Sarker, Farnebo, & Sjöberg, 2013; Medow, Minson, & Stewart, 2005). Throughout the rest period, participants were able to move around freely and were asked to hydrate. Before the start of exercise, participants were requested to void. Participants were asked to refrain from drinking for the remainder of the session. However, water was available upon request. None of the participants requested water throughout the visit.

Participants were fitted with a heart rate monitor (M1, Suunto, Finland) and two LDF probes (Probe 457, Perimed, Sweden). One LDF probe was placed on the same site where L-NAME was administered, and another probe was placed on a different, optimal site on the same forearm, which went without L-NAME influence (control site). Both probes were secured with Transpore 3M tape. Skin temperature probes were also fitted on the participant to record mean skin temperature. Body mass was then measured.

Participants were then comfortably seated in a chair in an upright position and were asked to abstain from any movement of the left arm or any large movements in the rest of the body. Steady, normal breathing was also encouraged. The LDF and temperature probes measured SkBF and $\bar{T}_{sk}$ (see Measurements, below), respectively, throughout the remaining duration of the experimental session.

Baseline measurements were recorded for 10 min, including manual blood pressure (BP), HR, and RPE. Thermal comfort (TC) using an adapted four-point scale from the Bedford seven-point rating scale and thermal sensation (TS) using the ASHRAE seven-point scale were also recorded (Appendix D). After 10 min, body mass was measured and then the exercise began. Participants were seated on the SRCE (Corival recumbent cpet, Lode, Netherlands) and cycled for 30 min at 60% of their $\dot{V}O_2$max, determined from the first visit. The 30 min exercise served as the systemic heat stress stimulus. The exercise intensity and duration were adapted from Rowland et al. (2008), who compared boys and men cycling at 65% $\dot{V}O_2$max in the heat and among other things, demonstrated that both groups were able to cycle for the same duration, with a similar increase in body temperature. Every 5 min, BP, HR, RPE, $\bar{T}_{sk}$, TC, and TS were recorded.

Upon completion of the 30-min cycling, body mass was measured, and then local heating was performed using the same LDF probes to determine maximum SkBF, while the participant was seated in the upright position. The local heating protocol began at a set temperature of 33°C, which is the typical thermoneutral temperature of the skin surface. The temperature was increased at a rate of 1°C·20s^{-1} until 42°C and 1°C·min^{-1} until 44°C. Thereafter, data (SkBF, Tsk,) were recorded for 30 min (at 44°C). HR, $\bar{T}_{sk}$, TC, and TS were

recorded every 5 min. BP was recorded at 5 min and at 30 min. Finally, body mass was measured and marked the completion of the experiment.

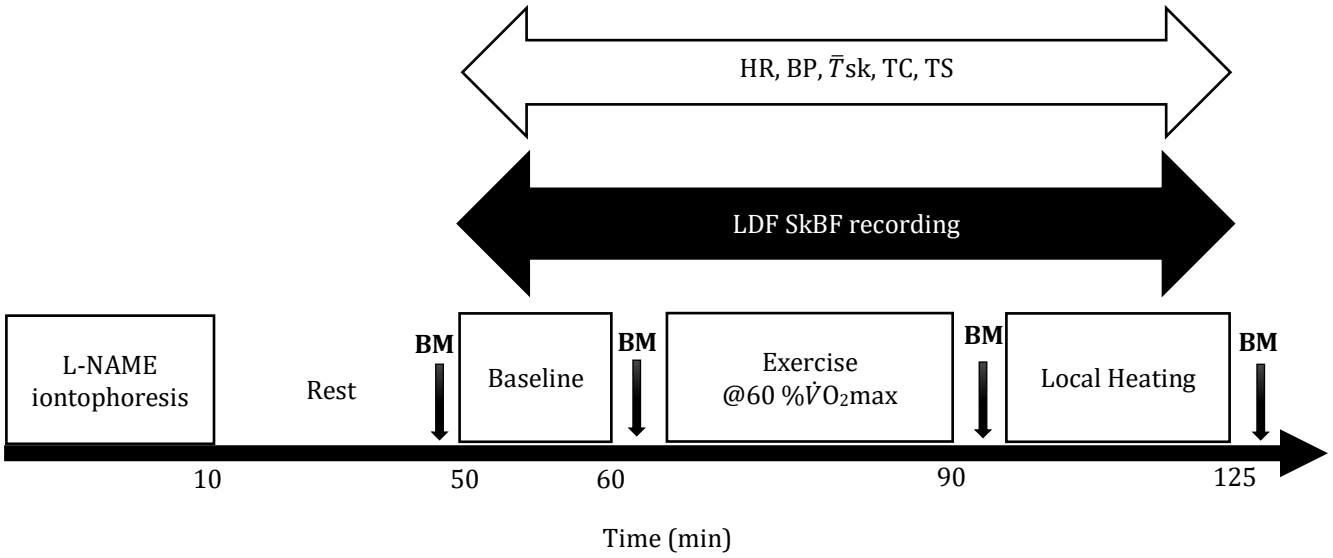


Figure 1. Timeline of *Visit 2*. Where BM = body mass (Kg), TC = thermal comfort, TS = Thermal sensation.

4.3 Measurements

4.3.1 Anthropometrics

Body mass was recorded to an accuracy of ±10 g (GFK 330aH, AE Adam, USA). Participants were barefoot (in the first visit only) and removed any excess clothing. Standing height was measured to the nearest 0.5 cm on a statiometer (Ellard Instrumentation Ltd., USA) while participants were barefoot. Body surface area was calculated according to Du Bois and Du Bois (1916).

4.3.2 Maturity

Somatic maturity was expressed as the years from the estimated age of peak height velocity. The age at peak height velocity was calculated based on age, mass, standing height, and seated height as described by Mirwald et al. (2002a). Sexual maturity was assessed using

secondary sex characteristics (pubic hair) (Tanner, 1962). Participants were provided images of male pubic hair at different stages of development and were asked to circle the image that most resembled them currently. In each instance, parents assisted the participants.

4.3.3 Body Composition

Skinfold thickness of the triceps and subscapula, was measured using a Harpenden skinfold caliper (Baty International, England) and was used to calculate percent body fat, using guidelines and equations by Slaughter et al. (1988).

4.3.4 Heart Rate

A heart rate monitor (M1, Suunto, Finland) was strapped onto a participant's chest, just below the pectoral muscles. During the submaximal test, HR was recorded in the last 10 s of every 4-min interval. During the incremental $\dot{V}O_2$max test, HR was recorded in the last 10 s of every 1-min interval. During the 30-min steady state cycling (visit 2), HR was recorded every 5 min.

4.3.5 Blood Pressure

Manual systolic and diastolic blood pressure of the brachial artery was recorded at rest, every 5 minutes during the 30-minute cycle exercise, and during local heating at 10 minutes and 30 minutes by the same investigator by auscultation.

4.3.6 Rate of Perceived Exertion

The Borg RPE Scale (Borg, 1973) is made up of 15 points (6-20). It was administered to participants during the submaximal phases, the progressive exercise phase to exhaustion (visit 1), and during the 30-minute cycling session (visit 2). During the submaximal and

maximal phases, the RPE was recorded at the last 10 s of every 4-min and 1-min intervals, respectively. During the 30-min cycling, RPE was recorded every 5 minutes.

4.3.7 Mean Skin Temperature

$\bar{T}_{sk}$ was recorded once at baseline and every 5 minutes during exercise and local heating with standard T-type thermocouples (PVC-T-24-190, Omega Environmental Inc., Laval, Canada) and estimated from a weighted average of four sites including the calf (20%), quadriceps (30%), biceps (20%) and chest (30%), as previously described by Ramanathan (1964).

4.3.8 Sweating rate

Sweating rate was estimated as the change in body mass from the beginning of the testing session (visit 2) until the end of exercise, divided by the duration (45 min). Participants did not consume any beverage during this time. Clothes were not weighed separately.

4.3.9 Exercise protocols

On the day of the test, the metabolic cart was calibrated before testing, using known gas mixtures. Expired gas was collected and analyzed breath-by-breath by a MOXUS metabolic cart (AEI Technologies, PA, USA). The semi-recumbent cycle ergometer was fitted to the participant.

Visit 1: After completing a 5 min warm-up, participants began with the submaximal phase that consisted of four 4-min incremental stages. Men began at 60 W and increased by 40 W/stage, while boys began between 20-30 W and increased by 15 W/stage. Participants maintained a cadence of 50-70 rpm. An average of the last minute of every stage measured every 15 s (i.e. four readings) was used to determine the $\dot{V}O2$ at that stage. A 10-min rest ensued before beginning the progressive exercise to exhaustion phase.

Typically, boys and men began the progressive $\dot{V}O_2$max protocol at 20-40 W and 60-80 W, respectively. The power then increased every minute by 10-30 W for the men and by 10-15 W for the boys. Cadence was maintained between 70-80 rpm. The test was concluded when the participant reached volitional exhaustion and could no longer maintain cadence, despite verbal encouragement. The average of the two highest consecutive readings was used to determine $\dot{V}O_2$max.

As suggested by Barker et al. (2011), approximately 10 min after the progressive phase was completed, a 'supramaximal' test was administered at 105% of the maximum power achieved during the progressive phase, in order to ensure that a true $\dot{V}O_2$max was measured. The participant began to pedal at 105% immediately until volitional exhaustion. The average of the two highest consecutive readings was used to determine $\dot{V}O_2$. None of the participants had a higher $\dot{V}O_2$max in the 'supramaximal' test than the maximal test.

Visit 2: The SRCE was fitted to the individual as determined in Visit 1. The wattage was set to 60% of each participant's $\dot{V}O_2$max, determined in Visit 1. Participants cycled for 30 min and maintained cadence between 60-70 rpm.

4.4 Data reduction

4.4.1 LDF Data

Raw laser-Doppler flowmetry data (in PU) during baseline, exercise, and local heating (Figure 1) (~75 min) were exported and analyzed off-line. The values during exercise and local heating were separately normalised to baseline values and presented as percent change from baseline, in PU. See Appendix E for raw data values. Typically, local heating is used with LDF to determine maximal vasodilation and normalize the data to the maximal response. However, in the present study, the 'maximal' response to local heating in the L-NAME-treated

site was not a true maximal response (since L-NAME blunts the SkBF response). Therefore, normalization to maximal vasodilation was not possible. For this reason, SkBF response was normalized to baseline values and expressed as percent change. Baseline values were similar for boys and men. Therefore, comparison of relative (%) changes from baseline between groups are not biased.

Data are presented as percent change from baseline PU. The data are also presented as cutaneous vascular conductance, which is calculated as perfusion units divided by mean arterial pressure ($CVC=PU \cdot MAP^{-1}$). In order to make comparisons between individuals (or in this case, between boys and men), CVC is used to normalize the data and consider individual or group differences in blood pressure.

