Mechanisms and Applications of Acute Heat Stress on Vascular Function

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Abstract

Passive heat stress shows promise as an effective strategy to reduce the risk of cardiovascular diseases (CVD) as an alternative or adjunct to exercise and/or medication. Improving function of the endothelium is one of the key targets underlying the reduction in CVD risk. Therefore, this thesis aimed to evaluate potential mechanisms of acute heat-related improvement of endothelial function and investigate the effectiveness of heat in selected clinical groups. Endothelial function was measured via the technique of flow-mediated dilation (FMD) and shear stress was estimated using duplex Doppler ultrasound imaging of large arteries. In study #1, we compared forearm vs. whole-body heating in in 12 young, healthy individuals. In addition to elevated shear stress, skin and core temperatures independently increased endothelial function. In study #2, we compared peripheral and cerebrovascular responses to whole-body heating with oral ingestion of an α-1 adrenergic antagonist (Prazosin) or placebo. Although α-1 adrenergic antagonism did not influence hyperthermia-induced reductions in cerebral blood flow or affect post-heating FMD, preliminary trends indicate a potential impact of sympathetic nervous system activity on FMD in the brachial artery. In order to test the effectiveness of passive heating in extremely sedentary individuals in study #3, we measured FMD in 15 participants with cervical spinal cord injuries (SCI) and 15 uninjured controls before and after acute leg heating. There was no change in either brachial or superficial femoral artery FMD in SCI or controls; however, systemically circulating microparticles reflective of endothelial activation were reduced by ~60% after heating in SCI but not controls. These reductions in endothelial microparticles were associated with improved shear patterns. Study #4 demonstrated that FMD was not different between older adults who are habitually exposed to passive heat stress (via regular sauna use) compared to those who are not. Furthermore, an exploratory analysis suggested there was no difference in FMD in
sauna users compared to non-users with coronary artery disease; these data help direct towards other mechanisms as factors underlying lower CVD risk with sauna use. Collectively, the findings within this thesis highlight thermal and hemodynamic responses to heating that acutely influence endothelial function in health and disease. These findings have implications on the design of future studies that will contribute to reducing CVD risk with aging and various disease states.
Lay Summary

The endothelium is the inner lining of blood vessels throughout the body and it plays an integral role in maintaining blood vessel as well as overall cardiovascular health. Since adequate amounts of exercise are rarely performed by the majority of the population, passive heat stress might be a useful alternative for improving endothelial function. The main findings of this thesis are that 1) increasing skin or body core temperatures can improve endothelial function in young adults; 2) the timing of measuring endothelial function after heating may be important due to effects of the ‘fight or flight’ system; 3) heat reduces biomarkers of endothelial dysfunction in individuals with limited exercise capacity (those with spinal cord injury) compared to uninjured individuals; and 4) endothelial function may not be the only factor contributing to lower disease risk in older adults (healthy and with heart disease) who are habitual sauna users.
Preface

All experimental chapters in this thesis were provided ethical approval (H17-01817, H19-02355, H18-00896, 2017-2179).

Chapter 1 was written by Geoff Coombs and edited by Prof. Ainslie.

Chapter 2 was written by Geoff Coombs and edited by Prof. Ainslie. No aspect of this work is currently under review for publication. For figures that were originally published elsewhere, permission for reproduction has been granted by their respective journals for use in this thesis.

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GBC and PNA conceived and designed the study. GBC, JCT, DAS, JAMJR, and DJW collected the data. GBC and AP analyzed the data. GBC drafted the manuscript. All authors interpreted results and approved the final version.
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List of Abbreviations

A-a, alveolar to arterial
ACE, angiotensin converting enzyme
AngII, angiotensin II
ANOVA, analysis of variance
AP, arterial pressure
ASIA, American Spinal Injury Association
BA, brachial artery
BMI, body mass index
CAD, coronary artery disease
Ca^{2+}, calcium
CBF, cerebral blood flow
cGMP, cyclic guanosine monophosphate
CHF, congestive heart failure
CON, control
COPD, chronic obstructive pulmonary disease
CSA, cross-sectional area
CVD, cardiovascular diseases
DBP, diastolic blood pressure
ECG, electrocardiogram
EDRF, endothelial-derived relaxation factor
EDTA, e9thylenediaminetetraacetic acid
EM, estimated means
EMP, endothelial microparticles
eNOS, endothelial nitric oxide synthase
FMD, flow-mediated dilation
HbA1c, glycated hemoglobin
HDL, high-density lipoprotein
Homa-IR, homeostatic model assessment of insulin resistance
HR, heart rate
HSP, heat shock protein
HUVECs, human umbilical vein endothelial cells
ICA, internal carotid artery
IL, interleukin
IV, intravenous
LBNP, lower body negative pressure
LDL, low-density lipoprotein
L-NMMA, N\textsuperscript{G}-Methyl-L-arginine
L-NNA, N-\(\omega\)-nitro-L-arginine
L-NAME, N(\(\omega\))-nitro-L-arginine methyl ester
MAP, mean arterial pressure
MBV, mean blood velocity
MCA, middle cerebral artery
MET, metabolic equivalent of task
NO, nitric oxide
NSAID, non-steroidal anti-inflammatory drugs
OGTT, oral glucose tolerance test
OSI, oscillatory shear index
P\textsubscript{a}, arterial partial pressure
PAD, peripheral artery disease
PCA, posterior cerebral artery
PCOS, polycystic ovary syndrome
P\textsubscript{ET}, end-tidal partial pressure
PWV, pulse wave velocity
RH, reactive hyperemia
ROS, reactive oxygen species
RPM, rating of perceived exertion
SBP, systolic blood pressure
SCI, spinal cord injury
SD, standard deviation
SE, standard error
SFA, superficial femoral artery
SkBF, skin blood flow
SNA, sympathetic nervous system activity
SR, shear rates
SRAUC, shear rate area under the curve
TCD, transcranial Doppler ultrasound
T_{es}, esophageal temperature
TG, triglycerides
T_{sk}, skin temperature
VA, vertebral artery
\dot{V}_E, minute ventilation
\dot{V}O_2_{peak}, peak rate of oxygen uptake
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Chapter 1: Introduction

1.1 Rationale

Among developed nations, the leading cause of death is cardiovascular diseases (CVD), including coronary artery disease, heart failure, peripheral arterial disease, and stroke. These CVD account for 22.7% of deaths in Canada (202, 271). Moreover, the economic burden related to direct and indirect costs is $22.2 billion in Canada, and about two thirds of that are due to lost productivity from disability or death (202). Some of the largest risk factors for CVD are advancing age, hypertension, dyslipidemia, obesity, and diabetes (146). The effects of exercise on preventing and reversing these risk factors is well known (191). However, accelerometer data from the 2007-2009 Canadian Health Measures Survey indicates that only 15% of Canadians meet the 150 min/week guideline for moderate intensity physical activity, and 65% of Canadians take less than 10 000 steps per day (54). This lack of engagement in physical activity contributes to the increasing prevalence of risk factors such as obesity and hypertension (144, 188). However, it has also been reported that reductions in traditional factors (e.g., blood pressure, cholesterol, lipids, diabetes, etc.) with physical activity only account for ~60% of the lower CVD risk (175); therefore, ~40% of the lower CVD risk must be attributable to some other factor(s). It has been postulated that improved endothelial function with exercise might a key protective factor of arterial stiffening, hypertension, and CVD (137).

It is readily apparent that alternative strategies to mitigate CVD and associated risk factors are necessary. Such alternative strategies to exercise might be particularly important for specific groups who experience difficulty exercising due to disability or disease (e.g., spinal cord injury, peripheral arterial disease, heart failure, COPD, etc.). The use of passive body heating has traditionally been used for thousands of years by various cultures; however, the scientific
evidence supporting the “therapeutic” use of approaches such as saunas, hot baths, sweat lodges, etc. was minimal. Recently, however, studies have demonstrated that heat therapy has the potential to reduce risk factors such as high blood pressure, obesity, and diabetes (33, 126). Importantly, a landmark study (the Finnish Kuopio Ischemic Heart Disease Risk Factor Study) reported that the risk of fatal CVD and all-cause mortality was lower with greater frequency of sauna use in 2315 men over a period of ~21 years (150). Several recent studies have also reported improvements in endothelial function with heat therapy (33, 209); however, elucidation of the mechanisms involved is required to inform future application of heat therapy. Therefore, the overall aim of this thesis was to determine the mechanisms of heat therapy on endothelial function and its potential application to healthy individuals and those at greater risk for CVD, which was achieved via four main experimental studies that comprise the body of this thesis.
Chapter 2: Literature review

2.1 Circulatory adjustments to passive heating

The two main autonomic thermoregulatory responses are sweating and skin blood flow. Redistribution of cardiac output to the cutaneous circulation is responsible for convection of heat from the core to the periphery where it can be exchanged (i.e., dissipated) with the external environment. This exchange occurs through conduction and radiation, depending on temperature gradients (e.g., skin to air or water), and also by evaporation of sweat from the skin surface depending on temperature and vapour pressure gradients. A constant supply of plasma volume is required to maintain interstitial fluid sources for evaporative heat loss. Thus, skin blood flow is an important component of thermoregulatory function for both direct heat loss and facilitation of sweating. Indeed, skin blood flow can increase by a maximum of 25-fold during passive heat stress, which is supported by a doubling of cardiac output (213) (see Figure 2.1).
Figure 2.1. Regional cardiac output redistribution during passive whole-body heating to mean skin temperature ~40°C. Delta (Δ) changes in cardiac output, splanchnic blood flow, renal blood flow, and muscle blood flow equal the total change in skin blood flow (i.e., +7.8 l/min).

Reproduced from (213) with permission from the publisher.
2.1.1 Cutaneous vasodilation

Skin blood flow is one of the largest and most important cardiovascular adjustments to body heating. Summation of changes in forearm blood flow, splanchnic, renal, and muscle blood flows suggest that skin blood flow can increase by up to 8 l/min during whole-body heating (213) (Figure 2.1). Forearm blood flow is considered to reflect changes in skin blood flow during passive heat stress where the muscles are not metabolically active. Indeed, relative changes in oxygen saturation of blood from deep compared to superficial forearm veins suggests that changes in blood flow are confined to the skin during heating (207). This observation was confirmed by intramuscular injections of a radioisotope demonstrating that muscle blood flow does not change during heating (166). Regional forearm blood flow measurements have been extrapolated to the entire surface area, supporting the earlier estimate by Rowell and demonstrating a striking skin blood flow of ~22 ml/100 ml/min during heating (230). Rises in skin blood flow are driven by both local (direct heating) and reflex (indirect heating)-mediated influences. For example, the initial changes in skin blood flow during passive heat stress are modulated by skin temperature and subsequent rises reflect the slower changes in core temperature (134).

In non-glabrous skin, the sympathetic nervous system controls cutaneous vasomotor tone through two branches: a vasoconstrictor system and an active vasodilator system. The sympathetic vasoconstrictor system releases norepinephrine to bind to post-junctional alpha-adrenergic receptors and it is withdrawal of this branch that is responsible for initial increases skin blood flow (168). When ambient conditions are thermoneutral, vasoconstrictor tone is already fully absent and active cutaneous vasodilation is responsible for the remainder of the rise in skin blood flow via cholinergic nerve activation in warm and hot conditions (84, 208).
Evidence for active vasodilation comes from studies where skin is treated with bretylium to prevent norepinephrine release from vasoconstrictor nerve terminals. During whole-body cooling (to induce systemic sympathetic vasoconstriction), skin blood flow was unchanged in the treated site compared to vasoconstriction at the control site; however, both skin sites increased blood flow similarly during heat stress (140), highlighting a separate vasodilator system (Figure 2.2).

The development of intradermal microdialysis has helped to more fully characterize the mechanisms of cutaneous vasomotor control. Kellogg et al. (138) demonstrated that reflex-mediated cutaneous vasodilation occurs via cholinergic nerve cotransmission (via an unknown neurotransmitter) when core temperature increases (138). Using intradermal microdialysis of L-NG-nitroarginine methyl ester (L-NAME; nitric oxide synthase inhibitor), Kellogg et al. (139) also demonstrated that reflex cutaneous vasodilation was attenuated but not abolished when nitric oxide (NO) production was blocked. However, when local afferents are stimulated by direct heating, a brief increase in skin blood flow due to neurally-mediated axon reflex occurs (169). This axon reflex is followed by a gradual and sustained increase in skin blood flow due to primarily NO stimulation of vascular smooth muscle cell relaxation (141, 169). Indeed, Minson et al. (169) demonstrated that the NO synthase (NOS) inhibition has a small effect on the initial peak of local heating induced cutaneous vasodilation and causes a large reduction in the skin blood flow plateau, whereas axon reflex block greatly reduces the initial peak but does not affect the plateau. Mechanisms dependent on NO are a minor component of reflex cutaneous vasodilation, accounting for approximately 20% of dilation in young subjects (169), compared to up to 80% during local heating protocols (50). Although local influences are sufficient to drive and modify skin blood flow responses, reflex cutaneous vasodilation dominates skin blood flow control where skin blood flow is closely linked to core temperature (134). Yet, it is important to
consider that the majority of skin surface area will be exposed to both direct heating and reflex vasodilation when whole-body heating is performed. Thus, the rapid increase in skin blood flow after the onset of heating matches the change in skin temperature, but gradual increases to maximum skin blood flow occur alongside increased core temperature (134).
Figure 2.2. The top panel demonstrates the lack of cutaneous vasoconstrictor response when treated with adrenergic blockade. In contrast, the bottom panel demonstrates that both treated and untreated skin sites vasodilate with heating, which indicates the existence of separate sympathetic vasoconstrictor and vasodilator systems. Reproduced with permission from the publisher from Johnson and Proppe (135), with data from (140).

2.1.2 Cardiac responses

Temperature can directly acts on cardiac nodal cells where the effects are both chronotropic (increased frequency of cardiac cycles) and domotropic (increased velocity of
pacemaker signal conduction). Jose et al. (136) demonstrated a 7 bpm increase in intrinsic heart rate per 1°C increase in body core temperature in humans using pharmacological autonomic blockade (i.e., propranolol and atropine). However, this response only accounts for 40% of the increase in heart rate during whole-body heat stress. Gorman and Proppe reported that the other 60% is governed by autonomic nervous system activation using anticholinergic and β-adrenergic antagonism in primates (105). This autonomic control of heart rate during heating is further broken down into 75% parasympathetic withdrawal and 25% sympathetic activation (105).

Although cardiac afterload is either maintained (102) or decreased (180), stroke volume is generally considered to remain unchanged during heat stress (60, 213). Sympathetic stimulation not only exerts a chronotropic effect but is often inotropic (increased contractility) as well (37, 101). However, at least during physiologically tolerable heat stress, increased contractility and reduced afterload appear sufficient to counteract reductions in cardiac preload. Plasma volume losses and interstitial fluid shifts resulting from sudomotor activity may contribute (159), but it is likely that the large volume stored in the cutaneous vascular compartment accounts for much of the reduction in preload during hyperthermia (76).

Indeed, right atrial pressure (atrial preload) and pulmonary capillary wedge pressure (ventricular preload) are both decreased during heat stress (216, 267). More recent technological advances support these responses with lower central blood volume by 17% measured using gamma scans of Tc⁹⁹ radiolabeled erythrocytes (61). Other mechanisms of preserved stroke volume include inotropy of the left ventricle and improved diastolic function. Diastolic function of the ventricle is a preload-dependent measurement. Therefore, the fact that diastolic function was reported to be maintained in heat stress suggests that diastolic was, in fact, enhanced (29). To further address this point, Brothers et al. (30) restored central venous pressure to
normothermic levels with plasma volume expansion (via combined colloid and saline infusion) and demonstrated that diastolic function was indeed increased during hyperthermia. The interaction of cardiac preload, afterload, inotropy, and diastolic function on stroke volume can be visualized with the Frank-Starling relation. Heat stress shifts the operating point up and leftward where the curve is steeper, representing a greater stroke volume at given ventricular filling pressure (266). When volume loaded to counteract the lower central blood volume, the operating point shifted to a flatter portion of the curve and also increases stroke volume (Figure 2.3), indicating an inotropic effect of heat (37).
Figure 2.3. Differences in the Frank-Starling relation during normothermia and heat stress. Arrows indicate baseline values before lower body negative pressure (i.e., operating point). The hyperthermic value is on a steeper portion of the Frank-Starling relation indicating an inotropic effect of heat. In other words, when matched for volume status, hyperthermia increases stroke volume stoke compared to normothermic baseline. Reproduced from (37) with permission from the publisher.
2.1.3 Systemic vascular responses

Cardiac output is also increased in order to offset the large decrease in systemic vascular resistance due to redistribution of blood volume to the skin. The large pressure gradient between the heart and limbs necessitates an increased vascular conductance to maintain flow to cutaneous vessels. Although central venous pressure and systemic vascular resistance are lower, mean arterial pressure is generally maintained or decreased by a small magnitude during whole-body heating (216). Increased resistance in other vascular beds likely contributes to this response – namely the splanchnic and renal circulations, which account for nearly 50% of cardiac output under resting, fasted conditions. Indeed, hepatic and renal blood flow measured via indocyanine green and sodium para-aminohippurate clearance, respectively, were both reduced by ~30% during heating (215). The 20-30% reductions of splanchnic blood flow during passive heating was confirmed via technetium-99m labeled autologous red blood cells and gamma imaging (61). Data from animal models with α-adrenergic blockade demonstrate that increased splanchnic vascular resistance with heating is sympathetically mediated (201). However, total reduction in blood flow to these regions amounts to ~1 litre, which does not nearly compensate for the redistribution of blood volume to the skin (up to 8 litres). Therefore, increased cardiac output is undoubtedly integral to maintenance of mean arterial pressure during whole-body heating.

The cerebral circulation is also often reported to decrease its flow during heat stress. Both intracranial artery velocities (measured via transcranial Doppler ultrasound) (15, 31, 99) and extracranial artery blood flow (via duplex Doppler ultrasound) (15) are reportedly lower during heat stress. However, these findings are not universal and may be subject to a thermal threshold where decreases in cerebral blood flow only occur above 0.5-1.2°C increases in core temperature. This threshold likely occurs due to hyperthermic hyperventilation where the
resultant hypocapnia causes cerebral vasoconstriction (90, 247). Yet, restoration of end-tidal 
PCO$_2$ during heating only partially mitigates reductions in cerebral blood flow in some studies 
(31, 246), whereas it fully restores it in others (15, 181). Thus, remaining mechanisms are 
speculative but sympathetically-mediated cerebral vasoconstriction is conceivable but currently 
untested.

2.2 Endothelium

The endothelium is the innermost layer of cells lining blood vessels of both the arterial 
and venous systems (Figure 2.4). Its role is multifaceted and integral to the function of blood 
vessels, as well as several other processes. Indeed, the endothelium is a selectively permeable 
barrier between the blood and extravascular compartments; it is responsible for inflammatory 
responses, angiogenesis, blood clotting, transport of macromolecules, and the control of vessel 
tone (39). In the context of cardiovascular health, normal function of the endothelium is 
important because impaired barrier permeability (i.e., endothelial dysfunction) permits entry of 
cholesterol loaded low density lipoprotein into the arterial wall where it accumulates (39). Thus 
endothelial dysfunction precedes the development of atherosclerotic plaques (164) and is 
considered predictive of future cardiovascular events (131, 274). The focus of this thesis will be 
endothelial-dependent dilation as other functions of the endothelial cells are reviewed in detail 
elsewhere (75).
Figure 2.4. Structure of an artery. This work is licensed under a Creative Commons Attribution-ShareAlike 3.0 Unported License. Retrieved from:


2.2.1 Endothelial function

A functional role for the endothelium in the control of vessel tone has been well established. A seminal study by Furchgott and Zawadzki in 1980 (100) demonstrated that relaxation of vascular smooth muscle cells in the isolated rabbit aorta by acetylcholine was dependent on intact endothelial cells which released an unknown “endothelial-derived relaxation factor (EDRF).” Pohl et al. (199) demonstrated in dogs that the femoral artery dilation response to increased flow was also abolished in response to acetylcholine following endothelial denudation, providing in vivo evidence for flow-mediated dilation dependent on the
endothelium. Further studies in dogs confirmed the requirement of an intact endothelium to cause vascular dilation in response to increased flow, and also suggested that a substance other than prostacyclin was released from endothelial cells because indomethacin did not prevent dilation (125, 217). It was discovered that the relaxation of tissues, changes in tone due to hemoglobin and superoxide dismutase exposure, inhibition of platelet aggregation and adhesion, did not differ between EDRF and NO (174, 189). Thus, NO was determined to be the previously unidentified EDRF.

The importance of the NO molecule has since been widely recognized for its role in vascular health through its vasodilatory, anti-inflammatory, and anti-atherogenic effects. Although, it was demonstrated that increased blood flow stimulates release of NO from endothelial cells, there are two main hemodynamic forces by which blood flow is sensed in the artery – shear stress and circumferential strain (114). Endothelial cells undergo circumferential strain across the cardiac cycle whereby the endothelial lining is stretched with pulse pressure propagation. Sensing and transduction of these mechanical stimuli occur through various chemical signalling pathways (e.g., matrix proteins, cation channels) that activate intracellular signalling cascades and can effect transcription factors of endothelial cell genes (70).

Shear stress is the tangential stress exerted on the luminal surface of the artery as a result of the friction of blood flow. It is expressed in units of force over surface area (dyne/cm²), and is proportional to the product of the velocity of flowing blood and its viscosity. Because shear stress is a relatively weaker force than pressure, there are multiple mechanosensors through which the endothelium detects shear stress (7). These include ion channels, receptor kinases, G-protein coupled receptors, junctional proteins, caveolae, and the endothelial glycocalyx (49). Junctional proteins (VE-cadherin, PECAM-1) are responsible for mechanotransduction of
signalling pathways that phosphorylate Akt and subsequently phosphorylation of endothelial nitric oxide synthase (eNOS) at Ser1177 (45, 276). Phosphorylation of eNOS leads to NO production via the conversion of L-arginine to L-citrulline and NO, in the presence of the eNOS cofactor tetrahydrobiopterin (BH4). Vasodilation then occurs following diffusion of NO into the smooth muscle cells where it binds to and activates the enzyme guanylyl cyclase, which catalyzes the dephosphorylation of guanosine triphosphate to cyclic guanosine monophosphate (cGMP). The second messenger cGMP is responsible for many downstream cellular functions, including smooth muscle relaxation (Figure 2.5).

Shear stress also modulates many other pathways which affect endothelial cell phenotype and ultimately NO bioavailability. For example, in vitro studies demonstrate that sustained exposure to increased shear stress promotes eNOS gene transcription (248, 251). Shear stress also increases the expression of antioxidant genes, increases production of prostacyclin, COX-1, PI2 synthase, and superoxide dismutase, which are important for endothelial-dependent vasodilation and NO bioavailability (148). Indeed, endothelial cells in areas with laminar shear stress demonstrate a quiescent phenotype where cells are elongated and aligned with the direction of flow. In contrast, atherosclerotic lesions preferentially develop at branch points of the vascular tree (40). These arterial regions are characteristic of disturbed flow patterns where turbulent flow (i.e., oscillatory shear stress) occurs particularly at the outer walls of vascular bifurcations (45, 160). Endothelial cells in atherosclerosis prone regions display altered gene expression leading to lower eNOS mRNA protein expression, increased endothelin-1 expression, increased expression of adhesion molecules (e.g., VCAM-1), increased expression of reactive oxygen species (ROS) producing enzymes (e.g., NADPH oxidase), and increased production of
These epigenetic changes lead to NO scavenging, platelet aggregation, and vasoconstrictor signalling – all of which can contribute to an atherogenic phenotype.

**Figure 2.5.** Schematic of shear stress activation of endothelial nitric oxide synthase (eNOS) and nitric oxide (NO) stimulation of cyclic guanosine monophosphate (cGMP) in smooth muscle cell (SMC) to elicit vasodilation. Created with BioRender.com

2.2.2 Methods of assessing endothelial function

There are several ways to directly measure or indirectly index endothelial function in humans. Probably the simplest measurement of conduit artery endothelial function is using pulse
wave velocity (PWV), where the pulse pressure transit time is recorded in the aorta. Resting PWV is clinically valid and strongly predicts future cardiovascular events (153, 257); however, vascular stiffness and tone are the primary determinants of this measure. Acute changes in PWV in response to reactive hyperemia may provide an index of endothelial health (177), but lacks integration of the functional response. The upside, however, of using stiffness measures as index for endothelial function is that there are several semi-automated devices which are repeatable and independent of operator proficiency. Direct stimulation of endothelial receptors using pharmacological activation can be coupled with techniques to assess the magnitude of change in blood flow. For example, venous occlusion plethysmography has been used extensively to measure forearm blood flow. This so-called isolated forearm model, when coupled with intra-arterial infusions of receptor agonists or antagonists, is considered one of the gold-standard techniques in vascular physiology (263). More recently, Doppler ultrasound has been used to assess downstream microvascular blood flow from the brachial artery; in combination with acetycholine and sodium nitroprusside infusions, endothelial-dependent and independent vasodilator responses can be assessed (121, 203). Additional co-infusion of NOS inhibitors (e.g., L-NMMA) can be used to further quantify the contribution of NO to such responses (111). Although these techniques provide direct measures of endothelial-dependent dilation and allows for mechanistic dissection of vasodilatory pathways, they are also expensive, invasive and associated with greater risks of infection, hematoma, and thrombosis.

In the late 1980s, studies reported the ability to non-invasively measure brachial artery dilation following arterial occlusion; this was termed flow-mediated dilation (FMD) (5, 226). In 1992, Celermajer et al. (43) demonstrated the potential of FMD to detect endothelial impairment in at-risk groups, and it was later demonstrated that NO was responsible for the FMD response
given it was abolished during intra-arterial infusion of L-NMMA (133). Since that time, FMD has been performed in thousands of healthy volunteers and patients, and a meta-analysis concluded that ~70% of the FMD response was mediated by NO (113). Thus, measuring FMD is a non-invasive technique that directly quantifies EDD and is mainly dependent on NO bioavailability (Figure 2.6).

![Figure 2.6](image)

**Figure 2.6.** Reductions in downstream vascular resistance following cuff release increase large artery blood flow – and thus shear stress (peak ~15 seconds) – leading to nitric oxide production and subsequent vasodilation (peak ~60 seconds). Reproduced from (232) with permission from the publisher.

2.2.3 Microparticles

Traditional biomarkers of CVD, such as natriuretic peptide, cardiac specific troponins, and C-reactive proteins, are plagued by high biological variability and low specificity (22). As a
result, efforts have been made to identify more specific biomarkers that could be linked directly to endothelial cells. Once thought to be cellular debris with importance for coagulation (268), microparticles are now recognized to be important in cell-to-cell communication, coagulation, inflammation, and angiogenesis. The term “platelet dust” was originally coined after the most abundant source of microparticles; however, they are released from all hematopoietic cells, including endothelial cells (119). The definition of a microparticle is an anucleoid, phospholipid particle containing RNA and micro RNA, with a diameter ranging between 100 nm and 1 µm (22, 71). These microparticles were first found to be elevated in vascular disease in 1999 (55), and are now considered as biomarkers of endothelial dysfunction with increased risk of atherothrombosis (22, 275). However, despite the pro-inflammatory, pro-apoptotic, and procoagulation properties of microparticles, it is important to note that microparticles are also involved in vascular repair and therefore are not exclusively biomarkers of an impaired endothelium (22, 71).

