DOCTOR OF PHILOSOPHY (2023) McMaster University
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HEAT THERAPY AND CARDIOVASCULAR HEALTH

THE IMPACT OF LOCAL HEAT THERAPY ON VASCULAR FUNCTION IN YOUNG, HEALTHY, RECREATIONALLY ACTIVE ADULTS

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LAY ABSTRACT

Regular participation in whole-body heat therapy can extend health and life span, but it is used infrequently because of a lack of feasibility from a cost, accessibility, and tolerability standpoint. This thesis explored whether local heat therapy in young healthy men and women would be effective for improving blood vessel health defined as endothelial function and arterial stiffness, both of which are linked to the risk of developing many chronic diseases. Furthermore, the effects of local heat therapy were compared to that of exercise training. We found that there were beneficial short- and long-term effects of lower limb hot water immersion that manifested in different areas of the body. Local heat therapy improved upper limb endothelial function and lower limb arterial stiffness immediately after a session, whereas with repeated exposure, it may have improved central arterial stiffness and cardiorespiratory fitness. Exercise training only had beneficial effects on the blood vessels when combined with heat therapy. Finally, short-term vascular responses can predict long-term vascular responses to both heat therapy and/or exercise training. Overall, our findings suggest that there may be some utility for local heat therapy to promote healthy blood vessels, but more work must be done to replicate our findings and explore its effects on other populations.

ABSTRACT

Heat therapy may be an alternative or adjunct intervention to exercise training for improving cardiovascular function and health. However, its prescription must be refined in order to overcome the feasibility and tolerability issues associated with current whole-body heating modes. There is substantial evidence to support the beneficial effects of high doses (e.g., frequency, duration, and intensity) of heating typically achieved using whole-body modes, but there is limited knowledge on whether lower doses of heating administered through local hot water immersion of the limbs can still have an impact on vascular function.

All studies were conducted in heathy young men and women. In the first study, we found that regardless of whether local heating was applied to the lower limbs up to the ankles or knees, upper limb endothelial function and lower limb arterial stiffness improved acutely. In the second study, we proceeded to prescribe ankle-level heating in a chronic intervention and compared its effects to that of moderate-intensity cycling exercise training. We observed no changes in endothelial function, but decreases in central arterial stiffness and increases in cardiorespiratory fitness in those who performed heat therapy and exercise training combined with heat therapy. In the third study, we evaluated the ability of acute vascular function responses to predict chronic vascular function responses with heating and exercise interventions, and found significant positive associations between the acute and chronic responses for absolute and relative brachial artery flow-mediated dilation and femoral-foot pulse wave velocity.

These findings suggest that, in healthy young men and women, local heating through ankle-level hot water immersion can improve indices of cardiovascular function both acutely and chronically, alone or combined with exercise training. Further, acute responses may be used to determine an individual's chronic responsiveness to a heat therapy and/or exercise training intervention. More research in larger, more diverse samples and with a longer duration of therapy and/or training should be conducted to determine if the results are replicable.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ACh	Acetylcholine
ACSM	American College of Sports Medicine
AD	Arterial diameter
AMPK	AMP-activated protein kinase
ANS	Autonomic nervous system
AUC	Area under the curve
BA	Brachial artery
BMI	Body mass index
BNP	B-type natriuretic peptide
BP	Blood pressure
CASRAI	Consortia Advancing Standards in Research Administration Information
CAVI	Cardio-ankle vascular index
CCA	Common carotid artery
cfPWV	Carotid-femoral pulse wave velocity
CHF	Chronic heart failure
CI	Confidence interval
cIMT	Carotid intima-media thickness
CO	Cardiac output
CON	Control
CONSORT	Consolidated Standards of Reporting Trials
CRediT	Contributor Roles Taxonomy
CVC	Cutaneous vascular conductance
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
ECG	Electrocardiogram
eHSP72	Extracellular heat shock protein-72
ELISA	Enzyme-linked immunosorbent assay
eNOS	Endothelial nitric oxide synthase
EX	Exercise training
ffPWV	Femoral-foot pulse wave velocity
FMD	Flow-mediated dilation
GLUT4	Glucose transporter type 4
HEAT	Heat therapy
HEATEX	Combined training and therapy
HR	Heart rate

HSP	Heat shock protein
IL-6	Interleukin-6
IMT	Intima-media thickness
IPAQ	International Physical Activity Questionnaire
IQR	Interquartile range
L-NAME	N^G-nitro-L-arginine methyl ester
L-NMMA	N-methylarginine
L-NNA	N^ω-nitro-L-arginine
LDF	Laser Doppler flowmetry
MAP	Mean arterial pressure
MAUI	Measurements from Arterial Ultrasound Imaging
MCP-1	Monocyte-chemoattractant protein-1
mRNA	Messenger ribonucleic acid
MSNA	Muscle sympathetic nerve activity
NBW	Nude body weight
NF-κB	Nuclear factor kappa B
NO	Nitric oxide
PACES	Physical Activity Enjoyment Scale
PAR-Q+	Physical Activity Readiness Questionnaire
PGC1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PNS	Parasympathetic nervous system
PPO	Peak power output
PWV	Pulse wave velocity
SBP	Systolic blood pressure
SFA	Superficial femoral artery
SNP	Sodium nitroprusside
SNS	Sympathetic nervous system
SR	Shear rate
SS	Shear stress
SSNA	Skin sympathetic nerve activity
SV	Stroke volume
T_{arm}	Arm skin temperature
TC	Thermal comfort
T_c or T_{core}	Core temperature
T_{calf}	Calf skin temperature
T_{chest}	Chest skin temperature
T_{foot}	Foot skin temperature
TS	Thermal sensation

T_{sk} or T_{skin}	Skin temperature
T_{thigh}	Thigh skin temperature
VO_2peak	Peak oxygen uptake
WBSR	Whole-body sweat rate

LIST OF EQUATIONS

Equation 1. $SS\ (dyn/cm^2) = \frac{2\mu V}{D}$

Equation 2. $SR\ (s^{-1}) = \frac{8V}{D}$

Equation 3. $FMD\ (mm) = D_{peak} - D_{base}$

Equation 4. $FMD\ (\%) = \left(\frac{D_{peak} - D_{base}}{D_{base}}\right) \times 100\%$

Equation 5. $Vascular\ conductance\ (ml/min \cdot mmHg) = \frac{BF}{MAP}$

Equation 6. $Distensibility\ (cm^2/mmHg) = \frac{(\pi\ r_{max}{}^2 - \pi\ r_{min}{}^2)}{(PP \times \pi\ r_{min}{}^2)}$

Equation 7. $PWV\ (m/s) = \frac{distance}{pulse\ transit\ time}$

Equation 8. $Scaled\ FMD\ mean = [(e^{EM} - 1) \times 100]$

Equation 9. $Scaled\ FMD\ standard\ deviation = [((e^{SE} - 1) \times 100) \times \sqrt{n}]$

Equation 10. $T_{skin}(°C) = 0.3T_{chest} + 0.3T_{arm} + 0.2T_{thigh} + 0.2T_{calf}$

Equation 11. $WBSR = \frac{((NBW_{Pre} - NBW_{Post}) + water\ consumed) \times 60}{intervention\ duration\ (\text{min})}$

Equation 12. $BF\ \left(\frac{ml}{\text{min}}\right) = (\pi r^2 \times V) \times 60$

where SS = shear stress, μ = blood viscosity, V = mean blood velocity, D = artery diameter, SR = shear rate, FMD = flow-mediated dilation, D_{peak} = peak artery diameter, D_{base} = baseline artery diameter, BF = blood flow, MAP = mean arterial pressure, r = radius, PP = pulse pressure, PWV = pulse wave velocity, EM = estimated means, SE = standard error, n = number of participants, T_{skin} = mean skin temperature, T_{chest} = chest skin temperature, T_{arm} = arm skin temperature, T_{thigh} = thigh skin temperature, T_{calf} = calf skin temperature, WBSR = whole-body sweat rate, NBW = nude body weight.

DECLARATION OF ACADEMIC ACHIEVEMENT

FORMAT AND ORGANIZATION OF THESIS

This thesis is prepared in the "sandwich" format as outlined in the School of Graduate Studies Guide for the Preparation of Master's and Doctoral Theses. It includes a general introduction (chapter 1), three studies prepared in journal article format (chapters 2-4), and an overall discussion (chapter 5). At the time of thesis preparation, a portion of chapter 1 and all of chapter 2 were published in peer-reviewed journals and chapters 3 and 4 were in preparation for submission. For all papers with multiple authorship, the contributions of the candidate and all co-authors are outlined below using the Consortia Advancing Standards in Research Administration Information (CASRAI) CRediT taxonomy (https://credit.niso.org/).

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CHAPTER 1

INTRODUCTION

STUDY 0

Effect of heat stress on vascular outcomes in humans

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1.1 Preamble

Many chronic diseases and global leading causes of death possess a vascular etiology, emphasizing how critical vascular function is for maintaining overall health (1–8). These conditions include, among many others, ischaemic heart disease, stroke, diabetes mellitus, and kidney diseases (World Health Organization Global Health Estimates, 2019). The vascular milieu is constantly balancing its response to the release of pro- and anti-atherogenic substances, and ultimately, a healthy environment is maintained when endothelial cells are in a quiescent state wherein harmful inflammatory and oxidative stress pathways are effectively silenced (9). Novel interventions such as heat therapy can support vascular function by eliciting cardiovascular (i.e., heart rate, shear rate), thermal (i.e., core and skin temperatures), and molecular (i.e., heat shock proteins) eustress, which triggers physiological signaling pathways that promote the release of vasoactive substances like nitric oxide (10). However, much work remains to be done with respect to the prescription of heat therapy, in order to develop protocols that are both effective for improving vascular function, and feasible, scalable, and accessible for the general population.

This introductory chapter will include (i) a synthesis of the existing literature that has examined the effects of passive heat stress on vascular function in young, healthy individuals (section 1.2) (11) and (ii) a summary of commonly used methods for the assessment of arterial function (section 1.3), culminating in (iii) an overview of the studies conducted for this PhD thesis, including clearly outlined objectives and hypotheses (section 1.4).

1.2 Effect of heat stress on vascular outcomes in humans

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REVIEW

Effect of heat stress on vascular outcomes in humans

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Cheng JL, MacDonald MJ. Effect of heat stress on vascular outcomes in humans. *J Appl Physiol* 126: 771–781, 2019. First published January 24, 2019; doi:10.1152/japplphysiol.00682.2018.—In addition to its role as an environmental stressor, scientists have recently demonstrated the potential for heat to be a therapy for improving or mitigating declines in arterial health. Many studies at both ends of the scientific controls spectrum (tightly controlled, experimental vs. practical) have demonstrated the beneficial effects of heating on microvascular function (e.g., reactive hyperemia, cutaneous vascular conductance); endothelial function (e.g., flow-mediated dilation); and arterial stiffness (e.g., pulse-wave velocity, compliance, β-stiffness index). It is important to note that findings of beneficial effects are not unanimous, likely owing to the varied methodology in both heating protocols and assessments of outcome measures. Mechanisms of action for the effects of both acute and chronic heating are also understudied. Heat science is a very promising area of human physiology research, as it has the potential to contribute to approaches addressing the global cardiovascular disease burden, particularly in aging and at risk populations, and those for whom exercise is not feasible or recommended.

arterial stiffness; endothelial function; heat stress; heat therapy; vascular physiology

INTRODUCTION

The search for innovative approaches to the management and prevention of cardiovascular disease (CVD) has created a potential niche for the use of heat as a therapeutic tool for improving vascular health outcomes. On a global scale, CVDs, ischemic heart disease and stroke in particular, are still the leading causes of death (World Health Organization, January 2017), and it is now known that endothelial dysfunction precedes the development of atherosclerotic plaques along the arterial wall that are characteristically associated with these diseases (22). The arterial wall is made up of three layers that are distinct in their composition and function. The tunica adventitia contains elastin, collagen, nerves, and blood vessels and provides the artery structural integrity; the tunica media contains smooth muscle cells and allows the artery to control blood flow; and the tunica intima is lined with endothelial cells that secrete substances that promote an antiatherogenic environment (80). Various vascular properties have emerged as indicators of CVD risk, including microvascular function (e.g., reactive hyperemia, cutaneous vascular conductance); endothelial function (e.g., flow-mediated dilation); and arterial stiffness (e.g., pulse-wave velocity, compliance, β-stiffness index) (Fig. 1).

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Heat stress is emerging as a potential alternative to exercise for improving cardiovascular health, because it too increases vascular shear stress, a critical stimulus for changing vascular function (94). Indeed, many studies have shown that heat, applied acutely or chronically, has the ability to alter a number of indices of vascular structure and function, which may be promising for the aging population, as well as at risk groups that may not be physically able to partake of the recommended amount of exercise. Activities such as Waon therapy, Japanese *onsen* bathing, Finnish sauna bathing, and Bikram yoga have been promoted in some venues for their heat-related benefits for many years (27, 31, 63, 81), but recent studies have also demonstrated that simpler alternatives, such as a hot foot spa or hot tub bath, may elicit similar positive effects on vascular health with regular use (8, 9, 89). Accordingly, the objectives of this paper are as follows: *1*) to describe currently used practical models of heat therapy and their effects on vascular structure and function; *2*) to outline the physiological changes in vascular structure and function previously observed in studies of chronic and acute heat exposure; and *3*) to propose potential avenues of future research on heat science as it relates to vascular health.

COMMON HEAT THERAPIES AND THEIR EFFECTS ON VASCULAR STRUCTURE AND FUNCTION

Commonly used heat therapies have been previously demonstrated to be successful at creating a general sense of well-being, whether it be through symptom alleviation, cultural inclusion, or physical fitness (27, 28, 63, 81, 87). These effects have contributed to the worldwide adoption of these heat

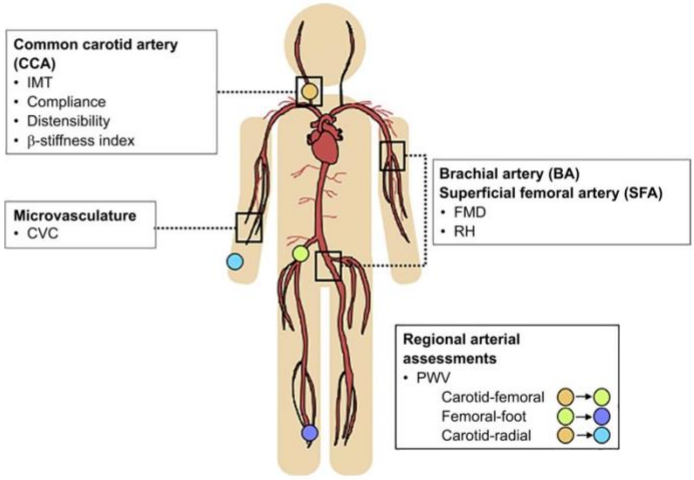

Fig. 1. Assessment methods for common vascular structure and function outcomes. Structural and functional characteristics are typically assessed at the common carotid artery (CCA), brachial artery (BA), superficial femoral artery (SFA), and the microvasculature. Microvascular function can be assessed through cutaneous vascular conductance (CVC) or reactive hyperemia (RH) response. Endothelial function can be assessed through flow-mediated dilation (FMD). Arterial stiffness can be assessed locally through compliance, distensibility, and the β-stiffness index, or regionally through pulse-wave velocity (PWV) at a variety of arterial sites. Arterial intima-media thickness (IMT) describes the thickness of the arterial wall, most typically at the CCA, and is a precursor to atherosclerosis.

therapies. Recent studies have highlighted the potential role of improved vascular health in contributing to the efficacy of these heat therapies (27, 31, 32, 37, 47, 67).

Waon Therapy

Waon therapy, which translated means "soothing warm therapy," was developed in Japan by Dr. Chuwa Tei in 1989 as a treatment for chronic heart failure (CHF) (63). It involves being seated for 15 min in a far infrared dry sauna set to 60°C, with the goal of increasing core temperature (T_c) by 1–1.5°C, followed by 30 min outside the sauna in supine rest while wrapped in blankets to retain heat. Water is provided ad libitum to prevent dehydration from sweating (63). This therapy has almost exclusively been used in CHF patients and individuals with risk factors for CVD, and its effect has not been explored in young, healthy individuals (63). Dr. Tei's research group has successfully shown that Waon therapy is able to improve cardiac hemodynamics and function, clinical symptoms, and prognosis of individuals with CHF (46, 48, 62, 88).