4.4.2 Time delay

The time delay of SkBF during the start of exercise (in seconds) represents the time from the start of exercise until a visible change in slope was apparent (Fig. 2). A single investigator was blinded to the treatment (L-NAME vs. control) and chose where the slope was perceived to increase (i.e., visual determination) for all the trials. This method has been previously used by Hodges et al. (2008). A coefficient of variation (CV) was used to assess the intra-observer reproducibility of determining the slope, which was done in triplicate. The CV was found to be 6.5% which is considered good (≤10%) (Iellamo et al., 1996).

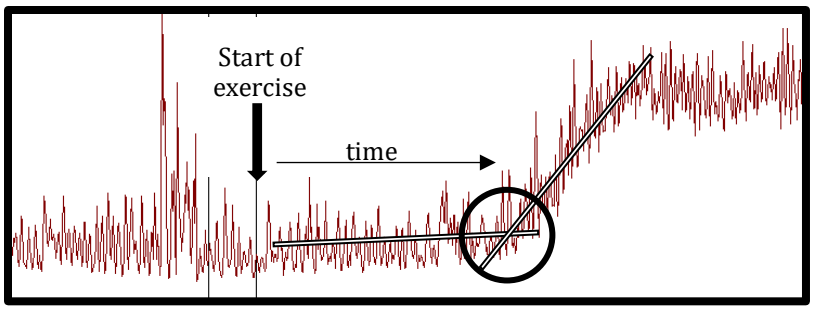


Figure 2. Visual determination of onset of increase in blood flow and assessment of time delay of SkBF response (s).

5.0 Statistical Analysis

All statistical analyses were performed using Excel 2017 or GraphPad Prism (GraphPad Software, Inc.). All data were normally distributed, as determined visually. Additionally, data were considered to be normally distributed if skewness was less than ±3 and a kurtosis was less than ±9, similar to previous studies using LDF (Mallette, Hodges, McGarr, Gabriel, & Cheung, 2016). Independent t-tests were used to determine differences between the two groups in physical characteristics, body mass changes, sweating rate, ambient temperature and humidity, and SkBF values at baseline. Separate two-way ANOVAs for repeated measures were used to determine main effects of Group (boys and men), Treatment (L-NAME vs. control) or Time (throughout exercise) and Interaction (group-by-treatment or group-by-time) on SkBF, heart rate, blood pressure, $\bar{T}$sk, thermal comfort, and thermal sensation. For all analyses, a Bonferroni *post hoc* correction was used for multiple comparisons. The acceptable level of significance for all tests was set to $p<0.05$. Data are presented as Mean and SD, unless otherwise indicated.

6.0 Results

All participants (*n*=24) completed both study visits. No injuries were reported as a result of study participation.

6.1 Baseline Characteristics

Absolute $\dot{V}O_2$max (in ml·min^{-1}) was higher (p<0.05) in men compared with boys. However, when adjusted for mass (Kg), or lean body mass only (Kg), there were no differences between groups. Similarly, absolute workload during visit 2 was greater in men compared with boys. However, relative to lean body mass, boys and men cycled at similar workloads. All boys reported pubertal stage (Tanner, 1962) and were classified as either stage 1 (*n*=9), stage 2 (*n*=1) or stage 3 (*n*=2). Years from age of peak height velocity was 2.79±0.76 yr. There was no difference in engagement in leisure activities between boys and men (p=0.23). In contrast, men had higher %BF (p<0.01).

Table 1 – Personal Characteristics of Boys and Men

	Boys	**Men**
Age (yr)	9.7 ± 1.2	22.2 ± 2.0
Height (cm)	144.8 ± 6.4	177.2 ± 7.5
Weight (Kg)	35.1 ± 4.9	79.9 ± 12.9
Body fat (%)	15.5 ± 2.7	20.5 ± 3.3*
$\dot{V}O_2$max (ml·min^{-1})	1620 ± 183	3674 ± 905*
$\dot{V}O_2$max (ml·kg^{-1}·min^{-1})	46.9 ± 7.0	45.7 ± 6.5
$\dot{V}O_2$max (ml·LBMkg^{-1}·min^{-1})	55.4 ± 7.2	57.5 ± 8.5
Leisure Activity Score	59.5 ± 14.5	66.6 ± 31.3
Workload (W) at 60% $\dot{V}O_2$max	62.1 ± 12.8	147.7 ± 33.7*
Workload (W) relative to LBM (Kg)	2.1 ± 0.5	2.3 ± 0.3

*=significant difference (p<0.05). Where LBM = lean body mass.

6.2 Ambient Air Temperature and Humidity

The ambient air temperature in the study room was warmer when the boys

(24.5±0.4°C) were tested, compared with the men (23.7±0.3°C). While the mean difference was less than 1°C, the difference was statistically significant (p<0.05). There was no difference (p>0.05) in relative humidity in the room when boys (31.4±4.3%) were tested compared with men (30.4±1.0%).

6.3 Thermal Comfort and Thermal Sensation

Thermal sensation increased similarly from the start to the end of exercise in boys (3.67±1.3 to 6.67±0.89) and men (3.83±0.83 to 6.17±0.58) (time effect; p<0.01). There was no difference between the boys and men (group effect; p>0.05) and there was no group-by-time interaction (p>0.05). Thermal comfort also significantly increased (p<0.05) from the start to the end of exercise in boys (1.67±0.49 to 2.92±1.0) and men (1.0±0 to 2.25±0.62) (time effect; p<0.01). Boys reported higher thermal comfort (i.e. feeling warmer) compared to the men (group effect; p<0.05). There was no group-by-time interaction (p>0.05).

Table 2 – Body Mass (BM) Changes and Estimated Sweating Rate (SR) in Boys and Men from the Start of the Experiment to End of Exercise (45 min)

	Boys	**Men**
ΔBM (g)	123 ± 57	296 ± 90*
ΔBM (%)	0.35 ± 0.17	0.37 ± 0.09
ΔBM ($g \cdot m^{-2}$)	104 ± 50	149 ± 40*
SR ($ml \cdot min^{-1}$)	2.7 ± 1.3	6.6 ± 2.0*
SR ($ml \cdot m^{-2} \cdot min^{-1}$)	1.1 ± 1.1	3.3 ± 0.9*

Where SR = sweating rate. *=significant difference (p<0.05).

6.4 Change in Body Mass, Sweating Rate

Body mass was measured pre-experiment and post-exercise. As demonstrated in Table 2, men had a greater decrease in body mass compared with boys, in absolute values or relative to BSA (in $g \cdot m^{-2}$) (calculated according to Du Bois & Du Bois, 1916) (p<0.01). However, there was no difference between groups when weight loss was expressed as a

percent of initial mass (p=0.42). The estimated sweating rate was greater in the men (p<0.01) compared with the boys.

6.5 Mean Skin Temperature

Mean skin temperature was recorded in 11/12 boys and 9/12 men due to technical issues with the thermocouples. At baseline, boys (32.3±1.24°C) had higher $\bar{T}$sk than men (30.7±0.50°C). Throughout the exercise, $\bar{T}$sk increased in both boys and men (Fig. 3). The increase in $\bar{T}$sk over the time of the exercise was significant in both groups (time effect; F(5, 108) = 4.13, p<0.01). Boys had higher $\bar{T}$sk compared with men (group effect; F(1, 108) = 53.63, p<0.01). There was no group-by-time interaction (F(5, 108) = 0.16, p=0.9).

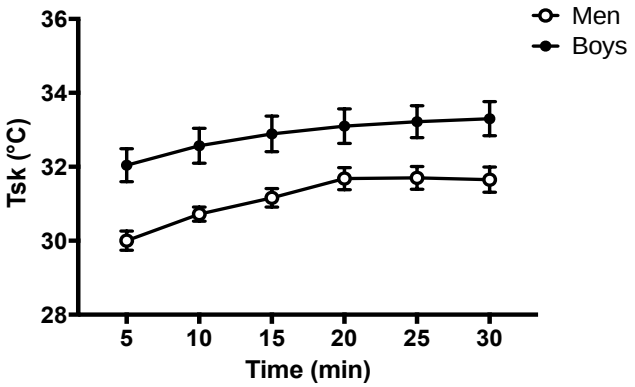


Figure 3. $\bar{T}$sk of boys (n=11) and men (n=9) from beginning to end of exercise. There was a significant Group (p<0.01), Time (p<0.01) effect, with no significant interaction. (Mean±SEM).

6.6 Heart Rate

Heart rate (bpm) increased from baseline until the end of exercise in both boys and men (Fig. 4). There was no difference between the boys and men (no group effect; F(1, 11) = 0.78, p=0.39) throughout the exercise. There was a difference from the start to the end of exercise (time effect; F(5, 55) = 78.42, p<0.001). There was no group-by-time interaction effect (F(5, 55) = 2.04, p=0.08).

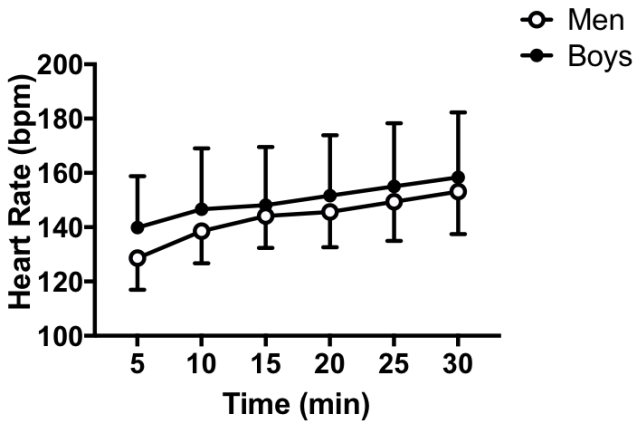


Figure 4. Heart Rate (bpm) in boys and men during 30 min of semi-recumbent cycling at 60% $\dot{V}O_2$max. (Mean±SD).

6.7 Blood Pressure

As expected, blood pressure (BP) increased during exercise in boys and men (Fig. 5). Men had higher systolic BP, higher diastolic BP, and higher mean arterial pressure (group effect; $F(1, 11) = 379.6$, $F(1, 11) = 69.65$, $F(1, 11) = 26.3$, $p<0.001$ for all, respectively). Throughout exercise, systolic BP increased (time effect; $F(5, 55) = 25.3$, $p<0.001$), diastolic BP decreased (time effect; $F(5, 55) = 4.71$, $p=0.001$), and mean arterial pressure increased (time effect; $F(5, 55) = 6.7$, $p<0.001$). There was a group-by-time interaction effect in systolic BP ($F(5, 55) = 3.11$, $p=0.02$), reflecting a higher increase in men compared with boys, with no interaction in diastolic BP ($F(5, 55) = 0.93$, $p=0.47$) or meant arterial pressure ($F(5, 55) = 0.78$, $p=0.57$).