Microparticles are shed (via blebbing) from the plasma membrane of origin cells during apoptosis and cellular activation. As such, there are two distinct endothelial microparticle phenotypes: 1) Apoptotic microparticles are stimulated by tumour necrosis factor-alpha (TNF-α), interleukin (IL)-2, IL-6, IL-8, IL-9, activated T-cells, and DNA damage; and 2) activated microparticles are stimulated by growth factors, vascular damage, inflammation, and shear stress (22). Shear stress in particular is important in the release of endothelial microparticles (e.g., CD144+, annexin V). Indeed, human umbilical vein endothelial cells (HUVECs) exposed to low shear stress (2 dyne/cm²) induce the release 2.5 fold more microparticles compared to high shear stress (20 dyne/cm²) (256). When L-NG-nitroarginine methyl ester (L-NAME; nitric oxide synthase inhibitor) was included the microparticles released during high shear stress increased by
3-fold, highlighting the importance of shear stress and NO as key regulators of endothelial microparticle release (Figure 2.7) (256).

**Figure 2.7.** Factors influencing microparticle (MP) shedding and the reciprocal effects nitric oxide (NO) bioavailability on endothelial function. Reproduced from (25) with permission from the publisher.

### 2.3 Influence of sympathetic nervous activity on endothelial-dependent dilation

The autonomic nervous system is divided into the parasympathetic and sympathetic components, and the majority of blood vessels are only under sympathetic control. Neural activity originating from the medulla is carried through the central nervous system via the spinal...
cord and exits through anterior roots giving rise to preganglionic nerve fibers, which are present at thoracolumbar levels in sympathetic nerves. These fibers then synapse to postganglionic nerves to connect with target organs. Preganglionic nerves are cholinergic and release acetylcholine as its neurotransmitter, whereas the postganglionic nerves synapse with target organs and are mainly adrenergic and release norepinephrine. In blood vessels, except for the skin circulation, alpha and beta-adrenergic receptors are responsible for vasoconstriction and vasodilation, respectively. Activation of baroreceptors and chemoreceptors increase sympathetic nervous system activity (SNA) to effect physiological adjustments, which often includes vasoconstrictor signalling. Indeed, endothelial function of the brachial artery (i.e., FMD) is acutely reduced under various conditions of high muscle SNA (e.g., exercise, lower body negative pressure [LBNP], hypoxia) (8, 122, 205, 231).

During whole-body heating, necessary increases in sympathetic outflow occur when core temperature rises >0.3°C (63–65). In the skin circulation, increases skin SNA are followed by active cutaneous vasodilation mediated via cholinergic nerves (63, 109). Simultaneously, functional sympatholysis occurs in the muscle where elevated SNA (101) during heat stress does not cause a change in skeletal muscle blood flow (78, 134). It is unknown how sympathetic activity influences the conduit arteries (e.g., brachial) and shear stress patterns during heat stress; however, improved shear patterns resulting from local heat stress can offset LBNP-induced impairment of FMD in the brachial artery despite increased SNA (231). Whether sympathetic vascular restraint occurs in the brachial artery during whole-body heat stress and the interaction with improved shear stress patterns is unknown.

2.4 Novel evidence for heat therapy
Following the seminal study of Finnish sauna users (150), many studies on the physiological effects of heating have been performed. For instance, it has been reported that acute bouts of heating can reduce arterial stiffness and blood pressure in some studies (152, 238), but not all (102). Moreover, several studies have demonstrated that endothelial function – measured via FMD – increases after acute passive heating in young, healthy participants (12, 33, 46, 117, 179, 239). Of these studies, only five performed acute bouts of heating (34, 117, 209, 237, 239), and the two studies that observed increased FMD both used local forearm heating (46, 239). In contrast, the studies that did not report improved FMD heated a greater surface area (e.g., lower body or whole-body heating), which increased core temperature (34, 209, 237). Therefore, the mechanisms of improved endothelial function during acute heat stress remain unclear and represent a paradoxical relationship with core temperature (Figure 2.8).
Figure 2.8. Forest plot of the mean difference (±95% confidence intervals) in flow-mediated dilation (FMD) following acute heating studies as a function of the change in body core temperature. *Indicates a significant change from baseline.

It has been suggested that endothelial dysfunction may be required to observe improvements with heating (209); however, this phenomenon is not always reported (56), thus the type (e.g., age, disease) and magnitude of dysfunction required are unknown. During long-term (e.g., 8 weeks), shear stress appears to be an important stimulus for both macro- and microvascular function, which is highlighted by an absence of improvement in a cuffed vs. non-cuffed arm during the intervention (42, 112, 179). Using a similar approach, it was also reported that increased skin temperature might be required as well, at least for microvascular improvements to occur (41). Additionally, the release of endothelial microparticles was reportedly reduced during whole-body heating (13) and intracellular heat shock protein 70 was greater in HUVECs cultured at an elevated temperature (36). Thus, both local temperature and
increases in shear appear to influence endothelial function acutely, but it is unknown how these responses differ between limb and whole-body heating (Figure 2.9).

Figure 2.9. Schematic of the potential mechanistic effects of heat on vascular function. Both local and whole-body heating increase blood flow and concomitant shear stress, which activates endothelial nitric oxide synthase (eNOS). High core temperature can also induce heat shock protein (HSP) expression, which protects against inflammatory and oxidative stress effects on nitric oxide (NO) bioavailability. Repeated exposures to heat may decrease sympathetic activity, which could reduce vascular tone. Created with BioRender.com
Longer term benefits of heat (e.g., FMD, quality of life) have been demonstrated in patients with congestive heart failure (142, 227); however, Brunt et al. (33) were the first to demonstrate improvements in endothelial function following a chronic heating intervention in young, healthy individuals. Indeed, hot water immersion 4-5 times per week to ≥38.5°C rectal temperature for eight weeks improved FMD, and reduced arterial stiffness and blood pressure compared to a thermoneutral sham condition (33). Similarly, Ely et al. (87) reported reduced sympathetic activity and cardiovascular risk profile in women with polycystic ovary syndrome (PCOS) following 8-10 weeks of a hot water immersion intervention. Although promising, the potential benefits of heat therapy on vascular function should not be viewed as a replacement of exercise training/physical activity. Indeed, passive heating as an adjunct to exercise might aid sports performance (277) and potentially add vascular benefits to training programs in individuals with low exercise capacity. Moreover, heat therapy might be particularly useful in those with extreme barriers to physical activity, such as individuals with spinal cord injury (SCI). Despite the absence of supraspinal sympathetic control of the circulation, individuals with SCI maintain the ability to vasodilate (254) below the level of injury and increase cardiac output (225) during heat stress, albeit to a lesser degree. Yet, it is unknown how an acute or long-term heating intervention will influence endothelial function in SCI. Although the main focus of this literature review is vascular function, it is becoming evident that there is promise for the effectiveness of heat on other outcomes For example, endothelial function, clinical symptoms, and quality of life were all improved with repeated bouts of passive heating in heart failure patients (130, 142, 227), and exercise tolerance was improved in patients with peripheral arterial disease after 12 weeks of heat therapy (4). There is also increasing evidence that heat therapy might be beneficial for individuals with obesity and/or type 2 diabetes. In 1999, Hooper reported
that HbA1c was reduced from 11.3% to 10.3% after three weeks of hot tub therapy in eight patients with type 2 diabetes (126); however, this study did not include a control condition. Acutely, the effects of passive heating on glucose control remain equivocal (161) with studies showing both no effect using an oral glucose tolerance test (OGTT) (206) and a reduced post-prandial peak glucose concentration following passive heating compared to exercise (92). More recently, chronic (2-8 weeks) have demonstrated that passive heating can reduce fasting insulin and glucose in overweight adults (124) as well as glucose and insulin control during an OGTT in women with PCOS (86).

Table 2.1. Summary of acute and chronic heating studies with measures of endothelial function in humans.

<table>
<thead>
<tr>
<th>First author and year of study</th>
<th>n</th>
<th>Group</th>
<th>Mode of heating</th>
<th>Vascular outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acute studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tinken 2009 (239)</td>
<td>10</td>
<td>Young, healthy males</td>
<td>30 min forearm heating</td>
<td>Improved BA FMD (+3.5%)</td>
</tr>
<tr>
<td>Greyling 2015 (117)</td>
<td>10</td>
<td>Older healthy males</td>
<td>30 min forearm heating</td>
<td>Improved BA FMD (+2.1%)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Older T2D males</td>
<td></td>
<td>Improved BA FMD (+1.7%)</td>
</tr>
<tr>
<td>Brunt 2016 (34)</td>
<td>10</td>
<td>Young, healthy males (5 female)</td>
<td>60 min hot water immersion (chest deep)</td>
<td>No change in BA FMD</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Duration</td>
<td>Intervention</td>
<td>Outcomes</td>
</tr>
<tr>
<td>----------------------</td>
<td>--------------</td>
<td>----------</td>
<td>--------------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Thomas 2016 (237)</td>
<td>Young, healthy (2 females)</td>
<td>30 min hot water immersion (waist deep)</td>
<td>No change in SFA FMD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Older healthy (2 females)</td>
<td>30 min hot water immersion (waist deep)</td>
<td>Decreased aortic PWV (-1.0 m/s)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Older PAD (4 females)</td>
<td></td>
<td>Decreased aortic PWV (-0.5 m/s)</td>
<td></td>
</tr>
<tr>
<td>Bain 2017 (13)</td>
<td>Young, healthy males</td>
<td>60 min water-perfused suit</td>
<td>Decreased EMPs (-30-45%)</td>
<td></td>
</tr>
<tr>
<td>Romero 2016 (209)</td>
<td>Young healthy (5 females)</td>
<td>45 min leg hot water immersion</td>
<td>No change in SFA FMD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Older healthy (5 females)</td>
<td></td>
<td>Improved SFA FMD (+1%)</td>
<td></td>
</tr>
<tr>
<td>Coombs 2018 (56)</td>
<td>Healthy (5 females)</td>
<td>60 min leg hot water immersion</td>
<td>No change in SFA FMD or BA FMD or aortic PWV</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SCI (5 females)</td>
<td></td>
<td>No change in EMP</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No change in SFA FMD or BA FMD or aortic PWV</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Decreased EMPs (-62%)</td>
<td></td>
</tr>
</tbody>
</table>
### Acute studies (continued)

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample Size</th>
<th>Intervention</th>
<th>Duration</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laukkanen 2018 (152)</td>
<td>102</td>
<td>CVD risk factor (46 females)</td>
<td>30 min Finnish sauna</td>
<td>Decreased aortic PWV (-1.2 m/s)</td>
</tr>
<tr>
<td>Cheng 2019 (46)</td>
<td>10</td>
<td>Young, healthy males</td>
<td>10 min forearm heating</td>
<td>Improved FMD (+2.6%)</td>
</tr>
<tr>
<td>Gravel 2019 (108)</td>
<td>21</td>
<td>Older, healthy (11 females)</td>
<td>10 min Finnish sauna</td>
<td>No change in BA FMD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20 min Finnish sauna</td>
<td>No change in BA FMD</td>
</tr>
<tr>
<td>Gravel 2020 (107)</td>
<td>22</td>
<td>Older, CAD (2 females)</td>
<td>20 min Finnish sauna</td>
<td>Improved BA FMD (+1.2%)</td>
</tr>
</tbody>
</table>

### Chronic studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample Size</th>
<th>Population</th>
<th>Intervention</th>
<th>Duration</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imamura 2001 (130)</td>
<td>25</td>
<td>Males with CVD risk factor</td>
<td>30 min IR sauna/day x 2 weeks</td>
<td>Improved BA FMD (+1.8%)</td>
<td></td>
</tr>
<tr>
<td>Kihara 2002 (142)</td>
<td>20</td>
<td>Older CHF (8 females)</td>
<td>15 min IR sauna/day x 2 weeks</td>
<td>Improved BA FMD (+1.3%)</td>
<td></td>
</tr>
<tr>
<td>Green 2010 (112)</td>
<td>10</td>
<td>Young, healthy males</td>
<td>30 min forearm heating 3/week x 8 weeks</td>
<td>Improved NO-dependent SkBF</td>
<td></td>
</tr>
<tr>
<td>Naylor 2011 (179)</td>
<td>9</td>
<td>Young, healthy males</td>
<td>30 min forearm heating 3/week x 8 weeks</td>
<td>Improved BA FMD (+2%)</td>
<td></td>
</tr>
<tr>
<td>Carter 2014 (42)</td>
<td>10</td>
<td>Young, healthy males</td>
<td>30 min leg hot water immersion 3/week x 8 weeks</td>
<td>Improved BA FMD (+2.5%)</td>
<td></td>
</tr>
<tr>
<td>Study (Year)</td>
<td>Age</td>
<td>Gender</td>
<td>Intervention</td>
<td>Outcome(s)</td>
<td></td>
</tr>
<tr>
<td>-------------</td>
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<td>--------------</td>
<td>------------</td>
<td></td>
</tr>
<tr>
<td>Carter 2014 (41)</td>
<td>10</td>
<td>Young, healthy males</td>
<td>30 min leg hot water immersion, 3/week x 8 weeks</td>
<td>Improved NO-dependent SkBF</td>
<td></td>
</tr>
<tr>
<td>Sobajima 2015 (227)</td>
<td>49</td>
<td>Older CHF (18 females)</td>
<td>45 min Waon therapy/day x 3 weeks</td>
<td>Improved BA FMD (+1.5%)</td>
<td></td>
</tr>
<tr>
<td>Bailey 2016 (12)</td>
<td>18</td>
<td>Young, healthy females</td>
<td>30 min hot water immersion, 3/week x 8 weeks</td>
<td>Improved BA FMD (+1.7%)</td>
<td></td>
</tr>
<tr>
<td>Brunt 2016 (32)</td>
<td>18</td>
<td>Young, sedentary (10 females)</td>
<td>60 min hot water immersion, 4-5/week x 8 weeks</td>
<td>Improved NO-dependent SkBF</td>
<td></td>
</tr>
<tr>
<td>Brunt 2016 (33)</td>
<td>20</td>
<td>Young, sedentary (12 females)</td>
<td>60 min hot water immersion, 4-5/week x 8 weeks</td>
<td>Improved BA FMD (+5.3%) Decreased aortic PWV (-1 m/s)</td>
<td></td>
</tr>
<tr>
<td>Ely 2019 (87)</td>
<td>18</td>
<td>Obese women with PCOS</td>
<td>60 min hot water immersion, 4/week x 8 weeks</td>
<td>Improved BA FMD (=3.9%) No change in aortic PWV</td>
<td></td>
</tr>
<tr>
<td>Akerman 2019 (4)</td>
<td>11</td>
<td>Older PAD (4 females)</td>
<td>30 min hot water immersion, 3-5/week x 12 weeks</td>
<td>No change in BA FMD</td>
<td></td>
</tr>
</tbody>
</table>
2.5 Summary of Literature Review

Heat stress evokes many cardiovascular and molecular responses that can acutely improve vascular function (Figure 2.9). If the heat stress is repeated over time, the majority of studies show favourable vascular adaptations. However, this finding is not universal and the exact mechanisms leading to improved vascular function are unclear. The acute and chronic studies using heat as a therapeutic tool for vascular function are summarized in Table 2.1. It is noteworthy that there is little consistency between modes of heating and very few studies that have included clinical populations. Therefore, it is important to identify the factors (e.g., shear stress, skin temperature, core temperature, increased cardiac output) that mediate vascular improvements in response to heat stress. The knowledge gained from these studies will help to build an evidence base that can be used to prescribe certain types of heat therapy for vascular improvement. Moreover, understanding the extraneous factors that might obscure or lead to contradictory outcomes (e.g., sympathetic vascular restraint) will provide data that can inform experimental design of future studies. Evaluating the safety and potential value of passive heating as a therapy in certain patients groups (e.g., SCI) is a priority to determine whether longer term interventions might improve vascular function and possibly quality of life in these groups. Finally, because passive heating may have a greater impact in those with some level of
vascular dysfunction (209), we conducted an exploratory analysis to determine whether better endothelial function mediates lower CVD risk with habitual sauna bathing. These gaps in our current understanding were addressed in four experimental chapters of this thesis that explored both within and between group responses to heat stress in healthy adults (young and older) as well as in various states of disease (e.g., SCI and CAD). The specific objectives of each chapter are as follows.

2.6 Objectives

1) Quantify the independent roles of local skin and body core temperatures on endothelial function following acute passive heat stress.

2) Investigate the effect of sympathetic activation following acute increases in core body temperature on endothelial function and cerebral blood flow.

3) Determine the effect of acute limb heating on endothelial function and circulating biomarkers of endothelial health in a group with low exercise capacity (e.g., SCI) compared to uninjured controls.

4) Investigate whether habitual exposures to heating (e.g., sauna) improves endothelial function in older adults with and without CAD.

2.7 Hypotheses

1) Skin and core temperatures will both increase endothelial function following acute heating independently of shear stress.
2) Endothelial function will increase following elevations in body core temperature with systemic adrenergic blockade, but not during placebo due to sympathetic restraint (i.e., increased sympathetic vascular tone).

3) Acute lower limb heating will improve endothelial function and reduce circulating biomarkers of endothelial damage in individuals with SCI to a greater extend than in uninjured individuals.

4) Endothelial function will be greater in a group of individuals who partake in habitual sauna bathing compared to those who do not partake in sauna bathing, and this difference will be augmented in patients with CAD.
Chapter 3: Distinct contributions of skin and core temperatures to flow-mediated dilation of the brachial artery following passive heating

3.1 Background

Recent reports from large-scale, prospective population studies suggest that regular exposure to passive heat stress via various methods (e.g., sauna, hot tubs) is associated with lower risk of cardiovascular diseases (CVD) and related mortality (150, 252). Several studies have investigated potential physiological mechanisms underlying the reduction in CVD risk with repeated heat exposures (33, 35, 124, 152). For example, Brunt et al. (33) demonstrated in a sham-controlled study that blood pressure and arterial stiffness were reduced and endothelial-dependent dilation (flow-mediated dilation, FMD) was improved following eight weeks of repeated whole-body hot water immersion via hot tub.

The precise mechanisms mediating improved cardiovascular function are unclear in part due to the difficulty of isolating the effects of temperature from the associated hemodynamic responses. Acutely, regional limb heating improves endothelial function of the brachial artery (46, 239) and whole-body heating additionally reduces blood pressure and aortic stiffness (108, 152). However, lower limb/body heating is often accompanied by increased core temperature (56, 209, 238) confounding interpretation of underlying mechanisms. The importance of shear stress as a hemodynamic stimuli has been highlighted (114) and it is widely reported as the main stimulus for heat-induced vascular adaptation (46, 209, 238). Some studies have used pneumatic cuff inflation around one limb to reduce regional flow with the aim of attenuating the increase in shear rate compared to the contralateral limb. For example, using this experimental paradigm, Tinken et al. (239) observed greater brachial artery FMD in the non-cuffed arm immediately following acute forearm heating thereby demonstrating a role for shear rate in vascular adaptations to passive heating. This finding was further supported by subsequent studies with chronic repeated increases...
in local shear rate (179) and moderate (+0.6°C) increases in core temperature (42); however, the FMD response of the brachial artery to acute and large elevations in core temperature remains unclear. Although both shear rate and skin temperature are involved in adaptation to eight weeks of local forearm heating in the cutaneous microvasculature (41), the interaction of local shear rate, skin temperature, and core temperature on brachial artery endothelium-dependent dilation requires further investigation. Indeed, rises in skin temperature increase local shear stress, but increased core temperature imposes a greater systemic cardiovascular stress similar to mild exercise, such that a larger cumulative increase in shear stress occurs.

Therefore, the primary aim of this study was to determine the thermal and hemodynamic factors that contribute to vascular responses to acute heat stress. To delineate these interactions, using a within-subject design, we bilaterally assessed vascular function in the brachial artery before and after: 1) bilateral local forearm heating (to increase shear rate and skin temperature); 2) whole-body heating (to increase shear rate, skin and core temperatures); and 3) thermoneutral time control. During each condition, forearm blood flow (and therefore shear rate) was attenuated to one limb via forearm cuff inflation. It was hypothesized that FMD will be greater following passive heating in a shear-dependent manner, and that the increases in FMD will be greater during whole-body heating compared to forearm heating, and in the non-cuffed arm compared to the cuffed arm. Furthermore, we hypothesized that FMD will be greater following forearm heating compared to whole-body heating in the cuffed arm due to higher skin temperature. As previously established, we also anticipated that FMD will be reduced in the time control condition where greater retrograde and low mean shear stress will be a result of immobilization (204) and cuff inflation (234).
3.2 Methods

3.2.1 Participants

Based on recently published data (46) reporting improvements in FMD after forearm heating [5.8% standard deviation (SD) = 2.2 vs. 8.4% SD=3.6], an a priori sample size estimate (α = 0.05, β = 0.80) using G*Power version 3.1 (University of Dusseldorf, Dusseldorf, Germany) required a minimum of 11 participants for this study (91). We therefore recruited 12 white male volunteers to account for potential loss of data inherent with vascular ultrasound. All participants [age: 25 (SD=5) years; BMI: 23.1 (2.6) kg/m²; \( \dot{V}O_{2\text{peak}} \): 41 (8) mg/kg/min] completed the study and provided written, informed consent prior to any experimental measures. Approval of the experimental protocol was obtained from the Clinical Research and Ethics Board at the University of British Columbia (H17-01817) and all procedures conformed to the Declaration of Helsinki, except for registration in a database. None of the participants reported a history of cardiovascular, respiratory, metabolic, or neurological disease and were not taking any medication for such conditions.

3.2.2 Measurements

Prior to baseline measurements, participants were instrumented with general purpose probes (RET-1, Physitemp Instruments, Clifton, New Jersey, USA) inserted ~40 cm into the esophagus for measurement of esophageal temperature (T_{es}). During the insertion of the esophageal probe, ~500 mL of water was consumed for participant comfort and to ensure euhydration prior to baseline measures. Skin temperature (T_{sk}) probes (MLT422/A, ADInstruments, Colorado Springs, CO, USA) were placed on the ventral surface of each forearm and secured with medical tape. Heart rate (HR) was monitored via lead II electrocardiogram and peripheral blood pressure was measured via automated brachial artery auscultation (BP5100, 36
Omron Healthcare Canada, Burlington, ON, Canada). A segmental cuff (SC5, Hokanson, Bellevue, WA, USA) was placed immediately distal to the elbow and antecubital IV catheter on the right arm to attenuate increases in blood flow to that arm. Core and skin temperature probes were interfaced with a data acquisition system (PowerLab 16/35, ADI) via thermistor T-type pods, respectively (ML309 and ML312, ADI), while the ECG signal was connected via a Dual Bio Amp (FE232, ADI).

All vascular sonography was performed using high resolution duplex ultrasound (Terson uSmart 3300/T3200, Teratech, Burlington, MA) interfaced with a 10-MHz linear array transducer (15L4 Smart Mark, Teratech). An angle of insonation of 60° was maintained throughout all scans. The same experienced sonographer performed all scans on the same arm during each trial for each participant. Endothelial-dependent dilation was assessed via FMD according to international guidelines (232, 233), which consisted of a 1-min baseline recording of brachial artery diameter and velocity followed by a forearm occlusion period of 5 min using rapid cuff inflation ≥220 mmHg. During occlusion recording was paused but imaging of the vessel was continued to ensure consistent location and angle of the image. Prior to the end of the occlusion period, recording was resumed and the cuff was rapidly deflated to induce reactive hyperemia while the recording was continued for 3 min. At each time point of the protocol, recordings of brachial artery diameter and velocity were performed for ~1 min or ≥20 cardiac cycles. Recordings were made using screen capture software (Camtasia Studio, TechSmith, Okemos, MI, USA) and saved for offline analysis.

3.2.3 Experimental protocol

The participants visited the laboratory for one preliminary visit where \( \dot{V}O_2 \) peak was determined while upright cycling. The protocol consisted of a 2-min warm up at 50 W with subsequent increases of 20 W every minute at a rate of 0.33 W/s until volitional exhaustion,
inability to maintain >50 RPM, or a plateau in oxygen consumption determined via expired gases (Vmax Encore Metabolic Cart, Carefusion, San Diego, CA, USA). The study consisted of three separate visits to the laboratory for experimental sessions. All participants arrived between 07:00 and 09:00 AM (except one at 12:00 PM) after ≥6 hours of fasting, having avoided alcohol and strenuous exercise for 24 hours and caffeine for 12 hours. The start time of the protocol was consistent within each participant for all visits, with at least 48 hours between sessions. After instrumentation, the participants rested supine for 20 min before baseline measures were performed, which included three blood pressure recordings on the left arm (i.e., non-cuffed) followed by simultaneous assessment of FMD on both arms. The participant was then raised into a semi-recumbent position on the bed and rested quietly for 5 min before three more blood pressure recordings, a 1-min brachial artery ultrasound recording. These measures were repeated halfway and at the end of the protocol. Immediately prior to commencing the experimental intervention, the cuff around the right forearm was inflated and maintained at ~90 mmHg throughout to attenuate the increase in blood flow during heating (41, 42, 179) and to induce disturbed flow patterns during the time control session (132, 234).

On the whole-body heating day, the participant donned a water-perfused suit (Med-End, Ottawa, ON, Canada) covering the entire skin surface area except for the head, feet, and arms below the shoulder. A Coghlan’s emergency blanket was placed on top to prevent any further heat losses. The suit was circulated with water maintained at 49°C using two heaters (A2.2-120V-US Sous Vide, Anova, San Francisco, CA, USA) and a magnetic drive pump (WMD-20RLT-492GPH, Iwaki America, Holliston, MA, USA) with the aim of increasing esophageal temperature by 1.5°C. Forearm heating was performed with the same method while only covering the forearms (including the occlusion cuff) with the aim of rapidly increasing forearm $T_{sk}$ to ~38°C. The whole-body and
forearm heating sessions were completed in a counterbalanced order to match the duration of whole-body heating (approximately 60 min) as closely as possible. The time control sessions were completed last in order to match the durations of the heating sessions. All trials were time-matched to the first visit for each participant to ensure there was no intra-individual variation in protocol duration. The forearm heating and time control sessions were performed wearing regular clothing (i.e., pants and t-shirt). Core temperatures were not measured during the time control because it is not expected that core temperature will change in a thermoneutral environment (33) and to maintain participant comfort. At the end of the intervention, the participant was returned to the supine position for 10 min prior to the final FMD measurement.