Improvements in cardiovascular health with Waon therapy may be attributable, in part, to improvements in vascular function with exposure to frequent therapeutic sessions (range: 5–7 times/wk for 2–5 wk). Using a modified Waon therapy protocol in Syrian gold hamsters and TO-2 cardiomyopathic hamsters, researchers showed that treatment upregulated endothelial nitric oxide synthase (eNOS) mRNA and protein content in endothelial cells (35, 36). In apolipoprotein E-deficient mice, Waon therapy increased limb perfusion, capillary density, and eNOS expression (1). Furthermore, angiogenesis (i.e., increased capillary density) was abolished with administration of N^G-nitro-L-arginine methyl ester (L-NAME) and in eNOS knockout mice, suggesting that these beneficial adaptations are heavily nitric oxide (NO) mediated (1). Reduced oxidative stress may also play a role in improved vascular function with Waon therapy, as the marker urinary 8-epi-$PGF_{2\alpha}$ decreased with 2 wk of daily treatment in humans with at least one coronary risk factor (57). Although most previous experiments examining the impact of Waon therapy were performed in animal models, the mechanisms of action in the animal experiments may provide an explanation for the observed improvements in endothelial function assessed by brachial artery (BA) flow-mediated dilation (FMD) in human intervention studies of Waon therapy in individuals with either CHF or at least one coronary risk factor (37, 47, 67). It should be noted that in these studies that have measured BA FMD in response to Waon therapy, methods used do not align perfectly with the established guidelines, which should be taken into consideration when their findings are interpreted (90).

Japanese Onsen Bathing

Onsen bathing is widely used in Japan regardless of age and disease status. Onsens are natural hot springs, which are now typically developed to include nearby bathing facilities and traditional inns (81). The temperature of water in an onsen, although not typically recorded, can vary between 25 and 45°C (81). The effects of onsen bathing on cardiovascular outcomes have scarcely been explored. Very recently, data have been published from the Shimanami Health Promoting Program, a longitudinal study evaluating the factors that contribute to cardiovascular disease, dementia, and death in an elderly Japanese population (49). In total, 873 participants filled out a questionnaire regarding their onsen bathing habits posted to them 1 yr after study cessation in December 2014. Participants bathed, on average, 5.8 ± 1.9 times/wk for 12.4 ± 9.9 min/session. Researchers observed that individuals who bathed more than or equal to five times per week had lower brachial-to-ankle pulse wave velocity (PWV), central pulse pressure, and plasma B-type natriuretic peptide (BNP). They also showed, in a subset of 166 participants with a mean follow-up duration of 4.9 yr, that maintaining these bathing habits (≥5 times/wk) eventually lowered resting levels of plasma BNP,

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which suggests improved blood pressure regulation (49). In another study conducted in the city of Beppu, Japan, a questionnaire regarding hot spa-bathing habits and disease history was administered to a random selection of 20,000 citizens aged 65 yr of age and older. Of the 9,252 valid responses received, analysis revealed that habitual onsen bathing may translate into the prevention of hypertension in women and cardiovascular disease in men, among a variety of other diseases surveyed (56). Some mechanisms of action have been demonstrated in a study in Sprague-Dawley rats (68) in which thickening of the intimal wall, a known precursor for atherosclerosis, was induced by surrounding right femoral arteries of rats with a polyethylene cuff. Following cuff treatment, rats underwent thermal therapy involving 15 min of hot water bathing (40.5–41.5°C) daily for 28 days. Rectal temperature was reported to have increased from 35 to 38°C from beginning to end of a hot water bathing session. Remarkably, thermal therapy decreased neointimal thickening and markers of atherosclerotic progression (i.e., ED-1, -2, -3-positive cells, MCP-1, and p22-phox); and increased the expression of heat shock protein (HSP) 72, which is thought to be a key component of the adaptive response to heat stress (68).

Finnish Sauna Bathing

In Finland, sauna bathing typically involves sitting in a room with 80–100°C dry, circulating air for anywhere between 5 and 20 min, interspersed with brief periods in a cold environment (27). Although common worldwide, sauna bathing is so pervasive in Finnish culture that when the Kuopio Ischemic Heart Disease risk factor study recruited middle-aged men between 42 and 61 yr old, sauna bathing habits were included in the baseline characteristics of participants. In recent publications using this data set, with a follow-up duration of at least 20 yr in >2,000 participants, researchers observed that increased sauna bathing frequency (4–7 times/wk) and duration (>19 min/session) were associated with a decreased risk of sudden cardiac death, coronary heart disease, cardiovascular disease, and all-cause mortality, for which the effect was amplified when combined with high cardiorespiratory fitness (50, 52). Frequent sauna bathing was also found to be associated with a lower risk of incident hypertension (104). Of note, sauna bathing habits were only assessed at baseline using a self-reported questionnaire; therefore, interpretation of the data relies on the assumption that these habits remained the same over the course of the succeeding 20+ yr. Currently, a mechanistic perspective on the risk-lowering effects of sauna bathing is lacking. One study has examined the acute changes in arterial stiffness with sauna bathing in 102 asymptomatic participants with at least one cardiovascular risk factor (54). Participants underwent a single session of sauna bathing at 73°C and 10–20% humidity for 30 min. Carotid-femoral (cf) PWV decreased immediately following sauna exposure, but returned to baseline levels after 30 min of recovery (54). The significance of this transient decrease in arterial stiffness remains to be determined, although it is likely more representative of a decrease in vascular tone.

Bikram Yoga

Bikram yoga was created and popularized by Bikram Choudhury in the early 1970s, and involves a set sequence of *pranayama* (deep breathing), *asanas* (poses), and *kapalabhati* (quick, strong exhalations) performed in a heated environment (35–42°C and 40% humidity) (28). Despite widespread adoption in Western cultures, there is a surprising lack of research examining the physiological impact of the practice of Bikram yoga. Previous studies on hatha (non-heated) yoga revealed no change in arterial stiffness [i.e., common carotid artery (CCA) compliance and β-stiffness index] or endothelial function (i.e., BA FMD) with regular practice (2–3 sessions/wk for 6–12 wk) (34, 85). The addition of the heated component in Bikram yoga yielded different results, but showed that the arterial response might differ based on factors such as age and training duration (31, 32). When groups of young and middle-aged to older adults practiced Bikram yoga for three times per week for 8 wk, CCA compliance and the β-stiffness index improved only in the younger group, while BA FMD improved only in the middle-aged to older group (31, 32). Interestingly, when a subsequent experiment was conducted by the same research group to directly compare heated and non-heated yoga in middle-aged to older adults only, BA FMD increased in the non-heated yoga group, but only trended toward an increase in the heated yoga group ($P = 0.056$) (33). Based on the available data, it appears that there is much greater variability around the BA FMD responses after heated vs. non-heated yoga, which may explain the lack of statistical significance observed in some of the previous studies. There is no clear consensus on the impact of Bikram (heated) yoga on arterial function, and it is possible that training duration (8 vs. 12 wk), which was different between the two studies in middle-aged to older adults, plays a role in the response.

While there is considerable evidence to suggest a general benefit of these commonly used heat therapies on vascular health and CVD risk, limited fundamental mechanistic work prompted researchers to expand on this area of research using controlled experiments to isolate the role of heat on the vasculature in the absence of other confounding elements such as muscular contraction.

PHYSIOLOGICAL RESPONSE TO HEATING

The hallmark physiological response to heat stress is the cutaneous vasodilation that allows for the necessary redirection of blood flow to the surface of the skin for heat dissipation (16) (Fig. 2). The role of vasodilators and vasoconstrictors changes as heat stress progresses in both duration and magnitude. Mild heat levels (T_c increase of ~0.5°C), characterized by isolated elevated skin temperature, generate minor adjustments in skin blood flow due to slight changes in both vasoconstrictor (decreased) and vasodilator (increased) input. Moderate heat levels (T_c increase of ~1.0–1.5°C), characterized by increases in T_c in conjunction with elevations in skin temperature, cause further increases in skin blood flow through exclusive vasodilator mechanisms in the absence of vasoconstrictor action. Severe heat levels (T_c >40°C) are characterized by significant increases in T_c that trigger active vasodilator pathways to further increase skin blood flow (16, 42). All modes of heat therapy aim to elicit a T_c increase between ~1.0 and 1.5°C; as such, the mechanisms and studies described throughout will reflect those of moderate heating. Complex neural and local mechanisms are involved in the cutaneous vasodilatory responses with heating, which are not necessarily the same as the

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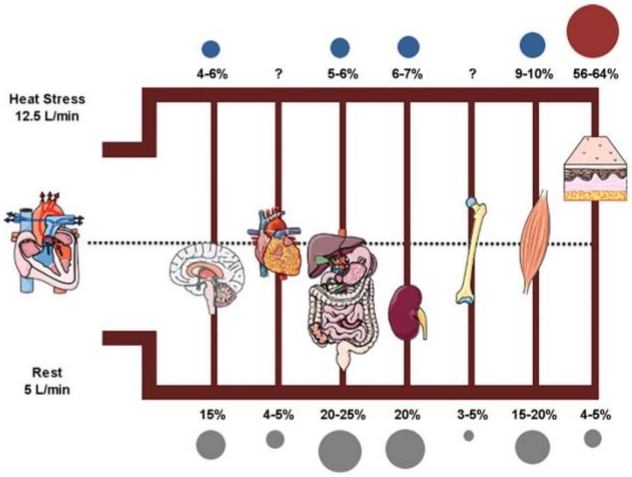

Fig. 2. Blood flow distribution at rest and during heat stress. During whole-body heat stress, cardiac output (CO) increases from ~5 to 12.5 l/min, a 2.5-fold increase. Blood flow is redirected to the skin (↑ 7–8 l/min) (39) from the splanchnic (↓ 40%) (61) and renal (↓ 15–30%) (72) regions, such that the organs comprise ~60, 5–6, and 6–7% of cardiac output with heating, respectively. Gray circles indicate the percent contribution to CO at rest, blue circles indicate a decrease in percent contribution to CO during heat stress, and red circles indicate an increase in percent contribution to CO during heat stress. Illustrations of human body organs produced by Servier Medical Art.

mechanisms that regulate conduit and peripheral arteries. Neural mechanisms of action remain unclear but are believed to exert their effects through a cotransmitter system, which suggests the release of multiple neurotransmitters to stimulate vasodilation (5, 45). Substances that are potentially involved in this mechanism include acetylcholine (45, 100), NO (43, 82, 84), vasoactive intestinal peptide (5, 99), substance P (neurokinin-1 receptors) (101), histamine (H1 receptors) (102), and prostaglandins (59). Local mechanisms create a two-tiered pattern of vasodilation in response to heating. Initial vasodilation occurs when temperature-sensitive vanilloid type 1 receptors in afferent cutaneous sensory nerves detect heat and prompt the reflex, antidromic release of vasodilatory neurotransmitter(s), the identity and action of which are currently unknown (60, 69, 86). Prolonged vasodilation occurs when HSP90 binds to, and subsequently activates, eNOS to generate NO, which diffuses to the smooth muscle layer of the arteriolar wall to cause it to relax (44, 60, 83). Well-controlled experiments using topical capsaicin (anesthetic) and L-NAME locally at the site of heating provide supporting evidence for the mechanisms of initial and prolonged vasodilation, respectively (44, 60, 86). Further information on this topic is detailed in other reviews (15, 40, 100).

With heating, the capacity for 7–8 l/min of increased skin blood flow with heating is facilitated predominantly by increased cardiac output (up to ~12.5 l/min) and decreased blood flow to the splanchnic (by ~40%) and renal (by ~15–30%) circulations (74) (Fig. 2). As a consequence, in the conduit (i.e., carotid) and peripheral (i.e., brachial, femoral) arteries, which are the focus of this review, heating results in increased blood flow and shear stress, particularly in the anterograde direction (forward, away from heart) (73, 91, 92, 94). The elevation in heart rate, concurrent with negligible changes in stroke volume, has been suggested to be mainly responsible for the increases in cardiac output of up to 2.5 times resting values. Changes in temperature and autonomic nervous system (ANS) activity with heat stress alter cardiac nodal cells such that action potentials are triggered more frequently (i.e., ↓ time to reach threshold for depolarization) and travel more quickly (i.e., ↑ conduction velocity) through cardiac myocytes (18, 23, 41, 98). Heating alters ANS balance, boosting sympathetic nervous system (SNS) activity and withdrawing parasympathetic nervous system (PNS) activity, to place the body in a global hyperadrenergic state that facilitates more frequent sinoatrial node firing. Although the goal of this mechanism is to satisfy the need for blood flow to the skin, it is very likely that these changes also influence the conduit and peripheral vascular environments, although it is currently unknown exactly how this happens. The reduction in splanchnic and renal blood flow has been attributed to decreased perfusion pressure and increased vasoconstriction in these vascular beds (20, 76–78). Overall, the redistribution of blood flow during heating allows for blood pressure to be maintained despite the substantial drop in vascular resistance to the skin blood vessels.

The SNS is a key component of the physiological response to heating in humans, controlling both the sweating and skin blood flow responses needed to dissipate heat (24). Additionally, the SNS is responsible for the compensatory increases in cardiac output and vascular resistance in noncutaneous beds necessary for the maintenance of blood pressure (75, 78). Approximately 80–95% of the increase in cutaneous blood flow with passive heat stress occurs through active vasodilation, a process that is mediated by cholinergic nerves evoking the release and action of acetylcholine and other neurotransmitters (42). In support of this idea, many studies have shown that whole-body heat stress sufficient to increase T_c by ~0.7 up to 1.3°C increases skin sympathetic nerve activity (SSNA) to trigger active vasodilation (24, 55). Muscle sympathetic nerve activity (MSNA), a more common index of sympathetic activation that is strongly associated with vasoconstriction, also increases by 40–90% to drive the increase in cardiac output and decrease in blood flow to the renal and splanchnic regions with whole-body heating (24, 55, 61). Changes in both SSNA and MSNA with heating are thought to occur through direct

J Appl Physiol • doi:10.1152/japplphysiol.00682.2018 • www.jappl.org

temperature-related mechanisms in an intensity-dependent manner (24).

IN VIVO HUMAN EXPERIMENTAL HEATING MODALITIES

Since practical heat therapies have amassed interest in recent years, researchers have begun to explore research questions on its effects in more controlled, experimental settings. Several methods have been used, thus far, to apply heat stress to the human body. Water immersion of various body parts is the most commonly used method as it is inexpensive and offers even heating of the immersed areas. Studies have employed this method to heat forearms (25, 65, 94), feet (30, 89), and lower legs (73), from the waist down (13, 14, 91, 92) and shoulders down (8–10). A limitation of this method may include the inability to collect any data involving electrical equipment (e.g., ECG), although protective seals can be applied to overcome this issue. Water-perfused tube-lined heating suits are also used, typically for whole-body heating (21, 64). There is also the option to leave an arm exposed to remove possible local skin-heating effects on measurements of arterial function. This option requires technical expertise for the construction of the suit, but has the benefit of leaving the skin surface dry for obtaining measurements. Our laboratory has previously used a heating pad/blanket to apply heat to the forearm, which, although inexpensive, is not ideal because of the uneven heating owing to difficulty wrapping fabric around irregularly shaped body parts as well as the fixed and subjective heat settings (e.g., low, medium, high). Other options include heat chambers and/or portable saunas, which would most closely replicate the conditions experienced in practical models of heat therapy.

The upcoming sections will summarize the acute and chronic effects of experimentally applied heat stress on the vasculature. Unless otherwise stated, findings highlighted are from cohorts of younger, healthy participants (both men and women) to describe the general observed response, rather than the influence of age and/or disease status.

ACUTE EFFECTS OF HEAT ON THE VASCULATURE

Few studies have examined the acute effect of a single exposure to heat on indices of vascular structure and function. Microvascular function has been shown to be improved in the lower limb following 45 min of lower leg immersion in water at 42°C (73), and unchanged in the upper limb following 60 min of combined whole-body and waist-down immersion in water at 40.5°C (10). In both studies, microvascular function was assessed indirectly using the reactive hyperemia peak and/or area under the curve response to a period of brief ischemia. More robust methods of microvascular function assessment, such as perfusion via laser Doppler, microdialysis, or contrast-enhanced ultrasound, should be used in this context for a more comprehensive evaluation. It might also be worth noting that Romero et al. (73) examined the superficial femoral artery (SFA) 30 min postheating while Brunt et al. (10) examined the BA 60 min postheating; either factor could explain the divergent findings.