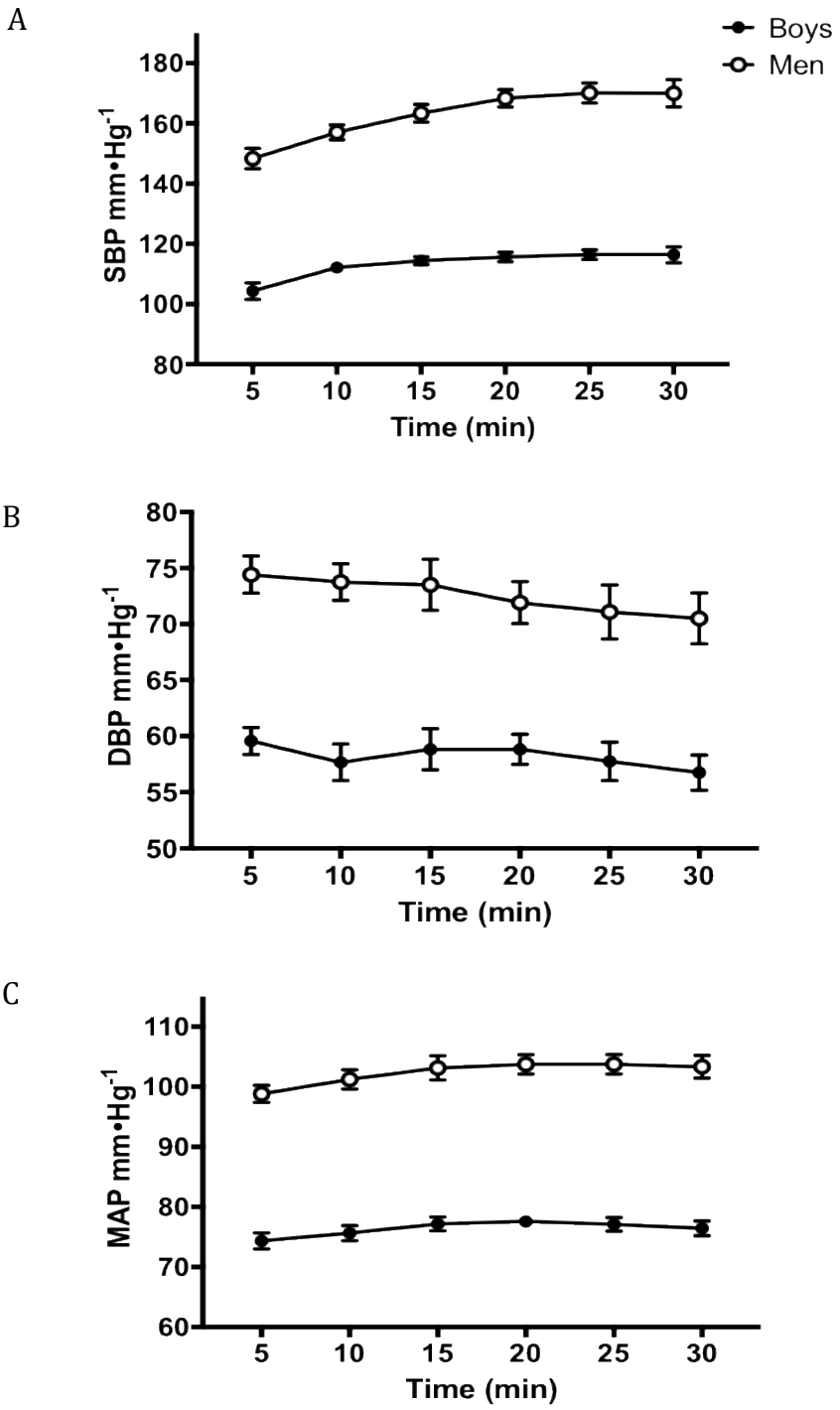


Figure 5. Systolic (Panel A), diastolic (Panel B), and mean arterial blood pressure (Panel C) during semi-recumbent cycling at 60% $\dot{V}O_2$max in men and boys. (Mean±SEM).

6.8 Skin Blood Flow during Exercise

During exercise there was an increase in SkBF in both boys and men (Fig. 6). The increase in the control site was significantly greater than at the L-NAME site in both groups in PU (treatment effect; $F(1, 11) = 32.82$, $p<0.001$) and in CVC (treatment effect; $F(1, 11) = 31.53$, $p<0.002$). The increase in SkBF in the boys was significantly greater than in the men, at both sites in PU (group effect; $F(1, 11) = 7.77$, $p=0.02$) and in CVC (group effect; $F(1, 11) = 6.57$, $p=0.03$). L-NAME influence on the group-by-treatment interaction did not reach significance in either PU or CVC $F(1, 11) = 1.30$, $F(1, 11) = 1.56$, $p>0.2$ for both, respectively).

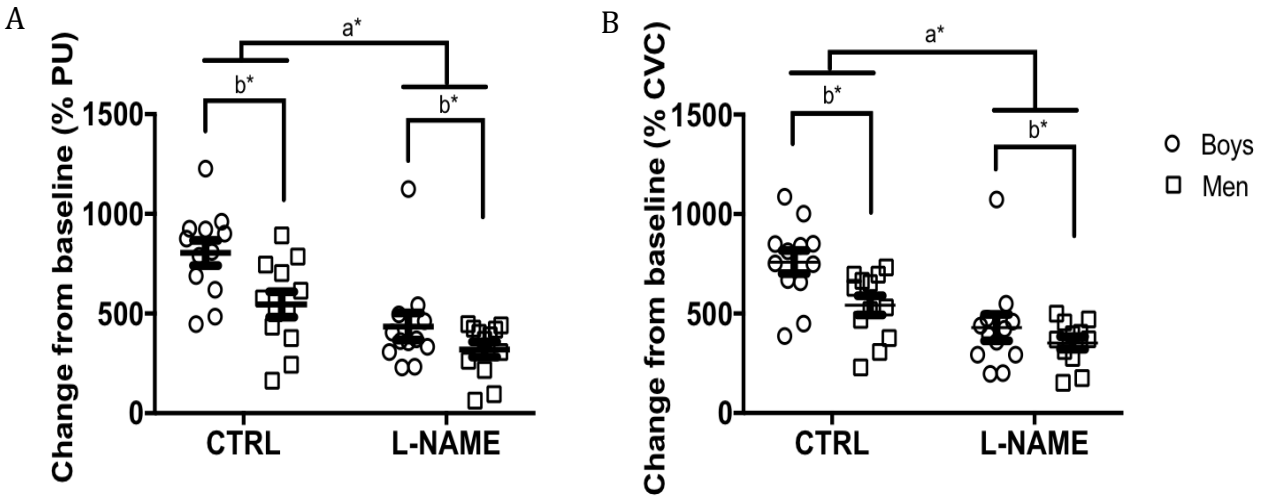


Figure 6. The percent change in SkBF from baseline to end of exercise in boys and men in perfusion units (PU) (A) and expressed in CVC (B). (Mean±SEM). * = significant difference ($p<0.05$). Group effect (b), Treatment effect (a). No interaction.

6.8.1 Time delay of SkBF during exercise `

The time delay of SkBF was measured from the start of exercise until the slope of SkBF began to visibly increase. Men had a longer time delay (s) (Fig. 7) than boys at the control site and at the L-NAME sites (group effect; $F(1, 11) = 16.08$, $p=0.002$). L-NAME increased the time delay in boys and men compared with the respective control sites (treatment effect; $F(1, 11) = 9.48$, $p=0.01$). There was no significant interaction ($F(1, 11) = 0.04$, $p=0.85$).

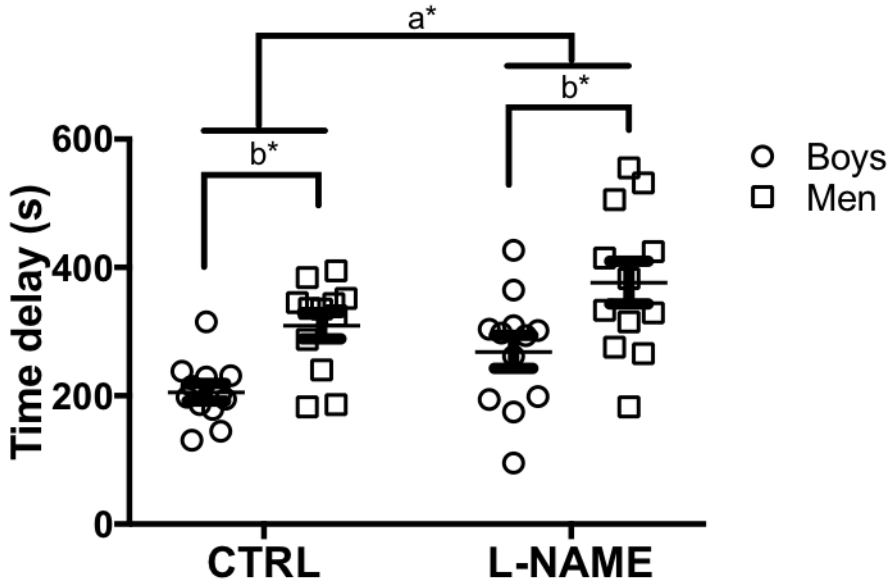


Figure 7. Time delay of SkBF (s) between boys and men at the start of semi-recumbent cycling at 60% $\dot{V}O_2$max. (Mean±SEM). *= significant (p<0.05). Group effect (b), Treatment effect (a). No interaction.

6.9 Skin Blood Flow during Local Heating

During local heating there was an increase in SkBF in both boys and men (Fig. 8). The increase in the control site was significantly greater than in the L-NAME site in both groups in PU % change from baseline (treatment effect; $F(1, 11) = 22.22$, $p<0.001$) and in CVC % change from baseline (treatment effect; $F(1, 11) = 21.07$, $p<0.001$). The increase in SkBF in the boys was significantly greater than in the men, at both sites in PU % change from baseline (group effect; $F(1, 11) = 21.65$, $p<0.001$) and in CVC % change from baseline (group effect; $F(1, 11) = 14.86$, $p=0.003$). Group-by-treatment interaction did not reach significance in either PU or CVC $F(1, 11) = 1.03$, $F(1, 11) = 0.92$, $p<0.3$ for both, respectively).

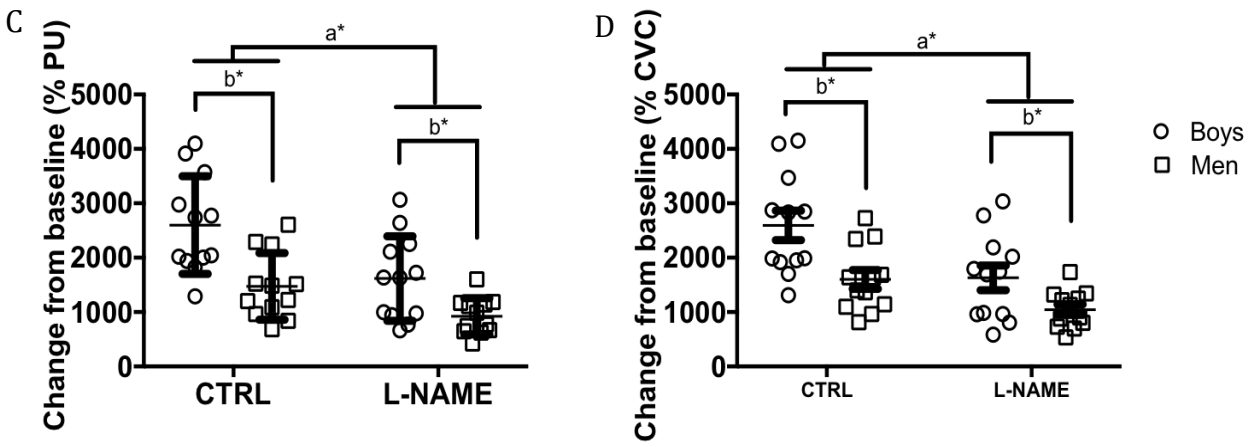


Figure 8. The percent change in SkBF from baseline to maximal SkBF (local heating) in boys and men in perfusion units (C) and expressed in CVC (D). (Mean±SEM). *= significant (p<0.05). Group effect (b), Treatment effect (a). No interaction.

