The effects of Type 1 Diabetes Mellitus on Heat Loss during Exercise in the Heat

M.Sc. Thesis

Submitted to the Faculty of Graduate and Postdoctoral Studies
in partial fulfillment of the requirements for the degree of
Master's of Science in Human Kinetics

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Acknowledgements

The development and completion of this thesis would not have been made possible without the guidance and support of the following individuals.

To Dr. Glen Kenny, thank you for giving me the opportunity to complete my Master's of Science degree under your supervision. Your patience, guidance and continuous push for excellence no matter what the activity is are life lessons that I will take with me. Without your guidance and support this project would never have been completed.

To my committee members Dr. Pascal Imbeault and Dr. Ron Sigal, thank you for your time, effort, and expertise in providing me with constructive feedback on my project.

To my fellow Human and Environmental Physiology Research Unit peers for their hours of physical and technical support and for creating an interesting work environment that allowed for much personal and professional growth.

A special thanks to Juliana Barrera for her assistance in data collection as well as Ryan McGinn, for his many hours as my pilot subject as well as his help with the data collection. Ryan, without you we would still be using the tilt table.

A special thanks to the individuals who volunteered as subjects for my project, your time and commitment towards my thesis is greatly appreciated; I only hope you learned as much from your experience as I did.

Finally, I would like to thank my mother, Lorraine, and my father, Jeff. You have always been there to support me and talk me back down from the fence, and without your support I would not be where, or who I am today.

Abstract

Studies show that vasomotor and sudomotor activity is compromised in individuals with type 1 Diabetes (T1DM) which could lead to altered thermoregulatory function. However, recent work suggests that the impairments may only be evidenced beyond a certain level of heat stress. We therefore examined T1DM-related differences in heat loss responses of sweating and skin blood flow (SkBF) during exercise performed at progressive increases in the requirement for heat loss. Participants were matched for age, sex, body surface area and fitness cycled at fixed rates of metabolic heat production of 200, 250, and 300 $W \cdot m^{-2}$ of body surface area, each rate being performed sequentially for 30 min. Local sweat rate (**LSR**), sweat gland activation (**SGA**), and sweat gland output (**SGO**) were measured on the upper back, chest and forearm while SkBF (laser-Doppler) was measured on the forearm and upper back only.

We found that despite a similar requirement for heat loss, LSR was lower in T1DM on the chest and forearm only, relative to Control and only different at the end of the second and third exercise period. Differences in chest LSR were due to reduced SGA whereas the decreased forearm LSR was the result of a decrease in SGO. SkBF did not differ between groups. The reduction in the sweating response in the T1DM group was paralleled by a greater increase in core temperature. We show that T1DM impairs heat dissipation as evidenced by reductions in LSR and not SkBF. A compromised thermoregulatory response during and following physical exertion is of considerable concern due to the associated increased risk of post-exertion heat-related injury.

Table of Contents

CHAPTER I: INTRODUCTION

1.1 Introduction

Human thermoregulation is the physiological process by which heat lost to the environment is matched by heat gained by the body. Through the balance of internal heat exchange from different tissues in the body and external heat exchange between the body and the environment, core temperature is maintained at a "set-point" of approximately 37°C. The pre-optic anterior hypothalamus (PO/AH) is the major thermoregulatory center of the body. Thermoafferent information from thermal sensors in the skin and core is carried to the PO/AH which then coordinates the appropriate efferent response (i.e. vasodilation/constriction). The PO/AH acts like a thermostat, integrating physiological information about core and skin temperature with the appropriate responses and through negative feedback loops thermal homeostasis is maintained (Charkoudian, 2003). In order to maintain heat balance when the body is exposed to a heat stress, thermoefferent activity, namely increased cutaneous perfusion of blood and sweating, increases the rate of heat dissipation thereby attenuating the rate of increase in core temperature. Cold exposure leads to vasoconstriction to minimize heat loss and if core temperature continues to drop, shivering to allow for heat generation.

Thermal homeostasis can be offset by both passive heat exposure (i.e. a hot ambient environment) as well as an increase in metabolic activity (i.e. exercise). The muscle contractions associated with exercise result in an increase in the metabolic demands of skeletal muscle and one of the by-products of these contractions is heat. Through conductive and convective heat transfer (from the working muscle, to the blood, to the rest of the body) core temperature rises. Through an increase in skin blood flow as well as the evaporation of sweat from the skin, heat is transferred from the body to the environment (Shibasaki, Wilson,

& Crandall, 2006). A sustained and elevated rate of metabolic heat production which is not completely offset by a parallel increase in the rate of heat loss will result in an increase in the body's core temperature.

Exercise results in a competition between the need to supply blood to the muscle to sustain the metabolic demands for O_2 and nutrients with the need to transfer the heat of the active musculature to the skin surface. This increased demand for tissue blood flow is mediated by an increase in cardiac output caused by an increase in stroke volume as well as heart rate. During exercise, the onset of cutaneous vasodilation is briefly delayed and the amount of blood flow to the skin is less than what would be achieved during a passive heat stress and this delay leads to the rapid increase in core temperature seen during exercise (Gonzalez-Alonso, Crandall, & Johnson, 2008). If the rate of heat loss by the body through skin vasodilation and sweating fails to match heat gained by the body from an increased rate of metabolic heat production and/or ambient temperature, the body is unable to achieve a thermal steady state condition and core temperature will rise. Continued exercise during uncompensable heat stress is potentially dangerous as an increasing core temperature can lead to heat related illnesses such as heat exhaustion and exertional heat stroke that may require hospitalization or even lead to death.

Though largely influenced by thermal stimuli (i.e. an increasing core or skin temperature) heat loss responses during exercise can also be influenced by non-thermal factors such as baroreceptors, metaboreceptors, central command (Kenny & Journeay, 2010) as well as aging, chronic disease and obesity (Kenny, Yardley, Brown, Sigal, & Jay, 2010). Studies have shown that the metabolic disorder, type 1 diabetes mellitus (T1DM), in which

the body fails to produce insulin, can affect both skin blood flow and sweat responses, potentially leading to a greater increase in core temperature during exercise when compared to healthy populations (Hoeldtke et al., 2001; Khan, Elhadd, Greene, & Belch, 2000).

During the 1995 Chicago Heat Wave, admission of diabetic patients to hospitals increased and like the 1966 New York Heat Wave, deaths of diabetics rose by approximately 117% (Schuman, 1972; Semenza, McCullough, Flanders, McGeehin, & Lumpkin, 1999). A better understanding of how diabetes effects thermoregulation during exercise is required to ensure the safety of T1DM populations.

Though research into exercise and thermoregulation in T1DM is limited, there are studies that have examined the functioning of the major avenues of heat loss (i.e. skin blood flow and sweating). Consistently elevated or poorly controlled blood glucose concentrations have been seen to damage the endothelium surrounding the blood vessels in populations with T1DM (Hurks et al., 2009). Khan et al., (2000) found that T1DM effects the cutaneous circulation of the skin and that both endothelium dependent (iontophoresis of acetylcholine) and independent vasodilation (iontophoresis of sodium nitroprusside) as well as maximal skin blood flow in response to heat is impaired early in the onset of T1DM. In one study after 30 minutes of passive heat exposure the core temperature of patients with diabetes increased by 1°C while the non-diabetic group saw an increase of only ≈0.5°C (Petrofsky, Besonis, Rivera, Schwab, & Lee, 2005). Most of the studies examining skin blood flow in T1DM that have used heat as the stimulus have just heated small sections of skin or heated the body passively. It remains to be seen if these vascular impairments in T1DM influence heat loss during exercise resulting in a higher, more rapid, increase in core temperature

during exercise in the heat than healthy populations.

Early in the disease T1DM is accompanied by changes in the autonomic nervous system which may influence thermoregulation during exercise by augmenting the sweat response to increasing core and skin temperatures. Anhidrosis of the lower body and hyperhidrosis of the upper body have been observed in T1DM populations (Asahina et al., 2008; Fealey, Low, & Thomas, 1989; Goodman, 1966; Hoeldtke et al., 2001; Provitera et al., 2010). Regional changes in sweat production may lead to reductions in the body's ability to dissipate heat through evaporative heat loss, however, it remains to be seen if sudomotor function is affected during exercise in T1DM.

There has been one study which recently examined whole body heat loss during a single exercise load in the heat using T1DM patients; it found no differences between control and type 1 diabetic participants in sweating, skin blood flow, core temperature and heat storage during exercise (Stapleton, Yardley, Boulay, Sigal, & Kenny, 2013). However, comparisons were limited to a single exercise intensity which may have been insufficient to exceed the physiological capacity of the body's heat loss responses. Studies examining differences in thermoregulation between sexes (Gagnon & Kenny, 2012) have found that differences occur at higher requirements for heat loss than those used in Stapleton *et al*. (2013), so it is plausible that differences in local heat loss responses in T1DM will only be seen at higher rates of metabolic heat production.

1.2 Rationale and Statement of the Problem

To date little is known about how thermoregulation is affected by T1DM. The mechanisms underlying the reflex response to increases in core temperature, namely

active vasodilation of blood vessels supplying the skin and sweating, are impaired in T1DM. The majority of studies have only examined local heat loss mechanisms and passive heat exposure (Hoeldtke et al., 2001; Khan et al., 2000) and these may not illustrate the whole body response during exercise (Minson, 2010) There has been one study which has examined whole body heat loss during low intensity exercise in the heat which found that regularly active T1DM patients have no reduced capacity to dissipate heat, however, the previous study was limited to a single exercise intensity which may have been insufficient to exceed the physiological capacity of the body's heat loss responses. Therefore, we decided to undertake a study to evaluate impairments in local heat loss responses in T1DM as a function of an increasing rate of metabolic heat production will show if during higher levels of heat stress differences in local heat loss capacity are evident. The effect(s) of chronic diseases, such as T1DM, on thermoregulation during exercise has not been explored enough to ensure the safety of T1DM populations. A compromised thermoregulatory response during physical exertion is of considerable concern within a working environment due to the associated increased risk of post-exertion heat-related injury.

1.3 Objectives

The purpose of the present study is to advance our understanding of the influence of T1DM on thermoregulatory function during exercise. The study aims to determine: 1) if differences between T1DM and non-diabetic participants in local sweat rate and skin blood flow are only evident above a certain requirement for heat loss; 2) if there are regional variations in diabetes-related impairments for heat loss; and 3) if T1DM participants have a greater increase in heat storage during exercise as evidenced by changes in core temperature. Therefore, to evaluate potential differences in local vasomotor and sudomotor activity in those with T1DM, in the present

study we measured the local heat loss responses of skin blood flow and sweating as well as changes in core temperature as a function of increasing rates of metabolic heat production.

1.4 Hypothesis

We tested the hypothesis that at higher rates of metabolic heat production, where the need to dissipate heat is greater, individuals with T1DM would exhibit lower local sweat rates compared to healthy controls. We also hypothesized that the impairments in heat loss responses would vary by region and would only be present at the highest requirements for heat loss. Based on previous experimentation (Stapleton et al., 2013), we also hypothesized that no differences in skin blood flow would be observed during exercise when expressed as a percentage of maximum. Furthermore, we tested the hypothesis that in comparison to matched healthy controls people with T1DM would have a greater increase in core temperature as a function of an increasing requirement for heat loss (i.e. greater rate of metabolic heat production).

1.5 Relevance

There is still much to be learned about the effects T1DM has on human thermoregulation, especially in regards to how the disease affects skin blood flow and sweating during exercise. Studies examining the reflex responses involved in heat dissipation at rest indicate that there may be impairments in the mechanisms of skin blood flow and sweating that underlie the control of core temperature in populations with T1DM (Khan et al., 2000; Stansberry et al., 1997). Part of the recommended treatment program for T1DM is regular physical activity (Riddell & Iscoe, 2006). However, the effects of T1DM on thermoregulation during exercise have not been explored to a great enough extent to ensure the safety of this population. Advancing our understanding of how T1DM influences thermoregulatory function during exercise is an important step in evaluating the risk of heat-

related injuries in individuals with type 1 diabetes.

1.6 Delimitations and Limitations

This study is testing non-habitually active individuals with T1DM between the ages 18- 35 who have uncomplicated T1DM with a minimum duration of 5 years. The results of this study cannot be extrapolated to older men with T1DM, children with T1DM, people with

T2DM or people with T1DM with diagnosed autonomic neuropathy. This study is focused on non endurance trained individuals with adequate glucose control ($HbA1_C$<10%) and cannot be extrapolated to physically active populations as there are indications that physical activity can improve heat loss responses (Tew *et al.*, 2011). Since the changes in the vasculature of people with diabetes can be likened to a state of accelerated ageing, the results of this study are limited to the age group examined (Stansberry *et al*., 1997).