Acute conduit artery endothelial function responses to a bout of passive heat stress have also been examined via FMD. An important consideration of findings is that the measurement of FMD is sensitive to baseline arterial diameter, and the appropriate statistical steps must be taken to account for acute diameter changes when responses pre- and postheating protocols are compared (2). Tinken et al. (94) demonstrated improvements in BA FMD without a change in baseline arterial diameter following 30 min of bilateral forearm heating in a water bath set to 40°C, but the lack of core and skin temperature data limits the characterization of the magnitude of heating stimulus (94). Brunt et al. (10) showed no change in BA FMD after 60 min of combination whole-body and waist-down hot water immersion at 40.5°C; but the postintervention FMD test was conducted 1 h after heating, and resting arterial diameter was observed to be increased at this time point compared with the preintervention FMD test (3.24 ± 0.24 to 3.46 ± 0.25 mm); therefore, it is possible that the expected change was missed (10). Romero et al. (73) and Thomas et al. (91) assessed acute responses to heat exposure via SFA FMD, which although relevant due to the atherosclerosis-prone nature of the SFA, has not been validated as a surrogate for coronary endothelial function or as an indicator of cardiovascular disease risk. Nevertheless, neither 45 min of waist-down water immersion nor 30 min of lower limb (up to 33 cm) water immersion, both at a temperature of 42°C, were sufficient to change SFA FMD, despite using allometric scaling statistical procedures (73, 92). In addition to the inconsistencies in statistically accounting for changes in baseline arterial diameter across studies, disparities in heating protocols and location of an artery examined preclude any overarching conclusions with respect to the acute impacts of local limb heating on conduit artery endothelial function.

Arterial stiffness responses to acute heat stress have previously been assessed via PWV and cardio-ankle vascular index (CAVI) and are likely reflective of transient changes in blood pressure and vascular tone with heating. There is no consensus with respect to the current literature, with findings ranging from increased (64), decreased (30), and no change (21, 64) in stiffness metrics with whole-body or lower limb heating. Examining special populations seemed to follow the same lack of trend, with the increased cfPWV in Moyen et al. (64) also observed in smokers and the decreased CAVI in Hu et al. (30) also observed in older patients (64). Additionally, Thomas et al. (92) observed decreases in both cfPWV and carotid-radial PWV in older individuals and those with peripheral arterial disease (91). All protocols except for that used in Hu et al. (30), which saw a T_c change of ~0.3–0.4°C, generated increases in T_c in the range of 1.5–1.8°C (21, 30, 64, 91). Discrepancies in the results of these four studies may be attributable to the various populations examined and assessment techniques used. The lack of change in Ganio et al. (21) may have been due to an underpowered sample size ($n = 8$) and the inclusion of both men and women (without control of menstrual phase). For comparison, Moyen et al. (64) had 26 men, with 13 in each of the smoking and nonsmoking groups, and Hu et al. (30) had 32 women, with 16 in each of the younger and older age groups. Timing of arterial stiffness assessment may have also contributed to the divergent findings in these studies. Moyen et al. (64) measured PWV while the body was still under heat stress, in which case it is plausible that the SNS resulted in increased vascular tone and arterial stiffness. On the other hand, Hu et al. (30) measured CAVI in the absence of heat stress, albeit immediately after water immersion, and found transiently decreased arterial stiffness that returned to approximate resting

J Appl Physiol • doi:10.1152/japplphysiol.00682.2018 • www.jappl.org

levels after 30 min. However, to further complicate the issue, Thomas et al. (92) observed decreased PWV despite assessment 30 min after the heating intervention. It is worth mentioning that only Thomas et al. (92) followed the guidelines on the recommended method of arterial stiffness assessment (7), which should be considered when findings between studies are compared.

Overall, regardless of the vascular outcome examined, the literature with respect to acute vascular responses to heating is sparse, and results are equivocal at best. Understanding the acute vascular responses to heating are important for informing the mechanisms by which heating may influence the vasculature with repeated use and should be studied further.

CHRONIC EFFECTS OF HEAT ON THE VASCULATURE

Recent research conducted in controlled experimental settings has begun to focus on the effects of chronic application of heat on measures of micro- and macrovascular function in cohorts of young, healthy men and women. In previous studies, microvascular function has been commonly assessed as the cutaneous vascular conductance (CVC) response to a heat stimulus using laser Doppler array probes. A series of two studies employed 8-wk heat training protocols where participants came in for 30-min sessions three times a week for hot water immersion (40–42°C). Green et al. (25) used a bilateral forearm heating model intended to increase local temperature and found increased forearm CVC after 4 wk, and Carter et al. (13) used a waist-down heating model intended to increase T_c and found increased forearm CVC after 8 wk (13, 25). Restricting the normal blood flow and shear stress elevations to the BA, where assessment was conducted, negated the improvements in CVC. In the study by Carter et al. (13), skin temperature at the nonheated forearms also appeared to play a key role in modulating the microvascular function response to lower limb heat training, as CVC was found to decrease when the natural rise in skin temperature was restricted and instead clamped at resting levels. Taking it a step further, Brunt et al. (8) used the same laser Doppler microvascular function assessment technique but layered on a microdialysis approach to determine the mechanisms underlying the observed changes in microvascular function with 8 wk of a combination of whole-body and waist-down heat training. Before and after the training period, a heat challenge was applied to several sites on the ventral forearm where microdialysis fibers had been inserted. Microdialysis fibers received either lactated Ringer (control), the NOS inhibitor N^{ω}-nitro-L-arginine (L-NNA) to reduce NO production, or the superoxide dismutase mimetic tempol to reduce oxidative stress (8). Administration of L-NNA decreased CVC, while tempol increased CVC but not above that of the control condition, demonstrating that improved NO bioavailability is the key heat training-induced change responsible for improved microvascular function (8). Most recently, Francisco et al. (19) tested whether microvascular adaptations could be elicited by 10 days of 60 min/day forearm heating using a cylindrical water-spray device. Throughout the heating sessions, the contralateral arm was submerged in a thermoneutral (32°C) water bath to serve as a within-subject control. The T_c remained unchanged throughout forearm heating in a subset of two subjects in whom this outcome was assessed (19). When comparing microvascular function before and after short-term acclimation, researchers found that 10 days of repeated heating was insufficient to elicit changes in the cutaneous vascular responses to local heating, acetylcholine administration, or prolonged forearm heating, suggesting that either a longer acclimation period or less localized stimulus is needed (19).

In previous examinations of vascular responses to heat training, macrovascular function was most typically assessed as endothelial function using a FMD test. In separate studies from the Green research group, BA FMD was examined before, during, and after 8 wk of 3 times/wk 30-min limb hot water (40–42°C) immersion sessions. Naylor et al. (65) heated both forearms and observed an increase in BA FMD after 2 wk of heat training (65), while Carter et al. (14) heated from the waist down and observed an increase in BA FMD after 4 wk. In both cases, BA endothelial function improved early on, but reverted to baseline levels by the end of heat training, which is consistent with the proposed time course of vascular adaptations to exercise training (93). Accompanying structural outcomes would have given a more complete picture, although the observed lack of change in resting arterial diameter in either study at any time point during the 8 wk of heat training suggests no structural remodeling in the form of increased arterial dimension occurs (14, 65). It also seems that the location of heating may impact the time course of change in FMD (i.e., increase after 2 vs. 4 wk). Because these earlier studies were designed for the purpose of investigating the effect of shear stress alterations rather than heating, skin and T_c measurements were absent or limited, making it difficult to characterize the magnitude of the heating stimulus. The most recent study by Brunt et al. (9) is the most comprehensive and robust study on passive heat stress training to date, including measures of arterial wall thickness and stiffness in addition to endothelial function. A combination whole-body and waist-down water immersion protocol was used to heat young, sedentary but otherwise healthy men and women, with the ultimate goal of maintaining a T_c between 38.5 and 39°C (~1.5°C above resting) for up to 90 min (9). For comparison, the heating protocol used by Carter et al. (14) increased T_c by only ~0.5°C by the end of the 30 min. Heat training in the Brunt et al. (9) study was also more rigorous with 4–5 sessions per week of heating for a total of 36 sessions throughout the 8-wk period. Improvements were observed in BA FMD, SFA compliance, SFA β-stiffness index, cfPWV, CCA intima-media thickness (IMT), and blood pressure (BP) at various time points throughout heat training (9). Increases in BA FMD (after 2 wk), SFA compliance (after 4 wk), and mean BP (after 2 wk) persisted until the end of the 8-wk training period. Decreases in diastolic BP were observed at *weeks 4* and *8* only. Decreases in the SFA β-stiffness index, cfPWV, and CCA IMT were observed only at the end of the 8 wk (9). The IMT findings in this study should be interpreted with caution, as calipers were used for analysis (96). Otherwise, gold standard methodologies were used for all other vascular outcome measures (7, 53, 90). The differential adaptations in different arterial locations (e.g., CCA vs. SFA) is very interesting and suggests that different mechanisms may be involved in the response to chronic heating. Furthermore, the maintenance of improved endothelial function up to 8 wk of training surpasses the time course of adaptations suggested by Tinken et al. (93), which may point to alternate signaling pathways being activated with chronic combined local and core temperature ele-

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vation compared with other interventions, such as local heating alone and exercise (14, 65, 93). Recently, Bailey et al. (3) demonstrated that 8 wk (30-min sessions, 3 times/wk) of whole-body hot water immersion improved BA FMD to the same extent as moderate-intensity cycling training, providing support for the eventual use of heat stress as an alternative or adjunct therapy to exercise (3). Certainly, more research is warranted as this study was conducted in a group of young recreationally active women, which represents only a fraction of the world population.

POTENTIAL MECHANISMS AND FUTURE DIRECTIONS

Shear stress is a critical physiological stimulus thought to be imperative for eliciting vascular adaptation in response to stressors through its ability to trigger the production of substances (e.g., NO) that are vasodilatory and thought to be atheroprotective (Fig. 3) (17, 26, 51). Endothelial cells detect shear stress through the alteration and deformation of the morphology of their stress fibers, which subsequently trigger the production of vasodilators or vasoconstrictors. The effects of shear stress on vascular function have been demonstrated in cells, animals, and humans (12, 26, 51, 66, 97). Seminal work by Pohl et al. (70) demonstrated, through their removal, that endothelial cells are essential to the FMD response. In this study conducted in vivo in canine femoral arteries, the responses to nitroglycerine and norepinephrine, both vasoactive agonists, were preserved, while FMD was lost after intimal denudation (70). In experiments that followed by Laughlin and colleagues (38), researchers observed tight coupling between shear stress induced by increased flow and FMD ex vivo in rat superficial femoral arteries. Additionally, they demonstrated that higher vs. lower or no flow resulted in greater mRNA

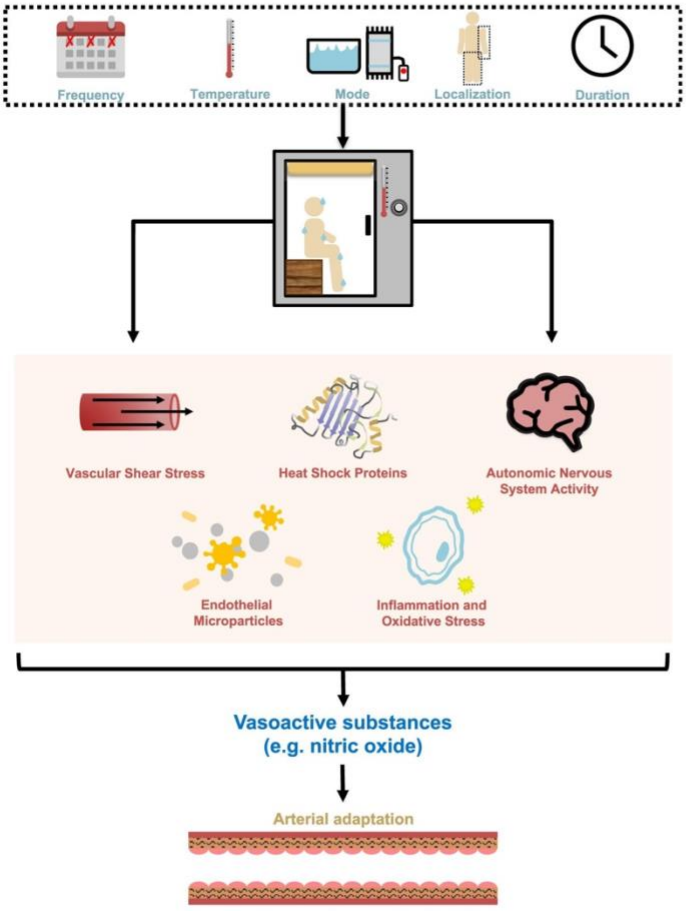

Fig. 3. Potential control mechanisms for the effect of heat stress on the vasculature. Heat stress can be characterized by frequency, temperature, mode, localization, and duration, to determine the magnitude and intensity of the stimulus. Heat alters vascular shear stress, heat shock protein content, autonomic nervous system activity, endothelial cell damage pathways, and inflammation and oxidative stress, all of which are associated with the regulation of secreted vasoactive substances to mediate arterial adaptation.

J Appl Physiol • doi:10.1152/japplphysiol.00682.2018 • www.jappl.org

expression of eNOS, an enzyme needed to produce NO, and Cu/Zn superoxide dismutase, an enzyme that scavenges superoxide (O_2^-), thereby slowing down the rate of degradation of NO (103). With regard to heat stress, it is known that vascular shear stress is increased during heating to satisfy the demand for blood flow at the skin surface; and it has been postulated that these repeated increases in shear stress drive the improvements in micro- and macrovascular artery function that arise with chronic heat exposure (94). Recent work in humans in vivo has recapitulated some of the early shear stress work conducted in cells and animals, with a series of experiments repeatedly demonstrating that the improvement in FMD and CVC in response to heating, as well as a number of other interventions, is abolished when a cuff is inflated to 80–100 mmHg throughout the intervention to prevent the naturally occurring increase in shear stress (6, 25, 94, 95). The obligatory role of increased shear stress on the heat-induced improvements in vascular function has become accepted in the literature, as it is one of the most well-studied and reproduced phenomenon in this research area.

Changes in arterial stiffness with acute and chronic heating are more challenging to explain, as both passive (i.e., deposition of elastin and collagen in arterial wall) and active (i.e., vascular tone) components of the arterial wall can contribute to measured values (79). Currently available assessment methods and outcome measures, such as PWV, compliance, and the β-stiffness index, are not capable of discerning which of the two contributors is responsible for observed changes in the stiffness profile, although duration of intervention or training can help to inform this question. Modifications to passive stiffness are likely restricted to chronic intervention durations owing to the time course for deposition of new elastin or collagen protein, while active stiffness can reasonably fluctuate over a shorter period of time and may be transient, since neural drive is a primary effector of this type of change (Fig. 3) (79). It may be worthwhile to assess sympathetic tone through muscle or skin sympathetic nerve activity in future studies to provide additional insight on arterial stiffness changes, particularly with chronic heat stress.

Much of the recent work alludes to the potential roles of increased HSPs and decreased inflammation and oxidative stress in mediating changes in vascular function (Fig. 3). HSPs play an important role in activating eNOS, as supported by data that show that the HSP90 inhibitor geldanamycin attenuates the NO-mediated vasodilation in skin (83). In vitro experiments in bovine coronary endothelial cells have demonstrated that acute heat activates HSP90, yielding increases in circulating NO through elevated eNOS activity (71), whereas ex vivo experiments in peripheral blood mononuclear cells from humans have shown that chronic heat increases the amount of HSP72 and HSP90 protein (58). HSPs also work to stabilize and activate other proteins, and those involved in inflammatory and oxidative stress responses are likely relevant to vascular function. Although the main purpose was to examine responses to hypoxia-reoxygenation, recent work by Brunt et al. (11) showed that in human umbilical vein endothelial cells incubated for 24 h in "mild heating" (39°C) media or with serum from participants that have undergone an 8-wk heat therapy intervention, there was reduced production of O_2^- and elevated production of manganese superoxide dismutase (O_2^- scavenger) at rest compared with sham conditions (37°C media and thermoneutral water immersion therapy, respectively). These differences coincided with increased HSP70 protein levels in mild heating-pretreated cells and peripheral blood mononuclear cells isolated from venous blood collected from human participants (11). Additionally, Bain et al. (4) show in humans that arterial concentrations of activation- and apoptosis-derived endothelial microparticles and platelet microparticles are reduced after ~1 h of whole-body heat stress (4). These outcome measurements indicate endothelial cell damage and activation of inflammatory and thrombotic pathways, respectively, which provide further information on mechanisms by which heating improves vascular function (Fig. 3). Further work in this area should concurrently explore the changes in HSPs, inflammation, and oxidative stress, and indices of cardiovascular disease risk in response to heating interventions.

As some of the literature in both practical and experimental models of heat therapy suggests, age and disease risk status may be moderators of the effect of heat on the vasculature. It would be advantageous to extend the acute and chronic heating findings in experimental studies to groups of individuals that are unable to perform traditional physical activities, or undergo periods of reduced physical activity, such as older adults and clinical populations. Very few tightly controlled, experimental studies have been conducted in these populations for whom heat therapy may be most transformative in terms of health outcomes (73, 91). In a recent study, Teixeira et al. (89) showed, albeit in a young healthy population, that immersing one foot in a hot bath for 30 min, three times a day, can mitigate the impairment in SFA endothelial function that occurs with just 5 days of reduced physical activity (89). This study should be replicated in clinical groups, since it is reasonable to expect even greater positive results considering they are likely beginning with reduced endothelial function and have more to gain from the heating intervention.