7.0 Discussion

7.1 Summary

Laser-Doppler flowmetry was used to determine the SkBF response to heat stress (exercise) in boys and men to better understand children's thermoregulatory strategy during heat stress. In addition, L-NAME iontophoresis was used to examine potential age-related differences in cutaneous vascular function. The SkBF response (% change from baseline) during exercise and local heating was greater in boys compared with men, as hypothesized. A higher microvascular reactivity may allow children to rely more on dry heat dissipation during heat stress compared with adults. L-NAME blunted the SkBF response during exercise and local heating to a similar extent in both groups, suggesting that nitric oxide plays a similar role in both age groups. As well, men had a longer time delay in SkBF response from the start of exercise compared with boys, which was lengthened similarly in both groups with L-NAME influence. These data suggest that boys experience faster increases in SkBF during exercise and local heating compared with men. These findings contradict my hypothesis in that the effect of NO on vascular reactivity during exercise and local heat stress was similar in boys and men.

There are a limited number of studies that have compared SkBF responses in children versus adults (Drinkwater et al., 1977; Falk et al., 1992; Martin et al., 1995; Rivera-Brown et al., 2006; Shibasaki et al., 1999, 1997); only one of those studies measured forearm SkBF in boys and men *during* exercise (Shibasaki et al., 1997). Further, no previous study examined the potential mechanism for greater SkBF utilization in children compared to adults. In the current study, L-NAME iontophoresis was used to assess cutaneous microvasculature function in healthy children and adults.

7.2 Factors that affect SkBF

As outlined in the literature review, factors such as: acclimatization, fitness level or maximal aerobic power, and plasma volume or hydration status could all impact the SkBF response to heat stress. In my study, an attempt was made to minimize the effect of these factors. Participants completed both visits of this study within 2 weeks to minimize any lifestyle modifications (i.e. going on vacation, increasing activity level). All testing was completed in the Winter or early Spring, so we assume participants were not acclimatized to heat. As well, participants were told to arrive to both visits hydrated. During the second visit, although participants were allowed to drink during the study, no one did, which could perhaps be as a result of consistently reminding participants to drink during the rest portion of the visit. In doing so, it not only allowed for controlling hydration, but also to minimize error when estimating sweating rate. Finally, maximal aerobic power (VO2max) was similar in the two groups, when expressed relative to body size.

7.3 SkBF during Exercise

Boys had a greater SkBF response during 30 min of cycling at 60% $\dot{V}O_2$max compared with men. This finding is in line with the findings by Falk et al. (1992), who showed decreasing SkBF with increasing levels of maturity during exercise (50% $\dot{V}$O2max) in the heat. In addition, Martin et al. (1995) found higher SkBF in boys compared with men, albeit it was at rest during local heating. The SkBF response pattern during exercise in the present study was similar to the SkBF response during local heating, whether expressed in PU or as CVC (i.e., accounting for differences in blood pressure). These findings support the notion that children's thermoregulatory 'strategy' to dissipate heat relies more on SkBF compared with adults, who have shown to rely more on sweat evaporation (Falk & Dotan, 2008). Core

temperature was not measured in this study. Therefore, heat balance cannot be calculated. However, based on Rowland et al (2008), who utilized a similar exercise protocol in environmental conditions similar to the current study, it is assumed that heat storage was similar in the two groups.

This study's finding is not in line with previous studies that measured forearm SkBF in children compared with adults yet is considered consistent with their strategy for heat dissipation. Shibasaki et al. (1997) found no differences between boys and men in forearm SkBF while exercising at 40% $\dot{V}O_2$max in the heat, although boys had higher SkBF on the trunk. Drinkwater et al. (1977) also reported no differences in FBF between girls and women while exercising on a treadmill at 30% $\dot{V}O_2$max in the heat. However, their SkBF methodology was different: following the exercise portion, a 10 min recovery period ensued, where FBF was measured (VOP) at the first 3 min and last 2 min of that period. The authors did not include a detailed description of their method, but perhaps the break after exercise completion was long enough to blunt any potential findings and the method itself may have involved high variability between subjects. Secondly, their sample size was very small (n=5 per group), which may have influenced the power to detect a difference between groups. Lastly, they examined females, which tend to demonstrate less pronounced differences in the thermoregulatory response pre- to post-puberty, compared with males.

In the two previous studies exercise intensity was lower than the one used in the present study (60% $\dot{V}$O2max), although in both of these studies, exercise was performed in the heat, which could be a sufficient heat stress on its own. Rivera-Brown et al. (2006) measured SkBF in females, using VOP, cycling at 60% $\dot{V}O_2$max. Despite using the same intensity as in the present study, no differences in forearm SkBF were found. Similar to

Drinkwater et al. (1977), Rivera-Brown et al. (2006) may not have found significant differences due to high variability as a result of their methodology: following the exercise portion (on an upright cycle ergometer), participants were asked to stop pedaling for 1 min to reduce movement artifact before three measurements (used to calculate an average) of FBF were recorded. It is possible that following 1 min recovery, group differences were no longer apparent. Although the authors did not comment on whether or not high variability had influenced their findings, compared to the present study (in which a semi-recumbent cycle ergometer was used), the participants were not in the ideal position to reduce variability.

Although group differences were found at the control and treatment sites during exercise and local heating, we expected a greater effect of L-NAME in boys compared with men. Based on the literature in adults, 60% of the increase in SkBF is due to NO production (Kellogg et al., 1999; Minson et al., 2001), while 40% is unaccounted for. One study showed that EDHF may explain part of the 40% (Brunt & Minson, 2012) but EDHF was not examined in the present study. If children do in fact rely more on SkBF compared with adults to dissipate heat, it would be reasonable to suggest that by blunting NO, there would be a more pronounced difference in the SkBF response between the boys and men, though that was not found. It is possible that in children, other factors (e.g. neural drive, EDHF) have a more pronounced effect on the SkBF response. It is also possible, that given our sample size and variability, we did not have the power to detect a group-by-treatment interaction in our analysis, resulting in a type-II error. After completing a *post hoc* power analysis, it was determined that 29 participants per group would be needed to show a statistical difference between the *% change* from baseline during exercise in boys and men with and without L-

NAME treatment. However, it is important to note that this was not the primary objective of the study.

L-NAME blunted the vasodilatory effect of exercise (submaximal) and during local heating (maximal) in both groups, as expected. Prior studies have demonstrated the effectiveness of L-NAME in blunting CVC during exercise (Welch et al., 2009) and local heating (Casey et al., 2013; Dreyfuss et al., 2013) in adults, only. Thus, this study extends previous studies in adults, demonstrating the important role NO plays in the SkBF response to systemic and local heat in children.

Older adults have lower cutaneous microvascular reactivity, (Tew, George, Cable, & Hodges, 2012) and less NO-mediated cutaneous vasodilation, compared with younger adults (Hodges et al., 2017; Minson, Holowatz, Wong, Kenney, & Wilkins, 2002) . Similarly, Hodges et al. (2010) showed lower forearm SkBF, using VOP at rest, with ageing. Thus, in view of the decrease in cutaneous microvasculature reactivity seen with age in adults, we expected a more pronounced L-NAME-related blunting of the SkBF in children. There are some possible reasons as to why this was not the case. NO is accountable for 60% of the increase in SkBF (in adults) (Kellogg et al., 1999; Minson et al., 2001)It is possible that other factors affecting microvascular reactivity (e.g., catecholamines, sympathetic tone, EDHF) are responsible for the observed age-related difference in SkBF response. These were not examined in this study. Additionally, as mentioned above, our original sample size estimation was focused on our main outcome variable, namely the SkBF response. Therefore, we may not have had the statistical power to detect true group differences in the mechanistic response of NO.

7.2.1 Time Delay of SkBF

At the start of the exercise, it took men a longer time to demonstrate an increase in SkBF compared with boys. This is consistent with adults' greater reliance on sweat evaporation and children's greater reliance on dry heat dissipation during heat stress. That is, the boys increased SkBF sooner, in order to dissipate the heat, compared with the men, who likely increased the sweating response. While the time delay or kinetics of the sweating response were not examined, men demonstrated a higher sweating rate compared with the boys. This is in line with previous studies examining sweating rate in different age groups (Rees & Shuster, 1981). Our findings are supported by a recent study from our lab which demonstrated that the vasodilatory response in boys is greater than that of men at rest and during submaximal stimuli, such as local heating (39°C). The authors attributed a greater contribution of endothelial mediators in boys compared with men as the cause for greater vasodilation. Hodges et al. (2018) also demonstrated that forearm skin vascular conductance increases more in boys compared with men because of NO-dependent and NO-independent mediators while performing an isometric handgrip exercise. However, the SkBF response to steady-state exercise, i.e., systemic heat stress, was not examined.

The results of the present study are in line with and extend the findings of Hodges et al. (2018). During whole-body, dynamic exercise, as expected, boys had a greater change from baseline in SkBF, suggesting again, that boys depend more on heat dissipation via increased SkBF compared with men. In the present study, the potential mechanism of cutaneous vasodilation was explored by inhibiting NO (via L-NAME). In the control site, boys had a shorter time delay (or a faster increase of SkBF) compared with men, suggesting NO-dependent mediators were activated in boys sooner (or in a larger amount) than men. Similarly, when NO was inhibited, both groups had an increase in time delay, yet again, boys

took less time than the men. It is possible that NO-independent mediators of microvascular reactivity, which are not affected by L-NAME, are more active in boys.

7.4 Cardiovascular Outcomes

7.4.1 Blood Pressure

Diastolic BP significantly decreased during exercise in the present study in both groups, which is typically only seen in individuals with compromised cardiovascular health (Tverdal, 1987). Though speculative, the slight decrease in DBP observed during exercise may be a result of the participants' body position on the SRCE. If more blood volume returns to the heart in the supine position, it would lower central venous pressure (CVP) causing less blood to be available before cardiac contraction, which would decrease stroke volume. Low stroke volume would mean more blood leftover in the heart after contraction, causing lower pressure (lower DBP). The DBP decreases over the course of exercise but the participants do not move from upright to supine throughout the exercise to match the gradual change. Perhaps the CVP begins to decrease slightly due to positioning (as opposed to it normally having to increase when working upright) it may take ~30 min to see changes. It should be noted that the above is a speculative explanation for the statistically significant reduction in DBP over time. Importantly, the biological significance of the decrease (~3 mmHg) is not clear.