CHAPTER II: REVIEW OF LITERATURE

2.1 Human Thermoregulation

The human body is able to function in a wide range of environmental conditions and independent of the environment. Through the balance of thermoafferent sensory information and thermoefferent responses, core temperature is maintained at a set point of approximately 37±1°C (Johnson & Park, 1981). The pre-optic anterior hypothalmus (PO/AH) acts as the thermoregulatory command center as it integrates both central and peripheral somatosensory information from spine and skin thermoreceptors while generating the appropriate effector response(s) (Charkoudian, 2003).

In order to maintain a core temperature of 37°C, balance between heat produced in the body and heat transferred between the environment and the body must be achieved. When a healthy human is exposed to a cold stimulus, skin vasoconstriction is increased, reducing the amount of convective heat transfer from the body to the environment. If core temperature continues to drop, shivering will begin as this reflex series of muscle contractions generates heat to prevent a further drop in core temperature. In response to an increasing core temperature, through either a change in internal or external conditions, vasodilation occurs increasing the amount of convective heat transfer from the skin to the environment. In parallel with an increase in blood flowing to the skin, sweat is released from acetylcholine stimulated eccrine glands. Due to its high latent heat (2426 $J \cdot g\ sweat^{-1}$, at 30°C) (Wenger, 1972) as sweat is evaporated from the skin, the skin is cooled allowing for the blood in the dilated vessels to be cooled before returning to the core, decreasing core temperature. Failing to respond to either an increase or a decrease in core temperature can result in hyper or hypothermia respectively which can potentially lead to hospitalization or death.

The metabolic activity occurring in tissues at rest generates heat. To maintain thermohomeostasis, heat produced by the body at rest in thermoneutral conditions (i.e. room temperature) is balanced through a combination of conduction, convection and radiation. This is referred to as "dry heat exchange" and it relies on a temperature gradient that allows heat produced by the body to dissipate into the surrounding environment. In situations where the ambient temperature is higher than the temperature of the body, the body will gain heat from the environment through dry heat exchange which causes evaporative heat loss to become increasingly important in maintaining thermal homeostasis.

2.1a Heat Balance

Heat balance in humans is achieved by matching heat dissipation with heat generation and can be described by the equation below:

$$M-W = (K \pm C \pm R - E_{sk}) \pm (C_{res} + {}_{Eres)} + S$$

M = rate of metabolic heat production.
W = rate of mechanical work.
K = rate of conductive heat exchange.
C = rate of convective heat exchange from skin.
R = rate of radiative heat exchange from skin.
E_{sk} = rate of evaporative heat loss from skin.
C_{res} = rate of convective heat exchange from respiration. E_{res} = rate of evaporative heat exchange from respiration.
S = rate of body heat storage.

(All units are $W \cdot m^2$) (ASHRAE, 1989)

When all variables in the heat balance equation above are balanced, the amount of heat storage will be zero and core temperature will not increase. During exercise, an increase in metabolic heat production (M±W) that is not matched by heat loss mechanisms (K ± C ± R –

E_{sk}), will result in a continuous rise in core temperature. If left unchecked, this rise in core temperature can result in hyperthermia and potentially hospitalization and ultimately death.

2.2 Heat Loss Responses During Exercise

Heat can be dissipated from the body through dry heat exchange; a combination of conduction, convection, radiation as well as evaporative heat loss from the skin. Conductive heat transfer (**K**) is the transfer of heat between two surfaces in direct contact. The amount of energy transferred is dependent upon the temperature gradient between surfaces. Convective heat exchange (**C**) is the mechanism by which heat is transferred by the movement of a fluid medium from a high to low thermal gradient. This allows for heat to be transferred from a surface to a liquid or gas as is the case in heat exchange between human skin and the environment. Radiative heat transfer (**R**) is the mechanism by which heat is transferred via electromagnetic waves from the body to the environment. Evaporative heat loss ($_{(}E_{sk)}$) is the loss of heat through the vaporization of water. In hot and dry environmental conditions and/or during high rates of metabolic heat production, evaporative heat loss through increased sweat production and evaporation has the greatest potential to dissipate heat as dry heat exchange becomes insufficient to match increases in body heat content. Therefore, the major avenues of thermoregulation during heat stress are increased skin blood flow (increased convective heat transfer) and sweating (evaporative heat loss).

Physical activity increases the rate of metabolic heat production 10-20 fold during exercise as humans are at best 30% efficient at converting chemical energy into mechanical

energy (Joyner & Coyle, 2008). This leads to an increased need to dissipate heat to maintain thermal homeostasis. Increases in cutaneous circulation and sweat production are proportional to increases in core temperature and will continue until either a steady state core temperature is reached or the maximum responsiveness of skin blood flow and sweating have been achieved. The temperatures at which cutaneous vasodilation and sweating begin are referred to as "thresholds" and the slope of the skin blood flow/sweat response to increases in internal temperature describes the "gain" or "sensitivity" of the skin vasodilator or sweat response (Charkoudian, 2003). Core temperature regulation as mediated by changes in skin blood flow and sweating is largely determined by thermoafferent input from skin and core. However, non-thermal factors have been shown to have an effect on heat loss responses by modulating their activity during exercise which will be discussed in section 2.2c (Kenny & Journeay, 2010).

2.2a Skin blood flow

The manipulation of skin blood flow is one of the major effector responses involved in thermoregulation and is the only way to redistribute heat from the core of the body to the environment (Rowell, 1977). Increasing or decreasing skin blood flow serves to alter the amount of convective heat transfer from the body to the environment, as during heat stress reflex cutaneous vasodilation allows for a greater amount of blood to be moved from the core to the skin where cooling can occur. At rest, in thermoneutral conditions, ≈500ml·min^{-1} of blood (5-10% of cardiac output) is supplying the skin (Lossius, Eriksen, & WallÃ¸e, 1993). During heat stress up to 6-8L·min^{-1} of blood (50-70% of cardiac output) is directed to the cutaneous circulation. This increase is due to a combined effect of increased cardiac output as well as a redistribution of that cardiac output (i.e. the splanchnic and renal

system at rest receives 20% of cardiac output, during heat stress it can fall to 1% of total cardiac output (Brubaker, Kaminsky, & Whaley, 2002).

Altered vascular responses of the skin can be caused by an increased threshold for the response, a reduced sensitivity of the response or by some combination of the two. Human nonglabrous or "hairy" skin (i.e. skin of the forearm, legs, torso) and is largely responsible for heat exchange with the environment. Non-glabrous skin is under the control of noradrenergic sympathetic vasoconstrictor nerves, and cholinergic active vasodilator nerves. It can also be influenced by local sensory information (i.e. cooling of a small area of warmed skin). At rest, in thermoneutral conditions, norepeniphrine (released from sympathetic vasoconstrictor nerves) as well as a noradrenergic co-transmitter, maintain a basal level of vasomotor tone (Kellogg, 2006). During heat stress the withdrawal of this nervous activity is responsible for 10-20% of the increased skin blood flow (Charkoudian, 2003).

In thermoneutral conditions the active vasodilator system is inactive. In contrast, during heat stress (i.e. passive heating or exercise); activity of this system is responsible for up to 90% of increased skin perfusion seen during whole body heating. The neurotransmitter(s) responsible for active cutaneous vasodilation have yet to be determined. It has been suggested that nitric oxide (NO), prostaglandin, vaso-active intestinal peptide (VIP) and endothelium derived hyperpolarizing factor may all play roles in increasing skin blood flow during heat stress (Golay et al., 2004; Shastry, Dietz, Halliwill, Reed, & Joyner, 1998; Shastry, Minson, Wilson, Dietz, & Joyner, 2000). The vascular response to an increasing core temperature caused by increased metabolic heat production is different than

what occurs during local passive heating. During exercise, redirection of blood flow to the skin still occurs but not to the same degree as it would at rest at the same core temperature. At the onset of exercise there is a brief increase in sympathetic vasoconstrictor activity as skin blood flow is sacrificed to allow for increased blood flow to the working muscles. In addition to this slight initial reduction in skin blood flow during exercise, there is an increase in the threshold core temperature required for active vasodilation. There is also a plateau in the skin blood flow response at a core temperature of 38°C during exercise not seen during passive heat exposure. Ultimately, during exercise skin blood flow is sacrificed to meet the metabolic requirements of active muscle and evaporative heat loss becomes increasingly important for the maintenance of core temperature.

2.2b Eccrine Sweating

When the ambient temperature is higher than that of the skin or during high rates of heat production, evaporative heat loss becomes increasingly important for maintaining a stable core temperature. Heat is carried from the working muscles by the blood to the body's core and eventually to the skin, where the production of sweat on the skin surface, and the subsequent evaporation of the sweat (which has a latent heat of 2426 $J \cdot g\ sweat^{-1}$, at 30°C (Wenger, 1972)cool the surface of the skin, ultimately leading to an increase in heat exchange from the body to the environment. During high levels of heat stress (with adequate hydration), whole body sweat output can reach 1-2.5 litres per hour. As the evaporation of sweat from skin takes energy away from the body, sweat production is a major avenue of heat loss (Kenny & Journeay, 2010).

Sweat production for thermoregulation is accomplished by the activation of some or all of the 2-4 million eccrine sweat glands distributed all over the body's surface. Regional distribution

can vary between individuals, but sweat gland density tends to be highest in the forehead, then the arms, torso and legs (Sato & Dobson, 1970). The central nervous system relays efferent information through an increasing frequency of nerve pulses which leads to the release of acetylcholine from the sudomotor junction. The acetylcholine binds to muscarinic receptors found on the sweat gland. By increasing the frequency of nerve pulses, the number of glands as well as the amount of sweat produced per gland increases. The onset of sweat production occurs across the body at the same time. However, there are regional differences in the amount of sweat produced per gland (Taylor, Caldwell, & Mekjavic, 2006).

The sweat response can be influenced by changes in the mean skin temperature as well as the local skin temperature, as both local and mean skin temperatures can influence both the threshold and/or sensitivity of sweating (Nadel, Mitchell, Saltin, & Stolwijk, 1971). Like skin blood flow, increases in the sweat response are primarily influenced by an increase in core temperature above a certain threshold. The initial increase in sweat production during heat stress is caused by increased sweat gland activation, while further increases in sweat production are related to increasing the amount of sweat per gland. Therefore, differences in sweat production between groups could be caused by either an increase in the number of sweat glands activated or the amount of sweat produced per gland. Any pathologic process, such as autonomic neuropathy, that impairs the sudomotor response resulting in a decrease in sweat production is referred to as anhydrosis. Anhydrosis is most likely the result of damage to the peripheral nerves and may impair thermoregulation (Goodman, 1966).

2.2c Non-thermal influence on heat loss responses

Skin blood flow and sweating can be influenced by factors other than the need to dissipate heat. Non-thermal afferent sensory information, in particular sensory information from baroreceptors, can influence skin blood flow and sweating during. The baroreceptor is an elastic stretch or mechanoreceptor involved in the regulation of blood pressure. There are two divisions of baroreceptors in the human body: 1) arterial baroreceptors, located in the carotid sinus and aortic arch and are high pressure sensors; and 2) cardiopulmonary baroreceptors or low pressure sensors which are located in the atria, ventricles and pulmonary vessels (Mark, Abboud, & Fitz, 1978). The baroreceptor acts as a short term buffer against sudden swings in blood pressure (Brubaker et al., 2002) and during exercise blood pressure increases in response to an increase in demand and delivery of oxygen by working muscle. It was originally believed that the baroreceptor was turned off at the onset of exercise to allow for this to happen. This is not the case. Experiments have shown that during exercise the baroreceptor is still active, but its operating point is reset to a higher level to better control for blood pressure changes at this higher operating point (Ogoh et al., 2005). During exercise baroreceptor sensitivity (BRS) is reduced (Niemela et al., 2008).

T1DM has been implicated in changes to skin blood flow and sweating as well as changes in baroreflex sensitivity and cardiovascular function at rest. As T1DM can influence both the effector responses involved in thermoregulation as well the systems that can influence the reflex response (i.e. the baroreceptors), T1DM could have a major impact on whole body heat loss during exercise in the heat.

2.3 Diabetes

Diabetes mellitus is a metabolic disease in which the body fails to regulate glucose properly and is characterized by consistently elevated blood glucose concentrations. Diabetes mellitus affects 150 million people worldwide and that number is forecast to rise to 592 million by 2030 (*IDF diabetes atlas,* 2013). According to the Public Heal Agency of Canada as of 2006, 19 million Canadians suffered from some sort of this form this metabolic disorder and the number is steadily increasing. Type 1 diabetes mellitus (T1DM) is a condition where it is believed that the autoimmune system attacks the insulin producing beta-islet cells of the pancreas, killing them and preventing the production of endogenous insulin. It affects approximately 5-10% of those diagnosed with diabetes. Type 2 Diabetes Mellitus (T2DM) is a condition in which the body produces some insulin, but not enough to maintain euglycemia. In addition, there is often insulin resistance (Dai et al., 2011).