CONCLUSIONS

Based on a wealth of evidence in Waon therapy, Japanese onsen bathing, Finnish Sauna bathing, Bikram yoga, and other heat-related activities, it seems that chronic exposure to heat in a therapeutic paradigm has a beneficial effect on vascular health (27, 31, 63). Recent efforts to characterize the vascular responses to acute and chronic heating using experimental models have generally demonstrated improvements across all aspects of vascular health, including IMT, compliance, β-stiffness index, FMD, and CVC with chronic heat exposure. In contrast, there is less consensus regarding the responses to acute heat exposure due to a variety of factors, but most notably suboptimal and inconsistent assessment methods and protocols. For instance, a major challenge of acute investigations is the wide range in the definition of the acute phase, anywhere between 0 min to 24 h postintervention. With most research groups assessing outcomes at just one or two time points, this makes it extremely difficult to compare findings and determine which responses are scientifically relevant. A study aiming to establish a time course of change in vascular outcomes in response to acute heat stress is needed to further progress in this area. Another major limitation of the current literature is the extensive array of heating protocols and assessment methodologies, making it difficult to arrive at a consensus for any given effect (e.g., acute vs. training, degree

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of heating, location of heating, duration of heating). The future of heat stress therapies may benefit from utilizing the F.I.T.T. (frequency, intensity, type, time) principle more commonly used in exercise, as studies will need to consider these aspects of the stimulus as moderating factors of the effect (Fig. 3). The literature on Finnish sauna bathing is likely the most advanced in this regard, with studies having demonstrated negative dose-dependent relationships between frequency and/or duration of sauna use and CVD and all-cause mortality risk (50, 52). It is reasonable to believe that indicators of vascular dysfunction that precede many CVDs would be even more sensitive to detect the effects of nuances in these heating parameters. Although not much has been done to explore the impact of heating intensity (i.e., temperature), many heating mechanisms are thought to only be activated beyond certain threshold temperatures, which suggests that milder heating may not be as effective as moderate heating at triggering the atheroprotective signaling pathways known to be beneficial for vascular function. Overall, important caveats to many of the observations made, as well as the limited number of studies in both acute and chronic heating models, necessitate additional studies be conducted that are comprehensive and well controlled.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.L.C. and M.J.M. conceived and designed research; J.L.C. and M.J.M. prepared figures; J.L.C. and M.J.M. drafted manuscript; J.L.C. and M.J.M. edited and revised manuscript; J.L.C. and M.J.M. approved final version of manuscript.

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1.3 Methods of arterial function assessment

In this thesis, the term arterial function is used broadly to refer to endothelial function, arterial stiffness, and arterial structure. There are a variety of methods to assess each of these outcome measures. My studies used reference standard methods where possible, including flow-mediated dilation for the assessment of endothelial function and pulse wave velocity and carotid artery distensibility for the assessment of arterial stiffness. These techniques were chosen because they are non-invasive, technically feasible, and provide an indication of future CVD risk. Other methods are outlined in this chapter to allow the reader to contextualize our findings with respect to that of previous literature.

1.3.1 Assessment of endothelial function

Endothelial function refers to the capacity of the endothelial cells lining the arterial wall to release substances that regulate vasomotion, cellular adhesion, thromboresistance, smooth muscle cell proliferation, and vessel wall inflammation (9). Most methods of endothelial function assessment focus on the measurement of arterial vasodilation, as it is more observable and quantifiable *in vivo* compared to the other processes listed above. Greater increases in artery diameter or perfusion in response to pharmacological or shear-induced vasodilator release are indicative of better endothelial function (9, 12). Endothelial function can be assessed in both the macro- and microvasculature.

1.3.1.1 Cardiac catheterization

Cardiac catheterization is a well-controlled, but invasive, method of assessing endothelial function. It involves inserting a catheter into the coronary arteries and infusing pharmacological, vasoactive agents such as acetylcholine or N-methylarginine (L-NMMA) and then subsequently observing changes in artery diameter using quantitative angiography (12, 13). Due to the medical expertise required and risks involved, this procedure is now rarely used in research settings, especially considering that many other less invasive surrogate measures are adequate indicators of coronary endothelial function (12).

1.3.1.2 Venous occlusion plethysmography

Venous occlusion plethysmography similarly uses changes in forearm or leg volume – a surrogate for perfusion – under different pharmacological conditions as an indicator of endothelial function (9, 12, 14). In this technique, pneumatic cuffs are inflated around the distal and proximal limb such that arterial inflow is permitted but venous outflow is blocked. Strain gauges are wrapped around the widest part of the forearm to measure changes in resistance corresponding to changes in limb volume in response to the administration of different stimuli. Examples of stimuli include vasoactive agonists and antagonists, limb height adjustments, postural changes, and isolated muscular contractions (14). Increased limb volume is indicative of increased vasodilation, and therefore, improved endothelial function. While less invasive than cardiac catheterization, venous occlusion plethysmography is technically challenging and

difficult to standardize across labs; and its clinical relevance has been called into question because changes in forearm blood flow reflect the microvascular circulation rather than the disease-susceptible peripheral conduit arteries (9).

1.3.1.3 Flow-mediated dilation

Flow-mediated dilation (FMD) is the reference standard assessment of macrovascular endothelial function. In an FMD test, ultrasound imaging is used to capture conduit artery diameter to quantify the degree to which the artery is able to expand or dilate in response to increased shear stress. A pneumatic cuff wrapped distal to the artery site of interest is inflated to suprasystolic levels (*e.g.* 200 mmHg) for 5 minutes to generate an ischemic stimulus that results in a dramatic increase in blood flow, and consequently shear stress, to the distal limb when the cuff is released. Ultrasound images are obtained for 30 to 60 seconds at rest, prior to cuff inflation, as well as 3 minutes after cuff release to ensure that peak vasodilation is captured. FMD is typically expressed as a percentage change in arterial diameter (AD) relative to baseline AD (15), but allometric scaling procedures that account for the logarithmic relationship between resting AD and changes in AD can be applied during statistical analysis to permit assessments across individuals with different caliber arteries (16, 17). The FMD test is completely non-invasive and can be performed on several peripheral arteries, including the brachial, superficial femoral, and popliteal arteries. However, it is most commonly conducted on the brachial artery because of its clinical significance in this site. Epidemiological studies

show that BA FMD is predictive of future cardiovascular events, such that every 1% increase in FMD is associated with a 13% reduction in risk (18, 19).

1.3.1.4 Laser doppler flowmetry and iontophoresis

Laser doppler flowmetry (LDF) coupled with iontophoresis of acetylcholine (ACh) and sodium nitroprusside (SNP) can provide an indication of microvascular endothelial function. ACh and SNP are endothelium-dependent and independent vasodilators, respectively; therefore, a decrease in perfusion with ACh without a concomitant change in perfusion with SNP can signify endothelial dysfunction (20). Changes in perfusion using this method are quantified by arbitrary units of flux; this value can also be expressed as a percentage of maximal vasodilation either by iontophoresis of a nitrovasodilator or in response to 42-44 °C local heating, or a percentage of maximal cutaneous vascular conductance calculated as flow divided by mean arterial pressure (20).

1.3.1.5 Circulating factors

Other methods to assess endothelial function that focus on functions other than vasodilation involve measuring circulating factors in blood serum or plasma or in cell exposure studies. Endothelial function is maintained by the appropriate balance of anti-atherogenic (e.g., nitric oxide, endothelium-derived hyperpolarizing factor, prostacyclin) and pro-atherogenic (e.g., endothelin-1, NF-κB, prostanoids, angiotensin converting enzyme) factors in the arterial milieu, all of which can be measured using various assays targeting phosphorylation status or protein or mRNA levels (9). While these factors are

valuable for providing mechanistic insight, there are some drawbacks to their use and application. Serum and plasma measures have been criticized for reflecting the systemic circulation more broadly and it is difficult to ascertain that any changes observed or differences are directly and exclusively attributed to endothelial cell function (21). Circulating factors can also be measured in plated and cultured endothelial cells – usually from human umbilical veins – which allows for the administration of experimental protocols that use chemicals or stimuli that might otherwise be lethal *in vivo* (22–25). However, the tight experimental control afforded by this method comes at the cost of not knowing whether any responses seen will translate to *in vivo* scenarios which involve the integration of all body systems.

1.3.2 Assessment of arterial stiffness

Arterial stiffness describes the elasticity of the arterial wall. Techniques used to measure arterial stiffness are based on either the transmission/propagation model or pulsation/distension model (26). Changes in arterial stiffness can occur through either structural alterations in the elastin and collagen composition of the arterial wall, or functional alterations in vascular smooth muscle tone as a result of cellular signaling favouring the release of constrictors (e.g., angiotension II, endothelin, oxygenases, etc.) rather than dilators (e.g., nitric oxide, endothelium-derived hyperpolarizing factor) (27). Most methods of assessing arterial stiffness do not allow for the discrimination of the mechanism responsible for change, but some information can be gleaned based on the time frame of assessment. Regulation of the synthesis and breakdown of structural

components of the arterial wall is a dynamic but slow process (27). Therefore, it can be inferred that if arterial stiffness changes acutely, it is likely due to cellular signaling and changes in vascular tone; whereas if arterial stiffness changes chronically, it can be due to either of the mechanisms or a combination of both.

1.3.2.1 Arterial compliance, distensibility, and β-stiffness index

Compliance, distensibility, and β-stiffness index are all measures of local arterial stiffness, and are most commonly assessed in the common carotid artery (CCA) (28). These measures fall under the pulsation/distension model of arterial stiffness assessment. Compliance is the absolute change in CCA diameter for a given change in pressure, whereas distensibility further accounts for minimum artery diameter (28). β-stiffness index is the logarithmic value of relative pressure divided by the relative change in arterial diameter (29). In the lab, these outcomes are measured using ultrasound imaging and applanation tonometry in tandem on opposing sides of the body to collect images and pressure waveforms, respectively, from the CCAs in the neck. Data from 10 heart cycles is used to calculate each of the stiffness metrics (28). Greater compliance and distensibility are indicative of a more elastic artery, while greater β-stiffness index is indicative of a less elastic artery.

1.3.2.2 Pulse wave velocity

Pulse wave velocity (PWV) is the speed at which a pulse travels through an arterial segment, and it is a measure of regional arterial stiffness. This measure falls under

the transmission/propagation model of arterial stiffness assessment. Carotid to femoral PWV is most commonly reported and established as the reference standard assessment of arterial stiffness because it holds clinical significance. Those who possess a cfPWV ≥ 10 m/s are thought to be at risk for developing CV disease, and every 1 m/s increase in cfPWV is associated with 14%, 15%, and 15% increases in risk of total CV events, CV mortality, and all-cause mortality, respectively (30, 31). However, PWV can also be assessed in the upper and lower limbs between the carotid to radial arteries and femoral to tibialis posterior or dorsalis pedis arteries. The reference standard protocol for the measurement of PWV involves two applanation tonometers and the simultaneous collection of approximately 30 heart cycles' worth of high-quality pressure waveforms. The surface distance between the two pulse sites of interest are divided by the average time delay between the foot of the waveforms for each heart cycle to yield a PWV value (30).

1.3.2.3. Cardio-ankle vascular index

Cardio-ankle vascular index (CAVI) is a non-invasive metric of arterial stiffness that combines the principles of pulse wave velocity and β-stiffness index. In essence, it is an expansion of β-stiffness index in that it is a measure that is independent of blood pressure, but indicative of whole-body stiffness, from the heart to the ankle (32, 33). CAVI is a commonly used measure in Japan where portable machines that can provide this stiffness estimate are widely distributed (32). The method involves placing ECG electrodes on both wrists, a microphone on the sternum, and four blood pressure cuffs to

wrap around each extremity. Data collection is automated and does not require technical expertise (32, 33). CAVI is theoretically appealing because of the ability to account for blood pressure, which may influence PWV; however, in practice, it is a weaker predictor of health outcomes likely because blood pressure is such a strong predictor of mortality (34). For this reason, PWV remains the recommended method of arterial stiffness assessment.

1.3.3 Assessment of arterial structure

1.3.3.1 Carotid intima-media thickness (cIMT)

Arterial structure describes the composition of the layers of the arterial wall. In many chronic disease conditions, the final stage of arterial deterioration that occurs prior to the accumulation of plaque is the thickening of the intima-media layer of the carotid arterial wall which can be measured using ultrasound imaging (35). cIMT increases with age and is greater in those that suffer with chronic diseases like type 2 diabetes, hypercholesterolemia, hypertension, and obesity (36–41). Unlike metrics of endothelial function and arterial stiffness, changes in cIMT occur over the span of years. Rarely are improvements in cIMT seen in the relatively short-term (i.e., 4-12 weeks) interventional studies that are conducted in physiology research. Changes in cIMT are clinically significant: every 0.1 mm increase in cIMT is associated with a 10-15% increased risk of having a myocardial infarction and a 13-18% increased risk of having a stroke (42). cIMT and arterial stiffness are correlated and share the same risk factors, but still provide unique risk profiles because of the reliance of stiffness measures on blood pressure (43).

In the disease progression towards atherosclerosis, arterial stiffness generally precedes increasing cIMT, which represents the cumulative burden of risk factors accumulated over the life span (43).

1.4 Study objectives and hypotheses

The overarching goal of this thesis is to evaluate the effectiveness of local heat therapy as a vascular health-promoting intervention both acutely and chronically, compared to and in conjunction with exercise training. This research was conducted in young, healthy recreationally active adults. In **Chapter 2 (Study 1)**, our objective was to determine whether ankle- or knee-level hot water immersion would be sufficiently stimulating to change vascular function assessed both locally and systemically in the acute time frame. We hypothesized that both protocols would result in improved vascular function but with greater magnitude changes elicited by the knee-level condition. In **Chapter 3 (Study 2)**, our objective was to compare the vascular function responses to 8 weeks of local heat therapy, aerobic exercise training, or combined training and therapy. We hypothesized that, compared to a control condition, all therapy and/or training groups would have improved vascular function, but that combined therapy and training would be superior to either intervention alone. In **Chapter 4 (Study 3)**, our objective was to determine if any relationships exist between the acute and chronic vascular function responses to our interventions as a means of understanding whether measures such as flow-mediated dilation and pulse wave velocity have predictive potential for lifestyle prescription. We hypothesized that acute vascular function responses to a bout of an

intervention pre-therapy and/or training would be able to predict chronic vascular function responses post-therapy and/or training.

1.5 References

Note: Section 1.2 has been published in the Journal of Applied Physiology (126:771-781, 2019) and contains its own reference list. For the purposes of brevity, this section includes only citations made in Sections 1.1 Preamble and 1.3 Methods of arterial function assessment.

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CHAPTER 2

STUDY 1

Improvements in vascular function in response to acute lower limb heating in young healthy males and females

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RESEARCH ARTICLE

Physiology of Thermal Therapy

Improvements in vascular function in response to acute lower limb heating in young healthy males and females

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Abstract

Regular exposure to passive heat stress improves vascular function, but the optimal heating prescription remains undefined. Local limb heating is more feasible than whole body heating, but the evidence demonstrating its efficacy is lacking. The purpose of this study was to determine whether acute improvements in vascular function can be achieved with lower limb heating in 16 young healthy individuals (8 female, 8 male). In separate visits, participants underwent 45 min of ankle- and knee-level hot water immersion (45°C). A subset of seven participants also participated in a time-control visit. Endothelial function was assessed through simultaneous brachial and superficial femoral artery flow-mediated dilation (FMD) tests. Macrovascular function was quantified by %FMD, whereas microvascular function was quantified by vascular conductance during reactive hyperemia. Arterial stiffness was assessed through carotid-femoral and femoral-foot pulse wave velocity (PWV). Plasma concentrations of interleukin-6 and extracellular heat shock protein-72 (eHSP72) were used as indicators of inflammation. Our findings showed that 45 min of lower limb heating—regardless of condition—acutely improved upper limb macrovascular endothelial function (i.e., brachial %FMD; Pre: 4.6 ± 1.7 vs. Post: 5.4 ± 2.0%; $P = 0.004$) and lower limb arterial stiffness (i.e., femoral-foot PWV; Pre: 8.4 ± 1.2 vs. Post: 7.7 ± 1.1 m/s; $P = 0.011$). However, only knee-level heating increased upper limb microvascular function (i.e., brachial peak vascular conductance; Pre: 6.3 ± 2.7 vs. Post: 7.8 ± 3.5 mL/min s^2 mmHg; $P \leq 0.050$) and plasma eHSP72 concentration (Pre: 12.4 ± 9.4 vs. Post: 14.8 ± 9.8 ng/mL; $P \leq 0.050$). These findings show that local lower limb heating acutely improves vascular function in younger individuals, with knee-level heating improving more outcome measures.