7.5 Thermoregulatory Outcomes

7.5.1 Body Mass and Sweating Rate

Boys and men did not differ in % change in BM, but when corrected for BSA, men lost more mass. Thus, as expected, the calculated absolute and relative SR was greater in men compared with boys. Lower SR in children compared with adults has been demonstrated in

previous studies (Bar-Or, 1980; Falk, 1998; Inoue et al., 2004; Rees & Shuster, 1981). Falk et al. (1991) showed a higher sweating rate with increasing maturity, suggesting that larger differences would be seen in pre-pubertal children and mature adults. Thus, our SR results are consistent with previous studies.

7.5.2 Mean Skin Temperature

Boys had consistently higher $\bar{T}$sk compared with men. This is contrary to findings by Falk et al. (1992) who reported an increase over time but no differences between maturity groups, although that study was performed in hot conditions. Indeed, a higher ambient temperature during the boys' experimental visits is likely reflected in the boys' higher $\bar{T}$sk compared with the men. This difference in $\bar{T}$sk and ambient temperature may explain the slightly higher thermal (dis)comfort score reported by the boys. In fact, thermal comfort, although indicating a statistically significant difference, was only ~1 unit apart, which may have little if any practical or biological significance. Additionally, it is possible that the boys' higher $\bar{T}$sk at the beginning of exercise also resulted in their earlier increase in SkBF. Nonetheless, it is also possible, as has been previously postulated, that in boys, a higher $\bar{T}$sk facilitates dry heat transfer, by increasing SkBF (Falk & Dotan, 2008).

8.0 Conclusion

The present study aimed to divulge differences in the SkBF response during exercise in boys and men. The SkBF response was greater in boys compared with men and L-NAME decreased the SkBF response in both groups. The study tried to uncover a mechanistic explanation for child-adult differences in the thermoregulatory 'strategy'. Exercise and local heating were chosen to elicit thermoregulatory responses as they reflect a systemic and a local stimulus, respectively. The boys' response to both stimuli was greater than the men's. Additionally, in response to both stimuli, L-NAME, resulted in a similar effect (blunting of the SkBF response) in both groups. This suggests that NO is at least partly responsible for the vasodilation seen in systemic and local heat stress, in both children and adults. The effect of L-NAME was not different between groups, suggesting that NO plays a similar role in both children and adults. Thus, other factors or mediators likely affect the age-related difference in the SkBF response.

9.0 Strengths, Limitations and Future Directions

9.1 Strengths

Careful consideration was given to the type of instrumentation and the physical design of the study. A SRCE was chosen to stabilize the participants to minimize any movement artifact from the arm, and still provide an exercise stress in a familiar way. As well, the timing between visit 1 and visit 2 for all participants was less than 15 days, in order to negate any influence of lifestyle changes.

9.2 Limitations

The time of day at which visit 1 and visit 2 took place was inconsistent within and between individuals, which could have impacted our findings. For both visits, a greater percentage of men was tested in the morning compared with boys. Indeed, Aoki et al. (2001) showed that SkBF (via LDF) with whole body heating was lower in the morning (0630 h) compared with afternoon (1630 h). Further, the present study did not conduct the experiment in a controlled environment (chamber), which could have minimized the variability and differences that were observed in ambient temperature.

The boys' group was not homogeneous in terms of pubertal level. It is possible that a more pronounced difference would be found in the interaction effects between boys and men with regards to SkBF during exercise and local heating if all the boys were pre-pubertal. However, there are no apparent differences in the results between the prepubertal and pubertal boys. Further, although males were used in order to minimize the effects of hormonal influence on thermoregulation, hormone levels were not measured nor controlled for in any way throughout the present study.

Sweating rate is presented as an estimate in the present study due to a lack of precision when conducting the measurement as oppose to the recommendations by Armstrong (2007), Maughan, Shirreffs, & Leiper (2007), and Cheuvront, Haymes, & Sawka (2002). The participants' nude body mass and clothes were not measured, and they did not vigilantly towel-dry before body mass measures were recorded. Further, the sweating rate was calculated using a standard, estimated time length of 45 min (10 min baseline, 5 min instrumentation, and 30 min exercise).

When determining the slope to identify the time delay of SkBF at the start of exercise, the investigator was not blinded to the group. Therefore, there may have been a bias towards a longer time delay in men compared with boys.

NO activity was examined by inhibiting the NOS enzyme via L-NAME. NO dilatory effects in the smooth muscle of the microvasculature were not measured. Perhaps differences in vasodilatory responses between boys and men would be apparent if NO effects in the smooth muscle within the vessel wall were examined.

Core body temperature was not measured. Thus, we were unable to calculate heat balance. However, based on Rowland et al (2008), who utilized a similar exercise protocol in environmental conditions similar to the current study, it is assumed that heat storage was similar in the two groups.

9.3 Future Directions

In addition to considering the limitations of the present study, future studies should consider several next steps. First, factors affecting SkBF other than (or in addition to) NO should be examined, such as: prostaglandins, EDHFs, sex hormones, and sympathetic/neuronal drive. Second, in studies in which the mechanism of vasodilation is

examined, a larger sample size is required, based on our *post hoc* power calculation. Third, in the present study, distinct sex and age groups were selected. Future studies could consider other populations (i.e. females, different age groups, a range of maturity and hormonal levels) to map out the transition in microvascular reactivity and the SkBF response to heat stress across the lifespan. Fourth, in studies where participants vary widely in body composition, workload should be assigned relative to muscle mass (or lean body mass) in order to normalize the heat production. Fifth, various chronic conditions or diseases may affect microvascular reactivity or vascular health. For example, obesity has numerous negative effects on vascular health (Hedvall Kallerman et al., 2014). Future studies should examine the role of NO and other mediators in the thermoregulatory response and specifically, in the SkBF and dry heat exchange response in child and adult populations with chronic conditions known to affect thermoregulation.

References

Alexander, C. M., Kasza, I., Yen, C.-L. E., Reeder, S. B., Hernando, D., Gallo, R. L., … MacDougald, O. A. (2015). Dermal white adipose tissue: a new component of the thermogenic response. *Journal of Lipid Research*, *56*(11), 2061–2069. https://doi.org/10.1194/jlr.R062893

Aoki, K., Stephens, D. P., & Johnson, J. M. (2001). Diurnal variation in cutaneous vasodilator and vasoconstrictor systems during heat stress. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *281*(2), R591–R595. https://doi.org/10.1152/ajpregu.2001.281.2.R591

Araki, T., Toda, Y., Matsushita, K., & Tsujino, A. (1979). AGE DIFFERENCES IN SWEATING DURING MUSCULAR EXERCISE. *Japanese Journal of Physical Fitness and Sports Medicine*, *28*(3), 239–248. https://doi.org/10.7600/jspfsm1949.28.239

Armstrong, L. E. (2007). Assessing hydration status: the elusive gold standard. *Journal of the American College of Nutrition*, *26*(5 Suppl), 575S-584S.

Barker, A. R., Williams, C. A., Jones, A. M., & Armstrong, N. (2011). Establishing maximal oxygen uptake in young people during a ramp cycle test to exhaustion. *British Journal of Sports Medicine*, *45*(6), 498–503. https://doi.org/10.1136/bjsm.2009.063180

Bar-Or, O. (1980). Climate and the Exercising Child - A Review. *International Journal of Sports Medicine*, *01*(2), 53–65. https://doi.org/10.1055/s-2008-1034631

Benzinger, T. H., Pratt, A. W., & Kitzinger, C. (1961). THE THERMOSTATIC CONTROL OF HUMAN METABOLIC HEAT PRODUCTION. *Proceedings of the National Academy of Sciences of the United States of America*, *47*(5), 730–739.

Boas, D. A., & Dunn, A. K. (2010). Laser speckle contrast imaging in biomedical optics. *Journal of Biomedical Optics, 15*(1), 011109. https://doi.org/10.1117/1.3285504

Borg, G. A. (1973). Perceived exertion: a note on 'history' and methods. *Medicine and Science in Sports, 5*(2), 90–93.

Brunt, V. E., & Minson, C. T. (2012). KCa channels and epoxyeicosatrienoic acids: major contributors to thermal hyperaemia in human skin. *The Journal of Physiology, 590*(15), 3523–3534. https://doi.org/10.1113/jphysiol.2012.236398

Casey, D. P., Walker, B. G., Ranadive, S. M., Taylor, J. L., & Joyner, M. J. (2013). Contribution of nitric oxide in the contraction-induced rapid vasodilation in young and older adults. *Journal of Applied Physiology (Bethesda, Md.: 1985), 115*(4), 446–455. https://doi.org/10.1152/japplphysiol.00446.2013

Charkoudian, N., & Johnson, J. M. (2000). Female reproductive hormones and thermoregulatory control of skin blood flow. *Exercise and Sport Sciences Reviews, 28*(3), 108–112.

Charkoudian, N., & Stachenfeld, N. S. (2014). Reproductive hormone influences on thermoregulation in women. *Comprehensive Physiology, 4*(2), 793–804. https://doi.org/10.1002/cphy.c130029

Cheatham, C. C., Mahon, A. D., Brown, J. D., & Bolster, D. R. (2000). Cardiovascular responses during prolonged exercise at ventilatory threshold in boys and men. *Medicine and Science in Sports and Exercise, 32*(6), 1080–1087.

Cheuvront, S. N., Haymes, E. M., & Sawka, M. N. (2002). Comparison of sweat loss estimates for women during prolonged high-intensity running. *Medicine and Science in Sports and Exercise, 34*(8), 1344–1350.

Cracowski, J. L., Gaillard-Bigot, F., Cracowski, C., Roustit, M., & Millet, C. (2011). Skin microdialysis coupled with laser speckle contrast imaging to assess microvascular reactivity. *Microvascular Research*, *82*(3), 333–338. https://doi.org/10.1016/j.mvr.2011.09.009

Cracowski, J. L., Minson, C. T., Salvat-Melis, M., & Halliwill, J. R. (2006). Methodological issues in the assessment of skin microvascular endothelial function in humans. *Trends in Pharmacological Sciences*, *27*(9), 503–508. https://doi.org/10.1016/j.tips.2006.07.008

Dahl, J. C., & Glent-Madsen, L. (1989). Treatment of hyperhidrosis manuum by tap water iontophoresis. *Acta Dermato-Venereologica*, *69*(4), 346–348.