The treatment paradigm for T1DM can be viewed as a "triad" composed of nutrition, insulin and physical activity to control blood glucose levels. Exercise has both physiological and psychological benefits for people with T1DM. However, exercise in T1DM populations can lead to life threatening consequences such as hypoglycaemia, hyperglycemia and ketoacidosis. As these potentially fatal consequences of exercise have to do with blood glucose control and insulin concentrations, much of the work examining T1DM and exercise has focused on glucoregulation.

Though the majority of studies examining T1DM and exercise have focused on glucoregulation, some studies have looked at how T1DM affects the body's ability to increase cardiac output and oxygen delivery during exercise. It has been found that good glucose control in people with T1DM can allow for maintenance of cardiorespiratory

function and allow for similar performance during exercise testing (Baldi, Cassuto, Foxx-Lupo, Wheatley, & Snyder, 2010). Tight glucose control as measured by HbA1c, a test that measures the average amount of sugar attached to haemoglobin in the blood, must be maintained as adults with diabetes are two to four times more likely to develop cardiovascular disease than non-diabetic counterparts (Booth, Slaughter, & Laupacis, 2003). T1DM patients are also seen to undergo significant changes to the macro and micro-vasculature that can lead to the development of blindness, end-stage renal disease as well as the loss or deadening of some neural signals through neuropathy. Neuropathy associated with T1DM has been implicated in the development of early atherosclerosis (Schalkwijk & Stehouwer, 2005), anhydrosis of the lower body (Hoeldtke et al., 2001), loss of control of the microcirculation (Tibirica, Rodrigues, Cobas, & Gomes, 2007) and changes in autonomic nervous system's control of blood pressure and heart rate (Dalla Pozza et al., 2007; Weston et al., 1996; Weston et al., 1998). Endothelial dysfunction precedes the development of major vascular complications in T1DM and these changes may also affect skin blood flow, ultimately affecting heat transfer (Schalkwijk & Stehouwer, 2005).

2.3a Diabetes and Thermoregulation

It is not yet understood if T1DM impairs the body's capacity to dissipate heat during exercise due to impairments in skin blood flow and sweating. There are several theories as to why and how diabetes may interfere with this process. In response to pharmacological stimulation (sodium nitroprusside, acetylcholine, nitro-glycerine, C-peptide infusion) the responsiveness of the endothelium is reduced in people with T1DM (Forst et al., 1998; Khan et al., 2000; Sorensen, Mathiesen, Clausen, Flyvbjerg, & Feldt-

Rasmussen, 2005). Experiments examining the vascular response to occlusion in T1DM have found a reduction in flow mediated vasodilation (Hurks et al., 2009; Meyer & Schatz, 1998; Sorensen et al., 2005; Tibirica et al., 2007). There is also a large amount of damage caused by free, non-oxygenated radicals in the vasculature of people with T1DM including increased DNA damage due to reactive oxygen species (Dandona et al., 1996; West, 2000).

Advanced glycosylated end products, essentially proteins that are glycosylated through a series of dehydration reactions, are increased in expression in T1DM due to an abundance of free radicals which drive this reaction (Bucala, Tracey, & Cerami, 1991). These AGEs are able to create more free radicals which can lead to further endothelial damage. Buccala *et al*. (1991) showed that AGEs, through a chemical reaction that results in NO becoming inactivated, impairs endothelial functioning in diabetics and this in turn could impact thermoregulation in diabetic patients by preventing NO release and reducing the bioavailability of the this and other neurotransmitters purported to have a role in cutaneous vasodilation in response to heating. Some reports suggest that improving glucose control will restore endothelial functioning (Enderle, Benda, Schmuelling, Haering, & Pfohl, 1998), however, (Eberl et al., 2005) found that even after a pancreas and kidney transplant the maximal increase in skin perfusion is less than in healthy age matched controls. Based on the results from these studies it is believed that major microvascular changes occur due to continuously elevated blood glucose concentrations, or periodic glycemic elevations, that lead to permanent changes which may impair whole body heat loss.

Early in the disease, T1DM is accompanied by changes in the autonomic nervous

system which may influence thermoregulation during exercise. Anhydrosis of the lower body and gustatory sweating as well as hyperhidrosis of the upper body have been observed in T1DM populations (Asahina et al., 2008; Hoeldtke et al., 2001; Provitera et al., 2010). Regional changes in sweat production may lead to impairments in the body's ability to dissipate heat through evaporative heat loss. Hoeldtke and colleagues (2001) examined the sweat response of newly diagnosed T1DM patients to an infusion of acetylcholine and observed an augmented sweating response. At the time of the first evaluation they saw a redistribution of sudomotor responses as evidenced by increased sweating in T1DM patients at the forearm compared to controls and a decrease in sweating in the lower limbs. They also reported an increase in the total amount of sweat at the first evaluation. However, studies that have examined sweat responses to whole body heating have found regions of anhidrosis in long duration T1DM (Fealey et al., 1989; Goodman, 1966).

Only one study (prior to the present one) has examined the effects of T1DM on thermoregulatory function during exercise. Stapleton *et al*., (2013) showed that individuals with T1DM maintain a similar rate of whole body heat loss as their young healthy counterparts when performing exercise at a low to moderate rate of heat production (≈200 $W \cdot m^{-2}$). These findings could be over generalized to indicate that diabetes impairments in vasomotor and sudomotor function do not occur during exercise. The limiting factor of this study is that comparisons were limited to a single exercise intensity which may have been insufficient to exceed the physiological capacity of the body's heat loss responses. As such it remains unclear if during higher levels of heat stress, differences in capacity would be evident between T1DM and matched healthy controls. It is plausible that differences may occur at a higher requirement for heat loss as defined by a greater rate of metabolic heat

production (Gagnon & Kenny, 2012).

In summary, studies demonstrate impairments in skin blood flow and sweating function between T1DM patients and people without diabetes. These impairments may affect the body's ability to dissipate heat, thereby leading to elevated core temperature responses during heat stress as compared to healthy individuals. The only previous study to examine heat loss responses in individuals T1DM found no changes in thermoregulatory function but the heat load may not have been great enough to see differences in response as previous work in healthy populations has shown differences in heat loss responses may be a function of heat load.The ultimate effect of T1DM on heat loss during exercise remains to be clarified.

CHAPTER III: METHODS AND RESULTS

Type 1 diabetes-related impairments in heat dissipation are only evidenced beyond a certain requirement for heat loss

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Running Head: Type 1 diabetes and local heat loss

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ABSTRACT ***(274 words)***

Studies show that vasomotor and sudomotor activity is compromised in individuals with type 1 Diabetes (T1DM) which could lead to altered thermoregulatory function. However, recent work suggests that the impairments may only be evidenced beyond a certain level of heat stress. **PURPOSE**: We examined T1DM-related differences in heat loss responses of sweating and skin blood flow (SkBF) during exercise performed at progressive increases in the requirement for heat loss. **METHODS**: Eight (5 males and 3 females) adults with and without T1DM matched for age, sex, body surface area and fitness cycled at fixed rates of metabolic heat production of 200, 250, and 300 $W \cdot m^{-2}$ of body surface area, each rate being performed sequentially for 30 min. Local sweat rate (**LSR**, ventilated capsule), sweat gland activation (**SGA**, modified iodine-paper technique), and sweat gland output (**SGO**) were measured on the upper back, chest and forearm while SkBF (laser-Doppler) was measured on the forearm and upper back only. **RESULTS**: Despite a similar requirement for heat loss, LSR was lower in T1DM on the chest and forearm only relative to Control. Reductions were measured in the second (Chest: 0.58±0.08 vs. 0.82±0.12 $mg \cdot min^{-1} \cdot cm^{-2}$; Forearm: 0.68±0.14 vs. 0.85±0.11 $mg \cdot min^{-1} \cdot cm^{-2}$) and third (Chest: 0.66±0.1 vs. 1.02±0.16 $mg \cdot min^{-1} \cdot cm^{-2}$; Forearm: 0.75±0.11 vs. 0.98±0.12 $mg \cdot min^{-1} \cdot cm^{-2}$) exercise bouts. Differences in chest LSR were due to reduced SGA whereas the decrease forearm LSR was the result of a decrease in SGO. SkBF did not differ between groups. **Conclusion**: We show that T1DM impairs heat dissipation as evidenced by reductions in LSR and not SkBF. However, these differences are only evidenced beyond a certain requirement for heat loss.

Key Words: sweating; skin blood flow; core temperature; thermosensitivity; chronic disease.

INTRODUCTION

Paragraph 1 Physical activity is considered to be an important part of the management and overall well-being for patients with type 1 diabetes mellitus (T1DM) as engaging in regular physical activity reduces mortality and cardiovascular disease risk (4). During physical activity, however, the increase in metabolic rate above resting levels increases the rate at which heat must be dissipated to the environment to prevent a dangerous rise in core temperature. When physical activity is performed in environmental conditions where the skin temperature is lower than the ambient air temperature, the body begins to gain heat via dry heat exchange. This further increases the requirements for sweating and circulatory responses to achieve a rate of heat dissipation that will maintain heat balance. However, studies suggest that otherwise healthy individuals with T1DM may have a reduced capacity to dissipate heat as evidenced by reductions in skin blood flow (12, 16, 17, 30, 32) and attenuated sweat production (6, 15, 18, 24) in response to local heating and/or pharmacological stimulus. Less well understood is the consequence of T1DM on thermoregulatory function during exercise in the heat.

Paragraph 2 The limited studies examining skin blood flow in T1DM show that microvascular reactivity of the skin in individuals with T1DM is impaired leading to lower maximal levels of skin blood flow (32). This is thought to be the result of both an endothelium-dependent and endothelium-independent vasodilation mediated impairment of skin blood flow. These impairments have been observed in both the upper and lower body of type 1 diabetic individuals. Studies examining the sudomotor response in individuals with T1DM have been limited to the evaluation of the sweating response to assess the level of

autonomic neuropathy. These studies show that sweating abnormalities in type 1 diabetes include regional hypohydrosis and early hyperhydrosis (15), with complete anhydrosis in extreme situations (6, 18), which can lead to global anhydrosis (13). The level of impairment in both vasomotor and sudomotor function is exacerbated in individuals with longer duration diabetes (16, 21), poor glucose control (13, 16, 32) and greater degree of neuropathy (35).

Paragraph 3 To date there remains a lack of information regarding the consequences of the type 1 diabetes-related impairments on the body's physiological capacity to dissipate heat during exercise. A recent study by Stapleton *et al*. (33) reported no differences in whole-body heat dissipation between relatively healthy T1DM and non-diabetic controls during mild intensity exercise (~42% of maximal oxygen consumption) performed at a fixed rate of heat production (~200 $W \cdot m^{-2}$). However, whole-body heat loss responses were only measured during a single heat load. The level of whole-body heat loss required to achieve heat balance during exercise, particularly in the heat, is determined by the sum of metabolic and environmental heat load (dry heat gain from the environment). Thus, the greater the heat load, the greater the rate of whole-body heat loss needed for heat balance (and therefore a stable core temperature) (20). As such, it remains unclear if Type 1 diabetes-related differences in heat dissipation may only be evidenced above a certain requirement for heat loss and therefore heat load.

Paragraph 4 The following study was conducted to evaluate if Type 1 diabetes-related differences in local heat loss responses of skin blood flow and sweating are only evidenced above a certain requirement for heat loss during exercise in the heat. In view of

the recent findings by Stapleton *et al*. (33), that no differences in heat dissipation between T1DM and healthy controls were observed at a heat load of 200 $W \cdot m^{-2}$, we hypothesized that individuals with T1DM would exhibit lower skin blood flow and sweat rates compared to their healthy counterparts at levels of metabolic heat production exceeding 200 $W \cdot m^{-2}$. Further, we evaluated the hypothesis that Type 1 diabetes-related differences were more pronounced with increasing requirements for heat loss, and therefore heat loads.

METHODS

Ethical Approval

Paragraph 5 The experimental protocol was approved by the University of Ottawa Health Sciences and Science Research Ethics Board, in accordance with the Declaration of Helsinki. Written informed consent was obtained from all volunteers prior to their participation in the study.

Participants

Paragraph 6 Eight participants with T1DM (5 males, 3 females) were matched for sex, height, body mass, body surface area, body composition, physical fitness and training status with eight healthy control participants. Participant characteristics are presented in Table 1. Hemoglobin A_{1c} (HbA_{1c}) for the group with diabetes was 8.5±0.4% and ranged from 6.6 to 9.8%. To avoid differences in hormonal status, all female participants were tested between the first and tenth day of self-reported menses. On the day of the experiment, a venous blood sample was obtained from female participants to confirm that the session occurred during the follicular phase of the menstrual cycle. Hormonal status was confirmed by taking a venous blood sample on the day of each experimental session. Blood samples

were collected with a SST Vacutainer (BD Vacutainer, Franklin Lakes, NJ) for the determination of plasma 17β-estradiol and progesterone. Plasma concentrations were quantified by an independent external laboratory (Gamma-Dynacare Medical Laboratories, Ottawa, ON, Canada). None of the experimental sessions for female participants had to be withdrawn or repeated based on blood sample results. Two T1DM participants (1 male, 1 female) were taking Levothyroxine (0.125 $mg \cdot day^{-1}$) and Eltroxine (0.1 $mg \cdot day^{-1}$) for under-producing thyroid and self-reported normal hormone levels and the female participant in addition to synthetic thyroid hormone was also taking metformin (500 $mg \cdot day^{-1}$) for polycystic ovarian syndrome. All other T1DM and control participants were non-smoking and free of any cardiovascular, respiratory, and other metabolic diseases.