NEW & NOTEWORTHY This study demonstrates that lower limb hot water immersion is an effective strategy for acutely improving vascular function in young, healthy males and females, thereby encouraging the development of accessible modes of heat therapy for vascular health.

arterial stiffness; endothelial function; heat therapy; passive heat stress; perception

INTRODUCTION

Experimental research on repeated exposure to passive heat stress (i.e., a chronic model) has consistently demonstrated beneficial effects on vascular function in young, healthy individuals (1–7). Despite these positive findings, passive heat stress is relatively underutilized as a health-promoting intervention. Most existing heat therapies involve whole body protocols, which are not ideal for several reasons. The equipment required for whole body heating is typically expensive (e.g., sauna or hot tub) or accessible only with additional subscriptions (e.g., gym membership). Furthermore, whole body heating as performed in experimental studies has been reported as thermally uncomfortable, which may limit longer-term adherence to this therapy (8–10). Similar to exercise prescription, heat stress can be modified based on several parameters to determine the magnitude of physiological stress (11). Stimulus localization is a prescription parameter that contributes to heating intensity and may be leveraged to tackle the barriers that prevent widespread adoption of heat therapy. For example, lower limb heating is an appealing alternative to whole body heating because it may be more feasible and tolerable for the general public, and some research has shown that only a limited proportion of body mass needs to be heated to observe some of the beneficial vascular responses to heating (12–14). However, the exact amount of body mass that needs to be heated to elicit improved vascular function remains undetermined but may be linked to a minimum magnitude of heating-induced core temperature (T_c) change required, as seen in the cutaneous vasculature (15).

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Owing to protocol variability, studies examining the acute impact of passive heat stress yield mixed results. With acute heating, macrovascular endothelial function has been shown to increase in some (16–18) but not all instances (19–21) in the brachial artery (BA), whereas the superficial femoral artery (SFA) appears resistant to change (13, 20, 22). In contrast, microvascular function has been shown to increase in some (13, 17) but not all instances (19–21) in both the BA and SFA. Arterial stiffness has also been shown to decrease when examined in the upper limb (23) or whole body (12), but not in the central or lower limb segments (23). Another factor that may impact vascular function is the inflammatory profile. Although elevated basal concentrations of inflammatory markers such as interleukin-6 (IL-6) and extracellular heat shock protein 72 (eHSP72) are linked to cardiovascular disease, acute transient elevations through exercise or heat therapy may improve the basal inflammatory profile, and thus, vascular function (24, 25).

To overcome the barriers to participation in heat therapy, it is important to demonstrate the efficacy of acute local lower limb heating protocols. Determining the minimum effective dose of heating needed to elicit an acute vascular response may contribute to the development of heat therapies for those who are more sensitive to thermal stimuli. Therefore, the purpose of this study was to compare the acute vascular function responses to ankle- and knee-level lower limb heating. We hypothesized that both lower limb heating protocols would elicit acute improvements in vascular function, but that the magnitude of change would be greater with the knee versus the ankle condition.

METHODS

All experimental testing sessions took place at the Vascular Dynamics Lab at McMaster University in Hamilton, Ontario, Canada. This study was approved by the Hamilton Integrated Research Ethics Board (reference no. 5269) and registered with ClinicalTrials.gov (identifier no. NCT03618524). Participants provided informed written consent before undergoing any part of the study.

Participants

Sixteen young healthy recreationally active adults (8 male, 8 female; 24 ± 2 yr old) between the ages of 18–35 yr old participated in this study. All were classified as normal weight and normotensive (Table 1). Females were tested either during the early follicular phase (i.e., *days 1–7*) of their natural menstrual cycle (N = 1) or during the "no hormone/placebo" phase of their hormonal contraceptive cycle (pill: N = 5, ring: N = 2) when they would be experiencing withdrawal bleeding. Exclusion criteria included current smoking and/or drug use; a history of cardiovascular, musculoskeletal, or metabolic disease; and for females, having an irregular menstrual cycle (> 40 days) (26). Sample size was calculated a priori based on prior research by Tinken et al. (18), who observed large-sized effects (d = 0.90) of local forearm heating on the acute BA FMD response in young, healthy men (mean ± SD: 4.6 ± 0.9 vs. 8.1 ± 5.4%). For the current study, using the same baseline value of 4.6%, more conservative anticipated responses of 1% (ankle) and 2% (knee) with a common standard deviation of 1.2 calculated from prior work in our laboratory (16) was chosen to reflect the potential impact of a more distant local leg heating protocol. Given >80% power to detect differences of this magnitude and α = 0.05, 16 participants were required.

Table 1. *Participant characteristics*

Variable	N = 16
Sex, male/female	8/8
Age, yr	24 ± 2
Height, m	1.7 ± 0.1
Body mass, kg	71.1 ± 13.3
BMI, kg/m^2	23.3 ± 3.8
Resting SBP, mmHg	110 ± 6
Resting DBP, mmHg	63 ± 4
Resting MAP, mmHg	81 ± 4
Resting HR, bpm	55 ± 9

All data are expressed as mean ± SD. N = 16 participants. BMI, body mass index; DBP, diastolic blood pressure; HR, heart rate; MAP, mean arterial pressure; SBP, systolic blood pressure. For each participant, resting blood pressure and HR were averaged across all their visits.

Experimental Design and Protocol

This study employed a within-subjects interventional design involving three visits to the laboratory. At the first visit, written informed consent was obtained followed by familiarization with the laboratory environment and protocols. The left BA and SFA were scanned to assess image quality, then simultaneous BA and SFA FMD tests were conducted to ensure tolerance during the data collection sessions. At the second and third visits, participants came to the laboratory to undergo the experimental heating interventions. Additionally, seven of our 16 participants also underwent a third time control condition (Con) on a fourth visit to assess the impact of supine, bent-leg lying alone (i.e., without water immersion) on our main vascular outcome measures. Prior to these visits, participants were instructed to do the following: 1) abstain from moderate to vigorous physical activity for ≥ 24 h, 2) abstain from alcohol, caffeine, and food for ≥ 8 h, and 3) ingest a wireless telemetric T_c pill with water 2–3 h before the scheduled visit time.

Upon arrival, anthropometric measurements were taken, and participants were asked to wear a fitted sensor belt (EQ02 + LifeMonitor; Equivital by Hidalgo, Cambridge, UK) around the chest before lying on the testing bed for 10 min of supine rest. During this period, participants were instrumented with two sets of single-lead ECG connected to each of the ultrasound machines (Vivid q; GE Medical Systems, Horten, Norway); three skin temperature (T_{sk}) probes affixed to the right foot, calf, and forearm (MLT422/AL probes with ML309 thermistor pods; AD Instruments, Colorado Springs, CO); and a finger cuff wrapped around the right middle finger for continuous photoplethysmographic hemodynamic measurements (Finometer MIDI and Beatscope software; Finapres Medical Systems, Amsterdam, The Netherlands). Additionally, the Equivital system provided an ECG signal and T_c reading from the wireless pill ingested. All raw data were synchronously collected using a data acquisition unit and linked software (PowerLab PL3516 and Labchart 8; AD Instruments; Colorado Springs, CO). After the rest period, discrete resting blood pressure was taken in triplicate

(Dinamap Carescape V100; GE Healthcare; Mississauga, ON, Canada).

The heating intervention involved lower limb hot water immersion at 45°C for 45 min with the water level either up to the ankles (Ankle, landmark: above malleoli) or knees (Knee, landmark: bottom border of patella) while in a supine bent-leg position, administered in a randomized order. These two local heating conditions were chosen to represent an incremental difference in the thermal load applied to the cardiovascular system, and to explore the concept of a minimum required physiological stimulus for generation of an acute vascular response. Prior work has shown that heating applied to the lower limbs can generate a T_c change in the range of Δ0.4–0.5°C, sufficient to increase heart rate and cardiac output (12, 13, 20). The heating apparatus consisted of a 10-gallon cylindrical container and a commercially-available sous vide immersion cooker (Wancle, Guangdong, China) attached to a custom-made adjustable-height wooden rig. BA and SFA flow profiles as well as perceptual measures were captured at rest and after 10, 15, 30, and 45 min of heating. Pre- and immediately postintervention in the straight-legged supine position, a venous blood draw followed in order by measurements of local and systemic arterial stiffness (within 15 min) and endothelial function (within 30 min) were performed.

Outcome Measures

Artery diameter and blood velocity.

Artery diameter and blood velocity were assessed at the left BA and SFA before and during each condition (at 10, 15, 30, 45 min) for 60 s at each timepoint. Duplex mode ultrasound (Vivid q; GE Medical Systems, Horten, Norway) was used with a 12 MHz linear array probe at 7.7 fps to allow for synchronized recording of artery diameter and blood velocity. The Doppler velocity gate was extended until it covered the entire arterial lumen with an insonation angle of 68°.

Shear stress and shear rate.

Shear stress (SS) is the tangential force produced by blood flow against the arterial wall and was calculated using the formula:

$$\text{SS}\ (\text{dyn/cm}^2) = \frac{2\mu V}{D} \quad (1)$$

Shear rate (SR), often reported as a surrogate for SS in the absence of blood viscosity was calculated using the formula:

$$\text{SR}\ (\text{s}^{-1}) = \frac{8V}{D} \quad (2)$$

For all formulas, μ is blood viscosity, V is mean blood velocity, and D is artery diameter. Mean SR was also divided into its component parts: anterograde and retrograde SR to quantify the shear stimulus in both directions. To do this, a series of arithmetic functions were applied to the mean blood velocity channel on Labchart (Labchart 8; AD Instruments, Colorado Springs, CO). These outcomes were assessed before, during, and after the interventions.

Flow-mediated dilation.

Simultaneous left BA and SFA FMD tests were conducted using a standardized protocol according to current guidelines (27). Each test began with a baseline phase wherein the arteries were imaged for at least 30 s to capture resting conditions, followed by the occlusion phase involving the rapid inflation of cuffs wrapped around the forearm and thigh to 200 mmHg using a rapid cuff inflator (E20 Rapid Cuff Inflator and AG101 Air Source; Hokanson, Bellevue, WA) to create an ischemic stimulus. After 5 min, the cuffs were released and the arteries imaged for 3 more min to capture peak arterial dilation and the total shear rate stimulus in the reactive hyperemia phase. Artery images were saved and analyzed using semi-automated edge-tracking software (Arterial Measurement System, Gothenburg, Sweden), whereas velocity traces were saved and analyzed using pixel-based tracking software (Measurements from Arterial Ultrasound Imaging; Hedgehog Medical, Waterloo, ON, Canada). To quantify macrovascular endothelial function, FMD was expressed as a percentage change in artery diameter relative to baseline artery diameter and calculated using the formula:

$$\text{FMD}\ (\text{mm}) = D_{peak} - D_{base} \quad (3)$$

$$\text{FMD}\ (\%) = \left(\frac{D_{peak} - D_{base}}{D_{base}}\right) \times 100\% \quad (4)$$

where D_{base} is baseline artery diameter and D_{peak} is peak artery diameter. Experienced ultrasound sonographers (J.W. and J.C.) performed all FMD tests at the brachial and superficial femoral arteries, respectively, with internal consistency such that one sonographer always conducted the FMD tests at one specific artery location. All FMD analyses were completed by an experienced rater (J.C.) who was blinded to condition.

Vascular conductance.

Vascular conductance was measured during the 3-min reactive hyperemia phase of the FMD test. To quantify microvascular function, peak and area under the curve (AUC) responses were calculated using the general formula for vascular conductance:

$$\text{Vascular conductance}\ (\text{mL/min} \cdot \text{mmHg}) = \frac{BF}{MAP} \quad (5)$$

The AUC calculation was performed on beat-by-beat conductance data in the postocclusion time period, and values calculated represent AUC above a theoretical zero.

Carotid distensibility.

Common carotid artery (CCA) distensibility was measured using a combination of simultaneous B-mode ultrasound imaging with a 12 MHz linear array probe on the left CCA and applanation tonometry (SPT-301 handheld tonometer; Millar Instruments, Houston, TX) to obtain pulse waveforms of the right CCA. These waveforms are subsequently converted to beat-by-beat forecasted pressures based on beat-by-beat finger pressures. The average arterial diameters and pulse pressures of 10 consecutive heart cycles were used to calculate arterial distensibility using the formula:

$$\text{Distensibility}\ (\text{cm}^2/\text{mmHg}) = \frac{(\pi\ r_{max}{}^2 - \pi\ r_{min}{}^2)}{(PP \times \pi\ r_{min}{}^2)} \quad (6)$$

where r is artery radius (maximum or minimum) and PP is pulse pressure. All analyses were completed by the same rater (J.W.) who was blinded to condition.

Pulse wave velocity.
PWV was quantified centrally between the carotid and femoral arteries (cfPWV) and peripherally between the femoral and dorsalis pedis arteries (ffPWV) using the most recent guidelines (28). In cases in which the dorsalis pedis could not be detected, the posterior tibial pulse was obtained instead. The same pulse sites were used across all visits within a participant. PWV was measured before and after heating using simultaneous applanation tonometry (SPT-301 handheld tonometer; Millar Instruments, Houston, TX) at the two artery sites of interest to obtain 20–30 pressure waveforms of sufficient quality. The distance between the two artery sites was taken using a straight and taut tape measure over the surface of the body. PWV was calculated using the formula:

$$\text{PWV (m/s)} = \frac{\text{distance}}{\text{pulse transit time}} \quad (7)$$

For cfPWV only, 80% of the direct carotid-femoral distance was used in the calculation as per the guidelines (28). All analyses were completed by the same rater (J.W.) who was blinded to condition.

Blood sampling and analysis.
Venous blood was drawn into 2 × 4-mL K_3-EDTA tubes, which were then spun in the centrifuge at 4°C and 4,000 rpm for 10 min. Plasma was aliquoted into 1.5-mL Eppendorf tubes and then stored at −20°C until batch analysis. Enzyme-linked immunosorbent assays (ELISA) were performed to determine plasma concentrations of IL-6 (Human IL-6 Quantikine HS, R&D Systems, Oakville, Ontario, Canada) and eHsp72 (AMP'D HSP70 High Sensitivity, Enzo, Cedarlane, Burlington, Ontario, Canada). Intraplate coefficients of variation were 5.4% and 7.1%, respectively. Hematocrit was assessed in duplicate through microcentrifugation (Adams micro-hematocrit reader, Clay-Adams Inc., NY). Hematocrit was used to calculate the flow and shear variables outlined above.

Perception.
Affect, thermal comfort (TC), and thermal sensation (TS) were assessed through perceptual scales. At rest and during intervention timepoints, participants were asked to indicate the number that most accurately represented their affect on the Feeling Scale ranging from −5 "Very Bad" to +5 "Very Good" (29). In addition, regarding the temperature experienced, participants were asked to describe how comfortable they felt (thermal comfort) on a scale ranging from −4 "Very uncomfortable (cold)" to +4 "Very uncomfortable (hot)" with 0 indicating the neutral "Comfortable", and how they would label the sensation (thermal sensation) on a scale ranging from 1 "Very Cold" to 9 "Very Hot" (30). Participants were instructed to provide these ratings for their whole body perceptions rather than that of the lower limbs alone.

Statistical Analysis

All statistical analyses were performed with IBM SPSS (v.20.0.0, IBM Corp., Armonk, NY). Normality was assessed with the Shapiro-Wilk test, and homogeneity of variance was assessed with Mauchly's test of sphericity. Where sphericity could not be assumed, the Greenhouse-Geisser correction was used. 2 × 6 (condition × time) repeated measures ANOVAs were used to compare T_c and T_{sk}, hemodynamics, and BA and SFA shear rate before, during, and after the heating interventions. A 2 × 5 (condition × time) repeated measures ANOVA was used to compare perceptual measures before and during the heating interventions. 2 × 2 (condition × time) repeated measures ANOVAs were used to compare the vascular function responses before and after heating. Control data were analyzed separately with one-way repeated measures ANOVAs and t-tests, as appropriate. Tukey's honest significant difference (HSD) was used as a post hoc analysis for significant interactions. All significant posthoc comparisons have a $P \leq 0.05$, and all data are reported as mean ± SD. With regard to the FMD analysis, data from one participant's BA and three participants' SFA were excluded due to insufficient image quality yielding $N = 15$ and $N = 13$, respectively. Any other missing data is due to malfunctions in equipment and/or data acquisition software.