Davies, C. T. (1981). Thermal responses to exercise in children. *Ergonomics*, *24*(1), 55–61. https://doi.org/10.1080/00140138108924830

Davis, M. A., Kazmi, S. M. S., & Dunn, A. K. (2014). Imaging depth and multiple scattering in laser speckle contrast imaging. *Journal of Biomedical Optics*, *19*(8), 086001. https://doi.org/10.1117/1.JBO.19.8.086001

DeSouza, C. A., Clevenger, C. M., Greiner, J. J., Smith, D. T., Hoetzer, G. L., Shapiro, L. F., & Stauffer, B. L. (2002). Evidence for agonist-specific endothelial vasodilator dysfunction with ageing in healthy humans. *The Journal of Physiology*, *542*(Pt 1), 255–262.

Draijer, M., Hondebrink, E., van Leeuwen, T., & Steenbergen, W. (2009). Review of laser speckle contrast techniques for visualizing tissue perfusion. *Lasers in Medical Science*, *24*(4), 639–651. https://doi.org/10.1007/s10103-008-0626-3

Dreyfuss, C., Wauters, A., Adamopoulos, D., Pochet, S., Azarkan, M., Berkenboom, G., … Argacha, J. F. (2013). L-NAME iontophoresis: a tool to assess NO-mediated vasoreactivity

during thermal hyperemic vasodilation in humans. *Journal of Cardiovascular Pharmacology*, *61*(5), 361–368. https://doi.org/10.1097/FJC.0b013e3182858f81

Drinkwater, B. L., Kupprat, I. C., Denton, J. E., Crist, J. L., & Horvath, S. M. (1977). Response of prepubertal girls and college women to work in the heat. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*, *43*(6), 1046–1053. https://doi.org/10.1152/jappl.1977.43.6.1046

Du Bois, D., & Du Bois, E. F. (1916). A formula to estimate the approximate surface area if height and weight be known. *Nutrition (Burbank, Los Angeles County, Calif.)*, *5*(5), 303–311; discussion 312-313.

Falk, B. (1998). Effects of thermal stress during rest and exercise in the paediatric population. *Sports Medicine (Auckland, N.Z.)*, *25*(4), 221–240.

Falk, B., Bar-Or, O., Calvert, R., & MacDougall, J. D. (1992). Sweat gland response to exercise in the heat among pre-, mid-, and late-pubertal boys. *Medicine and Science in Sports and Exercise*, *24*(3), 313–319.

Falk, B., Bar-Or, O., MacDougall, J. D., McGillis, L., Calvert, R., & Meyer, F. (1991). Sweat lactate in exercising children and adolescents of varying physical maturity. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *71*(5), 1735–1740. https://doi.org/10.1152/jappl.1991.71.5.1735

Falk, B., & Dotan, R. (2008). Children's thermoregulation during exercise in the heat: a revisit. *Applied Physiology, Nutrition, and Metabolism = Physiologie Appliquee, Nutrition Et Metabolisme*, *33*(2), 420–427. https://doi.org/10.1139/H07-185

Godin, G., & Shephard, R. J. (1985). A simple method to assess exercise behavior in the community. *Canadian Journal of Applied Sport Sciences. Journal Canadien Des Sciences Appliquees Au Sport*, *10*(3), 141–146.

González-Alonso, J., Quistorff, B., Krustrup, P., Bangsbo, J., & Saltin, B. (2000). Heat production in human skeletal muscle at the onset of intense dynamic exercise. *The Journal of Physiology*, *524 Pt 2*, 603–615.

González-Alonso, J., Crandall, C. G., & Johnson, J. M. (2008). The cardiovascular challenge of exercising in the heat. *The Journal of Physiology*, *586*(1), 45–53. https://doi.org/10.1113/jphysiol.2007.142158

Greenfield, A. D. (1960). Venous occlusion plethysmography. *Methods in Medical Research*, *8*, 293–301.

Hedvall Kallerman, P., Hagman, E., Edstedt Bonamy, A.-K., Zemack, H., Marcus, C., Norman, M., & Westerståhl, M. (2014). Obese children without comorbidities have impaired microvascular endothelial function. *Acta Paediatrica (Oslo, Norway: 1992)*, *103*(4), 411–417. https://doi.org/10.1111/apa.12549

Hodges, G. J., Mallette, M. M., Tew, G. A., Saxton, J. M., Moss, J., Ruddock, A. D., & Klonizakis, M. (2017). Effect of age on cutaneous vasomotor responses during local skin heating. *Microvascular Research*, *112*, 47–52. https://doi.org/10.1016/j.mvr.2017.03.002

Hodges, G. J., Mueller, M. C., Cheung, S. S., & Falk, B. (2018). Cutaneous vasomotor responses in boys and men. *Applied Physiology, Nutrition, and Metabolism = Physiologie Appliquee, Nutrition Et Metabolisme*, 1–8. https://doi.org/10.1139/apnm-2018-0083

Hodges, G. J., Sharp, L., Clements, R. E., Goldspink, D. F., George, K. P., & Cable, N. T. (2010). Influence of age, sex, and aerobic capacity on forearm and skin blood flow and vascular

conductance. *European Journal of Applied Physiology*, *109*(6), 1009–1015. https://doi.org/10.1007/s00421-010-1441-7

Iellamo, F., Legramante, J. M., Raimondi, G., Castrucci, F., Massaro, M., & Peruzzi, G. (1996). Evaluation of reproducibility of spontaneous baroreflex sensitivity at rest and during laboratory tests. *Journal of Hypertension*, *14*(9), 1099–1104.

Inbar, O., Bar-Or, O., Dotan, R., & Gutin, B. (1981). Conditioning versus exercise in heat as methods for acclimatizing 8- to 10-yr-old boys to dry heat. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*, *50*(2), 406–411. https://doi.org/10.1152/jappl.1981.50.2.406

Inoue, Y., Kuwahara, T., & Araki, T. (2004). Maturation- and aging-related changes in heat loss effector function. *Journal of Physiological Anthropology and Applied Human Science*, *23*(6), 289–294.

Iredahl, F., Löfberg, A., Sjöberg, F., Farnebo, S., & Tesselaar, E. (2015). Non-Invasive Measurement of Skin Microvascular Response during Pharmacological and Physiological Provocations. *PloS One*, *10*(8), e0133760. https://doi.org/10.1371/journal.pone.0133760

Iredahl, F., Tesselaar, E., Sarker, S., Farnebo, S., & Sjöberg, F. (2013). The microvascular response to transdermal iontophoresis of insulin is mediated by nitric oxide. *Microcirculation (New York, N.Y.: 1994)*, *20*(8), 717–723. https://doi.org/10.1111/micc.12071

Johnson, J. M., Minson, C. T., & Kellogg, D. L. (2014). Cutaneous vasodilator and vasoconstrictor mechanisms in temperature regulation. *Comprehensive Physiology*, *4*(1), 33–89. https://doi.org/10.1002/cphy.c130015

Kellogg, D. L., Liu, Y., Kosiba, I. F., & O'Donnell, D. (1999). Role of nitric oxide in the vascular effects of local warming of the skin in humans. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *86*(4), 1185–1190. https://doi.org/10.1152/jappl.1999.86.4.1185

Kellogg, D. L., Zhao, J. L., & Wu, Y. (2008). Endothelial nitric oxide synthase control mechanisms in the cutaneous vasculature of humans in vivo. *American Journal of Physiology. Heart and Circulatory Physiology*, *295*(1), H123-129. https://doi.org/10.1152/ajpheart.00082.2008

Kopincová, J., Púzserová, A., & Bernátová, I. (2012). L-NAME in the cardiovascular system - nitric oxide synthase activator? *Pharmacological Reports: PR*, *64*(3), 511–520.

Kuno, Y. (1956). *Human perspiration.* Springfield, Ill.: Thomas. Retrieved from http://catalog.hathitrust.org/api/volumes/oclc/1100428.html

Kvandal, P., Landsverk, S. A., Bernjak, A., Stefanovska, A., Kvernmo, H. D., & Kirkebøen, K. A. (2006). Low-frequency oscillations of the laser Doppler perfusion signal in human skin. *Microvascular Research*, *72*(3), 120–127. https://doi.org/10.1016/j.mvr.2006.05.006

LeGrys, V. A. (1996). Sweat testing for the diagnosis of cystic fibrosis: practical considerations. *The Journal of Pediatrics*, *129*(6), 892–897.

Leites, G. T., Cunha, G. S., Obeid, J., Wilk, B., Meyer, F., & Timmons, B. W. (2016). Thermoregulation in boys and men exercising at the same heat production per unit body mass. *European Journal of Applied Physiology*, *116*(7), 1411–1419. https://doi.org/10.1007/s00421-016-3400-4

Lindahl, F., Tesselaar, E., & Sjöberg, F. (2013). Assessing paediatric scald injuries using Laser Speckle Contrast Imaging. *Burns: Journal of the International Society for Burn Injuries*, *39*(4), 662–666. https://doi.org/10.1016/j.burns.2012.09.018

Loomba-Albrecht, L. A., & Styne, D. M. (2009). Effect of puberty on body composition. *Current Opinion in Endocrinology, Diabetes, and Obesity*, *16*(1), 10–15.

Mallette, M. M., Hodges, G. J., McGarr, G. W., Gabriel, D. A., & Cheung, S. S. (2016). Investigating the roles of core and local temperature on forearm skin blood flow. *Microvascular Research*, *106*, 88–95. https://doi.org/10.1016/j.mvr.2016.03.010

Martin, H. L., Loomis, J. L., & Kenney, W. L. (1995). Maximal skin vascular conductance in subjects aged 5-85 yr. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *79*(1), 297–301. https://doi.org/10.1152/jappl.1995.79.1.297

Maughan, R. J., Shirreffs, S. M., & Leiper, J. B. (2007). Errors in the estimation of hydration status from changes in body mass. *Journal of Sports Sciences*, *25*(7), 797–804. https://doi.org/10.1080/02640410600875143

McNamara, T. C., Keen, J. T., Simmons, G. H., Alexander, L. M., & Wong, B. J. (2014). Endothelial nitric oxide synthase mediates the nitric oxide component of reflex cutaneous vasodilatation during dynamic exercise in humans. *The Journal of Physiology*, *592*(23), 5317–5326. https://doi.org/10.1113/jphysiol.2014.272898

Medow, M. S., Minson, C. T., & Stewart, J. M. (2005). Decreased microvascular nitric oxide-dependent vasodilation in postural tachycardia syndrome. *Circulation*, *112*(17), 2611–2618. https://doi.org/10.1161/CIRCULATIONAHA.104.526764

Millet, C., Roustit, M., Blaise, S., & Cracowski, J. L. (2011). Comparison between laser speckle contrast imaging and laser Doppler imaging to assess skin blood flow in humans. *Microvascular Research*, *82*(2), 147–151. https://doi.org/10.1016/j.mvr.2011.06.006

Minson, C. T., Berry, L. T., & Joyner, M. J. (2001). Nitric oxide and neurally mediated regulation of skin blood flow during local heating. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *91*(4), 1619–1626. https://doi.org/10.1152/jappl.2001.91.4.1619

Minson, C. T., Holowatz, L. A., Wong, B. J., Kenney, W. L., & Wilkins, B. W. (2002). Decreased nitric oxide- and axon reflex-mediated cutaneous vasodilation with age during local heating. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *93*(5), 1644–1649. https://doi.org/10.1152/japplphysiol.00229.2002

Mirdell, R., Iredahl, F., Sjöberg, F., Farnebo, S., & Tesselaar, E. (2016). Microvascular blood flow in scalds in children and its relation to duration of wound healing: A study using laser speckle contrast imaging. *Burns: Journal of the International Society for Burn Injuries*, *42*(3), 648–654. https://doi.org/10.1016/j.burns.2015.12.005

Mirwald, R. L., Baxter-Jones, A. D. G., Bailey, D. A., & Beunen, G. P. (2002). An assessment of maturity from anthropometric measurements. *Medicine and Science in Sports and Exercise*, *34*(4), 689–694.