Experimental Design

Paragraph 7 All participants volunteered for one preliminary and one experimental session. During the preliminary session, training history, body height, mass, and density, as well as maximum oxygen uptake were determined. Training status was evaluated by having participants complete both the Kohl's Fitness Questionnaire and the Baecke Sport Index Questionnaire (1, 22). Body height was determined using a stadiometer (Detecto, model 2391, Webb City, MO), whereas body mass was measured using a digital high-performance weighing terminal (model CBU150X, Mettler Toledo, Mississauga, ON, Canada). Body surface area was subsequently calculated from the measurements of body height and mass (5). Body density was measured using the hydrostatic weighing technique and used to calculate body fat percentage (29). Maximum oxygen uptake was determined by indirect

calorimetry (MOXUS system, Applied Electrochemistry, Pittsburgh, PA) during a progressive incremental exercise protocol performed on an upright, seated, constant-load cycle ergometer (Corival, Lode BV, Groningen, the Netherlands). For each experimental session, participants reported to the laboratory between 7:00 and 11:00 a.m. The participants were asked to drink 500 ml of water the night before, as well as the morning of, the experimental session and to refrain from alcohol, caffeine, and exercise 24 h before experimentation. Participants were instructed to take their normal bolus of insulin adjusted for exercise and eat their usual breakfast prior to arriving at the laboratory.

Paragraph 8 On arrival at the laboratory the participants provided a urine sample, weighed themselves nude, and changed into shorts, sandals, as well as a sports bra for female participants. Blood samples were taken from an indwelling catheter at the end of baseline as well as the start of recovery to evaluate changes in plasma volume and osmolality during the exercise bout. They subsequently sat upright for a 60 min instrumentation period at an ambient room temperature of 24°C. Following instrumentation, the participants entered a thermal chamber regulated to an ambient air temperature of 35°C and a relative humidity of 20% where they remained resting in the upright seated posture for an additional 30 min. Thereafter they performed 90 min of continuous semi-recumbent cycling exercise. The exercise was performed at fixed rates of heat production equal to 200, 250, and 300 $W \cdot m^{-2}$ of body surface area with each level being 30 min in duration**.** At the end of the exercise test, participants underwent a 45 minute local heating during which time maximum skin blood flow at the mid-anterior forearm and upper back was determined by increasing the temperature of the heater housing the laser-Doppler probes to 44°C for 30 min. Participants

were then weighed nude and a urine sample was collected.

Measurements

Paragraph 9 Esophageal temperature was measured using thermocouple probes (Mallinckrodt Medical Inc., St-Louis, MO, USA) inserted 40 cm past the nostril while the participant sipped water (100-300 ml) through a straw. Rectal temperature was measured using a thermocouple temperature sensor (Mallinckrodt Medical Inc., St-Louis, MO, USA) inserted 12 cm past the anal sphincter. Skin temperature was measured at 4 sites using thermocouples (Concept Engineering, Old Saybrook, CT, USA) attached to the skin with surgical tape. Mean skin temperature was subsequently calculated using a 4 point weighting of the regional proportions determined by Ramanathan (25): chest (30%), arm (30%), calf (20%) and quadriceps (20%). All temperature data was collected using an HP Agilent data acquisition module (model 3497A) every 15 seconds. Data was simultaneously displayed graphically and recorded in spreadsheet format on a personal computer (IBM Think- Centre M50) with LabVIEW software (Version 7.0, National Instruments, TX, USA).

Paragraph 10 The ventilated capsule technique was used to measure local sweating. Sweat production on the left upper back, chest and forearm was measured from 3.8 cm^2 plastic capsules attached to the skin with adhesive rings and topical skin glue (Collodion HV, Mavidon Medical products, Lake Worth, FL, USA). Anhydrous compressed air was passed through each capsule at a rate of 1 $L \cdot min^{-1}$. Water content of the effluent air was measured using high precision dew point mirrors (model 473, RH systems, Albuquerque, NM, USA). Local sweat rate was calculated using the difference in water content between effluent and

influent air multiplied by the flow rate and normalized for the skin surface area under the capsule.

Paragraph 11 The number of active sweat glands was measured on the upper back, chest and forearm adjacent to each affixed sweat capsule at 30, 60 and 90 min of exercise using the modified-iodine paper technique with computer assisted analysis (8). The number of glands determined by computer analysis was subsequently divided by the surface area of the paper to give a value of active sweat glands per square centimeter. The sweat output per gland was calculated by dividing the sweat rate at the corresponding measurement period by the number of active sweat glands.

Paragraph 12 Local skin blood flow at the mid-anterior forearm and upper back adjacent to the sweat capsules was estimated using laser-Doppler velocimetry (PeriFlux System 5000, Perimed AB, Stockholm, Sweden). Prior to the start of the experimental trial, laser-Doppler flow probes (integrating probe 413, Perimed AB, Stockholm, Sweden) were affixed with an adhesive ring to the forearm in a site that demonstrated cardio-synchronous pulsatile activity. Skin blood flow response was expressed as a percentage of maximum, as determined during local heating.

Paragraph 13 Indirect calorimetry was used for the measurement of metabolic energy expenditure (23). Expired gas was analyzed for oxygen (error of ± 0.01%) and carbon dioxide (error of ± 0.02%) concentrations using electrochemical gas analyzers (AMETEK model S-3A/1 and CD 3A, Applied Electrochemistry, Pittsburgh, PA, USA). Prior to each session, gas mixtures of known concentrations were used to calibrate the gas analyzers and a 3 L syringe was used to calibrate the turbine ventilometer. Heart rate was measured

continuously by a Polar coded WearLink and transmitter, Polar RS400 interface, and Polar ProTrainer 5 software (Polar Electro Oy, Finland).

Paragraph 14 Plasma osmolality and plasma volume changes were determined using venous blood samples. An indwelling venous catheter was inserted in the antecubital vein of the left arm connected to a Luer-Lock extension (Microbore Extension, Clave™, Locking Spin Collar, Non-DEHP) and secured in place with a 6 x 7 cm film dressing (Tegaderm Film, 3M Health Care, St. Paul, MN, USA). Venous blood (approximately 10 mL) was collected without stasis into K2 EDTA™ and Serum™ vacutainers (BD Vacutainer, Franklin lakes, NJ, USA) for hematology and plasma osmolality analysis. Blood samples in the K2 EDTA™ vacutainer were immediately analyzed for hemoglobin (Hb) concentration and hematocrit (Hct) ratio. Both plasma and serum aliquots were immediately analyzed upon separation to determine osmolality using the freezing-point method (Osmometer, Advance Instruments). In addition, the change in body weight and urine specific gravity was assessed prior to and following exercise. Body mass was measured using a digital high-performance weighing terminal (model CBU150X, Mettler Toledo, Mississauga, ON, Canada). Urine specific gravity was determined in duplicate pre and post exercise using a handheld refractometer (TS400, Reichter, Depew, NY).

Glucose/Insulin Adjustments for Exercise

Paragraph 15 Participants with T1DM adjusted their insulin according to their regular pre-exercise routine. Blood glucose levels $\geq 5 mmol \cdot L^{-1}$ were required prior to the start of exercise. T1DM participants measured capillary glucose using personal hand-held

glucose meters 15 minutes into baseline, and at 5, 10, 15, 30, 45, 60, 75 minutes of exercise. Blood glucose was also measured from blood drawn from an indwelling catheter for both the T1DM and Controls at the start of exercise and during the final minute of the 90-min incremental exercise test. Gatorade was available throughout the session if necessary to increase blood glucose in the event of low blood sugars in T1DM participants. In order to ensure that potential differences in fluid balance at the end of exercise were caused by differences in sweating and not differences in fluid intake, participants with T1DM completed the experimental session before the matched control to ensure similar volume of Gatorade was provided to the matched Control participant and at the same time point during the session.

Data Analysis

Paragraph 16 All measurements were calculated into minute averages and the final minute of each workload was used to carry out statistical analyses on all variables. Due to volitional fatigue, one T1DM was unable to complete the full 30 min of the third and final exercise intensity (Exercise load 3; 300 $W \cdot m^{-2}$). As such, end exercise core temperature was assessed for 7 matched pairs only. Due to technical problems, local sweat rate of the upper back for one of the T1DM participant was not collected and therefore the data for the matched pair was not included in the analysis Mean body temperature was calculated as 0.9 · esophageal temperature + 0.1 · mean skin temperature (27). Group differences in the onset threshold and thermosensitivity of local sweat rate during each exercise period were determined using the linear portion of the response plotted against mean body temperature

and analyzed using segmental linear regression (3). As skin blood flow did not increase after the first exercise intensity, the onset threshold and thermosensitivity of the response was determined only for the first exercise bout (9). Due to technical problems with the measurement of esophageal temperature in one of the diabetic participants, it was not possible to calculate thermosensitivities for one matched pair.

Statistical Analysis

Paragraph 17 All dependent variables were compared between groups (T1DM vs. Control). Paired samples T-tests were used for comparisons between matched groups for physical characteristics, training status and maximal aerobic capacity. Minute averages were calculated for all continuous variables (esophageal, rectal and mean skin temperatures, local skin blood flow and local sweat rate). A two-way mixed model analysis of variance (ANOVA) was done using the repeated factor of time (4 levels: 0, 30, 60 and 90 minutes of exercise) and the non-repeated factor of group (2 levels: T1DM and Control). When a significant main effect was observed, post-hoc comparisons were carried out using paired samples T-tests. The level of significance for all analyses was set at an alpha level of $p \leq 0.05$. Statistical analyses were carried out using a commercially available statistical software package (SPSS 21.0 for Windows, SPSS, and Chicago, IL, USA) and segmented linear regression analysis was performed using GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA, USA) (3). All variables are reported as means ± standard error (SE) unless otherwise indicated.

RESULTS

Experimental Session

Paragraph 18 No differences were observed between T1DM and Control for local sweat rate, skin blood flow as well as core and mean skin temperatures during baseline resting. By design, the rate of metabolic heat production was the same for both participant groups across all three exercise intensities (p = 0.26). Workloads represented a similar percentage of VO_{2max} for T1DM and Control participants (200 $W \cdot m^{-2}$: 44.5 ±2.59 vs. 41.8 ± 1.89%; 250 $W \cdot m^{-2}$: 54.7 ± 3.1 vs. 51.7 ± 2.1%; 300 $W \cdot m^{-2}$: 66.2 ± 3.5 vs. 62.8 ± 2.7%, respectively, p = 0.85). Carbohydrate supplementation was required during exercise for 6 T1DM participants (21.9 ± 0.01 g of carbohydrate).

Local Sweat Rate

Paragraph 19 Despite exercise being performed at fixed requirements for heat loss, local sweat rate differed over time between T1DM and Control at the chest (p = 0.01) and forearm (p = 0.050); however, no differences were measured on the upper back (p = 0.65, Fig 1A). Chest sweat rate (Fig 1B) was similar between groups at the end of the first exercise period (p = 0.150) but, relative to control, the individuals with T1DM had a reduced sweat rate at the end of the second (p = 0.046) and third (p = 0.032) exercise periods. Similarly, forearm sweat rate did not differ between groups at the end of the first (*P* = 0.17) exercise period but was reduced relative to Control in T1DM during the second (p = 0.004) and third (p = 0.005) exercise periods. There were no differences measured between T1DM and Control in the onset threshold for local sweat rate at the chest, forearm and upper back

($P > 0.1$). During the first and second exercise period no differences in thermosensitivity were observed between T1DM and Control ($p > 0.1$, Table 2), while a greater thermosensitivity was measured at the chest site ($p = 0.030$ and 0.017 respectively) for those exercise periods. In contrast to the first and second heat loads, relative to Control, T1DM had a reduction in thermosensitivity during the final exercise period at all three sites (chest, $p = 0.005$; forearm, $p = 0.046$ and back $p = 0.044$, Table 2).