FMD data was corrected for both baseline arterial diameter and SR AUC by log-transforming baseline arterial diameter ($\ln D_{base}$), peak arterial diameter ($\ln D_{peak}$), and SR AUC (lnSRAUC). A generalized estimating equations analysis with an exchangeable correlation structure was then performed with $\ln D_{diff}$ (i.e., $\ln D_{peak} - \ln D_{base}$) as the dependent variable; and under Predictors, time (Pre vs. Post) and condition (Ankle vs. Knee) were selected as factors, and $\ln D_{base}$ and lnSRAUC were selected as covariates (31). For Con, the analysis was identical except that time was the only within-subjects factor included in the analysis. An independent correlation structure was also used for the control data in the SFA as the exchangeable correlation structure yielded poor goodness of fit (i.e., QIC). For each artery site, condition- and time-specific estimated means (EM) were back-transformed to obtain scaled FMD through the equation:

$$\text{Scaled FMD mean} = [(e^{EM} - 1) \times 100] \quad (8)$$

and estimated standard errors (SE) were back-transformed and used to estimate standard deviations using the equation:

$$\text{Scaled FMD standard deviation} = [((e^{SE} - 1) \times 100) \times \sqrt{n}] \quad (9)$$

where n is the group sample size.

To further explore the relationship between BA SR elicited during heating (independent variable) and Δ BA FMD% (dependent variable), robust regression analyses were conducted to account for the violation of the assumption of independence by adjusting the standard error of the model predictors. SPSS version 24 and the associated R Essentials extension package was used for these analyses. In separate analyses, the independent variables explored included peak mean SR, Δ mean SR, peak anterograde and retrograde SR, and Δ anterograde and retrograde SR. For all statistical tests, type I error rate was set a priori at $\alpha = 0.05$.

RESULTS

Physiological Responses to Heating

Core and skin temperature.
There was a condition by time interaction for T_c ($P = 0.045$). T_c increased by 15 min of Knee and 30 min of Ankle, and was greater in Knee versus Ankle at 45 min. Although T_c decreased

when heating ceased in both conditions, it had returned to baseline by the postheating timepoint in Ankle only (Fig. 1*A*). In Con, T_c decreased throughout all timepoints (Supplemental Table S1; all Supplemental material is available at https://doi.org/10.5683/SP2/1TBNBR). There was a condition by time interaction for forearm T_{sk} ($P < 0.001$). Forearm T_{sk} increased with both heating conditions at 30 and 45 min, and was greater in Knee versus Ankle at 45 min. Forearm T_{sk} decreased after heating in both conditions, but only returned to baseline in Ankle (Fig. 1*B*). In Con, there was a main effect of time for forearm T_{sk} ($P = 0.009$), but no significant post hoc comparisons (Supplemental Table S1). There was a main effect of time for foot T_{sk} ($P < 0.001$). Foot T_{sk} increased throughout heating and decreased but did not return to baseline by the post timepoint (Fig. 1*C*). In Con, foot T_{sk} decreased throughout all timepoints (Supplemental Table S1). There was a condition by time interaction for calf T_{sk} ($P < 0.001$). Calf T_{sk} increased throughout heating with both conditions and was greater in Knee versus Ankle at all timepoints. Calf T_{sk} decreased after heating in both interventions, but only returned to baseline in Ankle (all $P \leq 0.05$; Fig. 1*D*). In Con, calf T_{sk} did not change throughout all timepoints (Supplemental Table S1).

Central hemodynamics.

Blood pressure did not change during either of the heating conditions. There was a condition by time interaction for HR ($P = 0.054$). HR increased by 15 min of Knee and 30 min of Ankle. HR returned to baseline in both conditions post-heating (Table 2). There was a main effect of time ($P = 0.006$, no significant post hoc comparisons) and intervention ($P = 0.054$) for SV. SV was greater in Ankle versus Knee (Table 2). There was a main effect of time for CO ($P < 0.001$). CO increased at 30 and 45 min, and decreased to baseline post-heating (Table 2). None of the hemodynamic variables changed with Con.

Shear rate.

In the BA, there were condition by time interactions for mean and anterograde SR (both $P < 0.001$). BA mean and anterograde SR increased throughout heating and were greater in Knee versus Ankle, but both returned to baseline following heating (Fig. 2, *A* and *B*). There was a main effect of time for BA retrograde SR ($P = 0.013$), but no significant post hoc comparisons (Fig. 2*C*). In the SFA, there were condition by time interactions for mean and anterograde SR (both $P < 0.001$). SFA mean and anterograde SR increased throughout heating, and were greater in Knee versus Ankle. All variables remained elevated post- compared to preheating except anterograde SR in Ankle (Fig. 2, *D* and *E*). There was a condition by time interaction for SFA retrograde SR ($P = 0.001$), which decreased throughout heating in both conditions and returned to baseline post-heating in Ankle only ($P \leq 0.05$; Fig. 2*F*). Apart from a reduction in BA mean SR at post versus

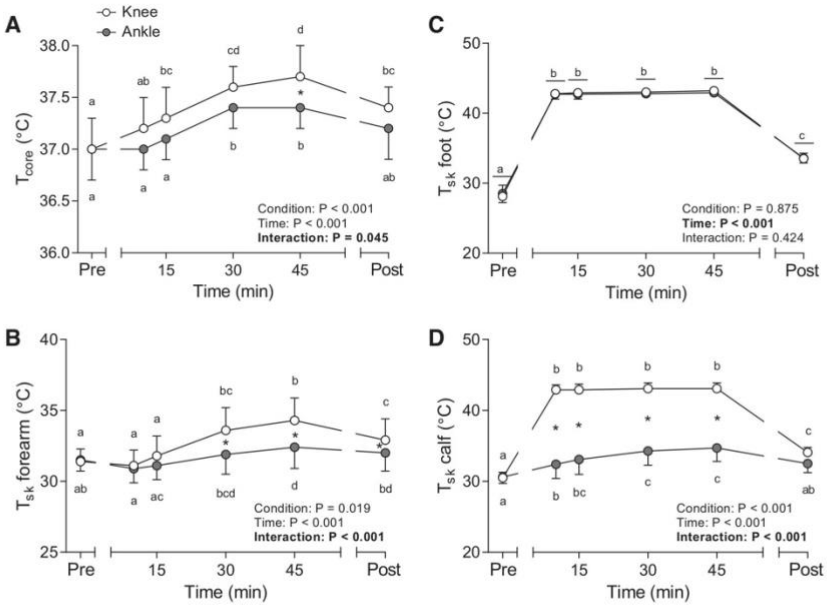

Figure 1. Core (T_{core}) and skin (T_{sk}) temperature responses to leg heating. A 2 × 6 (condition × time) repeated measures ANOVA was conducted to compare the core (*A*), forearm skin (*B*), foot skin (*C*), and calf skin (*D*) temperature responses to leg heating (all $N = 16$ participants). The Ankle condition is represented in gray and the Knee condition is represented in white. Within a condition, values with no common letters are different from each other; within a timepoint, a * indicates that the Ankle and Knee conditions are different from each other.

Table 2. *Hemodynamic responses to heating*

	N	Pre	10 Min	15 Min	30 Min	45 Min	Post		P
SBP, mmHg								Interaction	0.164
Ankle	15	113 ± 10	117 ± 11	117 ± 13	112 ± 12	111 ± 15	116 ± 12	Condition	0.187
Knee	15	114 ± 10	111 ± 8	108 ± 7	108 ± 10	106 ± 11	112 ± 14	Time	0.086
Control	6	108 ± 6	111 ± 6	111 ± 7	112 ± 6	117 ± 3	117 ± 5	Time	0.083
DBP, mmHg								Interaction	0.397
Ankle	15	61 ± 7	64 ± 9	66 ± 8	64 ± 7	63 ± 10	64 ± 8	Condition	0.512
Knee	15	61 ± 5	64 ± 4	62 ± 4	63 ± 5	62 ± 6	63 ± 7	Time	0.203
Control	6	61 ± 6	64 ± 5	63 ± 5	64 ± 4	66 ± 2	67 ± 2	Time	0.144
MAP, mmHg								Interaction	0.314
Ankle	15	80 ± 8	85 ± 9	85 ± 9	81 ± 8	81 ± 12	83 ± 9	Condition	0.293
Knee	15	80 ± 6	81 ± 5	79 ± 4	80 ± 6	79 ± 7	81 ± 9	Time	0.188
Control	6	76 ± 6	81 ± 6	80 ± 6	81 ± 5	83 ± 4	84 ± 6	Time	0.160
HR, bpm								Interaction	**0.054**
Ankle	15	58 ± 8^{a}	64 ± 10^{ab}	67 ± 10^{ac}	73 ± 12^{bc}	77 ± 13^{c}	63 ± 10^{ab}	Condition	0.002
Knee	15	59 ± 8^{a}	70 ± 12^{ab}	74 ± 14^{bc}	82 ± 13^{cd}	88 ± 19^{d}	66 ± 9^{ab}	Time	<0.001
Control	6	57 ± 15	57 ± 8	56 ± 10	56 ± 10	56 ± 10	57 ± 12	Time	0.952
SV, mL								Interaction	0.162
Ankle*	15	94.1 ± 18.1	91.9 ± 16.2	90.9 ± 16.5	88.7 ± 18.2	89.6 ± 22.0	96.5 ± 19.6	Condition	**0.054**
Knee	15	91.1 ± 21.8	82.6 ± 17.4	82.6 ± 17.3	80.2 ± 16.3	77.9 ± 16.9	86.1 ± 20.6	Time	**0.006**
Control	5	85.7 ± 14.5	92.3 ± 21.5	92.3 ± 23.6	89.3 ± 18.8	91.6 ± 19.8	93.9 ± 20.6	Time	0.590
CO, L/min								Interaction	0.304
Ankle	15	5.4 ± 1.1^{a}	5.8 ± 1.2^{ab}	6.0 ± 1.2^{ab}	6.3 ± 1.2^{b}	6.7 ± 1.5^{b}	6.0 ± 1.2^{ab}	Condition	0.504
Knee	15	5.4 ± 1.6^{a}	6.0 ± 1.5^{ab}	6.3 ± 1.8^{ab}	6.8 ± 1.7^{b}	7.0 ± 1.8^{b}	5.9 ± 1.5^{ab}	Time	**<0.001**
Control	5	4.4 ± 0.7	4.9 ± 0.6	4.7 ± 0.4	4.6 ± 0.3	4.7 ± 0.6	5.0 ± 1.1	Time	0.462

All data are expressed as mean ± SD. N = 15 participants (Ankle and Knee) or 5–6 participants (Control). A 2 × 6 (condition × time) repeated measures ANOVA was conducted to compare the hemodynamic responses to heating in the Ankle and Knee conditions. A separate one-way repeated measures ANOVA with six timepoints was conducted to track the hemodynamic changes over time with a control resting condition. Within a condition (Ankle/Knee), values with no common letters are different from each other; within a timepoint, a * indicates that Ankle and Knee conditions are different from each other. Significant main effects and interactions that apply for each variable are in bold. CO, cardiac output; DBP, diastolic blood pressure; HR, heart rate; MAP, mean arterial pressure; SBP, systolic blood pressure; SV, stroke volume.

30 min ($P \leq 0.05$), no variables changed with Con (Supplemental Tables S2 and S3).

Vascular Function Outcomes

Macrovascular endothelial function.

There was a main effect of time for BA FMD% (P = 0.004) and no change in SFA FMD%. BA FMD% increased pre- to postheating (Pre: 4.6 ± 1.7 vs. Post: 5.4 ± 2.0%; Fig. 3). These findings persisted even with corrections for baseline artery diameter and SR AUC applied; corrected BA FMD% increased pre- to postheating ($P < 0.001$) in both conditions (Pre: 4.5 ± 2.1 vs. Post: 5.4 ± 1.9%) and corrected SFA FMD% did not change (Table 3). There was no change in BA SR AUC; however, there was a main effect of time for SFA SR AUC (P = 0.015) such that SFA SR AUC was greater post- versus preheating (Pre: 40.0 ± 36.8 vs. Post: 53.3 ± 37.4 × 103 s^{-1}; Table 3). There were no differences in baseline AD, peak AD, or time to peak between timepoints or interventions. None of the variables assessed changed with Con (Table 3).

In robust regression analyses conducted with either BA peak mean SR, Δ mean SR, anterograde and retrograde SR, or Δ anterograde and retrograde SR as the independent variable and Δ BA FMD% as the dependent variable, no associations emerged as significant when conditions were pooled (both $P > 0.05$).

Microvascular function.

There was a condition by time interaction for BA peak vascular conductance (P = 0.030) such that conductance increased in Knee only (Pre: 6.3 ± 2.7 vs. Post: 7.8 ± 3.5 mL/min*mmHg; Table 3). There was a condition by time interaction for BA vascular conductance AUC (P = 0.023); however, none of the post hoc comparisons were significant (Table 3). There were no differences in peak vascular conductance or vascular conductance AUC in the SFA. None of the variables assessed changed with Con (Table 3).

Arterial stiffness.

There were no changes in CCA distensibility or cfPWV with heating in either condition, but there was a main effect of time for ffPWV (P = 0.011) such that it decreased pre- to postheating (Pre: 8.4 ± 1.2 vs. Post: 7.7 ± 1.1 m/s; Table 4). None of the variables assessed changed with Con (Table 4).

Blood markers.

There was a main effect of intervention for IL-6 (P = 0.046), such that higher concentrations were found in Knee versus Ankle. There was a condition by time interaction (P = 0.038) for eHSP72, such that plasma concentration increased following Knee only (Pre: 12.4 ± 9.4 vs. Post: 14.8 ± 9.8 ng/mL; Table 4).

Perception

There was a main effect of time for affect assessed by the Feeling Scale (P = 0.001); however, none of the post hoc comparisons were significant (Fig. 4*A*). Participants began each trial at an average reported thermal perception of "Slightly cool" (Both: TS = 4.1 ± 1.3) and "Comfortable" (Both: TC =

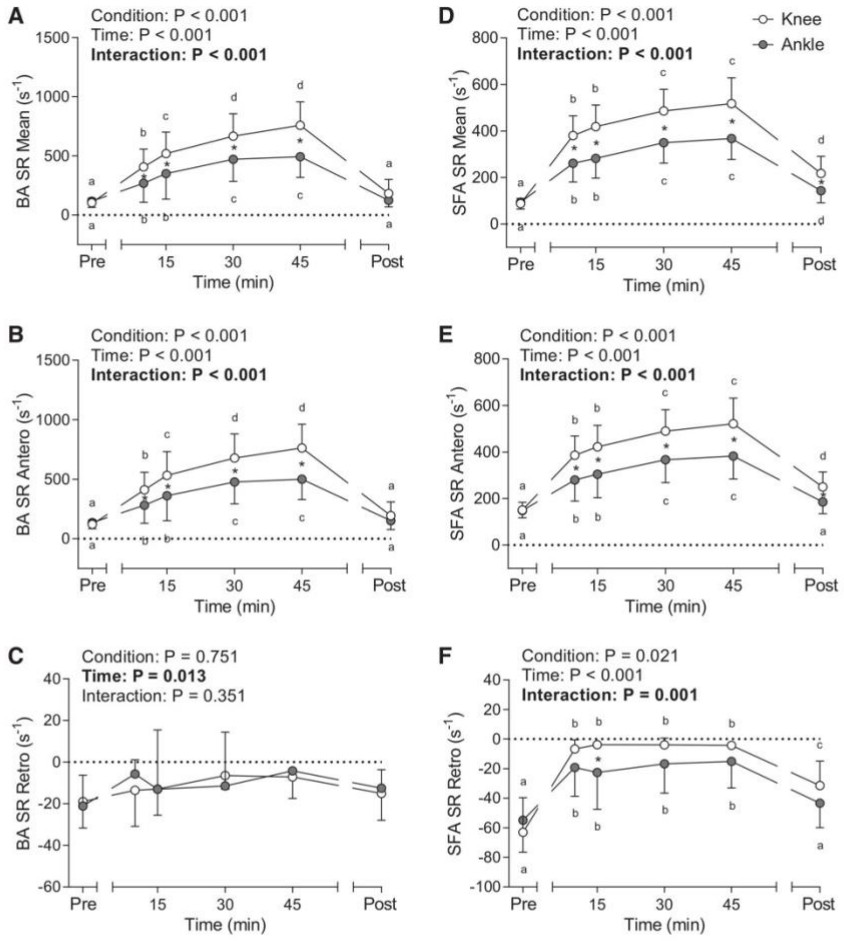

Figure 2. Shear rate responses to leg heating. A 2 × 6 (condition × time) repeated measures ANOVA was conducted to compare the mean, anterograde, and retrograde SR responses to leg heating in the BA (*A–C*) and SFA (*D–F*) (all *N* = 16 participants). The Ankle condition is represented in gray and the Knee condition is represented in white. Within a condition, values with no common letters are different from each other; within a timepoint, a * indicates that the Ankle and Knee conditions are different from each other. Antero, anterograde; BA, brachial artery; Retro, retrograde; SFA, superficial femoral artery; SR, shear rate.