Mora-Rodriguez, R., Del Coso, J., Hamouti, N., Estevez, E., & Ortega, J. F. (2010). Aerobically trained individuals have greater increases in rectal temperature than untrained ones during exercise in the heat at similar relative intensities. *European Journal of Applied Physiology*, *109*(5), 973–981. https://doi.org/10.1007/s00421-010-1436-4

Mora-Rodríguez, R., González-Alonso, J., Below, P. R., & Coyle, E. F. (1996). Plasma catecholamines and hyperglycaemia influence thermoregulation in man during prolonged exercise in the heat. *The Journal of Physiology*, *491 (Pt 2)*, 529–540.

Morrison, S. A., Sleivert, G. G., & Cheung, S. (2006). Aerobic influence on neuromuscular function and tolerance during passive hyperthermia. *Medicine and Science in Sports and Exercise*, *38*(10), 1754–1761. https://doi.org/10.1249/01.mss.0000230120.83641.98

Nadel, E. R., Mitchell, J. W., Saltin, B., & Stolwijk, J. A. (1971). Peripheral modifications to the central drive for sweating. *Journal of Applied Physiology*, *31*(6), 828–833. https://doi.org/10.1152/jappl.1971.31.6.828

Nielsen, B., Hales, J. R., Strange, S., Christensen, N. J., Warberg, J., & Saltin, B. (1993). Human circulatory and thermoregulatory adaptations with heat acclimation and exercise in a hot, dry environment. *The Journal of Physiology*, *460*, 467–485.

Oberg, P. A. (1990). Laser-Doppler flowmetry. *Critical Reviews in Biomedical Engineering*, *18*(2), 125–163.

Patterson, M. J., Stocks, J. M., & Taylor, N. A. S. (2004). Humid heat acclimation does not elicit a preferential sweat redistribution toward the limbs. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *286*(3), R512-518. https://doi.org/10.1152/ajpregu.00359.2003

Périard, J. D., Travers, G. J. S., Racinais, S., & Sawka, M. N. (2016). Cardiovascular adaptations supporting human exercise-heat acclimation. *Autonomic Neuroscience: Basic & Clinical*, *196*, 52–62. https://doi.org/10.1016/j.autneu.2016.02.002

Pfeiffer, S., Leopold, E., Schmidt, K., Brunner, F., & Mayer, B. (1996). Inhibition of nitric oxide synthesis by NG-nitro-L-arginine methyl ester (L-NAME): requirement for bioactivation to the free acid, NG-nitro-L-arginine. *British Journal of Pharmacology*, *118*(6), 1433–1440.

Puissant, C., Abraham, P., Durand, S., Humeau-Heurtier, A., Faure, S., Lefthériotis, G., … Mahé, G. (2013). Reproducibility of non-invasive assessment of skin endothelial function using laser Doppler flowmetry and laser speckle contrast imaging. *PloS One*, *8*(4), e61320. https://doi.org/10.1371/journal.pone.0061320

Pullinen, T., Mero, A., Huttunen, P., Pakarinen, A., & Komi, P. V. (2011). Resistance exercise-induced hormonal response under the influence of delayed onset muscle soreness in men and boys. *Scandinavian Journal of Medicine & Science in Sports*, *21*(6), e184-194. https://doi.org/10.1111/j.1600-0838.2010.01238.x

Pullinen, T., Mero, A., MacDonald, E., Pakarinen, A., & Komi, P. V. (1998). Plasma catecholamine and serum testosterone responses to four units of resistance exercise in young and adult male athletes. *European Journal of Applied Physiology and Occupational Physiology*, *77*(5), 413–420. https://doi.org/10.1007/s004210050353

Pullinen, T., Mero, A., Huttunen, P., Pakarinen, A., & Komi, P. V. (2002). Resistance exercise-induced hormonal responses in men, women, and pubescent boys. *Medicine and Science in Sports and Exercise*, *34*(5), 806–813.

Rees, J., & Shuster, S. (1981). Pubertal induction of sweat gland activity. *Clinical Science (London, England: 1979)*, *60*(6), 689–692.

Rivera-Brown, A. M., Rowland, T. W., Ramírez-Marrero, F. A., Santacana, G., & Vann, A. (2006). Exercise tolerance in a hot and humid climate in heat-acclimatized girls and women. *International Journal of Sports Medicine*, *27*(12), 943–950. https://doi.org/10.1055/s-2006-923863

Roustit, M., Millet, C., Blaise, S., Dufournet, B., & Cracowski, J. L. (2010). Excellent reproducibility of laser speckle contrast imaging to assess skin microvascular reactivity. *Microvascular Research*, *80*(3), 505–511. https://doi.org/10.1016/j.mvr.2010.05.012

Roustit, M., & Cracowski, J. L. (2012). Non-invasive assessment of skin microvascular function in humans: an insight into methods. *Microcirculation (New York, N.Y.: 1994)*, *19*(1), 47–64. https://doi.org/10.1111/j.1549-8719.2011.00129.x

Rowland, T. (2008). Thermoregulation during exercise in the heat in children: old concepts revisited. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *105*(2), 718–724. https://doi.org/10.1152/japplphysiol.01196.2007

Rowland, T., Hagenbuch, S., Pober, D., & Garrison, A. (2008). Exercise tolerance and thermoregulatory responses during cycling in boys and men. *Medicine and Science in Sports and Exercise*, *40*(2), 282–287. https://doi.org/10.1249/mss.0b013e31815a95a7

Rubin, D. A., Castner, D. M., Pham, H., Ng, J., Adams, E., & Judelson, D. A. (2014). Hormonal and metabolic responses to a resistance exercise protocol in lean children, obese children and lean adults. *Pediatric Exercise Science*, *26*(4), 444–454. https://doi.org/10.1123/pes.2014-0073

Saumet, J. L., Kellogg, D. L., Taylor, W. F., & Johnson, J. M. (1988). Cutaneous laser-Doppler flowmetry: influence of underlying muscle blood flow. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *65*(1), 478–481. https://doi.org/10.1152/jappl.1988.65.1.478

Sawka, M. N., Cheuvront, S. N., & Kenefick, R. W. (2015). Hypohydration and Human Performance: Impact of Environment and Physiological Mechanisms. *Sports Medicine (Auckland, N.Z.)*, *45 Suppl 1*, S51-60. https://doi.org/10.1007/s40279-015-0395-7

Shibasaki, M., Inoue, Y., Kondo, N., Aoki, K., & Hirata, K. (1999). Relationship between skin blood flow and sweating rate in prepubertal boys and young men. *Acta Physiologica Scandinavica*, *167*(2), 105–110. https://doi.org/10.1046/j.1365-201x.1999.00597.x

Shibasaki, M., Inoue, Y., Kondo, N., & Iwata, A. (1997a). Thermoregulatory responses of prepubertal boys and young men during moderate exercise. *European Journal of Applied Physiology and Occupational Physiology*, *75*(3), 212–218. https://doi.org/10.1007/s004210050150

Shibasaki, M., Inoue, Y., Kondo, N., & Iwata, A. (1997b). Thermoregulatory responses of prepubertal boys and young men during moderate exercise. *European Journal of Applied Physiology and Occupational Physiology*, *75*(3), 212–218. https://doi.org/10.1007/s004210050150

Slaughter, M. H., Lohman, T. G., Boileau, R. A., Horswill, C. A., Stillman, R. J., Van Loan, M. D., & Bemben, D. A. (1988). Skinfold equations for estimation of body fatness in children and youth. *Human Biology*, *60*(5), 709–723.

Sokolnicki, L. A., Khosla, S., & Charkoudian, N. (2007). Effects of testosterone and estradiol on cutaneous vasodilation during local warming in older men. *American Journal of Physiology. Endocrinology and Metabolism*, *293*(5), E1426-1429. https://doi.org/10.1152/ajpendo.00535.2007

Stephenson, L. A., & Kolka, M. A. (1999). Esophageal temperature threshold for sweating decreases before ovulation in premenopausal women. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *86*(1), 22–28. https://doi.org/10.1152/jappl.1999.86.1.22

Tanner, J. M. (1962). *Growth at adolescence: with a general consideration of the effects of hereditary and environmental factors upon growth and maturation from birth to maturity*

(2nd ed). Oxford: Blackwell Scientific Publications. Retrieved from https://trove.nla.gov.au/version/45424593

Tew, G. A., Saxton, J. M., & Hodges, G. J. (2012). Exercise training and the control of skin blood flow in older adults. *The Journal of Nutrition, Health & Aging*, *16*(3), 237–241.

Tew, G. A., George, K. P., Cable, N. T., & Hodges, G. J. (2012). Endurance exercise training enhances cutaneous microvascular reactivity in post-menopausal women. *Microvascular Research*, *83*(2), 223–228. https://doi.org/10.1016/j.mvr.2011.09.002

Tew, G. A., Klonizakis, M., Crank, H., Briers, J. D., & Hodges, G. J. (2011). Comparison of laser speckle contrast imaging with laser Doppler for assessing microvascular function. *Microvascular Research*, *82*(3), 326–332. https://doi.org/10.1016/j.mvr.2011.07.007

Tverdal, A. (1987). Systolic and diastolic blood pressures as predictors of coronary heart disease in middle aged Norwegian men. *British Medical Journal (Clinical Research Ed.)*, *294*(6573), 671–673.

Tyler, C. J., Reeve, T., Hodges, G. J., & Cheung, S. S. (2016). The Effects of Heat Adaptation on Physiology, Perception and Exercise Performance in the Heat: A Meta-Analysis. *Sports Medicine (Auckland, N.Z.)*, *46*(11), 1699–1724. https://doi.org/10.1007/s40279-016-0538-5

Wagner, J. A., Robinson, S., Tzankoff, S. P., & Marino, R. P. (1972). Heat tolerance and acclimatization to work in the heat in relation to age. *Journal of Applied Physiology*, *33*(5), 616–622. https://doi.org/10.1152/jappl.1972.33.5.616

Walling, H. W., & Swick, B. L. (2011). Treatment options for hyperhidrosis. *American Journal of Clinical Dermatology*, *12*(5), 285–295. https://doi.org/10.2165/11587870-000000000-00000

Welch, G., Foote, K. M., Hansen, C., & Mack, G. W. (2009). Nonselective NOS inhibition blunts the sweat response to exercise in a warm environment. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *106*(3), 796–803. https://doi.org/10.1152/japplphysiol.90809.2008

Whitney, R. J. (1953). The measurement of volume changes in human limbs. *The Journal of Physiology*, *121*(1), 1–27.