Paragraph 20 Changes in the number of active sweat glands on the chest significantly differed between T1DM and Control ($p = 0.036$, Table 3). Although, the number of active sweat glands did not differ at the end of the first ($p = 0.086$) and second ($p = 0.130$) exercise periods, fewer active sweat glands were measured in T1DM at the end of the third exercise period ($p = 0.049$). As such, the attenuated chest sweat rate in T1DM was due to a reduction in sweat gland activation as no differences between T1DM and Control were found in sweat gland output at the chest ($p = 0.351$, Table 3). In contrast, the number of active sweat glands on the forearm did not differ between groups ($p = 0.176$), however, sweat gland output significantly differed between T1DM and Control ($p = 0.002$). Sweat gland output was not different at the end of the first exercise period ($p = 0.754$, Table 3), however, sweat gland output was reduced in T1DM relative to Control during the second ($p = 0.004$) and third ($p = 0.012$, Table 3) exercise periods. Therefore, the attenuated sweat rate in T1DM at the forearm was primarily driven by a reduction in sweat gland output. No differences in local sweat rate between T1DM and Control were measured at the upper back site and this was paralleled by similar changes in the number of active sweat glands ($p = 0.809$, Table 3) and output per gland ($p = 0.994$, Table 3). Consistent with the attenuated

local sweat rates measured in the T1DM group, a lower change in body mass was measured in T1DM (-1.86 ± 0.09 %) relative to Control (–2.10 ± 0.14 %) ($p = 0.049$).

Skin Blood Flow

Paragraph 21 Skin blood flow at both skin sites increased significantly from baseline rest to end of the first exercise bout ($p < 0.01$). However, no further increase in skin blood flow was measured with the subsequent exercise bouts ($p > 0.1$). Skin blood flow response did not differ between groups (upper back, $p = 0.126$; forearm, $p = 0.660$). Similarly, no differences between groups were observed in the onset threshold for skin blood flow (upper back, $p = 0.828$; forearm, $p = 0.302$). However, a reduction in thermosensitivity of skin blood flow response was measured in the T1DM group at both the upper back ($p = 0.005$) and forearm ($p = 0.001$) skin sites during the first exercise period (Table 2).

Core and Mean Skin Temperatures

Paragraph 22 The change in esophageal temperature during the three exercise periods did not significantly differ between groups ($p = 0.344$, Fig 3A). However, T1DM had a significantly greater increase in rectal temperature over the 90 minute exercise period ($p = 0.005$). Baseline rectal temperatures were similar between T1DM and Control (37.00 ± 0.05 vs. 36.95 ± 0.09°C, $p = 0.45$). No differences in rectal temperature were found between groups at the end of the first ($p = 0.197$) and second ($p = 0.073$) exercise periods. However, the increase in rectal temperature was significantly greater in T1DM (1.47 ± 0.12 °C) compared to Controls (0.99 ± 0.1 °C, $p = 0.04$) during the last exercise period (Fig 3C). No

differences between groups in mean skin temperature response were observed ($p = 0.951$) (Fig 3B). During baseline and throughout the exercise period no differences in heart rate were found between T1DM and Control ($p = 0.532$).

Hydration status and blood glucose

Paragraph 23 No differences in urine specific gravity were measured between groups prior to the start (T1DM: 1.02 ± 0.004 vs. Control: 1.01 ± 0.003, $p > 0.05$) or at the end of the experimental session (T1DM: 1.02 ± 0.003 vs. Control: 1.02 ± 0.003, $p > 0.05$). Both blood and plasma volume decreased from baseline to the end of exercise with no significant differences found between groups ($p > 0.05$). Plasma osmolality was not significantly different at baseline between T1DM and Controls and there were no differences at the end of the 90-min exercise bout ($p > 0.05$). During the exercise period the change in blood glucose concentrations differed between groups ($p = 0.027$). At baseline, blood glucose concentration differed between groups (T1DM: 9.98 ± 1.03 vs. Control: 5.26 ± 0.17 $mmol \cdot L^{-1}$ $p = 0.004$), but by the end of exercise both groups had similar blood glucose concentrations (T1DM: 4.57 ± 0.99 vs. Control: 5.17 ± 0.27 $mmol \cdot L^{-1}$ $p = 0.223$).

DISCUSSION

Paragraph 24 We show for the first time that individuals with T1DM have a reduced capacity to dissipate heat as evidenced by the marked reduction in local sweating response. However, these differences were only evidenced beyond a certain requirement for heat loss. The lower sweat rate measured at the chest and forearm was paralleled by a reduced

thermosensitivity measured when the exercise heat load was equal to or greater than 250 $W \cdot m^{-2}$. However, the response was heterogeneous such that no differences were observed in the upper back with the exception that the thermosensitivity of the sweating response was reduced in T1DM at the highest requirement for heat loss (i.e., 300 $W \cdot m^{-2}$). We show that the underlying mechanisms associated with the lower sweat rate measured in T1DM also differed between sites such that the decrease in sweat rate at the forearm site was attributed to a lower sweat output per gland while the reduction in the number of activated sweat glands accounted for the lower sweat rate measured at the chest. Although reductions in thermosensitivity for skin blood flow were measured in the T1DM group at both the forearm and upper back, this was limited to the first exercise heat load. The attenuation in heat dissipation was paralleled by a greater increase in core temperature, and therefore level of thermal strain, measured at the end of the 90 minute exercise bout.

Paragraph 25 Studies show that the maximal skin blood flow responses to a passive stimulus (local heating or pharmacological) in individuals with T1DM is attenuated. Studies using pharmacological stimulation through the application of local vasoactive substances (i.e., sodium nitroprusside, acetylcholine, nitro-glycerine, or C-peptide infusion) and/or local heating report an attenuated skin vasodilatory response in individuals with T1DM (7, 21, 31). However, little is known about the consequences of these diabetes-related impairments in skin vascular control on skin blood flow response during exercise. To the best of our knowledge, only one study (33) has examined the effects of exercise on skin blood flow in individuals with T1DM and found no differences between T1DM and healthy controls. Consistent with Stapleton *et al*. (33) during exercise, we show that skin blood flow reached

similar levels in both the T1DM and Control groups at a moderate exercise-induced heat load (i.e., 200 $W \cdot m^{-2}$). However, in contrast to Stapleton et al. (33), thermosensitivity of the skin blood flow response was reduced in our T1DM relative to Control group at both measurement sites. Stapleton *et al*. (33) observed no differences in the thermosensitivity of skin blood flow response measured at the forearm site. The disparity in findings between the two studies may be the result of differences in glucoregulation and/or fitness. In the present study, our participants had a higher HbA_{1c} as compared to those T1DM participants in the study by Stapleton et al. (7.7 ±0.3 vs. 8.5 ± .04 % respectively). A higher HbA_{1C} in T1DM populations has been shown to lead to an increase in endothelial damage, the formation of advanced glycosylated end products and ultimately a reduced sensitivity to, or availability of, the vasodilator nitric oxide which is thought to impair skin blood flow (2, 32). Attenuation in skin blood flow in T1DM populations has been likened to an enhanced aging effect (32) and in addition to having a lower HbA_{1C}, the population studied by Stapleton *et al*. (33) also had a greater $VO_{2max.}$ This may in part explain the lack of a difference in skin blood flow response in the study by Stapleton *et al*. (33)in that her participants had a higher level of fitness relative the participants in the present study. A higher level of fitness has been linked to the preservation of the age-related attenuation of the skin blood flow response (34).

Paragraph 27 To date there remains a paucity information about the consequences of diabetes on the sweating response during heat stress. This is a critical knowledge gap as evaporative heat loss through sweating is the main avenue of heat exchange during exercise, especially in the heat. Thermoregulatory sweat tests which involve a rapidly induced state of hyperthermia (i.e., core temperature increase of 1ºC) using whole-body passive heating,

showed that individuals with T1DM exhibit regions of anhydrosis and in severe cases of diabetic neuropathy and longstanding diabetes global anhydrosis (13). A recent study by Stapleton *et al*., (33) reported that young adults with well controlled T1DM and no overt neuropathies do not demonstrate reductions in local sweating during exercise in the heat. However, Stapleton *et al*., (33) only measured local sweat at one site and by examining multiple sites we show regional variations in the extent to which diabetes may impair sweating. Both passive heating studies and studies using pharmaceutical stimulation have shown regional differences in the effects of T1DM on sweating (6, 18). Moreover, as the level of whole-body heat loss achieved during exercise in the heat depends upon the required evaporation for heat balance which is defined as the sum of metabolic heat production and dry heat exchange. When the combined environmental and metabolic heat load exceeds the individual's ability to achieve heat balance, the level of sudomotor activity achieved will be driven by the individual's maximum sweating capacity. As such, it is possible that the thermal challenge (i.e., environmental plus metabolic heat load) presented in the study by Stapleton *et al*. (33) was insufficient to exceed the T1DM group's capacity to dissipate heat. Our results support this point as at rates of metabolic heat production similar to those in Stapleton *et al*. (33) no differences in sweating were observed. However, at increasing levels of metabolic heat production and therefore an increasing requirement for heat loss, we show that individuals with T1DM exhibited marked attenuations in sweat rate. As noted above, regional variations in sweat rate were observed. Finally, in parallel to the lower sweat rates in our T1DM participants we observed a significantly lower percent change in body weight which suggests that whole body sweat rate and therefore evaporative heat loss was markedly

reduced in individuals with T1DM. As a consequence of the Type 1 diabetes related impairments in thermoregulatory function, we observed a greater increase in core temperature in our T1DM participants. Taken together these finding demonstrate that individuals with Type 1 diabetes may be at an elevated risk of experiencing heat-related injuries during exercise in the heat compared to healthy non-diabetic individuals.

Paragraph 28 Although these findings provide evidence that T1DM itself can modulate sweating, the precise mechanism(s) remain to be determined. Evaluating differences in the onsets and sensitivities of sweat rate between T1DM and healthy controls can provide additional insight into how T1DM affects sweat rate during exercise. Since the interpretation of thermoefferent activity, namely skin sympathetic nerve activity, is problematic between individuals or groups, the onset threshold and the thermosensitivity of thermoeffector responses currently represent the only viable means by which we can assess the effects of nonthermal factors such as T1DM on thermoregulatory control. Although both variables can represent a central and/or peripheral modulation of temperature regulation (14), it has been suggested that a parallel shift in the onset threshold of all effector responses must occur to be representative of a central modulation (11). As such, changes in the thermosensitivity of an effector response, without parallel changes in the onset threshold, likely imply a peripheral modulation. We observed a lower thermosensitivity, but no difference in the onset of the sweating response in T1DM compared to controls. Specifically, differences in thermosensitivity of local sweating only became evident at higher heat loads whereas no differences were observed at lower exercise intensities. These results suggest that T1DM impairments in sweating observed in the present study are caused by

peripheral changes. The mechanism underlying this peripheral change remains to be elucidated.

Paragraph 28 As described above, we observed a decrease in local sweat rate in our T1DM participants. This was due to a fewer number of activated sweat glands at the chest while at the forearm the lower local sweat rate was caused by a lower sweat output per gland. Previous studies have shown differences in the number of active glands between individuals with diabetes and healthy controls that became greater as the degree of autonomic neuropathy increases (19). Our participants did not have clinically diagnosed peripheral or autonomic neuropathies suggesting that the differences observed in the present study were related to other diabetes-related factors (i.e., glucoregulation, etc.). Irrespective of the underlying cause, differences in the number of active glands or output per gland may be the result of changes in thermoefferent neural activity, or the result of changes in the physical properties of the sweat glands themselves (26). This is in part supported by a study demonstrating that individuals with T1DM have a lower density of sweat gland nerve fibers, and potentially lower sudomotor activity (10).

Paragraph 30 Recently published guidelines (28) have recommended that individuals with T1DM who opt to perform exercise in the heat should do so with caution. This recommendation was partly based on epidemiological data which shows that individuals with T1DM face a greater risk of hospitalization and/or death during prolonged heat events as well as studies which indicated skin blood flow and sweating are impaired. The current study is the first study to show decreased local sweat rates and a greater increase in core temperature in individuals with T1DM during exercise. The population used in this study

was comprised of young, moderately active, non-neuropathic individuals with good blood glucose control and impairments during exercise were found in this group. Impairments in skin blood flow and sweating in previous studies have been linked to the severity of neuropathy and other diabetes complications (i.e. retinopathy and nephropathy) and therefore it is plausible that impairments in local heat loss responses during exercise may be even greater in T1DM populations with poorer blood glucose control and more co-morbidities. In addition to thermoregulatory responses we measured blood glucose responses during exercise and found that 75% of T1DM participants required exogenous glucose and of the 6 participants requiring carbohydrate supplementation 1 participant was unable to finish the final 30 minutes of exercise. Though exercise of increasing intensity was used as the stimulus in this study, a lighter exercise load with hotter environmental conditions could have produced a similar heat load. This study indicates that T1DM individuals may be disadvantaged when it comes to dissipating heat when performing moderate to high intensity exercise in a warm environment with low humidity. In this context further studies must be conducted to evaluate if diabetes related impairments are greater in individuals with longer duration or poorer control.