−0.3 ± 0.8). There was a condition by time interaction for TS (P = 0.014); TS was elevated and sustained throughout heating in both Ankle and Knee (Fig. 4*B*). There was a main effect of time for TC ($P < 0.001$); TC increased at 30 and 45 min of heating (Fig. 4*C*). At peak deviation from rest, which was at 45 min of heating, participants reported feeling "Warm" (Ankle: 7.1 ± 1.3) to "Hot" (Knee: 8.4 ± 0.9) and "Slightly uncomfortable (hot)" (Both: 2.3 ± 1.3) on average. There were no changes in affect, TC or TS during Con (Supplemental Table S1).

DISCUSSION

In this study, we sought to explore whether localized leg heating would generate a sufficient physiological stimulus to improve vascular function outcomes. Our findings demonstrate that both lower limb heating protocols generated an acute increase in upper limb macrovascular function and decrease in lower limb arterial stiffness, whereas only Knee was able to elicit increases in upper limb microvascular function and systemic, circulating heat shock protein-72.

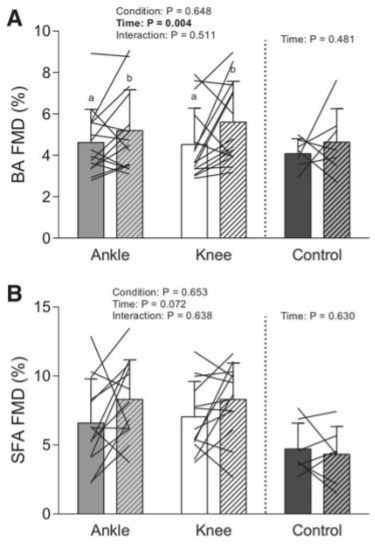

Figure 3. Endothelial function responses to leg heating. A 2 × 2 (condition × time) repeated measures ANOVA was conducted to compare the relative FMD responses to leg heating in the BA (*A*; *N* = 15 participants) and SFA (*B*; *N* = 13 participants). A separate paired samples *t* test was conducted to compare the FMD responses in the Control condition (*N* = 7 participants). The Ankle condition is represented in light gray, the Knee condition is represented in white, and the Control condition is represented in dark gray. Solid bars represent the Pre timepoint and hashed bars represent the Post timepoint. Individual lines represent the temporal responses of each participant within each condition. Within a condition (Ankle/Knee/Control), values with no common letters are different from each other. BA, brachial artery; FMD, flow-mediated dilation; SFA, superficial femoral artery.

Characterization of Heating Stimuli

The physiological responses to each heating condition show that, as might be expected, Knee was relatively more intense compared to Ankle, despite similar central cardiovascular strain. When compared to Ankle, Knee elicited a greater thermoregulatory stress with respect to both change in T_c magnitude (Δ0.7 vs. Δ0.4°C) and duration (≥30 vs. ≥15 min), such that the T_c stimulus was greater and more sustained. Likewise, the mean SR in Knee exceeded that of Ankle in both the BA (759 vs. 493 s^{-1}) and SFA (517 vs. 367 s^{-1}).

Prior literature suggests that there may be certain T_c and SR thresholds that must be achieved to trigger acute changes in vascular outcomes (15, 32–34). More comprehensive research in cutaneous vasculature suggests that active vasodilation involving the maximal release of the vasodilator nitric oxide occurs at ΔT_c of 1.0–1.5°C (15), though some studies posit that some dilatory responses may already be observed with lesser increases of ~Δ0.6°C (32). Currently, there is no firmly established minimum T_c threshold. In contrast, the idea of a SR threshold has been explored in the BA of young, healthy individuals, with two research groups arriving at the same target range of 250–300 s^{-1} (33, 34). No SR threshold has been established in the SFA.

Although both conditions surpass the BA SR threshold, only Knee potentially exceeds the suggested lower-end T_c threshold. Based on these variables alone, although both should be sufficient to elicit vascular function changes, it is reasonable to believe that Knee would be more effective as a stimulus for acute vascular change overall; this is indeed reflected in the current study.

Acute Changes in Vascular Function Outcomes

Both Ankle and Knee were effective at improving macrovascular endothelial function and arterial stiffness in specific areas. Lower limb heating resulted in an increase in BA FMD% alongside no change in SFA FMD%. These findings persisted even after accounting for both baseline artery diameter and SR AUC. Perhaps the most intriguing aspect of the BA FMD% findings is the similar magnitude of improvement with Ankle and Knee protocols despite considerable differences in the SR responses achieved during heating. Changes in SR are thought to be one of the main drivers of the acute FMD response (18). When comparing Ankle versus Knee, peak mean SR was 493 ± 176 versus 759 ± 199 s^{-1} and ΔSR was 376 ± 166 versus 655 ± 180 s^{-1}, respectively. Subsequent exploratory robust regression analyses affirm that in the current study, neither BA peak mean SR nor Δ mean SR is associated with any significant variance in Δ BA FMD%. In addition, we found that parsing out the mean SR response into its directional components (i.e., anterograde and retrograde SR) yielded no significant associations. These analyses provide more support for a regulatory framework of diminishing returns in the acute phase, wherein once a certain SR value has been surpassed, the gains in FMD with further increases in SR are negligible.

The BA is an artery site that appears to be more amenable to acute change, as evidenced by the current and other interventional studies using stimuli such as exercise, nutritional supplements, and pharmaceuticals (35–37). Some prior studies have demonstrated acute heating-induced improvements in BA FMD% in the range of 1.3–5.6% with varying water immersion protocols in cohorts of young, healthy males (16–18). The remaining studies, including some using lower limb heating protocols similar to the current study, did not observe a change in BA FMD, which may be explained by the different population group examined (i.e., middle-aged adults) (20) or the timing of the acute assessment (i.e., 60 min postheating) (19, 21). The BA is the most commonly assessed artery site for the FMD test because of its established association with the risk and incidence of cardiovascular events (38), and specifically the evidence showing that a 1% greater FMD is equivalent to a 13% lower risk of developing a cardiovascular disease (39). Although the magnitude of change in the current study is small at 0.8% and just shy of this 1% change, it does not necessarily mean that it is not meaningful, as small acute changes may still contribute to larger chronic changes with continued exposure. Such is the prevailing theory for how regular aerobic exercise beneficially impacts vascular function (35). Further studies must be conducted to determine whether these acute fluctuations amount to more permanent chronic changes over time.

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Table 3. *Endothelial function*

	Brachial Artery					Superficial Femoral Artery				
	N	Pre	Post		*P*	*N*	Pre	Post		*P*
FMD, mm				Interaction	0.549				Interaction	0.724
Ankle	15	0.19 ± 0.05[a]	0.21 ± 0.06[b]	Condition	0.476	13	0.40 ± 0.17	0.50 ± 0.16	Condition	0.768
Knee	15	0.19 ± 0.06[a]	0.23 ± 0.06[b]	Time	**0.004**	13	0.40 ± 0.16	0.48 ± 0.20	Time	0.062
Control	*7*	*0.18 ± 0.04*	*0.20 ± 0.06*	*Time*	*0.400*	*7*	*0.29 ± 0.12*	*0.27 ± 0.14*	*Time*	*0.687*
FMD, %				Interaction	0.511				Interaction	0.638
Ankle	15	4.6 ± 1.6[a]	5.2 ± 2.0[b]	Condition	0.648	13	6.6 ± 3.2	8.3 ± 2.9	Condition	0.653
Knee	15	4.5 ± 1.8[a]	5.6 ± 2.0[b]	Time	**0.004**	13	7.0 ± 2.6	8.3 ± 2.6	Time	0.072
Control	*7*	*4.1 ± 0.7*	*4.6 ± 1.6*	*Time*	*0.481*	*7*	*4.7 ± 1.9*	*4.3 ± 2.0*	*Time*	*0.630*
FMD Corrected, %				Interaction	0.386				Interaction	0.781
Ankle	15	4.6 ± 2.4[a]	5.1 ± 2.3[b]	Condition	0.447	13	7.1 ± 4.8	8.1 ± 4.9	Condition	0.313
Knee	15	4.5 ± 2.4[a]	5.7 ± 2.2[b]	Time	**<0.001**	13	6.7 ± 4.6	7.5 ± 5.0	Time	0.122
Control	*7*	*4.2 ± 1.4*	*4.6 ± 1.3*	*Time*	*0.511*	*7*	*4.5 ± 7.4*	*4.5 ± 7.9*	*Time*	*0.949*
BL arterial diameter, mm				Interaction	0.592				Interaction	0.979
Ankle	15	4.2 ± 0.6	4.2 ± 0.6	Condition	0.172	13	6.2 ± 0.6	6.1 ± 0.6	Condition	0.364
Knee	15	4.2 ± 0.6	4.3 ± 0.6	Time	0.804	13	6.1 ± 0.7	6.1 ± 0.7	Time	0.294
Control	*7*	*4.3 ± 0.7*	*4.3 ± 0.8*	*Time*	*0.975*	*7*	*6.3 ± 0.6*	*6.3 ± 0.6*	*Time*	*0.846*
PK arterial diameter, mm				Interaction	0.259				Interaction	0.623
Ankle	15	4.4 ± 0.6	4.4 ± 0.6	Condition	0.091	13	6.6 ± 0.6	6.6 ± 0.5	Condition	0.203
Knee	15	4.4 ± 0.6	4.5 ± 0.6	Time	0.093	13	6.6 ± 0.6	6.6 ± 0.7	Time	0.610
Control	*7*	*4.5 ± 0.7*	*4.5 ± 0.8*	*Time*	*0.690*	*7*	*6.6 ± 0.7*	*6.6 ± 0.7*	*Time*	*0.726*
AUC shear rate, 10^3 s^{-1}				Interaction	0.213				Interaction	0.629
Ankle	15	17.3 ± 19.2	12.9 ± 10.4	Condition	0.403	13	34.3 ± 33.2[a]	51.5 ± 37.1[b]	Condition	0.423
Knee	15	12.9 ± 13.8	13.8 ± 8.2	Time	0.539	13	45.8 ± 40.6[a]	55.1 ± 39.2[b]	Time	**0.015**
Control	*7*	*13.6 ± 15.3*	*13.1 ± 12.6*	*Time*	*0.840*	*7*	*60.5 ± 42.9*	*42.5 ± 39.1*	*Time*	*0.408*
TTP, s				Interaction	0.426				Interaction	0.707
Ankle	15	49 ± 26	46 ± 16	Condition	0.955	13	70 ± 49	89 ± 49	Condition	0.404
Knee	15	46 ± 19	50 ± 16	Time	0.883	13	87 ± 55	98 ± 54	Time	0.153
Control	*7*	*36 ± 16*	*42 ± 17*	*Time*	*0.253*	*7*	*95 ± 45*	*78 ± 57*	*Time*	*0.415*
PK VC, mL/min*mmHg				Interaction	**0.030**				Interaction	0.443
Ankle	16	6.7 ± 2.0[a]	6.8 ± 2.3[a]	Condition	0.506	15	23.5 ± 7.3	24.3 ± 8.5	Condition	0.661
Knee	16	6.3 ± 2.7[a]	7.8 ± 3.5[b]	Time	0.024	15	22.3 ± 8.4	24.3 ± 8.6	Time	0.077
Control	*5*	*5.2 ± 2.2*	*4.8 ± 1.1*	*Time*	*0.614*	*5*	*20.7 ± 4.6*	*19.7 ± 0.9*	*Time*	*0.623*
AUC VC, mL/mmHg				Interaction	**0.023**				Interaction	0.963
Ankle	16	9.9 ± 2.9	8.9 ± 2.8	Condition	0.795	15	35.9 ± 17.1	37.8 ± 14.9	Condition	0.506
Knee	16	8.9 ± 4.0	10.4 ± 4.8	Time	0.635	15	34.0 ± 19.7	36.1 ± 13.1	Time	0.544
Control	*7*	*8.4 ± 4.0*	*7.1 ± 2.5*	*Time*	*0.266*	*5*	*35.2 ± 10.3*	*35.0 ± 8.4*	*Time*	*0.975*

All data are expressed as mean ± SD. N = 15–16 (Ankle, Knee) or 5–7 (Control) participants. To compare the change in vascular outcomes with the Ankle and Knee conditions, 2 × 2 (condition × time) repeated measures ANOVAs were conducted. Paired samples t tests were conducted for data from the control resting condition. Within a condition (Ankle/Knee), values with no common letters are different from each other. Significant main effects and interactions that apply for each variable are in bold. Italics indicate that statistical analyses for the Control condition was performed separately from the Ankle and Knee conditions. AUC, area under the curve; BL, baseline; FMD, flow-mediated dilation; PK, peak; TTP, time to peak artery diameter; VC, vascular conductance.

In contrast, the SFA routinely experiences large and frequent fluctuations in blood flow and shear stress with habitual human activities such as walking or running, postural control, and changing body position (40–42). For young, healthy individuals that do not possess aging-induced endothelial dysfunction in this artery site, it appears that daily exposure to these shear fluctuations is sufficient to maintain a healthy vascular environment. It is likely that there is an absolute ceiling effect in the SFA FMD values that can be achieved in our participants, and that only those with existing vascular dysfunction would experience acute improvements with heating (13). Ultimately, the lack of change in SFA FMD observed in this study replicates prior research using lower limb hot water immersion (13, 22).

Along the same lines, lower limb heating resulted in a decrease in arterial stiffness peripherally but not centrally; ffPWV decreased by an average of 0.7 m/s postheating in both conditions, but cfPWV did not change. Similarly, CCA stiffness was also unchanged. The control of vascular tone during heating is precise, and neural signals are sent to simultaneously vasodilate at the skin to release heat, but also vasoconstrict at the internal organs to prevent severe drops in mean blood pressure (43). Previous studies show how the interpretation of acute arterial stiffness findings can be challenging given these competing interests. Ganio et al. (44) and Moyen et al. (45) observed either unchanged or increased PWV but performed their measurement during whole body heating when sympathetic nervous system and vasoconstrictor activity was likely high, making them moot comparators for the current study. Caldwell et al. (23) found no change in either cfPWV or ffPWV following heating using a whole body water-perfused suit, which may have generated a level of physiological stress in which vasoconstriction also trumps or negates vasodilation. The decrease in ffPWV in the current study is likely indicative of a transient reduction in arterial tone associated with local heat dissipation. This reduction occurred in the absence of changes in blood pressure, which is often a confounding factor for arterial stiffness in many interventional models since greater blood pressure amplifies the speed of pulse travel.

The remaining vascular function outcomes improved only with the relatively more intense Knee condition. Our microvascular function findings show that BA peak vascular conductance increased with Knee; and although there was a

Table 4. *Arterial stiffness and blood markers*

	N	Pre	Post		P
Distensibility, 10^{-3} cm^2/mmHg				Interaction	0.242
Ankle	16	5.9 ± 1.2	5.5 ± 1.2	Condition	0.843
Knee	16	5.6 ± 1.7	5.7 ± 1.6	Time	0.739
Control	7	6.0 ± 1.2	6.2 ± 1.5	Time	0.760
cfPWV, m/s				Interaction	0.742
Ankle	16	6.5 ± 0.5	6.6 ± 0.7	Condition	0.637
Knee	16	6.5 ± 0.6	6.6 ± 0.5	Time	0.546
Control	7	6.7 ± 0.7	6.9 ± 0.6	Time	0.148
ffPWV, m/s				Interaction	0.619
Ankle	16	8.6 ± 1.1[a]	7.8 ± 1.3[b]	Condition	0.305
Knee	16	8.3 ± 1.2[a]	7.7 ± 0.9[b]	Time	**0.011**
Control	7	8.6 ± 0.4	8.9 ± 0.4	Time	0.220
IL-6, pg/mL				Interaction	0.974
Ankle	15	0.71 ± 0.47	0.88 ± 0.78	Condition	**0.046**
Knee*	15	0.86 ± 0.48	1.03 ± 0.85	Time	0.143
Control				Time	
eHSP72, ng/mL				Interaction	**0.038**
Ankle	14	12.91 ± 8.98[a]	13.07 ± 9.11[a]	Condition	0.502
Knee	14	12.38 ± 9.36[a]	14.79 ± 9.85[b]	Time	0.002
Control				Time	

All data are expressed as mean ± SD. N = 14–16 (Ankle and Knee) or 7 (Control) participants. To compare the change in vascular outcomes with the Ankle and Knee conditions, 2 × 2 (condition × time) repeated measures ANOVAs were conducted. Paired samples t tests were conducted for data from the control resting condition. Within a condition (Ankle/Knee), values with no common letters are different from each other. A * indicates that Ankle and Knee conditions are different from each other. Significant main effects and interactions that apply for each variable are in bold. cfPWV, carotid-femoral pulse wave velocity; eHSP72, extracellular heat shock protein-72; ffPWV, femoral-foot pulse wave velocity; IL-6, interleukin-6.

condition by time interaction for BA vascular conductance AUC, no post hoc comparisons were significant. There were no changes in any variables in the SFA with either condition. These results are inconsistent with previous lower limb heating studies that have shown that reactive hyperemia does not change in the BA (20, 21) and increases in some instances in the SFA (13, 20). Our findings demonstrate that although the absolute peak perfusion capacity increased in the BA, there was no difference in total perfusion when examined over the fixed hyperemic period.