Wilk, B., & Bar-Or, O. (1996). Effect of drink flavor and NaCL on voluntary drinking and hydration in boys exercising in the heat. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *80*(4), 1112–1117. https://doi.org/10.1152/jappl.1996.80.4.1112

Wilk, B., Kriemler, S., Keller, H., & Bar-Or, O. (1998). Consistency in preventing voluntary dehydration in boys who drink a flavored carbohydrate-NaCl beverage during exercise in the heat. *International Journal of Sport Nutrition*, *8*(1), 1–9.

Wilk, B., Rivera-Brown, A. M., & Bar-Or, O. (2007). Voluntary drinking and hydration in non-acclimatized girls exercising in the heat. *European Journal of Applied Physiology*, *101*(6), 727–734. https://doi.org/10.1007/s00421-007-0539-z

Wilkinson, I. B., & Webb, D. J. (2001). Venous occlusion plethysmography in cardiovascular research: methodology and clinical applications. *British Journal of Clinical Pharmacology*, *52*(6), 631–646.

Appendices

Appendix A: Subject Screening and Medical History Questionnaire

APPLIED PHYSIOLOGY RESEARCH GROUP
DEPARTMENT OF KINESIOLOGY, BROCK UNIVERSITY

ID: _____ Date: _____

Your responses to this questionnaire are confidential and you are asked to complete it for your own health and safety. If you answer "YES" to any of the following questions, please give additional details in the space provided and discuss the matter with one of the investigators.

1. Have you ever been told that you have a heart problem?
   YES NO
2. Have you ever been told that you sometimes experience seizures?
   YES NO
3. Have you ever had any major joint instability or ongoing chronic pain such as in the knee, back or elbow?
   YES NO
4. Have you had any allergies to medication?
   YES NO
5. Have you had any allergies to food or environmental factors?
   YES NO
6. Have you had any stomach problems such as ulcers?
   YES NO
7. When you experience a cut do you take a long time to stop bleeding?
   YES NO
8. When you receive a blow to a muscle do you develop bruises easily?
   YES NO
9. Are you currently taking <u>any</u> medication (including aspirin) or have you taken any medication in the last two days?
   YES NO
10. Have been diagnosed by a physician with cardiovascular disease (e.g. atheroscolsis, high blood pressure)?
   YES NO
11. Have been diagnosed by a physician with respiratory issues (e.g. asthma, bronchitis)?
   YES NO
12. Have been diagnosed by a physician with neuromuscular disease (e.g. multiple sclerosis, mysasthenia gravis)?
   YES NO
13. Have been diagnosed by a physician with metabolic disease (e.g. diabetes, kidney or liver problems)?
   YES NO

Appendix B: Godin-Shephard Leisure Time Questionnaire

GODIN-SHEPHARD Leisure-Time Exercise Questionnaire

ID:_____ Date:_____

1. During a typical **7-Day period** (a week), how many times on the average do you do the following kinds of exercise for **more than 15 minutes** during your free time?

a) STRENUOUS EXERCISE
(HEART BEATS RAPIDLY)
(e.g., running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling)

Times per Week
_____

b) MODERATE EXERCISE
(NOT EXHAUSTING)
(e.g., fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing)

Times per Week
_____

c) MILD EXERCISE
(MINIMAL EFFORT)
(e.g., yoga, archery, fishing from river bank, bowling, horseshoes, golf, snowmobiling, easy walking)

Times per Week
_____

2. During a typical **7-Day period** (a week), in your leisure time, how often do you engage in any regular activity long enough to work up a sweat (heart beats rapidly)?

OFTEN

SOMETIMES

NEVER/RARELY

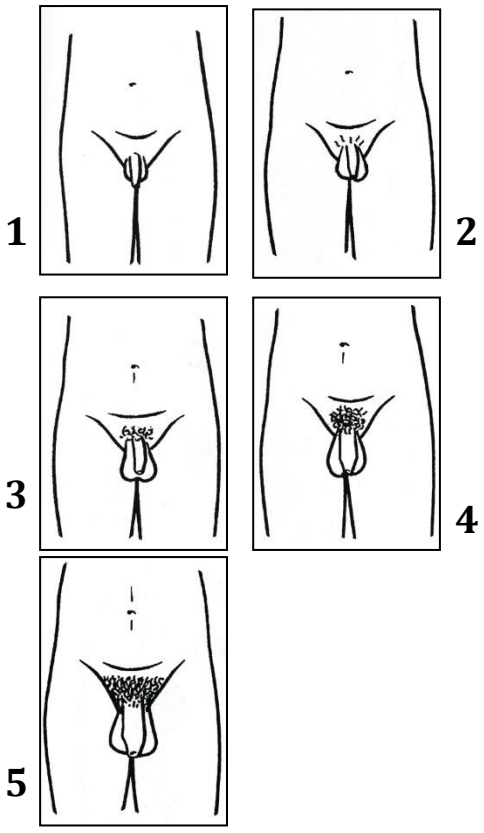
Appendix C: Pubertal Stage Questionnaire

Male Pubertal Stage

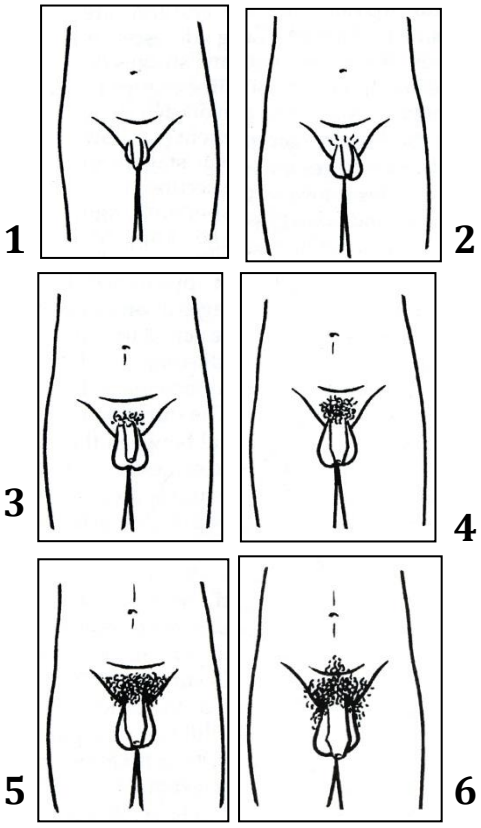
This survey will be used to assess the maturational levels of the participant.

ID: _____ Date: _____

Please circle the box below that looks most like you



- Please look at the **pubic hair only**
- Please circle the box that looks most like you



Appendix D: Bedford Thermal Comfort Scale, ASHRAE Thermal Sensation Scale

THERMAL COMFORT

1	Comfortable
2	Slightly uncomfortable
3	Uncomfortable
4	Very uncomfortable

THERMAL SENSATION

1	COLD
2	COOL
3	SLIGHTLY COOL
4	NEUTRAL
5	SLIGHTLY WARM
6	WARM
7	HOT

Appendix E: LDF Raw Data

Table 3 – Raw LDF Data (PU and CVC) during Baseline, Exercise, and Local Heating in Boys and Men

	LDF						CVC					
	L-NAME			Control			L-NAME			Control		
Condition	B	Ex	L	B	Ex	L	B	Ex	L	B	Ex	L
Boys	15.24	71.64	264.43	11.5	104.59	482.74	0.19	0.84	3.25	0.15	1.23	5.94
	9.53	43.40	260.89	21.27	152.88	409.55	0.13	0.56	3.65	0.29	1.96	5.72
	11.71	65.83	202.90	9.26	94.93	371.69	0.17	0.91	2.96	0.13	1.31	5.42
	28.92	133.62	311.01	19.32	193.40	414.11	0.45	1.75	4.29	0.30	2.53	5.71
	16.62	38.72	166.07	21.27	95.16	429.14	0.24	0.49	2.38	0.30	1.19	6.14
	33.54	77.11	317.14	12.60	110.36	349.26	0.54	1.06	5.24	0.20	1.52	5.77
	20.94	113.39	442.21	14.93	143.39	444.29	0.31	1.47	6.27	0.22	1.86	6.30
	25.14	124.65	432.98	25.98	125.71	335.49	0.33	1.52	5.78	0.34	1.54	4.48
	20.71	63.67	137.86	13.59	107.21	263.52	0.31	0.91	1.82	0.20	1.53	3.47
	24.34	81.09	187.26	11.81	108.92	323.69	0.37	1.08	2.98	0.18	1.46	5.15
	20.94	84.61	471.98	12.67	155.45	452.69	0.32	1.14	6.95	0.19	2.09	6.67
	11.71	131.69	358.63	12.98	89.34	260.08	0.16	1.69	4.79	0.17	1.15	3.47
Mean SD	19.95 ±7.3	85.79 ±32.8	296.11 ±112.9	15.60 ±5.10	123.45 ±31.57	378.02 ±72.92	0.29 ±0.12	1.12 ±0.42	4.20 ±1.62	0.22 ±0.07	1.61 ±0.42	5.35 ±1.04
Men	12.60	58.95	160.1	19.46	92.81	257.62	0.15	0.54	1.96	0.22	0.85	3.15
	12.21	66.78	63.72	14.40	102.88	135.07	0.13	0.67	0.72	0.16	1.04	1.53
	12.41	64.50	150.13	9.00	89.24	146.15	0.16	0.57	2.00	0.12	0.79	1.94
	20.78	65.75	175.09	17.13	91.49	223.28	0.22	0.62	1.96	0.18	0.86	2.50
	24.76	121.33	184.67	9.19	77.74	219.63	0.29	1.16	2.11	0.12	0.75	2.51
	7.94	15.55	102.00	26.73	92.72	285.00	0.09	0.16	1.19	0.30	0.93	3.32
	26.16	42.77	200.95	12.09	81.67	195.38	0.28	0.43	2.27	0.13	0.82	2.21
	19.20	96.57	237.69	8.85	78.34	239.48	0.21	0.85	2.67	0.10	0.69	2.69
	18.54	100.13	202.04	27.36	72.06	215.17	0.20	0.94	2.27	0.30	0.68	2.41
	21.20	86.32	185.28	14.05	112.85	222.69	0.24	0.88	2.14	0.16	1.15	2.57
	12.49	65.47	212.62	11.34	69.19	265.75	0.14	0.65	2.49	0.13	0.69	3.11
	20.22	73.32	146.37	13.62	82.03	161.99	0.24	0.74	1.65	0.16	0.83	1.83
Mean SD	17.38 ±5.10	71.45 ±22.36	168.39± 45.81	15.27 ±6.37	86.84 ±12.63	213.93± 43.05	0.20 ±0.06	0.68 ±0.26	1.95 ±0.54	0.17 ±0.07	0.84 ±0.14	2.48 ±0.55

Where B = baseline, Ex = exercise, L = local heating