Summary

Paragraph 31 In summary, non-neuropathic individuals with T1DM have reductions in local sweat rate as well as reduced sensitivity of both skin blood flow and sweating when compared to well-matched healthy control participants. These impairments become apparent at higher requirements for heat loss. However, there are regional variations in these responses. In our study as participants were matched for physical characteristics and training

history, relative as well as absolute workloads, any differences observed must be caused by differences in thermoeffector responses. During prolonged exercise of increasing intensity non-endurance trained patients with T1DM have a greater increase in core temperature than their healthy counterparts. Exercise, along with nutrition and blood glucose control are considered to be the cornerstones of self-care for individuals with T1DM and the effect(s) of chronic diseases, such as T1DM, on thermoregulation both during and following exercise have not been explored to a far enough degree to ensure the safety of T1DM populations. A compromised thermoregulatory response during and following physical exertion is of considerable concern within a working environment due to the associated increased risk of post-exertion heat-related injury.

Competing Interests

Paragraph 32 The authors declare that they have no competing interests.

Funding

Paragraph 33 This study was conducted in the Human and Environmental Physiology Research Unit and funded by the Natural Sciences and Engineering Research Council (grant# RGPIN-298159-2009), the Canadian Institutes of Health Research (grant # 286363) and Leaders Opportunity Fund from the Canada Foundation for Innovation (funds held by Dr. Kenny). Dr. Glen P. Kenny was supported by a University of Ottawa Research Chair Award. Mike Carter was supported by the Human and Environmental Physiology Research Unit.

Acknowledgments

Paragraph 34 The authors thank all the members of the Human and Environmental Physiology Research Unit who assisted with data collection. We would also like to thank all the participants who volunteered for the present study.

FIGURE CAPTIONS

Figure 1. Mean (±SE) Local Sweat Rate (LSR) for back (panel A, n = 7), chest (panel B, n =7) and forearm (panel C n = 8) sites during increasing levels of metabolic heat production. T1DM ○, Control (CON) ●. Dashed lines in panels A and C represent n = 7. Dashed line in panel B represents n = 6. Minute 90 represents the end exercise value for all participants. An asterisk (*) denotes significance between groups ($p \leq 0.05$)

Figure 2. Mean (±SE) skin blood flow (SkBF) during increasing levels of metabolic heat production. T1DM ○, Control (CON) ●. Dashed lines in panels A and B represent n = 7. An asterisk (*) denotes significance between groups ($p \leq 0.05$).

Figure 3. Mean (±SE) changes in esophageal (panel A), skin temperature (panel B), mean body temperature (panel C) and mean rectal temperature (panel D) during increasing levels of metabolic heat production. T1DM ○, Control (CON) ●. Dashed lines in panels A, B, and C represent n = 6. Minute 90 represents the end exercise value for all participants. An asterisk (*) denotes significance between groups ($p \leq 0.05$)

Table 1. Mean ± (SE) Participant Characteristics for T1DM and Control Participants

	Group	
	Diabetic	**Control**
Age (years)	22.3 (4.7)	23.5 (2.9)
Height (cm)	178.1 (8.2)	177.3 (10.1)
Body Mass (kg)	81.2 (15.8)	79.1 (15.6)
A_D **(m^2)**	1.99 (0.23)	1.97 (0.23)
Body Fat (%)	21.9 (0.93)	21.7 (1.8)
VO_{2max} **($L \cdot min^{-1}$)**	3.3 (0.7)	3.4 (0.7)
VO_{2max} **($ml \cdot kg^{-1} \cdot min^{-1}$)**	42.7 (6.9)	45.3 (4.7)
Baekes Sport Score	4.8 (2.9)	4.8 (2.4)
Physical Activity ($methrs \cdot week^{-1}$)	55.4 (38.8)	60.45 (22.7)
Hba_{1c} **(%)**	8.5 (0.39)	N/A
Disease Duration (years)	8.4 (2.9)	N/A
Body Weight Change %	-1.86 (0.09)*	-2.10 (0.14)

Values are means±SE. n/a, not applicable. $A_D(m^2)$, body surface area.*Significantly different from controls ($p \leq 0.05$).

Table 2. Mean ± SE for thermosensitivity of thermoeffector responses sweating and skin blood

	200 W·m^{-2}		**250 W·m^{-2}**		**300 W·m^{-2}**	
	Diabetic	Control	Diabetic	Control	Diabetic	Control
			Onset Skin Blood Flow (Δ˚C)			
Upper Back	0.09 ± 0.03	0.03±0.03	N/A	N/A	N/A	N/A
Forearm	0.16 ± 0.08	0.00±0.01	N/A	N/A	N/A	N/A
			Onset Local Sweat Rate (Δ˚C)			
Back	0.01±0.01	0.02±0.02	0.03 ± 0.03	0.01±0.01	0.04±0.05	0.01±0.03
Chest	0.02± 0.01	0.03±0.03	0.00 ± 0.01	0.01±0.01	0.01±0.02	0.06±0.05
Forearm	0.01±0.02	0.05±0.02	0.01 ± 0.02	0.02±0.01	0.00±0.02	0.03±0.02
			Thermosensitivity Skin Blood Flow (%max·˚C^{-1})			
Upper Back	25.6 ± 5.6*	65.5±8.8	N/A	N/A	N/A	N/A
Forearm	23.8 ± 5.9*	68.9±9.8	N/A	N/A	N/A	N/A
			Thermosensitivity Local Sweat Rate (mg·min^{-1}·cm^{-2}·˚C^{-1})			
Upper Back	0.92 ± 0.17	1.48±0.28	0.91± 0.23	1.09±0.63	0.24±0.09*	0.70±0.16
Chest	0.76 ± 0.09*	1.58±0.31	0.53 ± 0.1*	0.99±0.16	0.18±0.06*	0.89±0.11
Forearm	1.40 ± 0.51	1.55±0.18	0.93 ± 0.21	1.18±0.19	0.21±0.09*	0.58±0.16

Values are means ± SE. n/a, not applicable as the thermosensitivity of skin blood flow was only measured in the first exercise only. *Significantly different from controls ($p \leq 0.05$).

Table 3. Mean ± SE sweat gland activation and output

	200 $W \cdot m^{-2}$		250 $W \cdot m^{-2}$		300 $W \cdot m^{-2}$	
	Diabetic	Control	Diabetic	Control	Diabetic	Control
	Number of Active Glands per cm^2					
Back	44.2 ± 7.0	53.0 ± 10.2	58.4 ± 7.7	62.7 ± 6.1	59.6 ± 5.0	70.3 ± 4.3
Chest	34.1 ±7.4	45.7 ± 7.0	44.4 ± 5.7	60.19 ± 10.0	52.06 ± 4.0*	71.7 ± 7.2
Forearm	40.8 ± 5.7	49.5 ± 6.4	79.3 ± 9.2	72.9 ± 9.7	89.6 ± 11.2	70.4 ± 12.3
	SGO, µg/gland					
Back	13.0 ± 3.1	13.5 ± 4.3	12.9 ± 2.0	13.9 ±2.2	14.1 ± 2.1	13.2 ±1.9
Chest	17.33 ± 4.1	15.14 ± 2.8	15.8 ± 3	19.1 ± 3.6	14.8 ± 2.2	15.4 ± 3.5
Forearm	13.6 ± 3.2	13.1 ± 3.6	9.6 ± 3.2*	13.3 ± 2.7	8.9 ± 2.3*	15.7 ±3.3

Values are means ± SE. *Significantly different from controls ($p \leq 0.05$).

Fig 1.

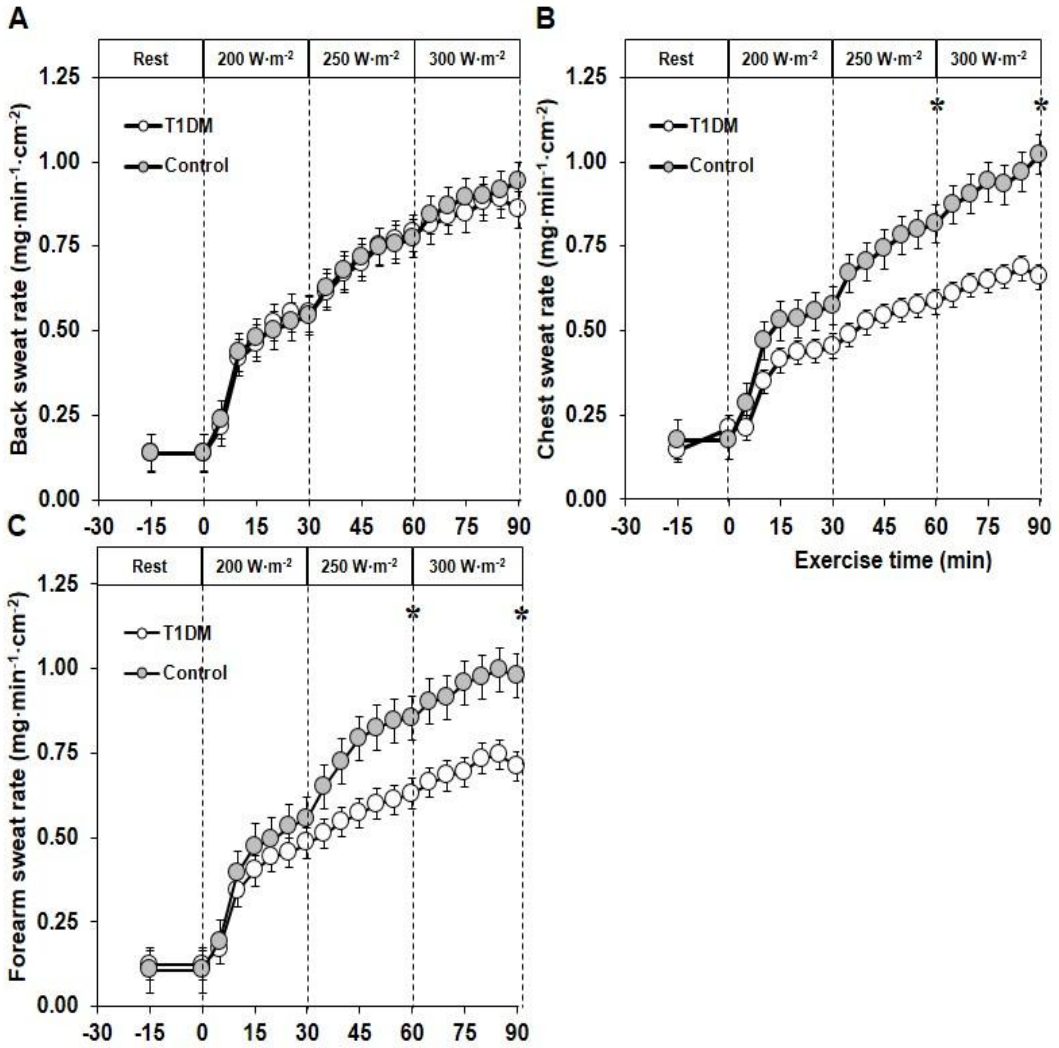

A
B
C
Rest
200 W·m-2
250 W·m-2
300 W·m-2
T1DM
Control
Back sweat rate (mg·min-1·cm-2)
Chest sweat rate (mg·min-1·cm-2)
Forearm sweat rate (mg·min-1·cm-2)
Exercise time (min)
1.25
1.00
0.75
0.50
0.25
0.00
-30
-15
0
15
30
45
60
75
90
*

Fig 2.