3.2.4 Data analysis

All temperature data and heart rate were sampled at 400 Hz from PowerLab into LabChart Pro software (Version 7, ADI); data are reported as 5-min averages. At each time point, blood pressure measurements were averaged and mean arterial pressure (MAP) was calculated as the sum of two thirds of diastolic pressure and one third of systolic pressure (Equation 3.1).

\[
\text{MAP} = \frac{1}{3} \times \text{SBP} + \frac{2}{3} \times \text{DBP} \text{ (mmHg)}
\]  \hspace{1cm} (Eq. 3.1)

The saved ultrasound recordings were analyzed using semi-automated edge detection software (FMD/BloodFlow, version 5.1, Reed C, Perth, WA, Australia) (270). Regions of interest were placed around the highest quality portion of the B-mode longitudinal image of the artery and the Doppler tracing of blood velocity. This software tracks the vessel walls and peak envelope velocity within their respective regions at 30 Hz. The product of calculated cross-sectional area (CSA; Equation 3.2) and mean blood velocity (MBV; Equation 3.3) were used to determine brachial blood flow (Equation 3.4).
CSA = \pi (0.5 \times \text{diameter})^2 \text{ (cm}^2\text{)} \quad \text{(Eq. 3.2)}

MBV = \left(\frac{\text{peak envelope velocity}}{2}\right) \times 60 \text{ (s/min)} \quad \text{(Eq. 3.3)}

Flow = \text{CSA} \times \text{MBV} \text{ (ml/min)} \quad \text{(Eq. 3.4)}

Diameter post-cuff occlusion was measured automatically using a 3-second moving window-smoothed average where the maximum median value was determined as the peak diameter and FMD was then calculated as the absolute and relative difference between peak and baseline diameters (Equation 3.5). Baseline for the post whole-body heating condition was considered the last 30 s of cuff occlusion considering the influence the large shear stimulus on pre-occlusion diameter. Post-cuff occlusion diameter and velocity were interpolated from 30 Hz into 3-s bins where peak reactive hyperemia was determined as an index of forearm microvascular function (211).

\[
\text{FMD} = \left(\frac{\text{peak} - \text{baseline diameter}}{\text{baseline}}\right) \times 100 \text{ (\%)} \quad \text{(Eq. 3.5)}
\]

Shear rates (SR) were estimated with Equation 3.6 using antegrade, retrograde, and mean blood velocities. The oscillatory shear index (OSI) was calculated per Equation 3.7.

\[
\text{SR} = 4 \times \text{MBV} / \text{artery diameter} \text{ (s}^{-1}) \quad \text{(Eq. 3.6)}
\]

\[
\text{OSI} = \frac{|\text{retrograde SR}|}{(|\text{antegrade SR}| + |\text{retrograde SR}|)} \text{ (au)} \quad \text{(Eq. 3.7)}
\]
Figure 3.1. Experimental set up demonstrating the location of ultrasound measurements of the brachial artery and the skin surface areas covered by the water-perfused suit in black. Created with BioRender.com.
3.2.5 Statistical analyses

All data are presented as means (SD). Data were analyzed using two factor linear mixed models with a compound symmetry covariance structure where time (pre/mid/post intervention) and arm (cuffed/non-cuffed limb) were repeated variables. Central hemodynamics and $T_{es}$ were compared between trials, whereas the forearm $T_{sk}$ and vascular responses were compared within trials only. A Bonferroni correction was used for multiple comparisons between main effects. When significance ($\alpha = 0.05$) was observed, simple main effects were determined with post hoc testing using a paired sample t-test with a Holm-Bonferroni correction. The model for FMD was run with logged changes in diameter as the dependent variable and baseline diameter and SRAUC as covariates. Corrected group means and SD for FMD were back calculated from the estimated means (EM) and standard errors of the model using the formula: $(e^{EM} - 1) \times 100$ (10, 223). The delta values for FMD between conditions were compared with a one-way ANOVA. Prior to correlational analyses, the data were screened for normality by calculating skewness and kurtosis, where values within $\pm 2.00$ were considered acceptable. Repeated measures correlations were performed between selected variables ($T_{es}$, forearm $T_{sk}$, HR, antegrade SR, retrograde SR, and OSI) and the change in uncorrected FMD using the rmcorr package for R (16) to determine the influence of intra-individual changes of the independent variables on FMD. A standard multiple linear regression analysis with the aforementioned independent variables was then performed on the change in uncorrected FMD values during each heating intervention. The absolute value of each standardized $\beta$ coefficient from the model was used to calculate the relative contribution of each independent variable as the quotient of $\beta$ for a given variable and the sum of $\beta$ coefficients from all variables [e.g., $\beta_a / (\beta_a + \beta_b + \beta_c) \times 100$] (255). To avoid unacceptable multicollinearity, only independent variables with tolerance $>$0.1 were accepted. Our Durbin-Watson value of 1.96
indicates that there is no autocorrelation between observations. All statistical analyses were performed in either SPSS version 24 (IBM, Armonk, NY, USA) or R, and figures 1-3 were generated with GraphPad version 6.0 (Prism, La Jolla, CA, USA).

3.3 Results

3.3.1 Central hemodynamics and thermometry

Heart rate, MAP, and T<sub>es</sub> are presented in Table 3.1. There was a time by arm interaction for heart rate (P<0.01), with greater increases during whole-body heating compared to forearm heating and time control. There were no differences in MAP between conditions or before and after heating (interaction P=0.83). There was also a time by arm interaction for T<sub>es</sub> (P<0.01), which increased more during whole-body heating [Δ1.3 (0.25)°C] compared to forearm heating [Δ0.11 (0.19)°C]. Skin temperatures are presented in Figure 3.2. During forearm heating, there was a main effect of time for forearm T<sub>sk</sub> (P<0.01) which increased by ~8°C on both arms with no time by arm interaction (P=0.424). During whole-body heating, there was a time by arm interaction (P=0.01) where forearm T<sub>sk</sub> increased by ~3°C in the non-cuffed arm compared to 0.8°C in the cuffed arm. During the time control, there was a time by arm interaction (P=0.02) where forearm T<sub>sk</sub> decreased by ~1.5°C in the cuffed arm but not in the non-cuffed arm.
### Table 3.1. Core temperature and cardiovascular variables.

<table>
<thead>
<tr>
<th></th>
<th>Whole-body heating</th>
<th></th>
<th>Forearm heating</th>
<th></th>
<th>Time control</th>
<th>ANOVA P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Mid</td>
<td>End</td>
<td>Pre</td>
<td>Mid</td>
<td>End</td>
</tr>
<tr>
<td><strong>Te</strong> (°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>36.99</td>
<td>37.70</td>
<td>38.28</td>
<td>36.98</td>
<td>36.99</td>
<td>37.10</td>
</tr>
<tr>
<td></td>
<td>(0.28)</td>
<td>(0.29)*</td>
<td>(0.50)*</td>
<td>(0.31)</td>
<td>(0.28)</td>
<td>(0.27)</td>
</tr>
<tr>
<td><strong>HR (bpm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>55 (8)</td>
<td>84 (17)*†</td>
<td>95 (16)*†</td>
<td>57 (8)</td>
<td>62 (9)</td>
<td>64 (11)*†</td>
</tr>
<tr>
<td></td>
<td>84 (7)</td>
<td>80 (9)</td>
<td>83 (7)</td>
<td>84 (4)</td>
<td>84 (6)</td>
<td>84 (6)</td>
</tr>
<tr>
<td><strong>MAP (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>84 (7)</td>
<td>80 (9)</td>
<td>83 (7)</td>
<td>84 (4)</td>
<td>84 (6)</td>
<td>84 (6)</td>
</tr>
</tbody>
</table>

Te, esophageal temperature; HR, heart rate; MAP, mean arterial pressure. Data are mean (SD). Te was not measured during time control for participant comfort. *P<0.05 vs. forearm heating. †P<0.05 vs. time control.
Figure 3.2. Forearm skin temperatures during each intervention in the cuffed and non-cuffed arms. *P<0.05 vs. contralateral arm.
3.3.2 Brachial artery hemodynamics

During whole-body heating, blood flow in the brachial artery increased more (interaction \( P<0.01 \)) in the non-cuffed arm [24 (13) to 240 (114) ml/min] compared to the cuffed arm [23 (10) to 115 (75) ml/min]. During forearm heating, brachial blood flow also increased more (interaction \( P<0.01 \)) in the non-cuffed arm [31 (28) to 142 (77) ml/min] compared to the cuffed arm [30 (19) to 59 (37) ml/min]. Brachial blood flow decreased more (interaction \( P=0.03 \)) during the time control protocol in the cuffed arm [32 (20) to 7 (12) ml/min] compared to the non-cuffed arm [42 (31) to 36 (24) ml/min].

Antegrade and retrograde SR responses are presented in Figure 3.3. During forearm heating, there was a time by arm interaction for antegrade SR (\( P<0.01 \)) where the cuffed arm increased by 57\% and the non-cuffed arm increased by 240\%. There was a time by arm interaction for retrograde SR (\( P<0.01 \)) where the cuffed arm increased by 217\% and the non-cuffed arm decreased by 99\%. There were also time by arm interactions during whole-body heating (both \( P<0.01 \)) for both antegrade and retrograde SR. In the cuffed arm, antegrade SR increased by 400\% and retrograde SR increased by 130\%. In the non-cuffed arm, antegrade SR increased by 641\% and retrograde SR decreased by 466\%. During the time control, antegrade SR did not change (\( P=0.52 \)), whereas there was a time by arm interaction for retrograde SR (\( P<0.01 \)). Retrograde SR increased in the cuffed arm by 488\% and by 33\% in the non-cuffed arm.
Figure 3.3. Antegrade (upper panels) and retrograde (lower panels) shear rates during each intervention in the cuffed and non-cuffed arms. *P<0.05 vs. contralateral arm.
3.3.3 Flow-mediated dilation

Brachial artery characteristics before and after each intervention are presented in Table 3.2. Individual values as well as allometrically scaled and shear-corrected mean FMD values are presented in Figure 3.4. There was a main effect of time where FMD increased by 1.5-2 percentage points in both arms after forearm heating (P<0.01). There was a main effect of time (P<0.01) where whole-body heating increased FMD by 5.5 percentage points in the non-cuffed arm 3 percentage points in the cuffed arm. There was a main effect of time where FMD decreased in both arms after the time control (P=0.03) by ~1.5 percentage points. In the non-cuffed arm, the change in FMD was greater with whole-body heating compared to forearm heating (P<0.01) and time control (P<0.01); however, forearm heating tended to induce a greater change in FMD compared to time control (P=0.06). In the cuffed arm, the change in FMD with whole-body heating was greater than time control (P<0.01) but not that induced by forearm heating (P=1.0); however, forearm heating induced a greater change in FMD compared to time control (P=0.04).
Figure 3.4. Flow-mediated dilation (FMD) before and after each intervention in the cuffed and non-cuffed arms. Upper panels display actual values (individual values in symbols and group means in grey bars) and the lower panels display the group means corrected for baseline diameter and the shear stimulus (i.e., SRAUC). *P<0.05 vs. pre-intervention. δP<0.05 vs. cuffed arm.
3.3.4 Reactive hyperemia

Post-occlusion peak reactive hyperemia (RH) values are presented in Table 3.2. There was a main effect of time (P<0.01) where peak RH increased by 33 and 23% in both the cuffed and non-cuffed arms, respectively, after forearm heating. There was also a main effect of time (P<0.01) where whole-body heating increased peak RH by 37 and 53% in both the cuffed and non-cuffed arms, respectively. During time control, there was a main effect of arm only (P<0.01) where peak RH was 23% higher in the non-cuffed arm compared to the cuffed arm.
Table 3.2. Brachial artery baseline characteristics and endothelium-dependent dilation before and after each intervention.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Whole-body heating</th>
<th>Forearm heating</th>
<th>Time Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arm</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td><strong>Baseline Diameter</strong> (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>3.97 (0.62)</td>
<td>4.28 (0.69)*</td>
<td>4.10 (0.68)</td>
</tr>
<tr>
<td>NC</td>
<td>3.93 (0.42)</td>
<td>4.12 (0.38)*</td>
<td>3.96 (0.32)</td>
</tr>
<tr>
<td><strong>FMD (mm)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.21 (0.10)</td>
<td>0.35 (0.17)*</td>
<td>0.18 (0.09)</td>
</tr>
<tr>
<td>NC</td>
<td>0.14 (0.09)</td>
<td>0.37 (0.12)*</td>
<td>0.20 (0.11)</td>
</tr>
<tr>
<td><strong>FMD (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>5.6 (3.0)</td>
<td>8.6 (4.9)</td>
<td>4.7 (2.9)</td>
</tr>
<tr>
<td>NC</td>
<td>3.6 (2.2)</td>
<td>9.2 (3.2)</td>
<td>5.1 (2.8)</td>
</tr>
<tr>
<td><strong>Corrected FMD (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>5.9 (3.5)</td>
<td>8.9 (3.1)*</td>
<td>3.8 (0.7)</td>
</tr>
<tr>
<td>NC</td>
<td>4.1 (3.1)</td>
<td>8.7 (3.5)*</td>
<td>4.6 (2.4)</td>
</tr>
<tr>
<td><strong>SRAUC</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>15855 (10256)</td>
<td>32797 (16922)*</td>
<td>20031 (9988)</td>
</tr>
<tr>
<td>NC</td>
<td>18568 (5564)</td>
<td>47399 (17470)*†</td>
<td>23644 (8267)†</td>
</tr>
<tr>
<td><strong>Peak RH (ml/min)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>247 (84)</td>
<td>338 (130)*</td>
<td>248 (68)</td>
</tr>
<tr>
<td>NC</td>
<td>258 (91)</td>
<td>395 (128)*</td>
<td>285 (84)</td>
</tr>
</tbody>
</table>

Data are mean (SD). Post hoc Bonferroni corrections: *P<0.05 vs. pre-intervention, †P<0.05 vs. contralateral arm. Abbreviations: C, cuffed; NC, Non-cuffed; FMD, flow-mediated dilation corrected for baseline diameter and shear stimulus; SRAUC, shear rate area under the curve; RH, reactive hyperemia.
### 3.3.5 Correlations of selected variables and regression analyses

The changes in FMD pooled from all three trials demonstrated significant positive correlations with changes in $T_{es}$, $T_{sk}$, HR, and antegrade SR, whereas there was a significant negative correlation with OSI and no correlation with retrograde SR (Figure 3.5). The multiple regression model (adjusted $r^2 = 0.421$, $P < 0.001$) shows that the relative contributions to the explained variance were 33.6% for $T_{es}$, 31.1% for forearm $T_{sk}$, 21.1% for HR, 12.2% for antegrade SR, 1.4% for retrograde SR, and 0.70% for OSI (Table 3.3).
**Figure 3.5.** Repeated measures correlations between selected variables on the x-axes and the change in FMD following each intervention. The circles represent individual participants and the dashed black line is the group average regression. $T_{es}$, esophageal temperature; $T_{sk}$, forearm skin temperature; HR, heart rate; Ante SR, antegrade shear rate; Retro SR, retrograde shear rate; OSI, oscillatory shear index.
3.4 Discussion

The purpose of this study was to determine the thermal and hemodynamic factors that contribute to vascular responses to acute heat stress. The main findings were that: 1) FMD improved following whole-body passive heating in the non-cuffed arm from 3.6 (2.2) to 9.2 (3.2)\% and in the cuffed arm from to 5.6 (3.0)\% to 8.6 (4.9)\%, irrespective of lower shear rates in the cuffed vs. non-cuffed arms; 2) FMD improved in the cuffed arm from 4.7 (2.9) to 6.8 (1.5)\% and in the non-cuffed arm from 5.1 (2.8) to 6.4 (2.6)\% following forearm heating-induced rises in skin temperature, irrespective of lower shear rates in the cuffed vs. non-cuffed arms; and 3) FMD decreased following a time-matched control condition where mean shear rates were reduced. Overall, in a multiple linear regression model with independent variables of both thermal and hemodynamic nature (Table 3.3), T_{es}, forearm T_{sk}, and HR were the largest predictors of the change in FMD during 60 min of whole-body or local heating and a time control period.

Our findings of increased FMD with forearm heating is consistent with existing literature in young adults (46, 239); however, the improved FMD with whole-body heating is a novel observation. The difference between the current study and others likely relates to differences in measured limbs (e.g., arm vs. leg) (34, 209, 237) and recovery time (e.g., immediate in the current study vs. 30-60 min in others) (34, 108, 209, 238). Previous studies have also demonstrated that increased shear stress is important for vascular adaptation with acute (239) and chronic forearm heating (179) as well as repeated and episodic increases in core temperature (42). The obligatory role of shear stress was demonstrated using similar experimental designs to the current study where increases in shear rates were attenuated in one arm via cuff inflation, and improvements in FMD were only observed in the non-cuffed arm. For instance, in the cutaneous circulation, both increases in skin blood flow (non-cuffed arm only) and skin temperature contribute to vascular
adaptation (41). However, the impact of changes in skin temperature on conduit arteries has not been previously investigated. Moreover, improved FMD in older but not young adults following local heating of the legs occurs alongside increased core temperature (209); therefore, direct comparison of increases in skin vs. core temperature is necessary in an attempt to delineate these mechanisms. Our current data reveal that attenuation of shear rates did not modify FMD for a given heating stimulus. For example, the increase in FMD following whole-body heating was not different whether shear rates were permitted to fully increase or not. The fact that – in the non-cuffed arm – the increase in FMD 2.5-fold greater following whole-body heating compared to forearm heating (P<0.01) further indicates an independent influence of core temperature on FMD (Figure 3.4).

The similar FMD responses to matched increases in forearm $T_{sk}$ but differing shear rates during forearm heating, suggest an independent influence of forearm $T_{sk}$ on FMD. However, $T_{es}$ explained the largest portion of variance of the multiple regression model (~34%), which is consistent with greater changes in FMD after whole-body heating. Increased bioavailability of nitric oxide (NO) due to high temperature (141, 169) also appears important for both macro and microvascular function (Table 3.2). The view that greater NO bioavailability drives increases in FMD is supported by in vivo cutaneous microvascular and in vitro studies. For example, inhibition of endothelial nitric oxide synthase (eNOS) with N-ω-nitro-l-arginine (L-NNA) abolished improvements in cutaneous microvascular dilator function following eight weeks of whole-body heating (32) as well as angiogenesis of endothelial cells cultured with serum taken from the same study participants (35). In that study, the increased endothelial tubule formation (i.e., angiogenesis) was related to greater abundance of eNOS protein (35). This finding suggests that circulating factors are integral to NO-mediated improvements in vascular function. Conversely, FMD was
attenuated with increased retrograde shear rates or decreased mean shear rates in the time control condition of the current study (Figure 3.4). These results support a role for shear stress in determining the hemodynamic milieu and predominant cell phenotype (49, 132, 148, 243).

Considering that the increase in forearm Tsk in the non-cuffed arm was about half of that during forearm heating, the larger improvement in FMD is likely due to the ~660% increase in antegrade SR in whole-body heating compared to the ~250% increase during forearm heating (Figure 3.3). However, the influence of SR on FMD is limited by design in the multiple regression model due to the attenuated SR in the cuffed arm. In fact, shear rates were not a major contributor to the explained variance of the model (~12%), but this should be interpreted cautiously considering the purpose of this statistical model was to quantify the contributions to FMD from factors other than shear stress. In the absence of appreciable changes in stroke volume during passive heat stress (60), changes in cardiac output are mediated by HR, and the change in HR explained ~21% of the model (Table 3.3). Moreover, the change in HR was related to the change in FMD (r=0.58, Figure 5C). As a result, whole-body heating appears to be a more potent stimulus for eliciting increased systemic shear stress (and potentially circulating factors related to temperature (36)), due to the greater increases in core temperature and HR (i.e., cardiac output). Indeed, whole-body heating increased HR by 40 bpm in comparison to an increase of 7 bpm during forearm heating (Table 3.1). Because shear stress increases with cardiac output, the obligatory rise in shear for vascular adaptation – which has been demonstrated many times (42, 110, 112, 116, 179) – should not be discounted or diminished in this study. Indeed, the primary outcome of this study is the change in FMD during our experimental manipulations of forearm Tsk and core temperature. Undue importance should not be placed on the outcomes of the multiple regression as this was performed in an exploratory attempt to determine significant contributors.
Table 3.3 Multiple regression model for changes in FMD across whole-body heating, forearm heating, and time control.

<table>
<thead>
<tr>
<th></th>
<th>Unstandardized β</th>
<th>SE</th>
<th>P value</th>
<th>Tolerance</th>
<th>Explained Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔTes (°C)</td>
<td>2.178</td>
<td>1.518</td>
<td>0.175</td>
<td>0.194</td>
<td>33.6%</td>
</tr>
<tr>
<td>ΔTsk (°C)</td>
<td>0.318</td>
<td>0.129</td>
<td>0.018</td>
<td>0.723</td>
<td>31.1%</td>
</tr>
<tr>
<td>ΔHR (bpm)</td>
<td>0.044</td>
<td>0.055</td>
<td>0.427</td>
<td>0.170</td>
<td>20.9%</td>
</tr>
<tr>
<td>ΔAnte shear rate (s⁻¹)</td>
<td>0.002</td>
<td>0.003</td>
<td>0.483</td>
<td>0.388</td>
<td>12.2%</td>
</tr>
<tr>
<td>ΔRetro shear rate (s⁻¹)</td>
<td>0.001</td>
<td>0.017</td>
<td>0.938</td>
<td>0.264</td>
<td>1.40%</td>
</tr>
<tr>
<td>ΔOSI (au)</td>
<td>0.148</td>
<td>4.600</td>
<td>0.974</td>
<td>0.360</td>
<td>0.70%</td>
</tr>
<tr>
<td>Adjusted r²</td>
<td>0.421</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

β, standardized regression coefficients; SE, standard error of the slope coefficient; r², partial contribution to total variance. Tolerance values >0.1 indicate acceptable collinearity.
Brunt et al. (36) showed that both temperature per se and circulating factors reduced basal superoxide production, whereas only temperature upregulated heat shock protein 70 in cultured human endothelial cells. The effects of heat shock proteins on macrovascular function are unclear, but heat shock protein 90, at least, is involved in the activation of eNOS (98, 222). Consistent with the findings of Brunt et al. (36), the current data support a role for a direct effect of temperature in vascular responses to heating. Indeed, $T_{es}$ explained 34% of the variance of the model (Table 3.3) and was related to the change in FMD ($r=0.59$, Figure 3.5). It has also been demonstrated that reactive oxygen species (ROS) impair FMD and prevention of ROS (via vitamin C) can reverse endothelial dysfunction in aging (272). There is evidence to suggest that heat shock protein 70 (which is upregulated by heat) may be protective against inflammatory and ROS responses to in vitro stress (e.g., hypoxia-reoxygenation) (36), and that heat therapy could therefore be protective against ischemia-reperfusion in vivo (34). However, though the role of heat shock proteins requires further research, it has been speculated that upregulation of heat shock proteins in patient groups via heat therapy could be beneficial for insulin resistance and related vascular dysfunction (165, 172).

It has been acknowledged that a better understanding of the factors that cause improvements in vascular function is required in order to prescribe optimal temperatures, durations, and modes of heating (47, 85). Indeed, passive heating has been evaluated in individuals with CVD [e.g., heart failure (142, 227), peripheral artery disease (4)] and those at risk of CVD [e.g., aging (209), coronary risk factors (130), obesity (124), polycystic ovary syndrome (87)]; however, there is little consistency between studies regarding passive heating protocols. Traditionally, heat acclimation protocols aim to increase core temperature above 38.5°C for ≥60 min (95, 229) and this approach has been used in short [e.g., 7 days (18)] and longer term [e.g., 8
weeks (33) studies to induce both thermoregulatory and vascular adaptations, respectively. However, such stringent protocols may be difficult to implement depending on the mode of heating, patient capacity/tolerance, or motivation. Therefore, it is important to determine the primary contributors to vascular adaptation in order to determine how best to modify acclimation parameters (temperature, mode, time) for various groups. We have demonstrated herein that protocols increasing skin temperature and core temperature – and not only shear – are likely to be beneficial. The implications of these observations indicate that interventions increasing skin temperature rapidly (e.g., sauna) but with low tolerance times may also be of benefit to vascular function compared to the traditional prolonged core temperature >38.5°C. However, evaluation of the time required to elicit responses are scarce. For example, as little as 10 to 30 min of forearm heating has been reported to increase FMD in young healthy participants (46, 239), whereas 10 to 20 min of sauna bathing did not change FMD in older healthy people (108). Conversely, 45-60 min of lower leg heating improved femoral artery FMD in older but not young adults (209) and may be of benefit in individuals with limited exercise capacity (e.g., individuals with spinal cord injury) (56). Clearly, the need for additional evidence on the duration and intensity of heating is important, but it is likely that even small doses of heat repeated over time are of benefit for reduction of CVD risk (142, 150).

3.4.1 Experimental Considerations: The inclusion of a time control condition in our study is an important strength. Not only do our data support previous reports of a detrimental impact of retrograde shear rates on endothelial function (132, 234), it also demonstrates that the improvements in FMD during heating are not simply due to time or other factors during our experimental protocol. Although FMD was measured supine before and after heating, hemodynamic responses were performed in a semi-recumbent position at baseline and during
heating to account for the effects of supine posture. Another important consideration in our study is that blood viscosity is a key determinant of shear stress at the arterial wall and this could be influenced by losses in plasma volume due to sweating. For instance, viscosity-related changes in FMD have been observed following removal of ~1 litre of blood volume (244), but the 1-2% loss of plasma volume during passive heat stress via sweating (18) is unremarkable in comparison. Moreover, the effects of hemoconcentration related to sweating are offset by hyperthermia resulting in no change of blood viscosity (38).

3.4.2 Limitations: The shear dependency of blood viscosity is well established (80), but it was recently demonstrated that blood exhibits shear-thinning properties where viscosity decreases at higher shear rates (155). Given that shear rates vary widely in heating protocols, future studies should aim to match the shear rates experienced in the study conditions to those used for viscosity measures using a range of shear rates and an exponential decay model. The current results are only relevant to the brachial artery and the forearm, thus differences could exist between limbs and these data should be replicated in the leg (e.g., superficial femoral, popliteal arteries). Although the use of a water-perfused suit is not a common mode of heating in everyday life, the passive heating of skin temperature resembles that of natural heating modes (e.g., sauna, hot baths) but the medium of heat transfer does not directly mimic air or water. Lastly, but certainly not least, we cannot extrapolate our findings to female participants or other racial groups. Although women demonstrate fluctuations in core temperature depending on the phase of the menstrual cycle (44), it is expected that the hemodynamic changes to heating would not be different to our results. However, sex differences exist in endothelial function following interventions which reduce shear stress (245, 258), so it is entirely possible that women could respond differently to our current protocols. Few studies have addressed racial differences in vascular function but evidence
indicates that cutaneous vasodilation in response to local heating (190) and vascular conductance to forearm exercise (17) are lower in black compared to white men. Further studies are required to replicate our findings in other ethnic and racial groups.