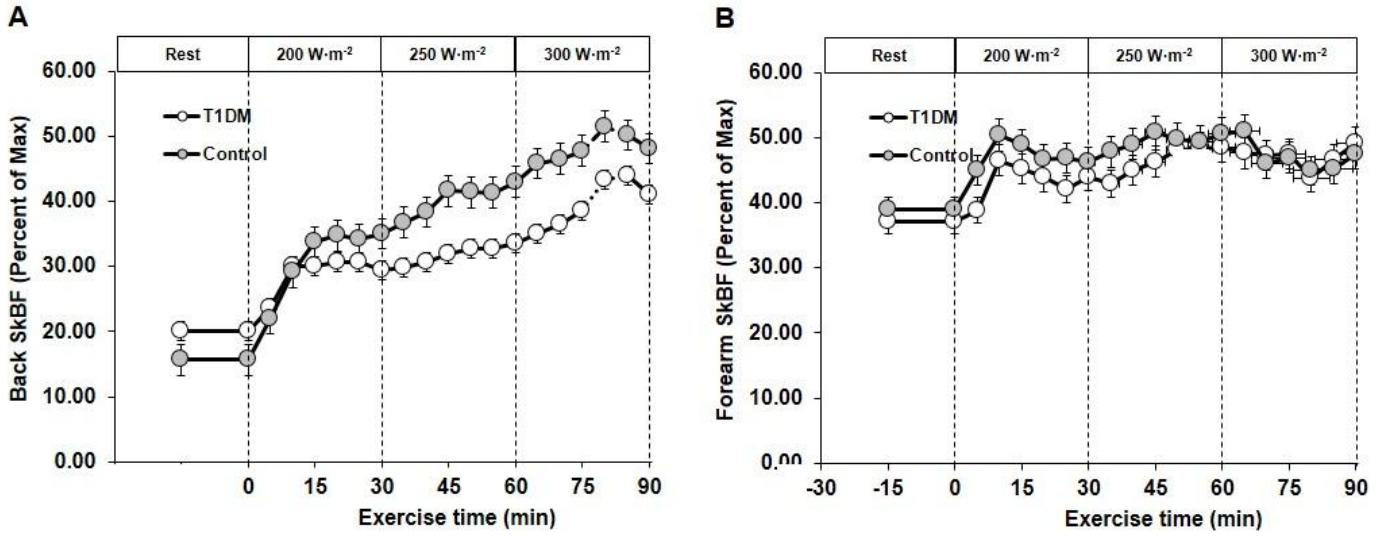
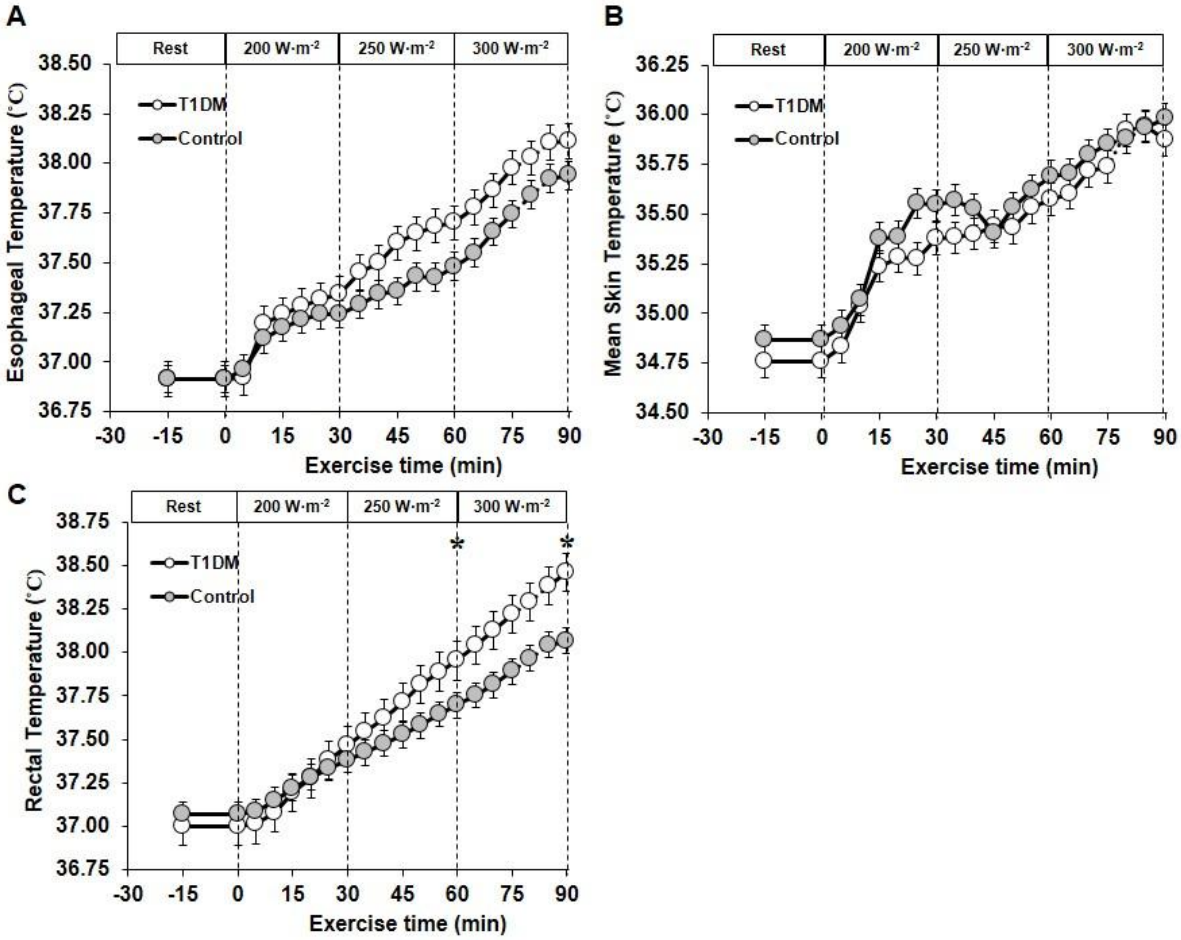

A
Rest
200 W·m-2
250 W·m-2
300 W·m-2
T1DM
Control
Back SkBF (Percent of Max)
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0
15
30
45
60
75
90
Exercise time (min)
B
Rest
200 W·m-2
250 W·m-2
300 W·m-2
T1DM
Control
Forearm SkBF (Percent of Max)
60.00
50.00
40.00
30.00
20.00
10.00
0.00
-30
-15
0
15
30
45
60
75
90
Exercise time (min)

Fig 3.


A
Rest
200 W·m-2
250 W·m-2
300 W·m-2
T1DM
Control
Esophageal Temperature (°C)
38.50
38.25
38.00
37.75
37.50
37.25
37.00
36.75
-30 -15 0 15 30 45 60 75 90
Exercise time (min)
B
Rest
200 W·m-2
250 W·m-2
300 W·m-2
T1DM
Control
Mean Skin Temperature (°C)
36.25
36.00
35.75
35.50
35.25
35.00
34.75
34.50
-30 -15 0 15 30 45 60 75 90
Exercise time (min)
C
Rest
200 W·m-2
250 W·m-2
300 W·m-2
T1DM
Control
*
*
Rectal Temperature (°C)
38.75
38.50
38.25
38.00
37.75
37.50
37.25
37.00
36.75
-30 -15 0 15 30 45 60 75 90
Exercise time (min)

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General Conclusions of the Thesis

The primary focus of this thesis was to determine if type 1 diabetes leads to impairments in local heat loss responses as a function of an increasing rate of metabolic heat production. In order to evaluate potential differences in local vasomotor and sudomotor activity we measured the local heat loss responses of skin blood flow and sweating during a continuous exercise bout separated into three consecutive periods of increasing levels of metabolic heat production.

The most important finding from this study is that compared to healthy controls, individuals with T1DM exhibit regions of impaired local sweating and higher core temperatures at the end of exercise. Additionally, thermosensitivity of both skin blood flow and the local sweat response was reduced in the group of T1DM participants. The mechanism underlying decreases in local sweat rate in T1DM patients differed by region as at the arm there was a reduction in the amount of sweat per gland while at the chest there was a reduction in the number of active sweat glands.

In summary, non-neuropathic individuals with T1DM have reductions in local sweat rate as well as reduced sensitivity of both skin blood flow and sweating when compared to well-matched healthy control participants. These impairments only become apparent at higher requirements for heat loss. During prolonged exercise of increasing intensity non-endurance trained patients with T1DM have a greater increase in core temperature than their healthy counterparts. In this study as participants were matched for physical characteristics and training history, relative as well as absolute workloads, any differences observed must be caused by differences in thermoeffector responses. This study indicates that T1DM individuals may be

disadvantaged when it comes to dissipating heat when performing moderate to high intensity exercise in a warm environment placing them at a greater risk for heat related injury.

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CHAPTER V: APPENDIX

Background letter and consent form

Thermal and non-thermal influences on post-exercise thermoregulation:
Type 1 Diabetes Mellitus
Investigators:

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Mr. Michael Carter (B.Sc.), MSc student

Ms. Jill Stapleton (M.Sc.), PhD student

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Background

Recent work has shown that non-thermal factors, associated with post-exercise blood pressure regulation may have profound influences the regulation of core temperature during exercise recovery. A compromised thermoregulatory response following physical exertion is of considerable concern within a working environment due to the associated increased risk of post-exertional heat-related injury.

Studies have shown that at rest baroreflex sensitivity is reduced in Type 1 Diabetes Mellitus (T1DM) populations and that this reduction may be influenced by the glucose control of patient as evidenced by the presence of microalbuminuria or elevated HbA1C% (Lefrandt et al. 1999, Weston et al. 1998, Dalla Pozza et al. 2007) . However, there has yet to be an examination of post-exercise hypotension in populations suffering from T1DM. The strategies employed by the body to manage its core temperature, namely sweating and active vasodilation of the blood vessels supplying the skin, are believed to be impaired in T1DM (Khan et al. 2007, Hoeldtke et al. 2001). In order to ensure the safety of T1DM populations advancing the understanding of how T1DM effects thermoregulation, cardiovascular function and the relationship between the two is an important first step.

Purpose

The following research study is therefore directed at understanding the cause of the post-exercise disturbance in core temperature regulation. Studies will be directed at investigating the separate and integrated influences of thermal and non-thermal factors on core temperature regulation during single and repeated bouts of exercise; and as a function of different biomedical factors which could alter this response (i.e., sex, age, physical fitness, heat acclimation, type 1 diabetes mellitus).

Information obtained from your participation in our study will help us increase our understanding of the health risks associated with physical work performed in the heat. By increasing our understanding of how a individuals responds to working in the heat, it will be possible to understand what steps and precautions a worker can take to avoid getting sick from working in the heat. This study is funded by the Natural Sciences and Engineering Research Council.

Subject profile

Participants must fall into one of two categories: 1) be physically active and non-obese (body fat percentage less than 30%) Type 1 Diabetic, that is to say exercise at least three times a week at a medium intensity, (12 or more on the Borg Scale) for at least 30 minutes and have an HBA1C%<7.5mmol/L or 2) be physically inactive, not meeting the above requirements for physical activity. Participants will be asked to list any medications they may be taking.

Inclusion criteria:

1. Type 1 diabetes mellitus as defined by the 2003 CDA guidelines requiring insulin therapy starting within one year of diagnosis and continuously thereafter.
2. Male or female, age >18 years
3. Non-smoking

Exclusion criteria:

1. Hypoglycemia unawareness, or severe hypoglycemia requiring assistance from another person within the previous 3 months
2. “Brittle” diabetes, characterized by frequent and unpredictable hypoglycemia (even if not requiring assistance from others) and hyperglycemia.
3. Restrictions in physical activity due to disease: intermittent claudication, severe peripheral neuropathy or active proliferative retinopathy, unstable cardiac or pulmonary disease, disabling stroke, severe arthritis.
4. Known or suspected clinically significant gastroparesis.

5. Body mass index >35 kg/m2.
6. Changes in medication judged by the patient or investigators to make participation in this study inadvisable.
7. Significant renal disease: serum creatinine >200 mEq/l. or proteinuria >1 g/24 hours.
8. Uncontrolled hypertension: BP >150 mm Hg systolic or >95 mm Hg diastolic in a sitting position.
9. Other illness, judged by the patient or investigators to make participation in this study inadvisable.
10. Cognitive deficit resulting in inability to understand or comply with instructions.
11. Unwillingness to sign informed consent
This study will be conducted in English only.

Preliminary session

Both the preliminary session and the experimental sessions will take place in at the Human Bioenergetics and Environmental Physiology Laboratory located on the main campus at the University of Ottawa. The time involvement will be approximately 45 min to 1 hour for the preliminary session. During the preliminary session, we will review all procedures with you. In addition, you will be introduced to all of the equipment and measuring devices that we will be using during the 4 different sessions. You will then be asked to complete a *Physical Activity Readiness Questionnaire (Par-Q)* and an AHA/ACSM Health/Fitness Facility Preparticipation Screening Questionnaire. These questionnaire are standard questionnaires that have been developed to help us evaluate your readiness for exercise. It is also used to assist us evaluate your general physical health and level of physical activity. At the end of the preliminary session, we will give you the opportunity read the Background and Informed consent document. If you agree to participate in the study, we will ask you to sign the informed consent below. Thereafter, we will complete some basic measurements including height and weight and resting blood pressure measurements. You will then be asked to perform a maximal incremental exercise test on a cycle ergometer (bicycle). During this exercise test, we will measure the amount of oxygen your body consumes until we achieve a maximal value as the intensity of the exercise is progressively increased. Typically, this test lasts no longer than 12 minutes. During this test, you will pedal at a speed of 80 revolutions per minute (rpm) while the resistance is increased 0.5 kp every two minutes until you choose to stop or until you can no longer maintain 80rpm. The measurement of oxygen consumption during the test is used to determine your maximal aerobic capacity. We also use this test to determine the work intensity that will be used during the experimental phase of the study.

Experimental sessions

You will participate in 1 preliminary and 2 experimental sessions.

During the preliminary session, we will review all procedures. You will be introduced to all of the equipment and measuring devices that we will be using. You will be asked to complete a health screening questionnaire as well as a physical activity assessment questionnaire, and sign an informed consent approved by the University of Ottawa Research Ethics Board. These questionnaires are standard questionnaires that have been developed to help us evaluate your readiness for exercise and are also used to assist us evaluate your general physical health and level of physical activity. Thereafter, we will complete some basic measurements including your height and body mass. Following these measures, you will be asked to perform a maximum oxygen consumption test on a cycle ergometer. This will consist of pedalling at a cadence of 80 rpm while the resistance is increased by 40 watts every two minutes until you can no longer maintain the required cadence (8-12 min).

Each experimental session will last approximately 4 to 5 hours. During these tests you will be asked to exercise in the heat (35°C). In each session, after a 60-min resting period, we will perform a head up tilt maneuver on a commercially available tilt table (Teeter Han-up table,

https://www.teetertv.com/). For this you will be tilted to a 70^{o} incline for 6 minutes. This tilt will be done 3 times with while returning you to the supine position for 10 minutes in between each tilt. Following this, on two you will be required to cycle on a on a cycle ergometer for 90-min at three increasing intensities (200, 250 and 300 $W \cdot m^{2}$). The trial will be repeated on two separate occasions separated by at least 72 hours.

Exercise will stop after your rectal/esophageal temperature reaches a steady value or if your esophageal temperature increases to 39.5ºC.

After you finish exercising you will then be required to rest quietly for 45 minutes in the supine (lying down) position. Following this you will remain resting but your position will be tilted to a 70^{o} incline for 6 minutes. This tilt will be performed 3 times with 10 minutes at supine resting position between each tilt.

You should be aware that at any point in the experimental protocol, the test will be terminated should you experience any distress or if the researchers feel that you are in distress.

In preparation for the experimental trials, you will be asked to abstain from alcohol and severe or prolonged physical activities for at least 12 hours prior to all sessions. It is highly recommended that you avoid eating for at least two hours before the trial. You are asked to ensure that you be properly hydrated by drinking at least 100 ml every waking hour prior to the experimental trials.

Female participants will be asked to participate in the follicular phase of their menstrual cycle, defined as between day 1 and 10 following the onset of their self-reported menstruation.

The following instruments will be used to monitor and record your physiological response during the experimental trial:

***Esophageal probe*:** In order to monitor central body temperature, the researcher will insert a flexible oesophageal temperature probe (2 mm in diameter) will be inserted through one of your nostril, during which time you will be asked to swallow sips of water. The tip of the probe, once fully inserted in your oesophagus (swallowing tube), will rest at the level of the heart. There can be mild discomfort and mild gagging reflex from swallowing the probe. However, this sensation soon passes (5-10 seconds).
***Rectal probe*:** You will be asked to insert a flexible probe though the anus into the rectum (10 cm). Proper instruction will be given to you on the placement of the rectal probe. This probe provides the researcher with an indication of the amount of heat stored in your body. You should be aware that there is some minimal risk associated with the insertion of a rectal probe. With the insertion, there is a risk of perforation of the rectum, and may cause some discomfort and minor irritation. However, proper instruction will be given to you on the placement of the rectal probe to ensure your safety and comfort. You will be responsible for the insertion of this probe.
***Tympanic probe*:** The researcher will insert a probe into your ear canal. The probe will be pushed gently until it touches the tympanic membrane. At this point, you will sense a slight discomfort and the probe will then be retracted slightly. The probe will be secured in its position by packing the ear with cotton balls held in place with surgical tape. The auditory canal temperature will be used as an index of brain and core temperature.
***Skin probes*:** Twelve skin probes will be taped to the skin surface with hypoallergenic tape. These probes give an indication of skin temperature and heat loss from the skin surface. Some hair may need to be shaved (by the use of disposable razors) in order to secure the probes adequately to the skin surface. Some discomfort may be experienced upon removing the tape.
***Sweat capsule*:** A small plastic capsule will be taped to the back of the shoulder (upper back). This capsule picks up humidity from the skin and provides an indication of sweat rate.
***Blood pressure*:** Blood pressure will only be monitored before and after the exercise period. Blood pressure will be monitored by a Finapress fingertip blood pressure monitor, as well as manually at pre-selected intervals with a sphygmomanometer and a stethoscope. You will feel a slight pressure on your finger while blood pressure will be

taken.

***Skin Blood flow*:** A flexible laser probe will measure skin blood flow non-invasively at the mid-forearm. This measuring device does not result in any discomfort or residual medical effects.

Lower leg and Forearm blood flow: Blood flow to your right lower leg and forearm will be measured using venous occlusion plethysmography controlled by an automated system. This measurement involves placing cuffs around your ankle, upper leg, wrist, and upper arm, as well as a strain gauge around the widest part of your lower leg and forearm. The measurement consists of inflating the ankle and wrist cuff at a pressure of 250 mmHg in order to occlude the circulation of blood to your foot and hand. Subsequently, the cuff around your arm will inflate (50 mmHg) and deflate in cycles of 15 seconds. Inflating the cuffs will impede venous return, without affecting arterial inflow of blood into your lower leg and forearm. Since venous return is impeded, but arterial inflow is left intact, the volume of your forearm will change and measured by the strain gauge. This change in volume is equal to the amount of blood entering your forearm. This measurement will be performed at specific time points during the experimental protocol and will be performed for a period of two minutes at a time. Therefore, the circulation to your hand will be arrested for only two minutes at a time. Although this procedure does not result in any residual medical effects, you may feel slight numbness in your foot and hand during the occlusion period. However, this sensation passes as soon as the cuff around your wrist is deflated.

***Oxygen consumption and Cardiac output*:** An automated metabolic cart (MOXUS system) will be used to assess oxygen consumption and cardiac output. You will be required to wear a breathing valve connected to the metabolic cart and a nose plug for the majority of the study. During the experiment you will be asked intermittently to follow a re-breathing procedure in order to assess cardiac output.

***Heart rate*:** Heart rate will be monitored by a strap placed around the chest (Polar Vantage heart rate monitor).

Blood glucose: will also be measured by means of a capillary test. Participants should already be familiar with this test (a.k.a finger stick test) as it is already part of daily blood glucose self-management for type 1 diabetic individuals. The test, which will be performed once prior to, once during, and once after exercise, involves pricking the end of a finger with a sterilized spring-loaded lancing device so that the resulting blood droplet may be placed on a test strip for insertion into a blood glucose meter. The resulting values will be recorded manually by the investigators. Participants will be asked to bring their regular blood glucose testing kit to the lab with them, and will perform the test themselves, as they would regularly do at home.

HbA1C: venous blood samples (approximately 10ml) will be collected through a butterfly vacutainer system during the preliminary session to evaluate the subjects HbA1C.

Tilt table: The tilt table is used to passively alter body posture and stimulate blood pressure responses. When the tilt table is flat (0^{o}), blood pressure is even throughout the body. By tilting the table in a positive angle you cause blood to pool in the lower legs which lowers blood pressure in the upper body and triggers an increase in heart rate.

Blood Sample: If you are a female, one venous blood sample will be collected through an indwelling plastic catheter in a superficial vein prior to the start of each experimental trial in order to confirm that the trial took place in the appropriate phase of the menstrual cycle (follicular). Blood samples (approximately 10ml) will be analyzed immediately following the experimental trials and will be discarded following the analysis. The appropriate phase of the menstrual cycle will be determined according to your self-reported onset of menstruation. The first day of menstrual flow will be taken as day 1 of the cycle, and you will be asked to perform the experimental trials between days 1 and 10 of your menstrual cycle. The blood sample will only be used to confirm that the you wre in the appropriate phase. The sample will be analyzed for progesterone and 17 β-estradiol concentrations. If, on the basis of the laboratory test results, it is found that the concentrations of these hormones did not match the appropriate phase of the menstrual cycle, you will be asked to come back and repeat that particular session. However, this only happens rarely.

Risks and discomforts

You should be aware that there are some physical risks associated with any form of exercise. There is essentially no major risk for healthy, active people while performing submaximal exercises. Some effects of maximal exercise testing are nausea, dizziness, fainting, abnormal blood pressure, chest pain and leg cramps. The incidence of cardiac arrest during maximal exercise tests is 1 in 10 000 tests. You should be aware that at any point during the maximal exercise test and the submaximal experimental exercise, you may stop at any time during these tests. To ensure your safety, all tests are conducted under standardized conditions for human exercise experiments as laid out by the Canadian Society for Exercise Physiology and the American College of Sports Medicine.

As described above, perforation of the oesophagus or oral or nasal cavities, as well as the rectum can occur during insertion of the oesophageal and rectal probes (potentially causing inflammation and infection). However, such an incident is very rare and no such incident has ever occurred in this laboratory. The risk of transmission of infectious disease is negligible as each subject has his own sterile probes that will be disposed of once all tests have been completed. There is also some risk of skin irritation and rash associated with the taping of the skin probes.

There are also certain risks that accompany an elevation of 1.5 to 2.0°C in your core temperature. These include: headache, extreme weakness, dizziness, nausea, hyperventilation, hypotension, confusion, diarrhoea, vomiting and loss of consciousness. An increase in your core temperature of this magnitude is unlikely under these experimental conditions. The risk of any ill-effect associated with an increase in your core temperature will be minimized by terminating the exercise at the first sign of distress. Immediately following the termination of the exercise, if deemed necessary by the researcher, we will immerse you in a cool water bath to bring your core temperature back down to your normal resting value.
Partaking in physical activity increases the risk of hypoglycemia both during and after exercise in type 1 diabetic individuals and in order to avoid hypoglycemia during exercise, participants will not be permitted to begin exercise unless their blood glucose levels, as measured by a pre-exercise finger stick test, are within a safe range (5 to 13.9 mmol/L). Participants with high blood glucose (>14 mmol/L) will be tested for ketones. Should ketones be present, they will be asked to inject a correction bolus of insulin, and exercise will be postponed until blood glucose levels drop below 14 mmol/L. If no ketones are present, the participant will wait 30 minutes before testing again, and exercise will commence when blood glucose is below 14 mmol/L. If blood glucose levels are low (i.e. near 5 mmol/L and showing a decreasing trend, or below 5 mmol/L) participants will be provided with 16g of glucose in tablet form. Participants will then be asked to check their blood glucose after 20 and 40 minutes to ensure that levels are greater than 5 mmol/L and stable before starting exercise. Should the correct range not be reached within 90 minutes, the experiment will be rescheduled. Participants will also perform a finger stick test at the end of the pre-exercise tilts, at the end of exercise, before the second round of three head up tilts and upon completion of the trial with the necessary insulin and glucose adjustments being made where necessary.

There is no risk associated with using the tilt table with an incline of 70 degrees. There is some risk that you might feel faint. The response you will feel is similar to standing up quickly.

Anonymity and Confidentiality

All raw data will be stored using alphanumeric coding system as such no one will be able to identify you as your name will not appear on these files. Data will be kept in the Laboratory of Human Bioenergetics and Environmental Physiology in locked file cabinets and only the researchers mentioned above will have access to your data. All computer files containing the secured data from the study are deleted from memory.

No records bearing your name will leave the institution. Only the researchers mentioned above will have access to your data. You are encouraged to request and discuss the results of the experimental trials at any time. The results of the preliminary session (body composition and VO_2 max test) will be available to the participant upon completion of the study.

The data collected in this study will be published in scientific journals.

For the entire duration of the study, it is fully understood that you may refuse to participate or withdraw from the study at any time, without question.

INFORMED CONSENT OF PARTICIPANT
Research involving human subject require written consent of the participants.

I, _____, hereby volunteer to participate as a subject in the study entitled **"**Thermal and non-thermal influences on post-exercise thermoregulation**"**. I have read the information presented in the above background information and I had the opportunity to ask questions to the investigators. I understand that my participation in this study, or indeed any research, may involve risks that are currently unforeseen.

I recognize that there will be no direct benefit to me from my participation in this study (besides receiving a fitness evaluation).

I understand that if I have any questions regarding the study, I may contact [redacted] at. If I have any questions with regards to the ethical conduct of this study, I may contact the Protocol Officer for Ethics in Research, University of Ottawa, [redacted],

I have been given a copy of this Background Letter and Consent Form for me to keep.

Signature of participant: _____ Date: _____

Signature of Researcher: _____ Date: _____

This is to confirm that the University of Ottawa Research Ethics Board identified above, which operates in accordance with the Tri-Council Policy Statement and other applicable laws and regulations in Ontario, has examined and approved the application for ethical approval for the above named research project as of the Ethics Approval Date indicated for the period above and subject to the conditions listed the section above entitled "Special Conditions / Comments".

During the course of the study the protocol may not be modified without prior written approval from the REB except when necessary to remove subjects from immediate endangerment or when the modification(s) pertain to only administrative or logistical components of the study (e.g. change of telephone number). Investigators must also promptly alert the REB of any changes which increase the risk to participant(s), any changes which considerably affect the conduct of the project, all unanticipated and harmful events that occur, and new information that may negatively affect the conduct of the project and safety of the participant(s). Modifications to the project, information/consent documentation, and/or recruitment documentation, should be submitted to this office for approval using the "Modification to research project" form available at:
http://www.research.uottawa.ca/ethics/forms.html

Please submit an annual status report to the Protocol Officer four weeks before the above-referenced expiry date to either close the file or request a renewal of ethics approval. This document can be found at:
http://www.research.uottawa.ca/ethics/forms.html

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

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