3.4.3 Conclusion: This study demonstrates that increases in forearm skin and core temperatures are important determinants of the vascular responses of the brachial artery to passive heat stress. In addition to heat-induced increases in shear stress, limb heating likely increases local factors such as nitric oxide to improve flow-mediated dilation. Future studies should address different combinations of skin/core temperatures and shear stress, with a range of acute and chronic durations, on vascular outcomes. Future focus should now be directed particularly to populations at risk of CVD and with limited exercise capacity.
Chapter 4: The effects of $\alpha_1$-adrenergic antagonism on peripheral and cerebral vascular tone during whole-body passive heat stress: a pilot study

4.1 Background

During whole-body heating, sympathetic nervous system activity (SNA) increases when core temperature rises in order to initiate cutaneous vasomotor and sudomotor thermoregulatory responses (63–65, 67, 69, 157). In the cutaneous circulation, elevated SNA directed to the skin elicits active cutaneous vasodilation mediated via cholinergic nerves (63, 69, 109). In contrast, functional sympatholysis prevents elevated muscle SNA (101) during heat stress from altering muscle blood flow (78, 134). It is unknown, however, how sympathetic activity influences the conduit vessels (e.g., brachial artery) during heat stress despite evidence that endothelial-dependent dilation of the brachial artery is reduced in normothermia under various conditions where muscle SNA is acutely increased e.g., exercise, lower body negative pressure, hypoxia) (8, 122, 205, 231).

The flow-mediated dilation (FMD) technique has commonly been used to test for improved endothelial-dependent dilation after passive heating (12, 33, 46, 56, 179, 209, 237, 239). These studies were, in part, conducted in order to discern the mechanisms of reduced CVD risk with repeated heat exposures (150, 252). During long-term protocols (e.g., 8 weeks), heat-induced shear stress was found to be an important stimulus for both macro- and microvascular adaptation with chronic repeated exposures to heat (42, 112, 179). However, only three studies have observed acute increases in FMD after heating in young, healthy participants. These studies used local forearm heating and measured FMD immediately in the heated limb (46, 117, 239), whereas other studies that increased core temperature and allowed a recovery period did not observe a change in FMD (56, 209, 238). In an exercise setting, Atkinson et al. (8) demonstrated that FMD was reduced
following acute moderate-intensity exercise (30 min of cycling at 75% of maximum heart rate), but this response was prevented by α-1 adrenergic antagonism (e.g., Prazosin ingestion). Recently, it was reported that acute lower body heating (45 min, +0.2°C core temperature) increased muscle SNA in the radial nerve by 38% for up to 30 min following heating in young adults (89). However, whether similar sympathetic vascular restraint impacts FMD in the brachial artery following whole-body heat stress is unknown.

In the cerebral circulation, a lack of specific cooling capacity (28) necessitates continuous arterial inflow as an avenue of heat dissipation – particularly with the high metabolic rate of the brain which ensures brain blood temperature is always greater than its arterial inflow (162, 186). However, it is consistently reported that cerebral blood flow (CBF) decreases when elevations in core temperature exceed ~0.5°C (15, 31, 90, 99, 181, 246). Thus, hyperthermia-induced reductions in CBF are counterintuitive from a thermoregulatory perspective. These decreases in CBF with heating appear to occur after a threshold where hyperventilation occurs (90, 247). Whether maintenance of isocapnia during heating completely (15, 181) or partially restores CBF with increased core temperature (31, 99, 246) is inconsistent. It has been speculated that sympathetic vasoconstriction of cerebral arteries contributes to the decrease in CBF (31, 246), but this hypothesis has not yet been directly investigated.

The purpose of this study, therefore, was to determine the influence of sympathetic activation on regional vascular responses during acute whole-body heat stress. We hypothesized that brachial artery FMD is restrained by elevated sympathetic activity following 30 min of acute whole-body heating and would be restored follow α-1 adrenergic blockade (via oral Prazosin administration) when compared to an placebo trial. To test the hypothesis that sympathetic activation partially contributes to reductions in CBF during acute heat stress, we reasoned that
CBF would be restored by $\alpha$-1 adrenergic blockade at the end of heating compared to the placebo trial.

### 4.2 Methods

#### 4.2.1 Participants

Using previously published data of the mean changes in FMD with acute SNA on Prazosin [+1.6 (2.2)%] vs. Placebo [-1.9 (2.2)%] (8), the minimum number of subjects required to detect significance ($\alpha = 0.05$, $\beta = 0.90$) was seven using G*Power. Prior to research curtailment due to COVID-19, we recruited five participants (one female) who were young, healthy volunteers [age: 27 (5) years; height: 1.78 (0.08) m; mass: 76 (11) kg]. All provided written informed consent and all procedures were approved by the Clinical Research Ethics Board at the University of British Columbia (H19-02355) and conformed to the principles set forth in the Declaration of Helsinki, except for registration in a database.

#### 4.2.2 Experimental design

Experimental days were randomized between placebo and systemic $\alpha$-1 adrenergic antagonist (Prazosin; 0.05 mg/kg) to blunt the effects of increases in sympathetic nervous system activity on the vasculature (11, 250). Upon arrival, the participant was provided the single-blinded capsules for that visit and baseline measures were performed 90 min after ingestion. After ingestion of the capsules, participants were clothed in a water-perfused suit and the remaining experimental set up ensued, including esophageal, rectal, and skin temperatures, lead II ECG, trans-cranial Doppler (TCD) ultrasound, and blood pressure (see below for details of measurements). After the 90-min period to achieve peak blood concentration of Prazosin (106), the participants remained in the supine position for the remainder of testing and blood pressure
was measured three times serially before circulating 49°C water through the water-perfused suit. Pre-heating FMD and CBF measures were then performed. After core temperature increased by ~1.2°C, CBF measurements were repeated and the heating was then terminated following these measurements. Extracranial ultrasound measurements were taken immediately prior to and at the end of heating while end-tidal PCO₂, or arterial PCO₂ when obtained, were restored to baseline values, and blood samples were drawn a final time. The post-heating FMD was performed 25 minutes following the end of heating.

4.2.3 Measurements

Prior to baseline measurements, right radial artery catheterization was successfully performed in two participants under local anaesthesia (Lidocaine, 1.0%) and ultrasound guidance, using a 20-gauge arterial catheter (Arrow, Markham, ON, Canada). At baseline and the end of heating, 1 mL of arterial blood was collected for immediate temperature-corrected measurement of arterial blood gases using the ABL90 Flex co-oximeter (Radiometer, Copenhagen, Denmark) to measure oxygen saturation, oxygen content, blood glucose, and blood lactate as well as several other haematological measures.

The participants were then instrumented with general purpose probes (RET-1, Physitemp Instruments, Clifton, New Jersey, USA) inserted ~40 cm into the esophagus for measurement of esophageal temperature (T_{es}). During the insertion of the esophageal probe, ~500 mL of water was consumed for participant comfort and to ensure euhydration prior to baseline measures. An indwelling catheter (Insyte Autoguard 18G, BD – Canada, Mississauga, ON, Canada) was inserted into an antecubital vein on the left arm for serial blood sampling. Skin temperature (T_{sk}) probes (MLT422/A, ADInstruments, Colorado Springs, CO, USA) were placed on the lateral aspect of the pectoralis major muscle and the right cheek and secured with medical tape. Heart rate (HR)
was monitored via lead II electrocardiogram and peripheral blood pressure was measured via automated brachial artery auscultation (BP5100, Omron Healthcare Canada, Burlington, ON, Canada) and radial artery pressure transducer. Core and skin temperature probes were interfaced with a data acquisition system (PowerLab 16/35, ADI) via thermistor T-type pods, respectively (ML309 and ML312, ADI), while the ECG signal was connected via a Dual Bio Amp (FE232, ADI).

All vascular sonography was performed as outlined in the methods section of Chapter 3. Measurements of extracranial blood flow through the right internal carotid artery (ICA) and left vertebral artery (VA) were estimated using the same methods and were summed and multiplied by two to estimate total CBF. Right external carotid artery blood flow was also measured. The ICA was insonated at least 1.5 cm from the carotid bifurcation to ensure that there is no turbulent or retrograde flow in the measurement and the VA was insonated between C5-C6. During these measurements the participant breathed through a mouthpiece and nose clip attached to an end-tidal forcing system to maintain CO$_2$ constant, because CO$_2$ is a major determinant of cerebral blood flow. Bilateral TCD was used to measure cerebral blood velocity in the right middle cerebral artery (MCA) and left posterior cerebral artery (PCA) using techniques previously described by Willie et al. (265) (Spencer Technologies, PMD150B). The use of 2 MHz probes connected to a headset, which emit sound waves through the temporal window that reflect off red blood cells within the vessel of interest, allow the resulting Doppler shift of these sound waves returning from moving red blood cells to be integrated into a measurement of blood velocity.

End-tidal PCO$_2$ values were maintained at baseline during cerebral blood flow measures using a dynamic end-tidal forcing system (AirForce) (249). One participant did not perform end-tidal forcing due to a previous adverse reaction to this forcing, and one other participant reached
volitional thermal tolerance prior to end-tidal forcing during the Prazosin trial. The end-tidal forcing system uses independent gas solenoid valves for \( O_2 \), \( CO_2 \), and \( N_2 \) for delivery of each gas. The system controls the volume of each gas being delivered to the inspiratory reservoir through a mixing and humidification chamber. End-tidal gases, tidal volume, breathing frequency and minute ventilation are determined for each breath online using specifically designed software (Labview 13.0, National Instruments, Austin, TX, USA).

4.2.4 Data analysis

All temperature data and heart rate were sampled at 400 Hz from PowerLab into LabChart Pro software (Version 7, ADI); data are reported as 5-min averages. At each time point, blood pressure measurements were averaged and mean arterial pressure (MAP) was calculated as the sum of two thirds of diastolic pressure and one third of systolic pressure (Equation 3.1), whereas mean radial arterial pressure (AP) was determined as the mean of the raw pressure tracing from the intra-arterial catheter. All blood flow and FMD data were blinded to the investigator and analyzed as outlined in the data analysis section of Chapter 3 using equations 3.1 to 3.6.

4.2.5 Statistical analyses

All data are presented as means (standard deviation, SD). All values were checked for normality with a Shapiro-Wilk test and if the distribution was non-normal, values were log transformed. Data were then analyzed using two factor linear mixed models with a compound symmetry covariance structure where time (pre/post intervention) and drug (placebo/Prazosin) were repeated variables. A Bonferroni correction was used for multiple comparisons between main effects. When significance (\( \alpha = 0.05 \)) was observed, simple main effects were determined with post hoc testing using a paired sample t-test with a Bonferroni correction. The model for FMD was
run with logged changes in diameter as the dependent variable and baseline diameter and SRAUC as covariates. Corrected group means and SD for FMD were back calculated from the estimated means (EM) and standard errors of the model using the formula: \( (e^{EM}-1) \times 100 \) (2, 48). All statistical analyses were performed in SPSS version 24 (IBM, Armonk, NY, USA) and figures were generated with GraphPad version 6.0 (Prism, La Jolla, CA, USA).

4.3 Results

4.3.1 Thermometry

The increase in esophageal temperature did not differ between conditions [Prazosin: \( \Delta 1.20 \) (0.43)°C vs. Placebo: \( \Delta 1.10 \) (0.35)°C; \( P=0.37 \)]. Similarly, the increases in skin temperature at the chest [Prazosin: \( \Delta 1.93 \) (0.83)°C vs. Placebo: \( \Delta 2.41 \) (1.66)°C; \( P=0.37 \)] and cheek [Prazosin: \( \Delta 3.81 \) (1.57)°C vs. Placebo: \( \Delta 1.50 \) (0.70)°C; \( P=0.37 \)] were not different between conditions.

4.3.2 Cardiorespiratory responses

All cardiorespiratory variables are presented in Table 4.1. There was a main effect of time where HR increased in both conditions (\( P<0.01 \)), but HR was higher in Prazosin throughout by ~10 bpm (condition \( P=0.01 \)). Brachial MAP did not change with heating (time \( P=0.44 \)), but it tended to be lower throughout in Prazosin by ~5 mmHg (condition \( P=0.06 \)). Radial AP decreased with heating by ~15 mmHg and was lower in Prazosin compared to Placebo throughout (n=2, no statistics). There was a main effect of time where poikilocapnic \( \dot{V}_E \) increased similarly in both conditions with heating (\( P<0.01 \)). During poikilocapnia, \( PaCO_2 \) decreased from baseline by ~6-9 mmHg with heating and returned to baseline values when isocapnia was restored (time \( P<0.01 \)). There was a main effect of time where isocapnic \( P_{ETCO_2} \) tended to be higher with heating (\( P=0.06 \)). There was also a main effect of condition where \( P_{ETCO_2} \) during poikilocapnia was ~4 mmHg.
higher during Placebo vs. Prazosin (P=0.04). There was a main effect of time where the A-a CO₂ gradient increased from negligible at baseline to ~2 mmHg at the end of heating in both conditions (P=0.01).

Table 4.1. Cardiorespiratory variables during whole-body heating with Prazosin or Placebo.

<table>
<thead>
<tr>
<th></th>
<th>HR (bpm)</th>
<th>MAP (mmHg)</th>
<th>AP* (mmHg)</th>
<th>V̇̇̇_̇̇̇_E (l/min)</th>
<th>P_{ET}CO₂ (mmHg)</th>
<th>P_{a}CO₂* (mmHg)</th>
<th>A-a CO₂* (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prazosin (n=4)</td>
<td>63 (10)</td>
<td>79 (5)</td>
<td>96 (8)</td>
<td>15 (5)</td>
<td>37 (3)</td>
<td>41 (0)</td>
<td>-0.5 (0)</td>
</tr>
<tr>
<td>Placebo (n=4)</td>
<td>55 (8)</td>
<td>83 (5)</td>
<td>101 (4)</td>
<td>14 (2)</td>
<td>41 (3)</td>
<td>41 (2)</td>
<td>-0.5 (0.3)</td>
</tr>
<tr>
<td><strong>Hot-poikilocapnic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prazosin (n=4)</td>
<td>98 (12)</td>
<td>70 (9)</td>
<td>77 (2)</td>
<td>16 (1)</td>
<td>34 (6)</td>
<td>35 (5)</td>
<td>2.3 (0.5)</td>
</tr>
<tr>
<td>Placebo (n=4)</td>
<td>80 (8)</td>
<td>83 (6)</td>
<td>87 (2)</td>
<td>13 (1)</td>
<td>39 (5)</td>
<td>32 (2)</td>
<td>2.3 (0.7)</td>
</tr>
<tr>
<td><strong>Hot-isocapnic</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Prazosin (n=2)</td>
<td>106 (18)</td>
<td>75 (4)</td>
<td>85 (0)</td>
<td>32 (11)</td>
<td>40 (2)</td>
<td>38 (0)</td>
<td>3.7 (0)</td>
</tr>
<tr>
<td>Placebo (n=3)</td>
<td>91 (9)</td>
<td>81 (1)</td>
<td>94 (6)</td>
<td>24 (12)</td>
<td>43 (2)</td>
<td>40 (1)</td>
<td>2.2 (0.2)</td>
</tr>
<tr>
<td><strong>P values</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>&lt;0.01</td>
<td>0.44</td>
<td>N/A</td>
<td>&lt;0.01</td>
<td>0.06</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Condition</td>
<td>0.01</td>
<td>0.06</td>
<td>N/A</td>
<td>0.23</td>
<td>0.04</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td>Time*condition</td>
<td>0.44</td>
<td>0.26</td>
<td>N/A</td>
<td>0.71</td>
<td>0.77</td>
<td>N/A</td>
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</tbody>
</table>

Data are mean (SD). HR, heart rate; MAP, mean arterial pressure; AP, arterial pressure; V̇̇̇_̇̇̇_E, minute ventilation; P_{ET}CO₂, end-tidal partial pressure of CO₂; P_{a}CO₂, arterial partial pressure of CO₂; A-a CO₂, alveolar to arterial CO₂ gradient. *Radial artery measures were only obtained in n=2 so statistics were not performed.
4.3.3 Flow-mediated dilation

Brachial artery responses to heating are presented in Table 2 and Figure 4.1. There were no significant interactions (P=0.18) or main effects on FMD. Average FMD values increased after heating in the Prazosin condition from 7.9 (5.8) to 10.9 (9.4)%, whereas FMD decreased after heating in the Placebo condition from 8.1 (2.7) to 6.4 (1.2)%. When baseline diameter and SRAUC were entered as statistical covariates (P=0.01 and P=0.68, respectively), similar results were observed (interaction P=0.27). There was a trend toward a time by condition interaction (P=0.08) where SRAUC increased after heating in the Prazosin but not Placebo condition. There was also a main effect of condition (P=0.02) where SRAUC was greater at both time points in Prazosin vs. Placebo. Peak reactive hyperemia (RH), an index of downstream microvascular function in the forearm, did not change with heating (time P=0.42); however, there was a trend toward a main effect of condition (P=0.06) where peak RH was higher in Placebo vs. Prazosin.
Table 4.2. Brachial artery hemodynamics and FMD before and after heating.

<table>
<thead>
<tr>
<th></th>
<th>PRAZOSIN (N=4)</th>
<th>PLACEBO (N=4)</th>
<th>P VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Baseline diameter (mm)</td>
<td>3.91 (0.63)</td>
<td>3.92 (0.64)</td>
<td>4.12 (0.27)</td>
</tr>
<tr>
<td>FMD (mm)</td>
<td>0.28 (0.17)</td>
<td>0.39 (0.27)</td>
<td>0.33 (0.10)</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>7.9 (5.8)</td>
<td>10.9 (9.4)</td>
<td>8.1 (2.7)</td>
</tr>
<tr>
<td>Corrected FMD (%)</td>
<td>6.1 (3.2)</td>
<td>8.4 (4.2)</td>
<td>9.9 (4.0)</td>
</tr>
<tr>
<td>SRAUC</td>
<td>32097 (12748)</td>
<td>40246 (7969)</td>
<td>19907 (4677)</td>
</tr>
<tr>
<td>Peak RH (ml/min)</td>
<td>272 (64)</td>
<td>294 (62)</td>
<td>367 (76)</td>
</tr>
</tbody>
</table>

Data are mean (SD). FMD, flow-mediated dilation corrected for baseline diameter and shear stimulus; SRAUC, shear rate area under the curve; RH, reactive hyperemia.
4.3.4 Cerebral blood flow

Figure 4.3 presents CBF data. There was a main effect of time where poikilocapnic gCBF decreased with heating by ~8% and returned to baseline values when isocapnia was restored (P=0.06). In the ICA, there was a main effect of time where isocapnic flow increased in both conditions (P=0.04). In the VA, flow did not significantly change during heating in either condition (interaction P=0.97). There was a main effect of time where poikilocapnic flow to the face and scalp through the ECA increased in both conditions (P=0.05).

Poikilocapnic MCA velocity trended to be lower in Prazosin (P=0.07); however, both conditions tended to decrease during heating (P=0.09) by ~8 cm/s. There was a main effect where poikilocapnic PCA velocity was lower during Prazosin compared to Placebo (P<0.01); however, both conditions tended to decrease similarly with heating by ~4 cm/s (P=0.09).

4.4 Discussion

The purpose of this study was to determine the effects of sympathetic α-1 adrenergic signaling on cerebral vascular responses to heat stress, and post-heating endothelial-dependent dilation. The preliminary findings of this study are that 1) post-heating FMD may not be affected by elevated α-1 adrenergic tone; however, trends indicate that reduced FMD 30 min after whole-body heating could be related to sympathetic activity and adrenergic signaling; and 2) poikilocapnia-related decreases in CBF during heat stress may not be influenced by α-1 adrenergic blockade and are driven by greater changes in the ICA compared to VA.
Figure 4.1. Individual flow-mediated dilation (FMD) values (A) and baseline diameter and shear stimulus-corrected FMD values (B).

4.4.1 Brachial artery

The trends observed in this study are preliminary and should therefore be judiciously interpreted. Indeed, our calculated effect size was moderate (d=0.58) resulting in an achieved power of 0.30, indicating that our sample is currently underpowered. Indeed, our a priori sample size calculation indicated seven participants were required; therefore, at least another three participants should be tested to in order to draw firm conclusions – at least for FMD. As such, the following discussion frames these findings in the context of the average trends observed and the existing literature. Hyperthermia has been considered a “hyperadrenergic state” (214) where increases in renal and splanchnic vascular resistance counteract the profound direct effects of local heating in the cutaneous circulation to maintain MAP (170). Indeed, direct recordings of muscle SNA have been observed to progressively increase (~40-90%) during whole-body heating (101, 109) and this response can be initiated by increases in core temperature of ~0.2°C (62). In the
peripheral circulation, arteries and large arterioles are highly responsive to $\alpha_1$ agonsim (147). It is therefore plausible that heightened adrenergic signaling could interfere with normal vascular function. For instance, a common sympatho-excitatory maneuver, lower body negative pressure, decreased FMD of the brachial artery (231). Since radial MSNA remained elevated for 30 min following mild heat stress in young adults (89), it is conceivable that whole-body heat stress would attenuate endothelial-dependent dilation of the brachial artery due to elevated sympathetic activity.

Although statistical significance of the effects of $\alpha_1$ antagonism was not observed in the current study, the large biological and technical variability of FMD is established (74) and likely contributes to the lack of effect in this small sample. However, preliminary trends were revealed which warrant discussion. Whole-body heating resulted in minimal change in FMD following 30 min of recovery from 8.4% to 6.9% in the Placebo condition, whereas FMD increased from 7.9% to 10.9% in the Prazosin condition (Table 2). This finding indicates that $\alpha_1$ adrenergic signaling may attenuate FMD during the Placebo trial where elevated plasma NE concentrations are expected (101). A similar phenomenon has previously been reported following exercise where FMD later returned to baseline following one hour of recovery (8). Thus, it is possible that improvements in FMD would be revealed with greater recovery following heat stress when sympathetic activation has dissipated. Although improved shear patterns with local heating offset reductions in FMD during sympathetic activation (231), this was not the case in the current study. The efficacy of the Prazosin dose appears evident from both the increased resting HR (Table 4.1) as well as the main effect of condition on SRAUC where higher values in Prazosin likely reflect attenuated vasoconstrictor tone in the muscle arterioles (Table 4.2).
Figure 4.2. Minute ventilation (left) and end-tidal PCO$_2$ (right) during whole-body heating with either orally ingested $\alpha$-1 adrenergic blockade (Prazosin) or Placebo. Time points of measurements were baseline (BL) and the end of heating with poikilocapnic (hot-poik) or isocapnic (hot-iso) breathing.

4.4.2 Cerebral blood flow

Several groups have reported reductions in MCA velocity during hyperthermia (15, 31, 99). Likewise, Low et al. (158) hypothesized that cerebrovascular sensitivity to CO$_2$ [~3% reduction per mmHg decrease of $P_a$CO$_2$ (264)] must be approximately doubled during hyperthermia if it were to fully explain the observed reductions in MCA velocity. Sympathetically-mediated cerebral vasoconstriction was subsequently thought to contribute to lower CBF during heat stress (31, 158). Further reports suggested that preventing hypocapnia or returning $P_{ET}$CO$_2$ to eucapnic levels during heat stress only partially (36-50%) restored MCA velocity to baseline values (31, 99, 246). Yet, it has also been demonstrated that restoring isocapnia during heat stress fully returns MCA and PCA velocities (15, 181) as well as global CBF (15) to baseline values. The current study demonstrates that MCA and PCA velocities decreased ~8 and 4 cm/s, respectively (Figure 4.4) and global CBF decreased almost 10% (Figure 4.3), but all were returned
to baseline values with end-tidal forcing to eucapnic levels. In fact, our data support the strong relationship previously reported between CBF and \(P_a\)\(CO_2\) during heat stress \((r^2=0.97)\) (181), with nearly full restoration of CBF when baseline \(P_{ET}CO_2\) levels were maintained. The present reductions in cerebral blood velocities and flow did not seem to be influenced by Prazosin administration, which could indicate that sympathetic activation via \(\alpha_1\) adrenergic receptors does not contribute to reductions in CBF during heat stress. These possibilities needs to be substantiated with more participants.

![Diagram](image)

**Figure 4.3.** Duplex ultrasound-derived estimates of global cerebral blood flow (A), internal carotid artery blood flow (B), vertebral artery blood flow (C), and external carotid artery flow (D) to the face and scalp. *\(P<0.05\) vs. baseline (BL, \(n=4\)). Hot, +1.5°C esophageal temperature; poik, poikilocapnic breathing (\(n=4\)); iso, isocapnia restored via end-tidal forcing (\(n=2\) Prazosin, \(n=3\) Placebo).
4.4.3 Considerations

The limitations of TCD as an index of CBF has been previously discussed (3); however, the results from global CBF and MCA/PCA are similar in the current study thereby lending support to the MCA and PCA velocity measures during heating. Prazosin is a selective α-1 receptor antagonist and, therefore, pharmacological blockade of other receptors (e.g., α-2, β), both alone and in combination, should be investigated in the future. Although we do not have microneurographic recordings of sympathetic activity, it is well established that MSNA increases during hyperthermia (66, 68, 101, 157). Moreover, Prazosin is a post-junctional receptor antagonist; therefore, its effects would not be observable via microneurography. However, the efficacy of Prazosin in our results is demonstrated by lower MAP through systemic reduction of α-1 adrenergic tone and thus higher baroreflex-mediated heart rates throughout heating (Table 4.1). Additionally, the dose provided to our participants was very close to the maximum single dose available (5 mg) and peak plasma concentrations occur at 2-3 hours after ingestion, which coincides with the end of heating in this study. Differences between the current results and others may arise from varying core temperatures, different end-tidal forcing methods, and different indexes of CBF (e.g., TCD vs. duplex ultrasound). It is also noteworthy that we observed a widening of the alveolar to arterial PCO₂ gradient from negligible at baseline to ~2 mmHg during hyperthermia (Table 4.1). The altered A-a CO₂ gradient might be relevant for studies where the level of hypocapnia was relatively minor (e.g., 4 mmHg) (31) and thus P_aCO₂ may not have been completely restored.
Figure 4.4. Middle cerebral artery velocity (MCAv; left) and posterior cerebral artery velocity (PCAv; right). BL, baseline (n=4); Hot, +1.5°C esophageal temperature; poik, poikilocapnic breathing (n=4); iso, isocapnia restored via end-tidal forcing (n=2 Prazosin, n=3 Placebo).

4.4.4 Conclusion

The main findings of this preliminary study are that sympathetic vasoconstriction may not occur in large cerebral arteries or brachial artery during hyperthermia, but preliminary trends indicate that post-heating FMD may be attenuated by sympathetic activation. Thus, the level of sympathetic activation and timing of vascular measures should be considered carefully when examining the mechanisms of improved vascular function with heat therapy.
Chapter 5: Acute heat stress reduces biomarkers of endothelial activation but not macro- or microvascular dysfunction in cervical spinal cord injury

5.1 Background

It is well appreciated that a spinal cord injury (SCI) can result in motor, sensory, and autonomic deficits. The loss of supraspinal sympathetic inputs to the heart commonly causes acute cardiac complications in SCI. Additionally, the risk of cardiovascular diseases (CVD) in chronic SCI is ~3-fold greater than the general population (59) and CVD account for ~40% of deaths in SCI (103). Moreover, the peripheral vasculature is also affected by many factors including the loss of sympathetic innervation, physical inactivity, and repeated exposures to autonomic dysreflexia (262). Due to extreme physical inactivity following SCI, rapid deconditioning of the vasculature occurs. For example, within weeks of SCI the common femoral artery diameter and leg blood flow have been reported to both be ~40% lower compared to uninjured controls (73). Impaired macrovascular (241) and microvascular (178) functions have been reported below the level of injury in SCI, but previous studies have also found that flow-mediated dilation (FMD) of the femoral artery was preserved (236) or even increased (72) following thoracic SCI. Conversely, arterial structure and function are generally maintained above the level of injury (235, 241), possibly indicating that preserved physical activity and intact sympathetic innervation preserves macrovascular function of local conduit arteries. However, cutaneous microvascular function has also been reported to be impaired in both the upper and lower limbs in thoracic SCI (254), which could indicate dysfunction of the small arterioles that precedes impairment of the conduit arteries. In uninjured individuals, acute reductions in physical activity were associated with impaired FMD in the lower limb and endothelial-derived apoptotic microparticles (27), highlighting a potential relationship between endothelial microparticles and macrovascular function. However, the types and concentrations of
circulating endothelial-derived microparticles have not been characterized in SCI. The majority of previous studies have tested low-level SCI (i.e., lumbar or thoracic injuries); therefore, the extent of potential peripheral vascular dysfunction is currently unknown in high level SCI (i.e., cervical injuries) in both the upper and lower limbs.

Regular exercise maintains or improves vascular function (77) and is dependent on greater levels of arterial shear stress (114, 148). Two main types characterize shear stress in arteries: antegrade, which is typically observed in physiologically forward moving laminar flow; and retrograde, which is oscillating low flow typically observed in turbulent regions such as artery bifurcations. Similar to exercise, heat stress raises antegrade shear stress and, importantly, it also reduces retrograde shear stress (209, 237). Indeed, it has been demonstrated that passive heating can increase antegrade shear stress on the endothelium by a greater magnitude than exercise (237) in the femoral artery. Several studies have reported both acute and long-term benefits of heat stress on important vascular measures including blood pressure (33, 152), central artery stiffness (33, 238), FMD (33, 179, 209), microvascular function (41, 209), and microparticles (i.e., lowered concentrations) (13). Moreover, recent evidence indicates that long-term repeated exposures to heat (i.e., sauna) is associated with lower fatal cardiovascular and all-cause mortality in middle-aged men (150). Passive heating, therefore, might be a useful strategy for improving vascular health in those with compromised exercise capacity, such as in SCI where physical activity levels are reduced (253). In a recent randomized control trial, following the physical activity guidelines specific to SCI was reportedly insufficient to improve vascular health (242), suggesting alternative strategies are needed to improve vascular function in SCI. The potential of heat therapy to improve vascular health has been demonstrated in various populations including older individuals (209), peripheral artery disease (238), and heart failure.
(142), but limited testing of this approach has been performed in individuals with SCI. Yet, existing evidence suggests that the acute cytokine response to hyperthermia is intact in SCI despite attenuated adrenergic activation (154).

The aims of this study were to determine the influence of cervical SCI on vascular function and whether the practical approach of lower limb heating has the potential to improve vascular function in SCI. It was hypothesized that individuals with SCI would display greater vascular dysfunction compared to uninjured control participants and that acute responses to a single bout of heating would improve these outcomes in the SCI group.

5.2 Materials and methods

Approval of this study was obtained by the Ethics Committee of the School of Medicine at the University of Split and all procedures conformed to the Declaration of Helsinki. Fifteen individuals with chronic cervical SCI and fifteen sex- and age-matched uninjured (i.e., able-bodied) controls (CON) were recruited. Participants in the SCI group were screened in accordance to the American Spinal Injury Association (ASIA) Impairment Scale (AIS) (143). All participants provided written, informed consent prior to completion of any data collection and were of Croatian nationality. Participant characteristics and medications are presented in Table 5.1.
Table 5.1. Participant characteristics, risk factors, and medications.

<table>
<thead>
<tr>
<th>Category</th>
<th>SCI</th>
<th>CON</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>10 M/5 F</td>
<td>10 M/5 F</td>
<td>-</td>
</tr>
<tr>
<td>Age (y)</td>
<td>43 (12)</td>
<td>42 (11)</td>
<td>0.74</td>
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<td>Mass (kg)</td>
<td>72 (13)</td>
<td>80 (12)</td>
<td>0.12</td>
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<td>BMI (kg/m²)</td>
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<td>25 (3)</td>
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<td>Time since injury (years)</td>
<td>21 (13)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Level of injury/severity</td>
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<td>Risk Factors</td>
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<tr>
<td>Total cholesterol (mg/dL)</td>
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<td>147 (38)</td>
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<tr>
<td>High density lipoprotein (mg/dL)</td>
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<td>0.77</td>
</tr>
<tr>
<td>Low density lipoprotein (mg/dL)</td>
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<td>Triglycerides (mg/dL)</td>
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<td>Glucose (mg/dL)</td>
<td>86 (11)</td>
<td>89 (19)</td>
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<td>Insulin (mIU/mL)</td>
<td>9.8 (7.9)</td>
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<td>2.1 (1.6)</td>
<td>1.00</td>
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<td>Medications</td>
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<td>Corticosteroids (asthma)</td>
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<td>Alprazolam</td>
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<td>Acetylsalicylic acid</td>
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</tr>
<tr>
<td>Lyrica</td>
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<td>Tramadol</td>
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<td>-</td>
</tr>
<tr>
<td>Ventolin</td>
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</tr>
<tr>
<td>Baclofen</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Data are presented as mean (SD). SCI severity was assessed using the American Spinal Injury Assessment (ASIA) impairment scale. Risk factors are based on n=11 SCI and n=15 CON. SCI, spinal cord injury; CON, uninjured control. P<0.05 difference between groups.
5.2.1 Experimental design

Participants were instructed to arrive at the laboratory fasted for at least six hours and well hydrated. The protocol comprised 60 min of passive lower limb heating in the supine position using a manually circulated water bath at 40°C (legs immersed to mid-calf level; ~30 cm) and an electric heating blanket covering the upper body. This protocol was chosen to be comparable in length with previous studies (34, 92, 154) as well as a practical method of heating similar to Romero et al. (209). Considering the length of the protocol, SCI participants rested supine with their knees flexed (~45°) and feet placed downward into the water bath to avoid hyperextension of the hips and consequent autonomic dysreflexia in the SCI group. Plastic covers were also placed over the feet and legs for appropriate skin protection. Prior to heating, the participants rested supine for ≥20 min before baseline measures of peripheral vascular function. Immediately prior to immersion in the 40°C bath, baseline thermo- and hemodynamics were measured after 5 min of leg immersion in thermoneutral water (33°C) to minimize the effects of hydrostatic pressure on these measurements. Central hemodynamics, as well as peripheral blood flow patterns, were measured at baseline and every 15 min thereafter until completion of the heating. Tests of vascular function were repeated approximately 30 min after the end of heating.

5.2.2 Thermometry

Core body temperature was measured via telemetric pill (HQInc, Palmetto, FL, USA) ingested ≥2 hours prior to data collection. Skin temperatures were measured using thermistor probes (ADInstruments, Colorado Springs, CO, USA) adhered to the skin with medical tape on the lateral deltoid (arm) and on the medial calf (leg), which was immersed in the water bath and
covered with Tegaderm (3M, St. Paul, MN, USA). Thermal sensation was assessed with a modified visual analogue scale (123) where -1 represented “slightly cool” and 5 represented “extremely hot.”

5.2.3 Hemodynamics

Heart rate (HR) was recorded via three-lead electrocardiogram (ECG) and beat-by-beat measurements of blood pressure were recorded using finger plethysmography (Finometer PRO, Finapres Medical Systems, Amsterdam, Netherlands), back-calibrated to manual or automated cuff readings of the brachial artery. All blood flow and FMD data were measured as outlined in the methods of Chapter 3 using equations 3.1 to 3.6.

5.2.4 Blood sampling

One 6 mL sample and three 5 mL samples of whole blood were drawn into vacutainers containing EDTA and sodium citrate, respectively, at baseline and at end-heating via an intravenous catheter. The whole blood sample (EDTA) was processed immediately for hematological parameters (AcT 8 Hematology Analyzer, Beckman Coulter, Brea, CA, USA). The sodium citrate samples were centrifuged at 1550 g for 10 min at room temperature to separate and freeze plasma at -80°C for future batch analysis. Plasma metabolic biomarkers (i.e., glucose, insulin, lipids, TGs, etc.; see Table 1) were assessed using standard techniques at a clinical laboratory and microparticles were analysed using flow cytometry (BD Biosciences FACSAria I High Speed Cell sorter and flow cytometer) as described in detail elsewhere (13, 96).

To characterize and quantify circulating microparticles, plasma was centrifuged at 13000 g for 2 min and 200 µL was transferred to a TruCount tube (BD Biosciences, Franklin Lakes, NJ,
USA). The EMP phenotype was determined by incubating samples with fluorochrome-labelled antibodies (BioLegend, San Diego, CA, USA) indicative of activation (CD62e+) and apoptosis (CD31+/42b−) for 20 min at room temperature in a dark room. Samples were then fixed with 2% paraformaldehyde (ChemCruz Biochemicals, Santa Cruz, CA, USA) and diluted with RNase-free PBS. The size threshold for microparticles was established using Megamix-Plus SSC calibrator beads (Biocytex, Marseille, France) and only events <1 μm in size and positively expressing markers of CD62e+ and CD31+/CD42b− were counted. Total number of circulating platelet, monocyte, and leukocyte-derived microparticles were also counted using platelet (CD62P, CD31+/42b−), monocyte, and leukocyte specific antibodies. The concentrations of MPs were determined using the following formula: \[
\left(\frac{\text{number of events in region containing microparticles}}{\text{number of events in absolute count bead region}}\right) \times \left(\frac{\text{total number of beads per test}}{\text{total volume of sample}}\right)
\] (184).

5.2.5 Statistics

All cardiovascular and skin temperature measurements were sampled at 1000 Hz using a digital-to-analog data acquisition system (PowerLab 880, ADinstruments, Colorado Springs, CO) interfaced with LabChart Pro software (Version 7.2, ADinstruments) and saved for offline analysis. Data were extracted from LabChart as 5-min averages at each 15-min interval. Core temperature was monitored continually via handheld device and values were recorded at 15-min intervals throughout heating.

Baseline participant characteristics, vascular, hemodynamic, and temperature values were compared between groups with a Mann-Whitney U test. All hemodynamic and thermodynamic values were compared with linear mixed-model analysis with a compound symmetry covariance
Logarithmically transformed diameter values were entered into the model for allometric scaling of FMD, where baseline artery diameter and SRAUC were entered as covariates (9, 187). Corrected FMD values and standard deviations were back-calculated from the linear mixed model estimated means (EM) and standard errors with the following equation: 
\[
\left( e^{EM} - 1 \right) \times 100
\]
Pre- and post-heating values for uncorrected FMD, microparticles, and blood biomarkers were compared with a mixed model ANOVA with the repeated factor of time. When significant interactions were detected, a Bonferroni correction was applied to multiple comparisons. Relationships between key variables were examined with simple linear regressions. All statistics were performed using SPSS v24 (IBM, Armonk, NY, USA) or GraphPad v6.0 (Prism, La Jolla, CA, USA). The level of statistical significance was set a priori at P<0.05. All data are presented as means and standard deviation (SD).

5.3 Results

5.3.1 Baseline variables

Participant characteristics and risk factors are presented in Table 5.1. The SCI and CON groups were matched for age, but BMI was significantly lower in SCI. Cholesterol, high density lipoprotein, low density lipoprotein, triglycerides, glucose, insulin, and Homa-IR were not different between groups (all P>0.05). The diameters of both the brachial (P<0.01) and femoral (P<0.01) arteries were significantly smaller in SCI by 26% and 17%, respectively, compared to CON (Table 2). Blood velocity tended (P=0.07) to be higher in the femoral artery and was higher (P=0.04) in the brachial artery of the SCI group, while blood flow and conductance were not
different between groups in either artery (all \(P>0.05\)). Consequently, antegrade shear rates were higher in the femoral (\(P=0.02\)) and tended to be higher in the brachial (\(P=0.07\)) artery in SCI vs. CON. However, retrograde shear rates were greater in CON vs. SCI in the brachial artery (\(P=0.02\)), whereas retrograde shear rates were not different between groups in the femoral artery (\(P=0.19\)). Although oscillatory shear index (OSI) in the brachial artery was not different between groups (\(P=0.32\)), OSI was greater in CON in the femoral artery compared to SCI (Table 5.2, \(P=0.02\)).
Table 5.2. Baseline vascular characteristics.

<table>
<thead>
<tr>
<th>Group</th>
<th>Diameter (mm)</th>
<th>Velocity (cm/s)</th>
<th>Flow (ml/min)</th>
<th>Conductance (ml/min/mmHg)</th>
<th>Antegrade Shear (s⁻¹)</th>
<th>Retrograde Shear (s⁻¹)</th>
<th>OSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCI</td>
<td>4.8 (0.8)</td>
<td>16.4 (10.8)</td>
<td>88 (58)</td>
<td>1.05 (0.71)</td>
<td>197 (95)</td>
<td>-44 (58)</td>
<td>0.17 (0.15)</td>
</tr>
<tr>
<td>CON</td>
<td>6.5 (1.0)</td>
<td>9.7 (6.3)</td>
<td>98 (54)</td>
<td>1.12 (0.62)</td>
<td>124 (50)</td>
<td>-47 (23)</td>
<td>0.31 (0.18)</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.01</td>
<td>0.07</td>
<td>0.53</td>
<td>0.59</td>
<td>0.02</td>
<td>0.19</td>
<td>0.02</td>
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</table>

**Femoral Artery**

<table>
<thead>
<tr>
<th>Group</th>
<th>Diameter (mm)</th>
<th>Velocity (cm/s)</th>
<th>Flow (ml/min)</th>
<th>Conductance (ml/min/mmHg)</th>
<th>Antegrade Shear (s⁻¹)</th>
<th>Retrograde Shear (s⁻¹)</th>
<th>OSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCI</td>
<td>3.5 (0.7)</td>
<td>27.6 (12.0)</td>
<td>83 (59)</td>
<td>0.97 (0.60)</td>
<td>355 (158)</td>
<td>-2 (5)</td>
<td>0.02 (0.04)</td>
</tr>
<tr>
<td>CON</td>
<td>4.2 (0.8)</td>
<td>17.9 (10.1)</td>
<td>81 (58)</td>
<td>0.95 (0.74)</td>
<td>232 (105)</td>
<td>-12 (21)</td>
<td>0.05 (0.09)</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.01</td>
<td>0.03</td>
<td>0.98</td>
<td>0.89</td>
<td>0.07</td>
<td>&lt;0.01</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Data are mean (SD) and based on n=15 SCI and n=15 CON for femoral hemodynamics, n=13 SCI and n=13 CON for brachial hemodynamics. SCI, spinal cord injury; CON, uninjured controls. P<0.05 difference between groups.
5.3.2 Thermodynamics

Core temperature was 0.73°C lower during the resting baseline in SCI compared to CON (P<0.01, Table 5.3) and increased to a greater extent in SCI than CON following 60 min of heat exposure [Figure 5.1; SCI: +0.68 (0.17) vs. CON: 0.34 (0.17)°C, interaction P<0.01)]. Skin temperature on the leg increased in both groups during heating [SCI: +6.2 (2.9) vs. CON: +6.3 (1.6)°C] with no between group difference (time P<0.01, interaction P=0.59). Skin temperature on the arm did not significantly change in either group with heating (P=0.18). The heating protocol was well tolerated in both groups with SCI reporting thermal sensation of “warm” compared to “hot” in CON. There was a main group effect where thermal sensation was lower (i.e., felt colder) in SCI (P=0.04). Core temperature data are absent from one CON participant due to connectivity issues with the telemetric pill, and skin temperature data are absent from two SCI participants and one CON participant due to technical difficulties.
Table 5.3. Hemodynamic and thermodynamic values before and after heating.

<table>
<thead>
<tr>
<th>Category</th>
<th>SCI Baseline</th>
<th>SCI End-heating</th>
<th>CON Baseline</th>
<th>CON End-heating</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hemodynamics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>55 (10)</td>
<td>58 (9)*</td>
<td>59 (11)</td>
<td>66 (9)*</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>120 (18)</td>
<td>118 (24)</td>
<td>126 (21)</td>
<td>121 (26)</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>68 (16)</td>
<td>66 (17)</td>
<td>73 (15)</td>
<td>65 (18)*</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>81 (10)</td>
<td>84 (15)</td>
<td>87 (12)</td>
<td>85 (10)</td>
</tr>
<tr>
<td>Brachial flow (ml·min⁻¹)</td>
<td>83 (59)</td>
<td>83 (44)</td>
<td>81 (58)</td>
<td>108 (51)</td>
</tr>
<tr>
<td>Femoral flow (ml·min⁻¹)</td>
<td>88 (58)</td>
<td>171 (81)*</td>
<td>98 (54)</td>
<td>199 (84)*</td>
</tr>
<tr>
<td><strong>Thermodynamics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Core temperature (°C)</td>
<td>36.37 (0.62)</td>
<td>37.05 (0.63)*</td>
<td>37.10 (0.26) †</td>
<td>37.45 (0.24)* †</td>
</tr>
<tr>
<td>Skin temperature – shoulder (°C)</td>
<td>32.12 (1.35)</td>
<td>32.67 (1.47)</td>
<td>32.84 (1.34)</td>
<td>32.85 (1.40)</td>
</tr>
<tr>
<td>Skin temperature – calf (°C)</td>
<td>31.78 (1.19)</td>
<td>38.81 (0.91)*</td>
<td>32.48 (0.92)</td>
<td>38.73 (0.88)*</td>
</tr>
</tbody>
</table>

SCI, spinal cord injury; CON, uninjured control. *different from baseline (P<0.05); †different from SCI (P<0.05).
**Figure 5.1.** Core (top), shoulder (middle), and calf (bottom) skin temperatures at 15-min intervals during lower leg hot water immersion (40°C). Data based on n=15 SCI and N=14 CON.

*Denotes statistical significance (P<0.05).
5.3.3. Hemodynamics

Data for central and femoral hemodynamics are presented for all participants in both SCI and CON groups. Brachial hemodynamics are based on n=13 for each group due to inadequate wall tracking of arterial diameters. Although heart rate increased slightly during heating in both groups (time P<0.01, interaction P=0.17), SBP (P=0.41) and MAP (P=0.18) did not change in either group while DBP decreased by 7 mmHg in the CON group only (Table 5.3; P=0.02).

Femoral blood flow (time P<0.01, interaction P=0.68) and conductance (time P<0.01, interaction P=0.61) increased by ~100% following heating in both the SCI and CON groups. In the brachial artery, blood flow (interaction P<0.01) and conductance (interaction P<0.01) increased with heating in CON but not SCI (Table 5.3). Femoral antegrade shear rates increased with heating to a greater extent in SCI vs. CON (Figure 5.2; +83% vs. +45%, respectively; interaction P=0.02). Similarly, greater increases in antegrade shear rates were also apparent in the brachial artery of the SCI group with heating (interaction P=0.04). Although retrograde shear rates in the femoral artery were reduced following heating in both groups, it was nearly abolished at 60-min of heating in SCI compared to CON [SCI: -3 (6) vs. CON: -23 (16) s⁻¹, time P<0.01, group P=0.03]. Conversely, brachial artery retrograde shear rates remained near zero in SCI throughout heating, while greater baseline retrograde shear in CON was attenuated with heating (interaction P=0.03). Femoral artery OSI decreased similarly in both groups but was lower in SCI vs. CON throughout heating (time P<0.01, group P=0.01). In the brachial artery, OSI was greater at baseline in CON compared to SCI and decreased with heating in SCI only (time P=0.02, interaction P=0.07).
**Figure 5.2.** Brachial (top) and femoral (bottom) artery shear rates at 15-min intervals during heating. Data based on n=15 SCI and N=14 CON. *Bonferroni-adjusted pairwise comparison between SCI and CON (P<0.05).

### 5.3.4 Macro- and microvascular function

Five FMD/reactive hyperemia measurements were excluded from the SCI group due to poor wall tracking and/or due to involuntary muscle spasms. Three and four participants for the femoral and brachial arteries, respectively, were excluded from the CON group due to
inadequate wall tracking or excessive angle shifts of the B mode image. There were no group differences in femoral FMD at baseline or after heating (interaction P=0.63, Figure 5.3). When corrected for baseline diameter and SRAUC there were no between group differences in femoral FMD (interaction P=0.70, Table 5.4). Conversely, brachial artery FMD was not different between groups or after heating (P=0.27), but it was 39% lower at baseline in SCI vs. CON after correction for baseline diameter and SRAUC (group P=0.04, Table 5.4). Brachial FMD did not change following heating in either group (interaction P=0.64).
Table 5.4. Endothelial-dependent vasodilation measured via flow-mediated dilation (FMD).

<table>
<thead>
<tr>
<th>Category</th>
<th>Pre-heat SCI</th>
<th>Pre-heat CON</th>
<th>Post-heat SCI</th>
<th>Post-heat CON</th>
<th>P-values</th>
<th>Time</th>
<th>Group</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Femoral Artery</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline diameter (mm)</td>
<td>4.80 (0.71)</td>
<td>6.32 (0.92)</td>
<td>4.94 (0.71)</td>
<td>6.20 (0.87)</td>
<td>0.77</td>
<td>&lt;0.01</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>Peak diameter (mm)</td>
<td>4.99 (0.72)</td>
<td>6.54 (0.93)</td>
<td>5.19 (0.68)</td>
<td>6.43 (0.85)</td>
<td>0.89</td>
<td>&lt;0.01</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>SRAUC</td>
<td>26705 (12885)</td>
<td>22344 (8784)</td>
<td>44330 (31640)</td>
<td>24473 (10098)</td>
<td>0.01</td>
<td>0.08</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Time to peak (s)</td>
<td>73 (59)</td>
<td>77 (48)</td>
<td>97 (51)</td>
<td>70 (30)</td>
<td>0.45</td>
<td>0.45</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>FMD (%)</td>
<td>4.2 (2.1)</td>
<td>3.5 (1.9)</td>
<td>5.2 (2.7)</td>
<td>3.8 (1.9)</td>
<td>0.26</td>
<td>0.17</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>Corrected FMD (%)</td>
<td>3.7 (2.6)</td>
<td>4.0 (2.1)</td>
<td>4.4 (2.3)</td>
<td>4.2 (2.1)</td>
<td>0.41</td>
<td>0.95</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td><strong>Brachial Artery</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline diameter (mm)</td>
<td>3.64 (0.64)</td>
<td>4.28 (0.73)</td>
<td>3.68 (0.65)</td>
<td>4.20 (0.76)</td>
<td>0.60</td>
<td>0.10</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Peak diameter (mm)</td>
<td>3.85 (0.63)</td>
<td>4.55 (0.75)</td>
<td>3.91 (0.65)</td>
<td>4.54 (0.77)</td>
<td>0.14</td>
<td>0.06</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>SRAUC</td>
<td>45439 (16385)</td>
<td>24250 (10130)</td>
<td>43907 (24585)</td>
<td>37543 (12835)</td>
<td>0.03</td>
<td>0.04</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Time to peak (s)</td>
<td>92 (22)</td>
<td>51 (15)</td>
<td>92 (42)</td>
<td>84 (34)</td>
<td>0.06</td>
<td>0.02</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>FMD (%)</td>
<td>5.8 (2.7)</td>
<td>6.5 (3.3)</td>
<td>6.4 (3.5)</td>
<td>8.4 (3.8)</td>
<td>0.09</td>
<td>0.30</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>Corrected FMD (%)</td>
<td>4.8 (3.2)</td>
<td>7.6 (3.4)</td>
<td>5.4 (2.9)</td>
<td>8.8 (3.0)</td>
<td>0.15</td>
<td>0.04</td>
<td>0.64</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean (SD) and based on n=10 SCI and n=12 CON for the femoral artery and n=10 SCI and n=11 CON for the brachial artery. SCI, spinal cord injury; CON, uninjured control; SRAUC, shear rate area under the curve.
Peak reactive hyperemia (Figure 5.4) was ~40% lower in SCI at baseline in both the femoral [SCI: 7.5 (3.8) vs. CON: 11.6 (2.9) mL/min/mmHg; group P<0.01] and brachial arteries [SCI: 2.7 (1.1) vs. CON: 4.4 (1.6) mL/min/mmHg; group P<0.01). Peak brachial conductance tended to increase with heating in the CON group only (time P=0.01, interaction P=0.15), whereas peak femoral conductance did not change with heating (interaction P=0.59). The 5-min area under the curve of reactive hyperemia (Table 5.4) was not different between groups in either the femoral [SCI: 13.4 (7.3) vs. CON: 15.2 (6.8) mL/mmHg; interaction P=0.92] or brachial [SCI: 7.6 (3.9) vs. CON: 8.1 (4.9) mL/mmHg; interaction P=0.12] arteries and were unchanged with heating.
5.3.5 Microparticles

Pre- and post-heating blood samples were unable to be obtained from five participants in the SCI group and three in the CON due to difficulties with IV catheter insertion. Baseline
concentrations of total circulating platelet [SCI: 217 (105) vs. CON: 208 (201) MP/µL; P=0.98], monocyte [SCI: 147 (82) vs. CON: 110 (86) MP/µL; P=0.46], and leukocyte-derived MPs [SCI: 582 (174) vs. CON: 583 (310) MP/µL; P=0.90] were not different between groups and were unaffected by heating. Additionally, there was no between-group difference of total endothelial-derived MPs [SCI: 105 (102) vs. CON: 56 (22) MP/µL; P=0.10]; however, when participants taking medications were excluded a difference between groups emerged [SCI: 152 (106) vs. CON: 58 (24) MP/µL; P=0.02]. Considering such an effect of medications, subtype analyses of endothelial-derived MPs were performed only on participants not taking medications. After stratifying for endothelial MP phenotype (i.e., activation vs. apoptosis) and removal of participants with potentially interactive medications, activation-derived MPs (CD62e⁺; Figure 5.5) were markedly elevated in SCI compared to CON [152 (106) vs. 58 (24) MP/µL] and were reduced by 62% to values similar to CON following heating [58 (18) vs. 43 (42) MP/µL, interaction P=0.05]. Conversely, apoptosis-derived MPs (CD31⁺/42b⁻) were not different between groups at baseline [SCI: 39 (17) vs. CON: 62 (30) MP/µL] and did not change with heating [SCI: 52 (21) vs. CON: 68 (31) MP/µL; interaction P=0.70].
**Figure 5.4.** Peak vascular conductance in the femoral (left) and brachial (right) arteries measured via Doppler ultrasound during reactive hyperemia before and after 60 min of lower limb heating. Data are based on n=10 SCI and n=12 CON. *Different from CON (P<0.05).

**Figure 5.5.** Individual responses of circulating endothelial microparticles (MP) before and after heat exposure. The left panel illustrates CD62e⁺ (i.e., cellular activation) and the right panel shows CD31⁺/42b⁻ (i.e., cellular apoptosis). SCI, spinal cord injury (n=6); CON, uninjured controls (n=10). *Different from CON (P<0.05). **Different from pre-heating (P<0.05).
5.3.6 Metabolism

As previously mentioned, all blood-based biomarkers of cardiovascular risk factors were not different between groups (Table 5.1, all P>0.05). Following heating, however, in both groups, circulating triglycerides were lowered by ~15-20% (time P<0.01), insulin by ~25% (time P=0.03), and hence HOMA-IR was lowered by ~20-30% (time P=0.02). Glucose levels were reduced with heating by 15% in SCI but did not change in CON (interaction P=0.03).

5.3.7 Relationship between selected variables

Linear regressions were calculated in participants in which complete data for both MPs and shear rates were present (n=6 SCI, n=10 CON). Negative correlations existed between the change in circulating CD62e+ and the change in femoral retrograde shear rate (P=0.02) and OSI (P=0.05), and there was a trend toward a relationship with the change in femoral antegrade shear rate (P=0.08). No relationships existed with the brachial artery. Moreover, no relationships existed between baseline MPs and age, BMI, or FMD, nor did one exist between the change in MPs and the change in core temperature with heating.

5.4 Discussion

The goal of this study was twofold: 1) to determine the influence of chronic cervical SCI on vascular function, and 2) to determine the effects of acute lower limb heating on the peripheral vascular function in SCI compared to healthy controls. We found that macrovascular function (i.e., FMD) was selectively reduced in the brachial, but not the femoral, artery in those with SCI. In both the femoral and brachial arteries, downstream microvascular function
measured via reactive hyperemia was ~40% lower in the SCI participants vs. controls. Additionally, circulating biomarkers of endothelial activation (CD62e⁺), but not apoptosis (CD31⁺/42b⁻), were significantly elevated in SCI. In response to heating, macrovascular and microvascular function remained unchanged, whereas increases and decreases in antegrade and retrograde shear rates, respectively, were associated with reductions of endothelial activation (i.e., [CD62e⁺]). The results of this study highlight the potential of acute heat therapy as a novel intervention to reduce biomarkers of endothelial disturbances in chronic SCI.

5.4.1 Conduit artery structure and function

The acute phase of SCI (i.e., 3-4 weeks post-injury) is characterized by inward vascular remodelling reflected by smaller artery diameters below the level of injury (i.e., lower limb) (72, 73), whereas the diameter of arteries above the level of injury (e.g., brachial artery) have been reported to be unchanged (73) or even increased (241). However, most of these studies tested paraplegic (i.e., thoracic injury, T1-T12) participants. Our study recruited cervical (level of injury C3-C6) SCI participants and we observed that both SFA and BA diameters were lower in SCI compared to CON by 26 and 17%, respectively (Table 5.2). De Groot et al. (72) reported that SFA diameter and leg volume were reduced by ~25% within 3 weeks following thoracic SCI, suggesting that vascular remodelling might be determined by metabolic demands of local tissues. Consistent with previous studies (73, 178), we also found that blood velocity was higher in SCI and blood flow was thus preserved despite smaller artery diameters (Table 5.2). Due to the reductions in artery diameter and increases in blood velocity, elevated shear stress on the artery walls below the level of injury is widely reported in SCI (24, 72, 73, 236). Consistently, in
the current study, antegrade shear rates in both the SFA and BA were higher in SCI vs. CON (Table 5.2). Thus, despite structural remodelling, peripheral hemodynamics appear to be relatively well maintained in chronic SCI.

Exposure to elevated arterial wall shear stress and inward vascular remodelling have been speculated as potential mechanisms of preserved endothelial function in SCI (236). For example, Thijssen et al. (236) reported that mean wall shear rate in the superficial femoral artery was ~4 times higher in thoracic SCI vs. controls. Moreover, in that study both endothelial-dependent (i.e., FMD) and independent (i.e., responsiveness to sodium nitroprusside infusion) dilation of the femoral artery were not different in SCI compared to controls (236). These findings indicate that both endothelial function and smooth muscle cell sensitivity to nitric oxide were normal following thoracic SCI. Other studies have similarly reported that FMD is maintained following SCI (72, 118). However, when corrected for the potentially confounding influence of baseline diameter, FMD was lower in the femoral, but not the brachial, artery in thoracic SCI compared to uninjured controls (241). This observation contrasts with the current study where we found that FMD was only lower in the brachial artery in SCI (Figure 5.3), but similar findings have previously been reported in low-level SCI as well (118). It is unclear why differences exist between studies; however, to the best of our knowledge, the current study is the first to measure both brachial and femoral FMD in cervical SCI participants. It is noteworthy that, despite the propensity of the femoral artery to develop atherosclerosis compared to the brachial, the brachial artery has been demonstrated to provide predictive value of future CVD events (115, 131), whereas this link has not yet been tested in the femoral artery. Additionally, our study participants included chronic SCI (i.e., >2 years; range 2-44 years), which might allow sufficient
time for vascular remodelling to reverse any physiological impairments from the acute phase of injury despite complete inactivity of the lower limbs.

5.4.2 Microvascular function

In addition to macrovascular FMD, we measured reactive hyperemia following 5-min artery occlusion as an index of microvascular function (167). Similar to FMD, reactive hyperemia has been reported to predict cardiovascular events in both healthy (6) and at-risk groups (129, 156). We found that peak vascular conductance in both the femoral and brachial arteries following cuff release was ~40% lower in the SCI group compared to CON (Figure 5.4), whereas the 5-min conductance area under the curve was not different between groups. The differences in peak but not total flow (i.e., area under the curve) suggest that microvascular function is impaired primarily due to the structure of resistance vessels (210) rather than an impaired production of vasodilating substances or resistance vessel responses to those substances (88). In support of our findings, several studies have reported impairments of microvascular function in SCI measured via both Doppler ultrasound of the femoral artery (178) and laser-Doppler flowmetry of the cutaneous microvessels (81, 182, 183). Importantly, Van Duijnhoven et al. (254) reported that microvascular function did not change after eight weeks of electrically stimulated cycling exercise in SCI (thoracic injuries). However, Nash et al. (178) observed that reductions in reactive hyperemia were offset in cervical SCI participants who had participated in weekly sessions of electrically stimulated cycling for a more prolonged period (at least five months and up to seven years). These data demonstrate the potential for long-term improvement in vascular function in SCI. In the context of SCI, where macrovascular dysfunction is likely...
unchanged due to vascular remodelling and exposure to high shear stress, microvascular function might provide an important early insight into continued CVD risk in this population.

5.4.3 Influence of heat on shear patterns and vascular function

In the current study, lower limb heating resulted in beneficial changes in shear patterns in both the SCI and CON groups (Figure 5.2). As a result of increased femoral blood flow and conductance, antegrade shear rates in the femoral artery were greater in SCI vs. CON. Heating also reduced retrograde shear rates to nearly zero in both groups. Previous research has demonstrated a detrimental impact of retrograde shear rates in uninjured individuals (132, 234) and SCI (241), as well as beneficial effects of increases in antegrade blood flow patterns (209, 238, 239). Inducing changes in shear patterns, at least in uninjured controls, via local heating has been demonstrated to improve FMD acutely when measured immediately post-heating (117, 239); however, the data are equivocal when allowing a recovery period post-heating (209, 237). Romero et al. (209) observed improvements of FMD in their older group only, but in both young and older participants for microvascular function. The age of participants in our study were younger compared to the older group studied by Romero et al. (209), so it follows that acute heating in our study may not have altered FMD. It is unclear, however, why we did not also observe similar improvements in microvascular function following heating. Despite a lack of improvement in macro- or microvascular functions following heating, we observed beneficial increases in antegrade and decreases in retrograde shear rates, which are considered to be important stimuli for maintaining vascular function (114, 160). Indeed, despite inconsistent findings between acute heating studies, repeated hemodynamic stimuli via chronic heating
protocols have proved effective at improving both macrovascular endothelial (32, 179) and microvascular (41) function in non-SCI groups. Thus, in order to explain when – and if – the favourable changes in shear patterns can improve overall vascular function, study of the chronic effects of heating in SCI are clearly warranted.

5.4.4 Microparticles

Experimental models that reduce mean and increase retrograde shear stress via cuff inflation have demonstrated impairments to endothelial function (234) and increased MP release indicating activation and apoptosis of the endothelium (132). Conversely, passive heat stress, which is generally associated with beneficial flow and shear patterns, has been demonstrated to reduce circulating MPs of both endothelial activation and apoptosis in healthy young men (13). In our study, concentrations of endothelial CD62e+ (activation), but not CD31+/42b− (apoptosis), were elevated in SCI vs. CON at baseline, but CD62e+ was reduced to levels similar to CON after heating (Figure 5.5). The unchanged MP response in CON and the fact that CD31+/42b− concentration did not change in SCI suggest that the reduction in CD62e+ is not only a function of greater clearance, via increased blood flow, but it is likely a reduction in the presence of these specific MPs. Endothelial-derived MPs have been reported to be elevated in several disease states including hypertension, coronary artery disease, metabolic syndrome, and have been implicated in the development of endothelial dysfunction and atherosclerosis by promoting inflammation and thrombosis, and reducing NO bioavailability (1, 22, 48). These data indicate that SCI is associated with chronic endothelial cell activation resulting in elevated concentrations of activation-derived endothelial MPs. However, SCI does not appear to confer a pro-apoptotic
influence on the endothelium as circulating apoptosis-derived endothelial microparticles were not significantly different compared with the uninjured adults. Endothelial activation and the subsequent release of activation-derived microparticles may contribute to the observed impairments of vascular function in the SCI group. Importantly, the effects of passive heating appear to be a promising strategy to counteract endothelial activation and its detrimental sequelae, including vascular inflammation and endothelial dysfunction.

5.4.5 Changes in metabolic markers

Although glucose, insulin, and HOMA-IR were all within the normal range and similar between SCI and CON in our study, glucose and insulin resistance are reported to be highly prevalent in SCI populations (19, 51). Thus, the clear reductions in these values, particularly in the SCI group for glucose, following heat stress potentially highlight clinically important metabolic responses. Indeed, at least in animal models, increases in temperature reduce insulin resistance and inflammation by augmenting the cell stress response and heat shock proteins (52, 127). The acute effects of heat on glucose control in humans, however, are less conclusive. For example, although one study reported that post-prandial glucose levels were attenuated following heat stress (+1°C core temperature) compared to the same core temperature change following exercise (92), several others have reported greater glucose and insulin levels at higher ambient temperatures (82, 93, 176). It is noteworthy that increases in core temperature in the latter studies were limited to <0.5°C. An important consideration when measuring glucose/insulin responses during whole-body heating is the increased forearm blood flow via cutaneous vasodilation, which might lead to an arterialization of venous blood samples (97). This could explain, in part,
the different responses of glucose concentrations between SCI and CON in our study given the slightly greater increases in brachial blood flow in CON. Although there is little data on the chronic effects of heating and glucose/insulin control, Hooper et al. (126) reported that HbA1c was reduced by one percentage point following three weeks of hot tub therapy in type II diabetic patients. The effects of long-term heating interventions on glucose and insulin resistance in SCI and pre-diabetic populations are therefore of great interest.

5.4.6 Experimental limitations

Although the sample size in the current study was larger than the majority of previous studies of peripheral vascular function in SCI, and the first in cervical SCI, the sample was still relatively small. The nature of this SCI population meant that many of the participants were on indicated medications (Table 5.1). On one hand, inclusion of SCI participants on medications is highly appropriate, and the majority of the findings persistent; however, some of the findings (e.g., endothelial microparticles) became clearer when isolated to those SCI patients not on medications. Although our study would benefit from extension into a larger cohort, the current results may provide some insight on knowledge gaps underlying CVD risk in SCI. Furthermore, we provide strong evidence for continued research on the therapeutic effects of passive heating. Although a 60-min bout of heating could be considered long, it might be a necessary stimulus to detect acute responses. However, evidence exists to suggest that shorter bouts of 30-min hot water immersions (12, 238) and 10 to 15-min sauna exposures (150), over long periods of times, confer vascular and health benefits. The implementation of longer-term heating interventions, as
used in young healthy volunteers (33), are especially warranted in the cervical SCI population who have a compromised capacity to exercise.

Another important consideration is the extent of thermoregulatory impairment in SCI. The lack of sweating and diminished cutaneous vasodilatory responses below the level of injury result in rapid increases in core temperature. Similar to previous reports (192), the observed core temperature increases were twofold greater with the same heat stimulus in SCI compared to CON (Figure 5.1). The differential changes in core temperature responses are important for the parsimonious interpretation of the physiological stimulus of vascular benefits from heating. The relatively small changes in core temperature in our CON group (+0.35°C) might explain the lack of changes in vascular function compared to other studies where increases in core temperature are generally >0.5°C. Repeated bouts of hemodynamic stimuli (e.g., heat-induced shear stress) have been widely reported to underlie improvements in vascular function (112, 239), but increases in temperature may also be an important stimulus. In particular, increases in core temperature may lead to improvements of metabolic function (i.e., glucose/insulin control) via heat-induced cellular stress. However, our intention with this experimental design was to test a practical and safe method of heating (i.e., lower limb water immersion), which would be feasibly implemented as a therapy for the SCI population.

5.4.7 Conclusion

In our participants with cervical SCI, we observed selectively impaired macrovascular function in the brachial artery and impaired microvascular function in both the brachial and femoral arteries. Circulating biomarkers of endothelial activation were also elevated in SCI.
Lower limb heating, however, induced beneficial changes to arterial shear patterns, which were associated with reductions in endothelial microparticles. This study provides evidence that, at least acutely, passive heating can be used as a safe and practical therapy for improving vascular function in a SCI population. This type of therapy might be of particular benefit, especially in individuals with high level SCI who experience greater difficulties exercising.
Chapter 6: The influence of habitual sauna bathing on flow-mediated dilation in older adults: a pilot study

6.1 Background

Advancing age is one of the dominant factors linked to the development of cardiovascular diseases. In addition to the accumulation of risk factors, changes in vascular structure and function are a key component of this age-disease interaction (146). Yet, a disconnect exists where the reduction in CVD is only partially explained by improvements in traditional risk factors due to exercise/physical activity. It was proposed that the additional benefits of exercise on endothelial function might partially mediate the lower CVD risk (137). Specifically, reduced NO bioavailability due to age-related increases in oxidative stress lead to endothelial cell senescence and consequently impaired endothelial-dependent dilation (79, 220). However, evidence suggests that habitual exercise can at least partially offset endothelial dysfunction (77, 212, 259). Despite existing evidence of the medical benefits of exercise (191), only a small portion of the population completes the recommended quantity of exercise (54). As a result, alternative strategies to prevent and reduce CVD in older adults, especially those limited exercise capacity, is a current research priority.

In a cohort of men aged 42–60 years, greater frequency of sauna bathing was related to reduced mortality associated with CVD (150), as well as all-cause mortality and the risk of stroke (145) and dementia/Alzheimer’s disease (151). Therefore, repeated exposures to heat stress may be an effective strategy to perturb the homeothermic physiology of sedentary humans (240). Although hemodynamic signals resulting from shear stress underlie vascular adaptations to exercise and heat interventions (23, 42, 148, 179), the physiological mechanisms that mediate lower risk of mortality are unknown. We hypothesized that greater endothelial-dependent
dilation, an index of endothelial cell function and NO bioavailability (113), is greater in healthy, older adults who regularly partake in sauna bathing compared to those who do not. To test whether sauna provides greater benefits to those with worse baseline vascular impairments, we additionally hypothesized that the improvement to endothelial-dependent dilation in regular sauna users compared to non-users is augmented in a group of older adults with coronary artery disease (CAD).

6.3 Methods

6.3.1 Ethical approval and participants

Participant characteristics, blood profiles, and medications are presented in Tables 1 and 2. This study was approved by the Montreal Heart Institute Research Ethics Committee (#2017-2179) and all participants provided written, informed consent prior to their participation in data collection. Based on FMD values in older adults (259) who were sedentary [0.15 (0.07) mm] vs. habitually exercise trained [0.26 (0.08) mm], we estimated that nine participants were required per group to detect a ~3% between-group difference in FMD using G*Power (v3, University of Duddeldorf, Germany) (91). Adults aged 50-80 years (n=16) were recruited from the community and all healthy participants were free from CVD as well as medications related to the primary or secondary prevention of CVD. Exclusion criteria were diagnoses of hypertension, diabetes, smoking within the last five years, or resting ECG abnormalities. A subset of participants with stable coronary artery disease (CAD) was also recruited (n=8). Stable CAD was defined as ≥70% narrowing of at least one major epicardial artery documented by angiography and treatment of percutaneous coronary intervention and/or coronary artery bypass graft >3 months prior to
enrollment, with stable medication $>1$ month prior to enrollment. Additional exclusion criteria for the CAD group was ejection fraction $<40\%$ or clinical evidence of heart failure, valvular disease, uncontrolled hypertension, diabetes, or ECG indication of ST segment abnormalities. All female participants were post-menopausal and were not on hormone replacement therapy. Physical activity (53) and sauna use levels were assessed via questionnaires.
Table 6.1. Baseline participant characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Age (years)</th>
<th>Sex (F/M)</th>
<th>BMI (kg/m²)</th>
<th>MAP (mmHg)</th>
<th>Physical Activity (MET·min/week)</th>
<th>Sauna Time (min/week)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Healthy adults</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Users</td>
<td>68 (9)</td>
<td>5/2</td>
<td>25.9 (2.8)</td>
<td>91 (7)</td>
<td>2297 (1417)</td>
<td>38.5 (16.4)†</td>
</tr>
<tr>
<td>Non-users</td>
<td>66 (6)</td>
<td>4/5</td>
<td>26.5 (2.6)</td>
<td>95 (9)</td>
<td>3433 (1521)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Stable CAD patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Users</td>
<td>66 (7)</td>
<td>0/4</td>
<td>29.9 (3.6)</td>
<td>91 (8)</td>
<td>4485 (1760)</td>
<td>26.9 (9.0)†</td>
</tr>
<tr>
<td>Non-users</td>
<td>66 (7)</td>
<td>0/4</td>
<td>28.7 (3.0)</td>
<td>95 (5)</td>
<td>2180 (1880)</td>
<td>0</td>
</tr>
<tr>
<td><strong>One-way ANOVA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.941</td>
<td>N/A</td>
<td>0.139</td>
<td>0.730</td>
<td>0.122</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

One-way ANOVA comparing values between all four groups. BMI, body mass index; MAP, mean arterial pressure; MET, metabolic equivalent of task. †P<0.05 vs. non-users.
6.3.2 Measurements and statistical analysis

All vascular measurements were performed as outlined in the methods section of Chapter 3, where ultrasound recordings were screen captured (Camtasia v9, TechSmith) from a remote computer via frame grabber (DVIUSB 3.0, Epiphan) and saved for offline analysis. Semi-automated edge tracking software (Cardiovascular Suite v3, Quipu SRL) provided time-averaged arterial diameter and blood velocity at a sampling rate of 30 Hz. Blood flow and FMD were calculated from the raw data using equations 1 to 6 from Chapter 3. All data are presented as means (standard deviation, SD). Data were checked for normality using the Shapiro-Wilk test and were then compared between all four groups using a one-way ANOVA performed in SPSS version 24 (IBM, Armonk, NY, USA). The critical P value was set at 0.05. When significance was identified, post-hoc tests were performed with a Bonferroni correction. Figures were generated with GraphPad version 6.0 (Prism, La Jolla, CA, USA).
Table 6.2. Baseline blood profiles and related medications.

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Healthy Adults</th>
<th>Stable CAD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sauna</td>
<td>Control</td>
<td>Sauna</td>
</tr>
<tr>
<td><strong>Total cholesterol (mmol/l)</strong></td>
<td>5.8 (0.3)†</td>
<td>4.9 (0.6)</td>
<td>3.1 (0.4)*</td>
</tr>
<tr>
<td><strong>LDL (mmol/l)</strong></td>
<td>3.0 (0.6)</td>
<td>2.6 (0.7)</td>
<td>1.3 (0.4)*</td>
</tr>
<tr>
<td><strong>HDL (mmol/l)</strong></td>
<td>2.2 (0.6)</td>
<td>1.8 (0.3)</td>
<td>1.3 (0.3)*</td>
</tr>
<tr>
<td><strong>TG (mmol/l)</strong></td>
<td>1.4 (0.4)</td>
<td>1.2 (0.6)</td>
<td>1.2 (0.4)</td>
</tr>
<tr>
<td><strong>Glucose (mmol/l)</strong></td>
<td>4.8 (0.2)</td>
<td>5.1 (0.6)</td>
<td>5.5 (0.8)</td>
</tr>
<tr>
<td><strong>HbA1c (%)</strong></td>
<td>5.5 (0.3)</td>
<td>5.4 (0.2)</td>
<td>6.2 (0.6)*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Medications</th>
<th>Healthy Adults</th>
<th>Stable CAD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sauna</td>
<td>Control</td>
</tr>
<tr>
<td><strong>Statins</strong></td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td><strong>Acetylsalicylic acid</strong></td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><strong>NSAID</strong></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Antiplatelets</strong></td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td><strong>Β blockers</strong></td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><strong>Ca²⁺ channel blockers</strong></td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><strong>ACE inhibitors</strong></td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td><strong>Hypoglycemic agents</strong></td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides; HbA1c, glycated hemoglobin; ACE, angiotensin converting enzyme; AngII, angiotensin II; NSAID, non-steroidal anti-inflammatory drugs; β, beta; Ca²⁺, calcium. *P<0.05 vs. healthy adults, †P<0.05 vs. non-users.
6.4 Results

By design, sauna time was greater in sauna users vs. non-users, with no difference between healthy adults and CAD patients (P<0.001, Table 6.1). All other baseline characteristics were not different between groups (all P>0.05) except for lower total cholesterol (P<0.001), LDL (P=0.001), and HDL (P=0.005) in CAD patients compared to healthy older adults, likely due to medications (Table 2). Habitual sauna users with CAD also had higher HbA1c compared to healthy older adults (P=0.014). Baseline brachial artery diameter (P=0.235) and conductance (P=0.812) were not different between groups, but reactive hyperemia SRAUC was lower in CAD patients compared to healthy older adults (P=0.013). However, neither variable significantly influenced FMD when included in the model as a covariate. Additionally, there were no differences between groups for FMD (P=0.224) or peak vascular conductance during reactive hyperemia (P=0.723).
Table 6.3. Brachial artery characteristics and endothelial-dependent dilation.

<table>
<thead>
<tr>
<th></th>
<th>Baseline diameter (mm)</th>
<th>Baseline conductance (ml/min/mmHg)</th>
<th>FMD (mm)</th>
<th>FMD (%)</th>
<th>Corrected FMD (%)</th>
<th>SRAUC (s)</th>
<th>Peak Conductance (ml/min/mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Healthy adults</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Users</td>
<td>4.35 (1.06)</td>
<td>1.17 (1.13)</td>
<td>0.18 (0.08)</td>
<td>4.4 (2.1)</td>
<td>3.9 (1.6)</td>
<td>15929 (8280)</td>
<td>4.5 (2.2)</td>
</tr>
<tr>
<td>Non-users</td>
<td>4.26 (0.89)</td>
<td>1.09 (1.14)</td>
<td>0.17 (0.08)</td>
<td>4.4 (2.6)</td>
<td>4.3 (1.8)</td>
<td>13266 (5223)</td>
<td>4.2 (2.2)</td>
</tr>
<tr>
<td><strong>Stable CAD patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Users</td>
<td>5.22 (0.70)</td>
<td>0.80 (0.26)</td>
<td>0.13 (0.07)</td>
<td>2.4 (1.5)</td>
<td>3.1 (2.0)</td>
<td>4588 (3808)*</td>
<td>4.0 (2.2)</td>
</tr>
<tr>
<td>Non-users</td>
<td>4.93 (0.11)</td>
<td>1.48 (0.64)</td>
<td>0.10 (0.05)</td>
<td>2.1 (1.1)</td>
<td>2.6 (1.8)</td>
<td>6020 (2183)</td>
<td>4.5 (1.0)</td>
</tr>
<tr>
<td><strong>One-way ANOVA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.235</td>
<td>0.812</td>
<td>0.301</td>
<td>0.224</td>
<td>0.568</td>
<td>0.013</td>
<td>0.723</td>
</tr>
</tbody>
</table>

One-way ANOVA comparing values between all four groups. FMD, flow-mediated dilation; SRAUC, shear rate area under the curve to peak diameter. *P<0.05 vs. healthy adults.
6.5 Discussion

The purpose of this study was to explore the potential relationship between habitual sauna bathing and endothelial-dependent dilation as a potential underlying mechanism of reduced mortality associated with CVD. The main findings were that FMD and peak vascular conductance, an index of microvascular function, were not different between older adults who regularly partake in sauna bathing compared to those who do not. Additionally, preliminary results provide no indication that FMD and peak vascular conductance were different in a subset (n=4) of older adults with stable coronary artery disease who regularly partake in sauna bathing compared to those who do not. Thus, factors other than endothelial function might underlie the reduction in CVD mortality associated with greater frequency of sauna bathing and will be discussed in the following paragraphs.

Our observations are consistent with those for aging and with clinical heart disease where endothelial-dependent dilation worsens with age and disease. For example, Wray et al. (272) reported FMD was ~30% lower in older, healthy adults compared to young adults and Manganaro et al. (163) reported that FMD is negatively related to disease severity in CAD patients. Indeed, we observed brachial FMD values of 4.4% in older, healthy adults (Table 6.3) compared to the typical ~6.5% observed in younger, healthy adults (104) and ~6% reported in Chapters 3 and 5. Moreover, the CAD sauna users and non-users exhibited FMD responses of 2.4% and 2.1%, respectively. It has been reported that FMD is predictive of future cardiovascular events (274) where a 1% increase in FMD was associated with a 13% reduction in the risk of a cardiovascular event (131). Moreover, a meta-analysis revealed that FMD is ~70% dependent on NO; thus it is plausible that this measure would relate to differences in risk (e.g., 22% lower risk of CVD-related mortality) with sauna bathing 2-3 times per week – which is similar to the
volume of sauna bathing in our participants (~30-40 min per week). Contrary to our hypothesis, however, regular sauna bathing was not associated with higher FMD. Although our sample size in the sauna group is underpowered by two participants in the sauna user group (based on an a-priori sample size calculation of between-group differences in FMD with exercise training), we did not observe a trend toward a difference between groups in healthy older adults. However, the absence of a mean difference between these groups results in an achieved power of 0.05. In the CAD groups, we would need to double the sample size in order to determine statistical difference from the healthy groups, based on an achieved power of 0.72. The minimal effect of sauna use, in both healthy and CAD groups (Figure 6.1), indicates that other physiological mechanisms might be responsible for the reduction in risk with sauna bathing.

Figure 6.1. Flow-mediated dilation (FMD) and peak vascular conductance of the brachial artery during reactive hyperemia.

For instance, arterial stiffness and other circulating factors (e.g., heat shock proteins) could be important mediators of the protective effects of repeated heat exposures. Both acute (83,
and longer term (33) heating interventions have reported reductions in central artery stiffness using pulse-wave velocity (PWV), and PWV is a strong predictor of future cardiovascular events (21, 26). The two main factors that determine PWV are vascular structure and neurovascular tone. Our data provide little indication of vascular remodelling or altered central hemodynamics, with no differences in baseline brachial diameters (Table 6.3) or MAP (Table 6.1). However, arterial stiffness was not measured so we cannot rule out potentially greater collagen deposition that could lead to stiffer vessels. In fact, lower cholesterol and LDL in the CAD group demonstrates a probable effect of medication use in this group compared to the healthy group (Table 6.2); therefore, MAP (and arterial stiffness) would possibly be higher in the CAD group without medication. Sauna bathing is also associated with lower systemic inflammation (149). However, if the non-sauna-user group experienced greater inflammation or reactive oxygen species (ROS) then it could be observable via FMD given their effects on NO bioavailability (219, 272).

The inclusion of the CAD group was to provided exploratory insight to determine if the effects of heat on vascular function were augmented in a group with baseline vascular dysfunction. For example, older healthy adults increased FMD following 45 minutes of limb heating whereas young adults did not (209). It was speculated that the age difference in this study might be reflective of a baseline vascular dysfunction in the older group that permitted improvements due to heating. However, in our study, there was no difference in FMD between the CAD and healthy groups, or between sauna users and non-users in the CAD group (Figure 6.1). On average, the FMD values appear to be lower in the CAD group, but conclusions from this comparison with healthy older adults are limited due to the small sample size of CAD patients. In the study by Romero et al. (209), microvascular function improved in the older,
healthy group as well as the young group, indicating that microvascular function may be a more sensitive measurement of acute changes in vascular function with heat stress. Another difference between our study and that of Romero et al. (209) is the measurement of FMD in the brachial compared to superficial femoral artery, respectively. These arteries differ in their baseline diameters, propensity for atherosclerosis, and their responses to local heating. For example, FMD in the brachial increases following forearm heating in young adults (46, 239), whereas superficial femoral artery FMD does not change following acute leg heating (56, 209).

The main limitation to this study is the cross-sectional design. A better approach would be to prospectively measure FMD and follow groups of individuals with varying sauna habits for several years in a longitudinal design. However, this approach would be logistically cumbersome, and our pilot study provides initial data to help direct the goals of such a study. The sample size is small, particularly in the CAD group; however, no trends exist in the healthy older group. Therefore, future studies should strongly consider measuring other aspects of vascular function including arterial stiffness, endothelium-independent dilation, and cardiac function. We also included both men and women in this study and all women were post-menopausal and not taking hormone replacement therapy. This latter point is important given the known effects of estrogen on vascular function (221). The majority of our participants were Caucasian, so testing the influence of habitual sauna use on vascular function in other racial groups would be of value, particularly in groups at greater risk of hypertension (e.g., African-American). The difference in sauna time per week between groups was large in this study, but it would be of interest to determine whether varying frequency and duration of sauna use impact vascular function as well. For instance, there was a progressive reduction in CVD risk with increasing sauna duration and frequency, but it is unclear with a stimulus-response relationship
exists for sauna time and FMD. Moreover, there were no between-group differences in the self-reported quantity of physical activity performed by the participants, which is an important confounder to control in order to isolate the influence of sauna habits on FMD.

6.5.1 Conclusion

The results of this pilot study indicate that endothelial-dependent dilation (measured via FMD) is not different between groups of habitual sauna users compared to non-users in older adults with and without CAD. Factors that affect CVD risk other than FMD should be considered as potential underlying mechanisms for reduced CVD mortality in habitual sauna users.
Chapter 7: General discussion

7.1 Overview of objectives and results

The overarching aim of this thesis was to determine selected mechanisms and applications of passive heating as an approach to improve endothelial function in healthy individuals and those at risk of CVD. The primary findings were that 1) in addition to elevated shear stress, skin and core temperatures independently increase endothelial function after heating; 2) although α-1 adrenergic antagonism did not influence reduced hyperthermic cerebral blood flow or post-heating FMD, preliminary trends indicate a possible impact of sympathetic nervous system activity on FMD in the brachial artery; 3) lower limb heating does not affect endothelial function in healthy individuals but it lowers biomarkers of endothelial cell inflammation in individuals with spinal cord injuries; and 4) at least in the exploratory cross-sectional study conducted, endothelial function is not improved in habitual sauna users compared to non-users.

7.2 Integration of key findings

Selected mechanisms and contributing factors to changes in FMD with passive heating were identified. These findings were then extended into groups at higher risk of CVD (e.g., SCI, aging, CAD). It is generally considered that increases in shear stress via passive heating increase NO bioavailability (35) and therefore FMD (33). However, when examining the existing literature on FMD following heating, directional changes in FMD consistently remain equivocal. Indeed, there appears to be a pattern where studies that do not increase core temperature (i.e., those employing limb-specific heating) report greater improvements in FMD compared to studies where core temperature is elevated by heating larger body surface areas (see Figure 7.1).
The differences in ΔFMD between these studies may arise from different modes of heating (i.e., limb vs. whole-body), and thus the magnitude of sympathetic activation. For example, isolated forearm heating leads to improved FMD (46, 117, 239) in some studies where changes in body core temperature are negligible, in contrast to an absence of change in FMD with moderate to severe increases in core temperature (34, 237). Accordingly, it is possible that elevated SNA following heat stress constrains FMD via α-adrenergic tone. The constraining effect of SNA on FMD also appears to outweigh the heat-induced reductions in oscillatory shear patterns (231). Furthermore, Engelland et al. (89) demonstrated that elevations of muscle SNA occur in young adults with increases in core temperature as little as 0.2°C, which could explain the lack of FMD improvement in studies using lower body heating where core temperature increases by ~0.4°C (56, 209). Moreover, elevated SNA persists for at least 30 min following increases in core temperature (89), indicating that the timing of FMD measurements after heating might also be an important factor to consider.
Figure 7.1. Updated forest plot of the mean difference (±95% confidence intervals) in flow-mediated dilation (FMD) following acute heating studies as a function of the change in body core temperature. The values above each study denote the increase in core temperature. Red points indicate data presented in this thesis. *Indicates a significant change from baseline.

In addition to differences in core temperature, isolated forearm heating (i.e., large increases in skin temperature, no increase in core temperature) studies have all measured FMD immediately after heating whereas whole-body heating studies generally provide a short (~30
min) period of recovery from heating before measuring post-heating FMD. This recovery period may allow the acute effects of shear stress to diminish before the FMD is performed while SNA remains elevated. In fact, acute increases in shear stress (~45%) can offset reductions in FMD due to elevated SNA (231), which might be the case for the results in Chapter 3. For instance, FMD increased by 5.6% (absolute change) immediately after whole-body heating compared to no changes in FMD in similar studies that provided a recovery period (34, 237). Moreover, it is possible that the protective/beneficial effects of increased shear stress are prolonged in the absence of elevations in SNA. Greyling et al. (117) reported that FMD remained increased for up to 90 min following forearm heating, yet FMD remained unchanged for up to 2 hours following whole-body heating (34). Few studies have assessed the time course of endothelial and neural responses following heat stress; therefore, more evidence is required before drawing conclusions regarding the temporal effects of acute heating on FMD. However, our data from Chapter 4 provide preliminary evidence of a potential role of SNA in the divergence of FMD responses following heating of varying modes (e.g., limb vs. whole-body) and timing of measurements (e.g., recovery vs. no recovery period). This was accomplished by comparing FMD following whole-body heating (with a 30-min recovery) between placebo and \(\alpha_1\) adrenergic blockade (via oral Prazosin ingestion). A potential trend emerged from this pilot study where FMD increased in the Prazosin condition compared to no change in the placebo condition. If confirmed in a larger data set, this finding indicates that potential effects of heating are present when sympathetic vascular restraint is absent.

Increases in FMD following local heating also suggest that skin temperature might be an important stimulus for vascular adjustments. This hypothesis is consistent with cutaneous vascular adaptations to heating where NO-dependent rises in skin blood flow are absent when
skin temperature of the forearms is clamped to baseline during increases in core temperature (41). The independent role of skin temperature on FMD, however, has not been dissected from other factors such as shear stress and core temperature, which occur concomitantly to changes in skin temperature and pose an experimental challenge. For example, in addition to local skin temperature, lower limb heating increases core temperature in several studies (41, 42, 56, 209).

In Chapter 3, we used a bilateral FMD design where one arm was cuffed to attenuate shear stress in order to independently modified core temperature, shear stress, and skin temperature during two separate heating protocols (forearm vs. whole-body heating). We demonstrated that FMD increased in both arms by 1.5-2% with high skin temperatures after forearm heating irrespective of ~50% lower shear stress in the cuffed arm – without changes in core temperature. Additionally, FMD increased similarly in both arms after whole-body heating despite attenuated shear stress and skin temperature in the cuffed arm. Multiple regression of the compiled FMD change scores from each protocol, including several thermal and hemodynamic factors as independent variables, indicated that core temperature explained the greatest variance of the model (34%) and skin temperature explained the second largest portion of variance (31%). When comparing only the non-cuffed arm, we also found that the changes in FMD were greatest after whole-body heating compared to forearm heating and a time-matched control, further supporting the established importance of shear stress as a major modulator of FMD. We therefore, experimentally and statistically, demonstrated independent effects of skin temperature, core temperature, and shear stress on acute responses of FMD to heating.

Delineation of the factors that contribute to FMD responses after heating in young, healthy individuals is important; however, these mechanisms likely differ in aging and disease. For example, FMD increased in older, but not young, adults following 45 min of lower limb
heating despite increased core temperature and a subsequent recovery period where SNA likely remained elevated (89). It was speculated that the benefits of heating may be augmented when some degree of vascular dysfunction exists at baseline (e.g., age-related decrements in FMD) (209). It is also important to apply our findings to groups that could benefit the most from improvements in vascular function. For example, individuals with SCI are among the least active individuals in our society and face many barriers to participation in physical activity (72, 253). Therefore, we tested whether a practical strategy of lower limb heating could improve FMD in individuals with high-level SCI compared to age- and sex-matched uninjured control participants (Chapter 5). Here, it was observed that no changes occurred in FMD following heating in our SCI or control groups. Although the aforementioned effects of increased core temperature could explain the lack of effect in the control group, sympathetic vasomotor control is absent in the SCI group and therefore cannot explain these results. The lack of supraspinal control in SCI exists at least in the femoral artery (below the level of injury), but the nature of the cervical level injuries caused varying degrees of sympathetic innervation in the brachial artery. Nevertheless, we demonstrated that systemically circulating endothelial MPs – which are elevated at baseline in SCI – were reduced following heating in the SCI group but not controls. The endothelial MP concentrations demonstrate the change in endothelial cell phenotype that occurs with increased temperature and/or shear stress from heating. Not only are MPs reflective of cell phenotype, but they are also important mediators of intercellular communication (71) via transportation of micro RNA, which are involved in the regulation of atherosclerosis (94). Additionally, MPs hold predictive value of future cardiovascular events in at-risk and diseased individuals (22, 71). It is possible that such changes repeated over time would manifest in a functional response as well (i.e., improved vascular function), but this has yet to be evaluated.
Whether improved endothelial function contributes to the reduction in risk of CVD associated with chronic sauna use (145, 150, 151) is unknown. We therefore compared FMD between older adults (with and without CAD) who do and do not engage in habitual sauna bathing in Chapter 6. In our preliminary results, however, we found no difference in FMD values between groups. The lack of difference suggests that endothelial function may not be the main factor contributing lower CVD risk with sauna use. Alternatives explanations, which were not measured in our study, could be related to lower arterial stiffness or circulating protective molecules (e.g., heat shock proteins); however, these have not been measured in sauna users. Although FMD is likely lower in CAD compared to healthy older adults, our data did not achieve statistical significance due to limitations in sample size. Indeed, it has previously been reported that heat therapy improves FMD in patients with coronary risk factors (130) and with heart disease (142). Whether higher FMD values would be demonstrated in habitual sauna users with CAD if the sample size was larger is unknown. Despite a lack of effect of heating on endothelial function, “heat therapy” remains promising due to other beneficial outcomes. For example, heart failure patients reported better quality of life after three weeks of Waon (i.e., infrared sauna) therapy (227). Moreover, Akerman et al. (4) demonstrated that 12 weeks of hot water immersion improved six-minute walk distance and pain-free walking distance in patients with peripheral artery disease. Thus, even in the absence of changes to endothelial function, passive heating is a potentially useful therapeutic tool to improve aspects of cardiovascular function as well as functional outcomes. For example, chronic heating has the potential to reduce arterial stiffness (33), muscle SNA (87), as well as glucose and insulin concentrations (86, 124).

7.2 Contributions of thesis work to field of study
The present findings advance the understanding of 1) the circumstances under which endothelial function is acutely improved by heating, and 2) the potential uses of passive heating as a therapeutic tool to attenuate the risk of CVD. Currently, there are no guidelines for the time, type, or intensity of passive heating to improve cardiovascular function. Fox et al. (95) demonstrated that elevating core temperature >38.5°C for one hour was an effective strategy to induce heat acclimation, and recent studies have adopted this approach to induce vascular adaptations (18, 33). However, limb heating studies demonstrate that benefits can occur without changes in core temperature and sauna studies suggest that much shorter durations may provide benefits as well. Our data from Chapter 3 identified that skin and core temperatures are practical measures that can be used as targets for heat therapy. Increasing shear stress by eliciting high skin temperatures improved FMD without changes in core temperature, and increasing core temperature below the 38.5°C “threshold” for one hour also improves FMD. Approximately 20-30 min are required to increase core temperature to ~38.5°C using hot water immersion, and therefore would require ~90 min to complete one session of heating using this model of heat acclimation. In order to make heat therapy less challenging and time consuming, the effectiveness of shorter duration sessions should be studied, particularly for individuals with impaired thermoregulatory and vascular response to heat stress (e.g., older adults). Changes in skin temperature can also be used to target a given stimulus in a less invasive manner such as with limb-specific heating.

Autonomic activity during heat stress is responsible for hemodynamic and sudomotor responses that facilitate thermoregulation. However, the impact of autonomic activity on endothelial function during heat stress has not been previously considered. Previously, debate occurred regarding the effect of acute exercise on endothelial function because impaired function
is often reported immediately following exercise (2, 218). However, Atkinson et al. (8) demonstrated that measurement of the beneficial effects of exercise (alongside likely increases in core temperature) are masked by vascular restraint due to sympathetic activation. Similarly, our preliminary results indicate that there may be an effect of SNA on FMD following an acute bout of heating. If this finding is substantiated in a completed data set it would be an important consideration for experimental design of studies examining the effects of heat on vascular function. For example, if FMD is restrained immediately following heat stress, then studies investigating the acute effects of heat on vascular function should consider performing post-heating measures several hours later, or perhaps even the following day.

The need for alternative or adjunct therapies to improve vascular function in population groups with limited abilities to perform exercise is paramount. Individuals with SCI are a prime example of a group with profound vascular dysfunction as well as many barriers to exercise, particularly with high level (e.g., cervical) injuries. Chapter 5 was the first study to evaluate effectiveness of acute heating on FMD in SCI. Although we found that acute heating does not change FMD in either the superficial femoral or brachial arteries, endothelial MPs were reduced following heating in the SCI group. This finding demonstrates that shear stress and/or high temperatures associated with acute heating may have benefits for endothelial cells, which warrants further study with chronic heating interventions. Finally, this thesis is also the first to test the mechanisms underlying reported benefits of habitual heat therapy. Although we did not find that endothelial function explains the reduction in CVD risk with habitual sauna bathing, our results could help to narrow the physiological targets of heat therapy.
7.3 Strengths of studies

Strengths that were consistently present throughout this thesis are the use of direct measures of core temperature and brachial artery FMD. Although invasive, esophageal and gastrointestinal temperature are important to accurately measure when assessing the contributions of thermal factors to vascular function with heat stress. We also measured FMD in the brachial artery of each study, which allows us to compare our results within chapters as well as to previously published work in the area largely performed in the brachial artery. However, we also measured superficial femoral artery FMD when logical. In chapter 5, it was important to measure endothelial function in the legs of individuals with SCI and because we used lower leg heating in that study. This experimental design was appropriate to address vascular function in immobile limbs, but it was also a practical method of heating that could be implemented as an at-home therapy due to its low cost and low level of expertise required for implementation. The inclusion of the time control in Chapter 3 was important to be confident that the changes in FMD were not due to other factors during the protocol. Furthermore, the time control confirmed previous reports that reductions in shear stress during periods of inactivity per se can decrease FMD.

7.4 Limitations to studies

The main limitation to this thesis is the small sample sizes in the exploratory chapters (Chapters 4 and 6). Although females were included in most of the studies, the majority of participants were male. The objectives of the thesis were not to examine sex differences in the vascular responses to heat stress, but greater representation of women is important and this is a logical area of study. We only measured blood viscosity in one study, but we do not expect changes in plasma volume with whole-body heating to change our findings due to increased red
blood cell deformability with hyperthermia (38). Nevertheless, blood viscosity is an important component of quantification of shear stress that should be included when possible to fully capture the true shear stimulus during reactive hyperemia. Viscosity might be particularly important when comparing limb vs. whole-body heating where changes in plasma volume and core temperature are vastly different.

A large portion of the heat therapy premise is based on the ability of heat to increase NO bioavailability; however, we do not have any direct measures of circulating markers of NO. We were also not able to quantify the magnitude of the NO contribution to vasodilation. In Chapter 6, the cross-sectional nature limits the ability to draw conclusions regarding the long term effects of habitual sauna bathing. Additionally, we did not measure endothelial independent dilation or arterial stiffness – two other measures of vascular function that could be associated with CVD risk independent of endothelial function. Therefore, broad measures of vascular function (not only endothelial function), including the influence of blood viscosity, should be a current research priority.

7.5 Application of findings

The findings of this thesis are important to inform future research and the potential development of guidelines on use of heat therapy to improve vascular function. Chapter 3 identified independent effects of skin and core temperatures on FMD. This is an important observation because skin and core temperatures are controllable factors that can be used to prescribe a given level of heating which will be beneficial for endothelial function. The data from the pilot study in Chapter 4 highlight a potential role of sympathetic activity on the measurement of FMD post-heating, which might be a consideration for the design of future studies addressing the mechanisms or effectiveness of acute heating interventions. The findings
of Chapter 5 demonstrate the potentially therapeutic effects of heat stress on endothelial cells in a group that has a low capacity to perform exercise. Although the findings of Chapter 6 are preliminary, the data highlight that mechanisms other than improved endothelial function may underlie the reduction in CVD risk with habitual heating. Therefore, these results will be useful in identifying further mechanisms of the vascular benefits of heat by informing future research design and providing data for sample size calculations.

7.6 Future directions

Future studies should attempt to further identify underlying factors mediating lower CVD risk with chronic heating. Once these physiological mechanisms have been more comprehensively determined, researchers can better target applied studies to optimize heating protocols toward health outcomes. For example, it would be valuable to investigate potential core and/or skin temperature thresholds, as well as various heating durations and modes and their effects on vascular outcomes. Indeed, habitual heating appears to confer benefits in small doses (e.g., 11-19 min) (150), however the dose-responsiveness and mechanisms are unknown, thus it remains difficult to characterize the minimum dose of heating that induces vascular benefits. Mechanisms related to NO should be of prime importance; these could be assessed via correlation of FMD to circulating markers of NO, or by pharmacological blockade of eNOS with concurrent measures of endothelial-dependent dilation (e.g., isolated forearm model). Additionally, whether benefits occur in varying clinical groups (e.g., hypertension, stroke, diabetes) is currently unknown, but should be a priority. Given the often impaired baseline vascular function in clinical groups, it is possible that the duration and level of heating differs compared to healthy individuals and thus should be assessed separately.
The majority of studies examining the role of heat on vascular function have included primarily male participants and, with some exceptions (86, 87), women have rarely been studied specifically. Therefore, identifying possible sex differences and characterizing vascular responses to heating in women should be an important component of future research. Likewise, the vascular responses to heat in different racial groups is very important, especially for groups at greater risk of hypertension (e.g., African-Americans) (20). Finally, the time course of FMD and neural responses following heating should be studied. Whether the improvements in FMD following acute heating last for one hour or one day is unknown but could provide valuable knowledge not only for the design of heating interventions, but for clinical groups who may benefit from the acute effects of heating on mobility and pain (e.g., PAD). The time course of neural responses following heating is also important because elevated SNA following heating may have competing influences on vasodilatory function.

7.7 Conclusion

The main findings from this thesis are that 1) skin and core temperatures both independently increase endothelial function in young, healthy individuals; 2) increased sympathetic nervous activity following heating might impact endothelial function; 3) leg heating does not acutely change endothelial function in healthy or individuals with spinal cord injury; however, elevated biomarkers of endothelial cell inflammation are reduced after heating in the spinal cord injury but not healthy group; 4) endothelial function may not fully explain lower cardiovascular disease risk with habitual sauna use. Overall, these findings advance our knowledge of the mechanisms contributing to improved endothelial function following acute heat stress, and the applied settings where heat may be beneficial to endothelial health.
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Appendices: other relevant publications

Appendix A: Passive heat therapy for cerebral protection: new ideas of age-old concepts

The roots of heat therapy extend far into history, with the earliest known use of heating as a therapy dating back to Egyptian physicians during the Fifth Century B.C. who harnessed the heat provided by the sun and natural volcanic hot air caverns. Passive heating is a tradition present today in a diverse range of cultures to promote health and well-being. Across the globe, these traditions differ in details and names – sauna or sweat lodge, onsen or hammam, banya or bath, jimjilbang or hot springs – yet share a unified approach: relaxation in a hot environment. Despite its ancient origins and applications, the association of passive heat therapy and health outcomes are only just emerging, with little understanding of the mechanistic underpinnings of the protective effects of regular heat therapy. In a recent article in *The Journal of Physiology*, Brunt *et al.* (36) sought to identify the potential mechanisms underlying the physiological benefits of passive heat therapy, specifically for protection against cellular stress. By developing an *in vitro* model of hypoxia-reoxygenation, Brunt *et al.* (36) isolated the effects of heat stress and the influence of circulating factors on endothelial cell protection. Simply heating endothelial cells mitigated the inflammatory and oxidative stress response to hypoxia-reoxygenation. Similar findings were observed after treating cells with serum from participants who completed either one 60-minute session or 8 weeks of passive heat therapy (90-minute sessions, 4-5 times per week). These results suggest that both local cell temperature and circulating factors confer cellular protection against hypoxia-reoxygenation stress.

*In vitro* hypoxia-reoxygenation models are often used to reflect ischaemia-reperfusion (I/R) *in vivo*, which is particularly relevant for simulating intra-arterial occlusion models of ischemic stroke. Stroke is the second leading cause of death worldwide and ischemic strokes are
Given the high metabolic rate relative to its mass and low substrate storage capacity, the brain is extremely vulnerable to disruptions in oxygen supply. The brain is particularly susceptible to ischaemia; yet, in stroke or following cardiac arrest, the brain can often endure long periods of ischaemia (e.g., only 20% of events arrive at hospital in <2 hours), which quickly lead to cellular hypoxia and neurological damage due to reactive oxygen species (ROS) production during the ischaemic cascade and subsequent reperfusion injury. The I/R injury damage can lead to endothelial dysfunction, paralysis, and disrupted abilities to perform activities of daily living if not reversed in a timely manner; thus, strategies are needed to help to protect neurons from I/R damage and the associated risk of dementia. This raises the question of whether the cellular protection observed by Brunt et al. (36) manifests in neurons or cerebral microvascular endothelial cells. An intriguing avenue of research may be emerging from perennial cultural practices. In >2000 men followed for ~20 years as part of the Kuopio Ischaemic Heart Disease Risk Factor Study, the risk of dementia and Alzheimer’s disease was three times lower in those who more regularly participated in Finnish sauna bathing (151). This finding suggests a neuroprotective effect of chronic heat therapy. Of course, the epidemiological nature of this study does not provide a physiological explanation but, importantly, the observations were adjusted for health risk factors, lifestyle, and socioeconomic status. Whether heat therapy promotes a resilient brain in humans merits investigation, and the recent data reported by Brunt et al. (36) provide an intriguing hypothesis regarding a potential mechanism to explain the association between sauna bathing and the risk of Alzheimer’s disease and cognitive decline (151).

Given the difficult nature of performing mechanistic studies in the human brain, important information must be gleaned from animal models regarding heat-related mechanisms
of cerebral protection following I/R. For example, data indicates that heat preconditioning (e.g., a single exposure to 42°C core temperature for 15 minutes) provides neuroprotection against cerebral ischaemia in rats via the attenuation of ROS accumulation (260). Such benefits appear to occur when heat shock protein (HSP) expression (e.g., HSP72) is increased but not after returning to basal levels, therefore suggesting that heat preconditioning might need to be recent (e.g., <1 week) or of sufficient exposure (e.g., several weeks) to induce chronic HSP elevation. A recent study demonstrated that increased levels of microvascular endothelial HSP27 (achieved using transgenic mice with overexpression of HSP27) preserves the integrity of the blood-brain barrier during I/R and prevents the infiltration of macrophages and neutrophils into the brain parenchyma (224). Yacobi et al. (273) also demonstrated the importance of glutamate receptors during hypoxic insults, which facilitate neuronal death via calcium penetration following a large glutamate surge. In this study, long term (30 days), but not short term (2 days), heat-acclimated rats exhibited lower presence of NMDA GluN1 proteins (i.e., lower receptor density) and reduced calcium permeability, highlighting different mechanisms by which heat acclimation-induced hypoxia cross-tolerance confers neuroprotection (273). Importantly, these results translated into improved functional outcomes of behavior assessment in the 30-day heat-acclimated rats following hypoxia, indicating preserved cognitive function (273). These studies provide evidence to support the hypothesis that, broadly similarly to Brunt et al. (36), both acute and chronic heat treatment induce beneficial cellular responses and afford at least some level of protection against cellular stress. However, the effects of heat therapy for cerebral protection in humans and its potential mechanisms (e.g., heat vs. circulating factors) remain unknown. Most importantly, whether heat has the capacity to produce clinically relevant outcomes in humans has yet to be tested.
The potentially beneficial effects of passive heating on the cerebrovasculature might be counterintuitive. For example, high brain temperatures can increase permeability of the blood-brain barrier and cause hyperthermia-induced hyperventilation leading to cerebral hypoperfusion. Therefore, restricting acute bouts of heating to moderate levels might be necessary to observe cerebrovascular benefits. However, the physiological effects of repeated exposures to heat stress (e.g., reduced blood pressure and artery stiffness, Q10 effect, etc.) could have important implications for preventing cerebral vascular decline. Moreover, physiological acclimation occurs with chronic heating such that basal levels of HSPs are upregulated. Indeed, it has also been suggested that benefits accrued from heat acclimation have a memory-like feature, permitting rapid restoration of previously-acquired protection even after the loss of acclimation (128). This fascinating feature of retention of some acclimation responses could prove very useful in the consideration of health across the lifespan. Could heat acclimation performed in young adulthood or middle age allow older individuals to more easily re-acclimate and reap the protective health benefits of heating against cognitive decline and dementia? Although it would not be possible to delineate the influences of exercise and heat in isolation, post-exercise heat acclimation protocols might be a potential method of eliciting both shear-mediated and temperature-induced mechanisms of vascular protection.

The impact of exercise on overall brain health and cognitive function is well known, but the effects of heat therapy for cerebral protection have not been fully clarified. Exercise offers many additional advantages (endocrine, metabolic, etc.) from heat therapy; therefore, physical activity is an important component of a healthy lifestyle that should not be overlooked. However, as more knowledge of the benefits of heat therapy emerge, its utility as an adjunct therapy may be important in an era of declining physical activity. Moreover, passive heating may be a
promising alternative to exercise in populations with larger barriers to exercise (e.g., spinal cord injury, peripheral artery disease, heart failure). Nevertheless, it is now appreciated that heat therapy can reduce cardiovascular risk factors (e.g., blood pressure, central artery stiffness), which are primary risk factors for stroke and cerebrovascular disease.

With the application of heat therapy as a traditional health practice spanning diverse cultures and thousands of years, the mounting evidence for its benefits is not surprising. However, it is important to recognize that the majority of the mechanistic data discussed above stem from animal or ex vivo studies, which may not be fully representative of human physiology. Indeed, different experimental conditions, suitability of the animal model, and disparity of experimental outcomes all contribute to the lack of translation from animal models to human research. Collectively, these data warrant further investigation of heat acclimation, HSP expression, and its effects on the human cerebral circulation. Regardless, isolating temperature from circulating factors and shear-mediated mechanisms in humans is difficult, if not impossible, and Brunt et al. (36) are therefore commended for their study combining in vitro models with human serum to provide insight on the mechanisms involved in heat therapy.
Appendix B: Cerebrovascular function is preserved during mild hyperthermia in cervical spinal cord injury

Introduction

Cerebrovascular diseases and the risk of stroke are 3-4 fold higher in individuals living with a spinal cord injury (SCI) compared to their uninjured counterparts (59). The loss of supraspinal control of autonomic function following SCI results in profound blood pressure lability, thereby leading to uncontrolled periods of cerebral hypoperfusion during orthostatic hypotension and hyperperfusion during bouts of autonomic dysreflexia (194, 196). Both hypo- and hyperperfusion of the brain are associated with cognitive decline and vascular damage (198, 269). Neurovascular coupling (NVC), which matches cerebral blood flow (CBF) to neural activity, is also reportedly impaired in SCI (197) and in some neurological diseases (e.g., stroke, Alzheimer’s) (193). Although low resting blood pressure is likely a primary cause of reduced cerebrovascular function in SCI (197, 261), impaired endothelial function, hyposensitivity to vasoconstriction, and profibrotic remodelling might also be contributing factors (195).

Given the reduced physical activity levels following SCI (253), identifying alternative strategies that can be used as an adjunct therapy to help offset elevated cerebrovascular risk are essential. To the best of our knowledge, no studies of cerebrovascular function during hyperthermia have been published in SCI; yet, many of the risk factors associated with cerebrovascular diseases have reportedly been improved using passive heat therapy in uninjured populations. For example, both acute (209) and chronic heating protocols (33) improve arterial stiffness and endothelial function in healthy young and old participants, as well as in peripheral artery disease (238). Additionally, passive heating causes an acute cytokine response and increases plasma nitrite in overweight men (124). This cytokine response also occurs acutely in SCI despite
attenuated adrenergic activation (120); however, the effects of heating on nitric oxide bioavailability in SCI are unknown.

The increases in core temperature from the aforementioned studies ranged from 0.4 – 1.8°C. Importantly, Bailey et al. (12) demonstrated that eight weeks of repeated lower body hot water immersion (+0.6°C core temperature, 30 min three times per week) were able to increase resting cerebrovascular conductance of the middle cerebral artery (MCA) and attenuate acute hypoperfusion from hyperthermia-induced hypocapnia. Thus, moderate increases in core temperature in the range of ~0.5-0.6°C induced by practical methods of immersing the lower limbs in hot water baths have been reported to benefit systemic and cerebral vascular function. Several neurological benefits have recently been reported from chronic heat exposures. The incidence of dementia and Alzheimer’s disease and the risk of stroke was observed to be lower in middle-aged men and women who more frequently participated in sauna bathing (145, 151). As such, passive heat stress may be a useful strategy to improve cerebrovascular function, particularly for those with SCI who experience greater barriers to physical activity. However, the cerebrovascular and ventilatory responses to heat stress in SCI are unknown and acute increases in core temperature >0.5°C often result in decreased CBF due to hyperventilation-induced hypocapnia in uninjured individuals (90). Therefore, the aim of this study was to determine the CBF and NVC responses following an acute bout of lower limb heating in SCI participants, with data from uninjured controls (CON) as a comparator. It was hypothesized that heat stress would decrease CBF and NVC due to hypocapnia from heat stress.

Methods
Ethical approval of this study was provided by the ethics board at the School of Medicine, University of Split, Croatia. Fifteen individuals (5 females) with chronic (i.e., >2 years) cervical (C3-C7, ASIA A-C) SCI [mean (standard deviation); age: 42 (12) years; BMI: 22 (3)] and fifteen age- and sex-matched uninjured control participants [CON; age: 42 (11) years; BMI: 25 (3)] completed this protocol. All participants signed informed consent prior to data collection and all procedures conformed to the Declaration of Helsinki. This study shares participants and some data (e.g., core temperature, heart rate, MAP, and baseline NVC) with other manuscripts (56, 228); however, the hypotheses and cerebrovascular outcomes related to heat stress are unique to the current study.

The protocol comprised 60 min of lower limb hot water immersion (40°C) up to the knee with blankets covering the upper body. Baseline measures were performed with the legs immersed in thermoneutral water (33°C) immediately prior to beginning heating. At the end of heating, the legs were removed from the water bath and CBF was recorded immediately with concurrent measures of end-tidal partial pressures of carbon dioxide (PETCO2). These measurements were followed by NVC and PWV ~5 and 15 min after heating, respectively. All post-heating measures were collected while core temperature remained elevated and the entire protocol was performed in the supine position. Core temperature (Tcore) was monitored with a telemetric pill (HQInc, Palmetto, FL, USA), heart rate was continuously monitored using a lead II ECG, PETCO2 was measured with a commercial gas analyzer, and arterial blood pressure was measured using finger photoplethysmography and verified every 15 min with an automated blood pressure cuff. Venous blood samples were processed using a hematology analyzer (AcT8 Hematology Analyzer, Beckman Coulter, Brea, CA, USA). Primary outcomes of CBF, PWV, and NVC were measured.
during supine rest (>15 min) prior to heating and within 10-15 min after the end of heating, with no measures collected during heating to facilitate participant comfort.

Transcranial Doppler ultrasound (Spencer Technologies, Redmond, WA, USA) was used to simultaneously measure the right middle cerebral artery (MCAv) and the left posterior cerebral artery (PCAv) velocities through acoustic windows located in the temporal region. The NVC response was quantified as the peak hyperemic value in the PCA during 30-second cycles of visual stimulation (i.e., eyes closed/eyes tracking a moving finger) over a 5-min period to evoke functional changes in cerebral perfusion. Cerebral artery pulsatility index (PI) was estimated in the MCA and PCA (e.g., [systolic MCAv – diastolic MCAv]/mean MCAv). Internal carotid artery (ICA) blood flow was measured using duplex ultrasound to provide an index of CBF. Blood flow was calculated from the one-minute screen-capture recordings as (peak envelope blood velocity / 2) * (π (0.5*diameter^2)) and analyzed using offline automated edge-detection software (270). Cerebral vascular conductance (CVC) was determined for MCAv, PCAv, and ICA flow by dividing values by mean arterial pressure (MAP). Carotid-femoral PWV (80% of distance between sites) was measured using a pulse wave tonometer (SPT-301, Millar, Houston, TX, USA) to estimate aortic stiffness, according to international guidelines (153). Data were analyzed using two-way mixed ANOVAs and are presented as means and standard deviation (SD).

**Results**

All cardiovascular and cerebrovascular data are presented in Table 1. Due to inadequate ultrasound image quality (e.g. automatic edge detection software could not properly track vessel walls or MCA/PCA recording did not display typical pulse wave), analyses were not performed on a small number of participants for some measures where indicated. Resting Tcore was 37.10
(0.26)°C in CON and 36.37 (0.62)°C in SCI. The increase in Tcore was two-fold greater in SCI compared to the change in CON [+0.68 (0.17) vs. +0.34 (0.17)°C; P<0.01]. There were no group-by-intervention interactions for any of the intracranial measures of MCAv, PCAv, PCA\textsubscript{CVC}, MCA\textsubscript{PI}, or PCA\textsubscript{PI} (n=14 SCI, n=13 CON; all P>0.05). However, MCA\textsubscript{CVC} decreased by 10% in SCI and increased by 12% in CON (P<0.01), reflecting divergent responses of MAP [SCI: +6(14) mmHg, CON: -8(12) mmHg; P=0.01]. Although the ICA diameter, flow, and ICA\textsubscript{CVC} (n=8 SCI, n=9 CON) were all greater in SCI vs. CON (18%, 33%, and 31%, respectively; all P<0.05), there were no group-by-intervention interactions (all P>0.05). There was no group-by-intervention interaction for carotid-femoral PWV (P=0.76), which may be consistent with recent studies of tetraplegic participants (171). Peak PCA\textsubscript{CVC} during visual stimulation also did not present a group-by-time interaction (n=13 SCI, n=10 CON; interaction P=0.22). The pre-heating PCA NVC response was 28.9 (9.0)% vs. 29.8 (8.8)% in SCI and CON, respectively, and remained unchanged after heating at 29.7 (8.6)% vs. 25.2 (8.8)% in SCI and CON, respectively (Figure 1). Hemoglobin and hematocrit did not change with heating (P>0.05), but there tended to be a main effect of time on white blood cell and platelet counts after heating (P<0.01 and P=0.08, respectively; Table 1).

**Discussion**

This study examined the cerebrovascular responses following acute heat stress in individuals with chronic SCI. The primary outcomes of this study were that CBF and NVC were maintained during increases in Tcore in SCI. Although it is established that CBF declines with heat stress as a result of hyperthermia-induced hypocapnia and consequent cerebral vasoconstriction [reviewed in Bain et al. (14)], we did not observe this response. This is likely because hyperventilation occurs during heat stress with a change in Tcore >0.5°C (90) and the SCI
group in this study was only moderately above this threshold and the CON group was below it. Moreover, $P_{ETCO_2}$ decreased (main effect of time $P=0.06$) by only 2 mmHg in both groups (Table 1), whereas slightly larger decreases might be required to reduce CBF(90). These results suggest that individuals with SCI may have a lower ventilatory sensitivity (i.e., hyperventilatory response) to heat stress considering the similar decreases in $P_{ETCO_2}$ with differing Tcore responses compared to CON. Indeed, $P_{ETCO_2}$ is inversely proportional to alveolar ventilation assuming a constant $VCO_2$, which is the case <1.0°C increase in Tcore(90). Yet, these results nonetheless demonstrate the safety and potential application of lower limb heating as a therapy in SCI. Caution should be applied, however, when exposing individuals with SCI to greater degrees of heat stress given the blunted sweating and cutaneous vasodilation responses below the level of injury, particularly in high level (i.e., tetraplegic) injuries (200). Individuals with SCI will store heat at a greater rate during external heating compared to CON (as observed in the current study) because of their smaller body mass, which also likely explains in large part their lower resting Tcore due to lower basal metabolic rate (173). Nevertheless, the use of the lower limb heating protocol (compared to whole-body heating) permitted high tolerance in SCI participants to mild-moderate increases in Tcore, and no adverse events occurred including any bouts of autonomic dysreflexia.

In contrast to some other studies of acute heat stress in uninjured participants, we observed smaller changes in MAP and no effect of heating on carotid-femoral PWV, which might be explained by the relatively mild changes in Tcore in this study. For example, 30 min of hot water immersion (up to the sternum) (238) induced reductions in MAP of up to 20 mmHg and reductions of PWV of ~1.0 m/s in uninjured populations. However, we observed increases in MAP similar to that reported by Shibasaki et al. (225) in participants with tetraplegia compared to both uninjured and individuals with paraplegia, although this response remains unexplained. It is noteworthy that
in the study reporting reduced MAP and PWV, Tcore increased by \( \sim 2.0^\circ C \) \( (238) \). Such extreme increases in Tcore highlight the acute benefits of passive heating on the cardiovascular system, but this magnitude of heating may not be necessary to induce chronic positive adaptations. Indeed, Bailey et al. (12) demonstrated that after eight weeks of repeated hot water immersion, with increases in Tcore \( \sim 0.6^\circ C \), MCAv was increased in uninjured young women. Moreover, it was recently reported that molecular responses to eight weeks of heat therapy (e.g., increased heat shock proteins) reduced inflammation and reactive oxygen species production during hypoxia-reoxygenation \( (36) \). These data indicate potentially promising results for endothelial cell protection during ischemia-reperfusion \textit{in vivo} and could be relevant for cerebral protection following strokes; yet, the translation to human cerebral endothelial function \textit{in vivo} has yet to be determined. Together, the indirect benefits to cardiovascular risk factors and direct effects of heat on the brain (e.g., \( Q_{10} \) effect, regionally increased metabolism\( (185) \), etc.) make passive heating a promising intervention for cerebral vascular health in SCI.

\textit{Limitations:} Compared to previous reports \( (197, 228) \), it is currently unclear why we did not observe lower NVC responses in the SCI group compared to CON at baseline. The supine position used throughout our protocol and similar blood pressures between groups \( (197) \) is likely the major factor since normalization of blood pressure in SCI while sitting has previously been shown to also normalize NVC \( (197) \). The lower statistical power of the ANOVA used in this current study could also explain the difference in baseline NVC between this study and other cross sectional studies \( (228) \), as well as the absence of changes in primary outcomes of CBF and NVC before and after heating in SCI. When pre- and post-heating data were analyzed using a paired t-test instead, there were no between-group difference in NVC \( (P=0.42) \), nor were there differences in CBF or NVC following heating in SCI only \( (P=0.88 \text{ and } P=0.71, \text{ respectively}) \).
Moreover, the nationality of the participants in the current study is another difference compared to previous studies (i.e., Croatian vs. Canadian). Considering their lower body mass and probable impairments of sweating and skin blood flow, it is not surprising that core temperature increased to a greater extent in the SCI group in the current study. We also observed no differences in the CBF or NVC responses following heating in either group, but the vascular responses to heating between groups should be carefully interpreted in view of the significant interaction between group and time on core temperature.

Nevertheless, the purpose of this study was to determine the effects of a practical form of heating on cerebrovascular function in SCI. After an hour of lower limb heating, Tcore increased by ~0.7°C in the SCI group indicating that this method of heating does not predispose participants to heat injury despite their thermoregulatory impairment. In uninjured individuals, other studies should aim to characterize NVC with greater increases in core temperature similar to that in the SCI group in the current study. The decreased MCA conductance (-10%) in SCI suggests that despite maintained CBF, intracranial conductance could be impaired in SCI. Finally, the mechanisms contributing to a lower ventilator sensitivity to heat in SCI warrants elucidation. Future studies should consider quantifying cerebrovascular conductance at more severe increases in Tcore to determine a possible threshold at which heat stress might be counterproductive in SCI.

**Conclusion:** The results of the present study indicate that a feasible method (i.e., lower limb heating) of mild acute heating does not impair or improve cerebrovascular function in SCI. Further study of the effects of chronic heating interventions are needed to establish any potential benefits for cerebrovascular health in SCI.
Legends

Table 1. Thermometric, cardiovascular, cerebrovascular, and hemodynamic variables before and after 60 min of lower limb hot water immersion. Data are mean (SD).

Figure 1. Average response of the posterior cerebral artery (PCA) during 30-sec periods of visual stimulation before and after heating. The top panels represent the average relative change in PCAv from a closed eyes baseline and the bottom panels represent the average absolute response. Data are presented as mean (SD) with values corrected for cerebrovascular conductance (CVC).
<table>
<thead>
<tr>
<th>Time</th>
<th>ANOVA</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>SCI</td>
<td>0.10</td>
<td>0.61</td>
<td>0.89</td>
<td>0.06</td>
<td>0.21</td>
<td>0.27</td>
<td>0.82</td>
<td>0.20</td>
<td>0.76</td>
<td>0.66</td>
<td>0.74</td>
</tr>
<tr>
<td>CON</td>
<td>0.33</td>
<td>0.80</td>
<td>0.99</td>
<td>0.48</td>
<td>0.88</td>
<td>0.25</td>
<td>0.43</td>
<td>0.87</td>
<td>0.03</td>
<td>0.03</td>
<td>0.45</td>
</tr>
<tr>
<td>Pre-heating</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCI</td>
<td>59 (7)</td>
<td>84 (14)</td>
<td>7.4 (1.8)</td>
<td>33 (5)</td>
<td>61 (13)</td>
<td>42 (12)</td>
<td>0.82 (0.08)</td>
<td>0.78 (0.12)</td>
<td>0.57 (0.12)</td>
<td>304 (107)</td>
<td>10 (3)</td>
</tr>
<tr>
<td>CON</td>
<td>62 (7)</td>
<td>89 (12)</td>
<td>7.6 (2.2)</td>
<td>34 (5)</td>
<td>60 (15)</td>
<td>37 (8)</td>
<td>0.79 (0.06)</td>
<td>0.78 (0.08)</td>
<td>0.46 (0.04)</td>
<td>221 (65)</td>
<td>10 (4)</td>
</tr>
<tr>
<td>Post-heating</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCI</td>
<td>61 (9)</td>
<td>89 (22)</td>
<td>7.5 (1.9)</td>
<td>31 (2)</td>
<td>58 (13)</td>
<td>41 (12)</td>
<td>0.82 (0.12)</td>
<td>0.77 (0.09)</td>
<td>0.56 (0.14)</td>
<td>313 (129)</td>
<td>10 (4)</td>
</tr>
<tr>
<td>CON</td>
<td>64 (7)</td>
<td>81 (12)</td>
<td>7.3 (1.0)</td>
<td>32 (3)</td>
<td>60 (13)</td>
<td>36 (8)</td>
<td>0.79 (0.10)</td>
<td>0.76 (0.08)</td>
<td>0.46 (0.04)</td>
<td>196 (67)</td>
<td>9 (3)</td>
</tr>
</tbody>
</table>
| Data are mean (SD) with the level of significance set to P=0.05. HR, heart rate; MAP, mean arterial pressure; cfPWV, carotid-femoral pulse-wave velocity; $P_{ETCO_2}$, end-tidal partial pressure of carbon dioxide; MCAv, middle cerebral artery velocity; PCAv, posterior cerebral artery velocity; ICA, internal carotid artery; PI, pulsatility index; Hb, hemoglobin; Hct, hematocrit; WBC, white blood cells; Plt, platelets.
Appendix C: Sauna use questionnaire

Sauna Use Questionnaire

1. Have you ever used a sauna before?
   Yes: No:

2. When was the last time you used a sauna?
   Years: Months: Days:

3. Do you regularly use a sauna?
   Yes: No:

   If you answered yes above for #3, please answer questions #4-8 below. If no, continue to question #9.

4. For how long have you been regularly using a sauna?
   Years: Months:

5. In a typical week, how many days do you use a sauna?
   Days:

6. In typical day, how many times do you use a sauna?
   Number:

7. How much time do you spend in a sauna on a typical day?
   Minutes:

8. In a typical sauna session, how many times will you enter and exit the sauna?
   Number:

9. Do you regularly participate in other forms of heat therapy?
   Yes: No:

   If you answered yes above for #9, please answer questions #10-11 below.

10. Hot yoga?
    Yes: No: Frequency:

11. Hot tubs?
    Yes: No: Frequency:

12. Other?
    Frequency:

Signature: Date:
Questionnaire d'utilisation de sauna

1. Avez-vous déjà utilisé un sauna auparavant?
   Oui:    Non:

2. À quand remonte la dernière fois que vous avez utilisé un sauna??
   Années:  Mois:  Days:

3. Utilisez-vous régulièrement un sauna?
   Oui:    Non:

*Si vous avez répondu oui ci-dessus pour le numéro 3, veuillez répondre aux questions 4 à 8 ci-dessous.*

4. Depuis combien de temps utilisez-vous régulièrement un sauna?
   Années:  Mois:

5. Dans une semaine typique, combien de jours utilisez-vous un sauna?
   Days:

6. Dans une journée typique, combien de fois utilisez-vous un sauna?
   Numéro:

8. Combien de temps passez-vous dans un sauna par jour?
   Minutes:

7. Dans une séance de sauna typique, combien de fois allez-vous entrer et sortie dans le sauna?
   Numéro:

9. Participez-vous régulièrement à d'autres formes de thermothérapie?
   Oui:    Non:

*Si vous avez répondu oui ci-dessus pour le numéro 9, veuillez répondre aux questions 10-11 ci-dessous.*

10. Yoga chaud?
    Oui:    Non:    La fréquence:

11. Jacuzzi?
    Yes:    No:    La fréquence:

12. Autre?
    La fréquence:

Signature:    Date:
Global Physical Activity Questionnaire (GPAQ)

WHO STEPwise approach to NCD risk factor surveillance

Surveillance and Population-Based Prevention
Prevention of Noncommunicable Diseases Department
World Health Organization
20 Avenue Appia, 1211 Geneva 27, Switzerland
Physical Activity

Next I am going to ask you about the time you spend doing different types of physical activity in a typical week. Please answer these questions even if you do not consider yourself to be a physically active person.

Think first about the time you spend doing work. Think of work as the things that you have to do such as paid or unpaid work, study/training, household chores, harvesting food/crops, fishing or hunting for food, seeking employment. [Insert other examples if needed]. In answering the following questions 'vigorous-intensity activities' are activities that require hard physical effort and cause large increases in breathing or heart rate, 'moderate-intensity activities' are activities that require moderate physical effort and cause small increases in breathing or heart rate.

<table>
<thead>
<tr>
<th>Questions</th>
<th>Response</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Activity at work</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Does your work involve vigorous-intensity activity that causes large increases in breathing or heart rate like [carrying or lifting heavy loads, digging or construction work] for at least 10 minutes continuously? [INSERT EXAMPLES] (USE SHOWCARD)</td>
<td>Yes 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No 2 If No, go to P 4</td>
</tr>
<tr>
<td>2</td>
<td>In a typical week, on how many days do you do vigorous-intensity activities as part of your work?</td>
<td>Number of days</td>
</tr>
<tr>
<td>3</td>
<td>How much time do you spend doing vigorous-intensity activities at work on a typical day?</td>
<td>Hours : minutes hrs mins</td>
</tr>
<tr>
<td>4</td>
<td>Does your work involve moderate-intensity activity that causes small increases in breathing or heart rate such as brisk walking [or carrying light loads] for at least 10 minutes continuously? [INSERT EXAMPLES] (USE SHOWCARD)</td>
<td>Yes 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No 2 If No, go to P 7</td>
</tr>
<tr>
<td>5</td>
<td>In a typical week, on how many days do you do moderate-intensity activities as part of your work?</td>
<td>Number of days</td>
</tr>
<tr>
<td>6</td>
<td>How much time do you spend doing moderate-intensity activities at work on a typical day?</td>
<td>Hours : minutes hrs mins</td>
</tr>
</tbody>
</table>

Travel to and from places

The next questions exclude the physical activities at work that you have already mentioned. Now I would like to ask you about the usual way you travel to and from places. For example to work, for shopping, to places of worship, places of work.

<table>
<thead>
<tr>
<th>Questions</th>
<th>Response</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Do you walk or use a bicycle (pedal cycle) for at least 10 minutes continuously to get to and from places?</td>
<td>Yes 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No 2 If No, go to P 10</td>
</tr>
<tr>
<td>8</td>
<td>In a typical week, on how many days do you walk or bicycle for at least 10 minutes continuously to get to and from places?</td>
<td>Number of days</td>
</tr>
<tr>
<td>9</td>
<td>How much time do you spend walking or bicycling for travel on a typical day?</td>
<td>Hours : minutes hrs mins</td>
</tr>
</tbody>
</table>

Recreational activities

The next questions exclude the work and transport activities that you have already mentioned.
Now I would like to ask you about sports, fitness and recreational activities (leisure), [insert relevant terms].

<table>
<thead>
<tr>
<th>Questions</th>
<th>Response</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>10  Do you do any vigorous-intensity sports, fitness or recreational (leisure) activities that cause large increases in breathing or heart rate like [running or football,] for at least 10 minutes continuously?</td>
<td>Yes 1</td>
<td>P10</td>
</tr>
<tr>
<td>[INSERT EXAMPLES] (USE SHOWCARD)</td>
<td>No 2 If No, go to P 13</td>
<td></td>
</tr>
<tr>
<td>11  In a typical week, on how many days do you do vigorous-intensity sports, fitness or recreational (leisure) activities?</td>
<td>Number of days</td>
<td>P11</td>
</tr>
<tr>
<td>12  How much time do you spend doing vigorous-intensity sports, fitness or recreational activities on a typical day?</td>
<td>Hours : minutes</td>
<td>P12</td>
</tr>
<tr>
<td>(a-b)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**GPAQ, Continued**

Physical Activity (recreational activities) contd.

<table>
<thead>
<tr>
<th>Questions</th>
<th>Response</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>13  Do you do any moderate-intensity sports, fitness or recreational (leisure) activities that causes a small increase in breathing or heart rate such as brisk walking, (cycling, swimming, volleyball) for at least 10 minutes continuously?</td>
<td>Yes 1</td>
<td>P13</td>
</tr>
<tr>
<td>[INSERT EXAMPLES] (USE SHOWCARD)</td>
<td>No 2 If No, go to P16</td>
<td></td>
</tr>
<tr>
<td>14  In a typical week, on how many days do you do moderate-intensity sports, fitness or recreational (leisure) activities?</td>
<td>Number of days</td>
<td>P14</td>
</tr>
<tr>
<td>15  How much time do you spend doing moderate-intensity sports, fitness or recreational (leisure) activities on a typical day?</td>
<td>Hours : minutes</td>
<td>P15</td>
</tr>
<tr>
<td>(a-b)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sedentary behaviour

The following question is about sitting or reclining at work, at home, getting to and from places, or with friends including time spent [sitting at a desk, sitting with friends, travelling in car, bus, train, reading, playing cards or watching television], but do not include time spent sleeping.

[INSERT EXAMPLES] (USE SHOWCARD)

<table>
<thead>
<tr>
<th>Questions</th>
<th>Hours : minutes</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>16  How much time do you usually spend sitting or reclining on a typical day?</td>
<td>hrs min s</td>
<td>P16</td>
</tr>
<tr>
<td>(a-b)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>