In exploring the acute systemic inflammatory response, our results show that eHSP72 increased after Knee only, while neither intervention elicited an IL-6 response. In the vasculature, heat shock protein 72 and others in the 70 kDa family can attenuate proinflammatory pathways by attenuating c-Jun N-Terminal Kinase activity and reduce free radicals by promoting the production of antioxidant enzymes such as superoxide dismutase (46, 47). Although its presence in circulation does not necessarily reflect intracellular HSP72 expression, where it is suggested to exert its beneficial effects (48), it does add further support to the efficacy of heat therapy to manipulate the expression of this cellular chaperone. The magnitude of the eHSP72 increase after Knee (~20%) is comparable to studies that have heated a larger part of the body (e.g., 1-h immersion up to the belly button in 40°C water) (25). Thus, although exercise studies suggest that the rise in T_c is an important determinant of the acute HSP72 response (49), the present study findings indicate that its circulating concentration can be elevated through relatively modest heat stress.

The changes in vascular function responses following localized heating indicates that future studies should consider both the type and location of the artery being investigated as each may have different regulatory factors and control mechanisms, some of which may demand a higher intensity thermal stimulus. The heat stress imposed in the present study affected all artery sites but resulted in increased dilatory and perfusion capacity in the upper limb and decreased vessel tone in the lower limb. Collectively, these physiological responses may contribute to improved vascular health overall with repeated exposure to lower limb heating. To be certain, future work should employ these lower limb heating protocols over a chronic period of time to determine whether these positive findings persist before implementing such interventions in practice.

Perception of Local Lower Limb Heating

The affective and thermal perceptual responses to heating are an important aspect of our investigation because tolerability is often cited as a barrier to adherence to whole body protocols (8, 9). A wealth of literature in exercise psychology suggests that affect is an important factor that drives human motivation to exercise. Studies have reported that those who experience negative feelings or displeasure during exercise tend to accumulate fewer exercise minutes and participate less in exercise behaviors over the longer term (50). Furthermore, there is an inverse relationship between exercise intensity and the affective response during a session (50). If similar theories can be applied to passive heat stress, the lack of change in affect in the current study may indicate that our local heating protocols are at an acceptable intensity. Additionally, thermal sensation was reported to be hotter in Knee compared to Ankle, yet thermal discomfort was reported as similarly uncomfortable in both conditions. It appears that thermal discomfort rather than sensation drives the desire for humans to thermally behave (e.g., changing the thermostat or turning on an electric fan) (51), so striking a balance between tolerability and an adequate physiological stimulus for vascular adaptation is key for public uptake of a heat therapy intervention.

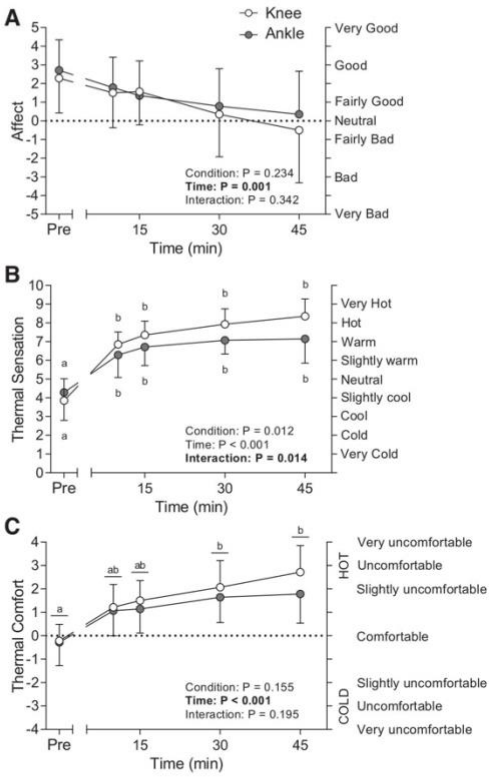

Figure 4. Perceptual responses to leg heating. A 2 × 5 (condition × time) repeated measures ANOVA was conducted to compare ratings of affect (*A*), thermal sensation (*B*), and thermal comfort (*C*) during leg heating (all *N* = 14 participants). The Ankle condition is represented in gray and the Knee condition is represented in white. Within a condition (Ankle/Knee), values with no common letters are different from each other.

These findings should be interpreted with appropriate consideration for the current study design. Indeed, adherence is multifactorial and thermal perception is likely just one of many variables that influence this outcome. Furthermore, acute perceptual responses do not always translate into chronic perceptual responses. In some instances, people come to derive pleasure and enjoyment out of an activity over continued participation. Therefore, future studies should determine the perceptual responses over a chronic period of local lower limb heating to determine if adherence is improved over time compared to whole body heating. Additionally, understanding perceptual outcomes can be challenging given limitations to the scale-based measurements themselves. With the lack of anchoring experiences to define the extremes of hot and cold in terms of both sensation and comfort, different protocols cannot be weighed against each other unless directly compared in the same study. As such, future work should directly compare the thermal perception between whole body and lower limb protocols to determine whether thermal discomfort is indeed alleviated by local heating (52).

Limitations

Our study was limited by an incomplete sample size for the time control condition and the lack of a whole body heating condition. Though we were unable to include these components due to logistical and resource constraints, they would have certainly strengthened the study and allowed for more definitive conclusions to be made with regards to the vascular function effects and thermal perception. With respect to the first limitation, currently available evidence on extended periods in supine laying and/or in bent leg postures suggests that vascular function would have either not changed or been negatively impacted in the time control condition (23, 53–56). Regarding the second limitation, future studies aiming to confirm the tolerability and feasibility of heat therapy should be designed specifically to answer this question to determine whether local lower limb heating may be used in lieu of whole body heating modes.

Future Directions and Applications

To build on this acute research, future work should determine the consequences of chronic lower limb heating. Such a study would be needed to confirm whether the transient fluctuations in vascular function outcomes, such as those demonstrated in the current study, are of sufficient magnitude to amount to positive structural and functional vascular remodeling with repeated heat therapy sessions. Future work should also explore any potential sex-specific responses to heating. In the current study, we achieved sex parity with a sample of eight males and eight females. However, sex-based subanalyses were not included as we were not adequately powered to explore this research question (57).

Although recent studies have focused on the application of heat as a beneficial treatment for compromised populations, it is important to remember that some of the most compelling evidence in support of heat therapies derive from lifelong participants whose sauna bathing habits prevented or delayed the development of cardiovascular diseases (58). As such, we would encourage that heating be recognized as a potential therapy or healthy lifestyle habit by the general population.

Conclusions

Our study demonstrates that 45 min of either ankle- or knee-level heating in 45°C water is a sufficient stimulus to acutely improve vascular function. Further work should aim to determine whether repeated exposures with either leg heating protocol will be a viable, tolerable, and feasible strategy to improve vascular health.

DATA

Supplemental Tables S1–S3: https://doi.org/10.5683/SP2/1TBNBR.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.L.C. and M.J.M. conceived and designed research; J.L.C., J.S.W. and S.P.H. performed experiments; J.L.C., J.S.W. and S.P.H. analyzed data; J.L.C., J.S.W., S.P.H., and M.J.M. interpreted results of experiments; J.L.C. prepared figures; J.L.C. drafted manuscript; J.L.C., J.S.W., S.P.H., and M.J.M. edited and revised manuscript; J.L.C., J.S.W., S.P.H., and M.J.M. approved final version of manuscript.

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Supplemental Table 1. Perceptual and temperature responses to heating

	N	Pre	10 min	15 min	30 min	45 min	Post		P
Perception									
Feeling scale								Interaction	0.342
Ankle	14	3 ± 2	2 ± 2	1 ± 2	1 ± 2	0 ± 2	- ± -	Condition	0.234
Knee	14	2 ± 2	2 ± 2	2 ± 2	0 ± 2	-1 ± 3	- ± -	Time	**0.001**
Control	7	3 ± 2	2 ± 2	2 ± 2	2 ± 2	2 ± 2	- ± -	Time	0.117
Thermal sensation								Interaction	**0.014**
Ankle	14	4 ± 1 ^{a}	6 ± 1 ^{b}	7 ± 1 ^{b}	7 ± 1 ^{b}	7 ± 1 ^{b}	- ± -	Condition	0.012
Knee	14	4 ± 1 ^{a}	7 ± 1 ^{b}	7 ± 1 ^{b}	8 ± 1 ^{b}	8 ± 1 ^{b}	- ± -	Time	<0.001
Control	7	4 ± 1	4 ± 1	4 ± 1	4 ± 2	4 ± 1	- ± -	Time	0.904
Thermal comfort								Interaction	0.195
Ankle	14	0 ± 1 ^{a}	1 ± 1 ^{ab}	1 ± 1 ^{ab}	2 ± 1 ^{b}	2 ± 1 ^{b}	- ± -	Condition	0.155
Knee	14	0 ± 1 ^{a}	1 ± 1 ^{ab}	2 ± 1 ^{ab}	2 ± 1 ^{b}	3 ± 1 ^{b}	- ± -	Time	**<0.001**
Control	7	-1 ± 1	-1 ± 1	-1 ± 1	-1 ± 1	-1 ± 1	- ± -	Time	0.427
Temperatures									
T_c (° C)								Interaction	**0.045**
Ankle	16	37.0 ± 0.3 ^{a}	37.0 ± 0.2 ^{a}	37.1 ± 0.2 ^{a}	37.4 ± 0.2 ^{b}	37.4 ± 0.2 ^{b}	37.2 ± 0.3 ^{ab}	Condition	<0.001
Knee	16	37.0 ± 0.3 ^{a}	37.2 ± 0.3 ^{ab}	37.3 ± 0.3 ^{bc}	37.5 ± 0.2 ^{cd}	37.7 ± 0.3 ^{d*}	37.4 ± 0.2 ^{bc}	Time	<0.001
Control	7	37.2 ± 0.1 ^{a}	37.0 ± 0.1 ^{b}	37.0 ± 0.1 ^{b}	37.0 ± 0.1 ^{b}	37.0 ± 0.1 ^{b}	37.0 ± 0.1 ^{b}	Time	**0.014**
T_{sk} forearm (° C)								Interaction	**<0.001**
Ankle	16	31.5 ± 0.8 ^{ab}	30.9 ± 1.0 ^{a}	31.1 ± 1.0 ^{ac}	31.9 ± 1.4 ^{bcd}	32.5 ± 1.5 ^{d}	32.0 ± 1.3 ^{bd}	Condition	0.019
Knee	16	31.4 ± 0.9 ^{a}	31.1 ± 1.1 ^{a}	31.8 ± 1.5 ^{a}	33.6 ± 1.6 ^{bc*}	34.4 ± 1.6 ^{b*}	32.9 ± 1.5 ^{c*}	Time	<0.001
Control	7	31.0 ± 0.8	30.2 ± 1.0	30.1 ± 1.0	30.1 ± 1.0	30.0 ± 0.7	29.8 ± 0.8	Time	**0.009**
T_{sk} foot (° C)								Interaction	0.424
Ankle	16	28.5 ± 1.3 ^{a}	42.7 ± 0.7 ^{b}	42.7 ± 0.7 ^{b}	42.8 ± 0.6 ^{b}	42.9 ± 0.6 ^{b}	33.6 ± 0.7 ^{c}	Condition	0.875
Knee	16	28.1 ± 1.6 ^{a}	42.8 ± 0.3 ^{b}	42.9 ± 0.4 ^{b}	43.0 ± 0.4 ^{b}	43.2 ± 0.5 ^{b}	33.5 ± 0.8 ^{c}	Time	**<0.001**
Control	7	28.3 ± 1.7 ^{a}	26.8 ± 1.2 ^{b}	26.7 ± 1.2 ^{b}	26.4 ± 1.2 ^{b}	26.0 ± 1.1 ^{b}	25.9 ± 1.1 ^{b}	Time	**<0.001**
T_{sk} calf (° C)								Interaction	**<0.001**
Ankle	16	30.7 ± 0.9 ^{a}	32.4 ± 2.0 ^{b}	33.1 ± 2.1 ^{bc}	34.3 ± 2.0 ^{c}	34.7 ± 1.9 ^{c}	32.5 ± 1.3 ^{ab}	Condition	<0.001
Knee	16	30.5 ± 0.8 ^{a}	42.9 ± 0.8 ^{b*}	42.9 ± 0.8 ^{b*}	43.1 ± 0.8 ^{b*}	43.1 ± 0.8 ^{b*}	34.1 ± 0.7 ^{c}	Time	<0.001
Control	7	29.5 ± 2.1	29.0 ± 1.2	28.8 ± 1.2	28.6 ± 0.7	28.3 ± 0.7	28.8 ± 0.8	Time	0.147

All data are expressed as mean ± standard deviation. A 2x5 and 2x6 (condition x time) repeated measures ANOVA was conducted to compare the perceptual and temperature responses, respectively, to heating in the Ankle and Knee conditions. A separate one-way repeated measures ANOVA with 5 and 6 timepoints, respectively, was conducted to track the perceptual and temperature changes over time with a Control resting condition, the results of which are highlighted in gray. Within a condition (Ankle/Knee), values with no common letters are different from each other; within a timepoint, a * indicates that Ankle and Knee conditions are different from each other. Significant main effects and interactions that apply for each variable are in **bold**. Abbreviations: T_c = core temperature, T_{sk} = skin temperature.

Supplemental Table 2. Brachial artery blood flow and shear responses to heating

	N	Pre			10 min			15 min			30 min			45 min			Post				P
BF (cm^3/min)																				Interaction	**<0.001**
Ankle	16	54.8	±	40.6 ^{a}	124.5	±	88.8 ^{b}	168.3	±	106.8 ^{b}	241.6	±	131.7 ^{c}	259.6	±	131.9 ^{c}	60.4	±	52.5 ^{a}	Condition	<0.001
Knee	16	46.0	±	21.2 ^{a}	194.8	±	90.8 ^{b*}	263.2	±	101.7 ^{c*}	391.5	±	180.5 ^{d*}	472.8	±	161.8 ^{e*}	92.3	±	85.1 ^{a}	Time	<0.001
Control	7	30.3	±	9.5	31.3	±	14.7	30.2	±	15.4	31.0	±	14.7	29.1	±	12.3	23.9	±	6.1	Time	0.192
Re (a.u.)																				Interaction	**<0.001**
Ankle	14	691	±	490 ^{a}	1653	±	1107 ^{b}	2361	±	1500 ^{b}	3277	±	1460 ^{c}	3467	±	1299 ^{c}	762	±	716 ^{a}	Condition	<0.001
Knee	14	624	±	346 ^{a}	2756	±	1024 ^{b*}	3629	±	967 ^{c*}	5223	±	1644 ^{d*}	6192	±	1489 ^{e*}	1050	±	837 ^{a}	Time	<0.001
Control	7	341	±	231	385	±	305	378	±	322	396	±	352	367	±	282	245	±	209	Time	0.080
SS (dyn/cm^2)																				Interaction	**<0.001**
Ankle	14	10.4	±	2.4 ^{a}	20.6	±	11.7 ^{ab}	28.1	±	17.2 ^{bc}	38.2	±	14.4 ^{cd}	40.0	±	12.9 ^{d}	10.4	±	3.2 ^{a}	Condition	<0.001
Knee	14	10.3	±	2.3 ^{a}	34.9	±	11.9 ^{b*}	44.8	±	14.8 ^{b*}	57.7	±	14.8 ^{c*}	65.3	±	14.8 ^{c*}	14.0	±	6.8 ^{a}	Time	<0.001
Control	7	8.6	±	1.2	8.6	±	1.1	8.6	±	1.1	9.3	±	2.3	8.8	±	1.6	8.4	±	1.3	Time	0.313
SR Mean (s^{-1})																				Interaction	**<0.001**
Ankle	16	116.3	±	52.4 ^{a}	268.6	±	161.1 ^{b}	349.4	±	215.8 ^{b}	470.6	±	187.2 ^{c}	492.7	±	175.5 ^{c}	124.5	±	56.4 ^{a}	Condition	<0.001
Knee	16	104.0	±	39.2 ^{a}	406.3	±	150.8 ^{b*}	519.5	±	182.1 ^{c*}	666.8	±	190.4 ^{d*}	758.8	±	198.6 ^{d*}	181.7	±	118.3 ^{a}	Time	<0.001
Control	7	69.6	±	37.3 ^{ab}	73.1	±	39.7 ^{ab}	71.6	±	40.2 ^{ab}	80.3	±	55.1 ^{a}	74.3	±	42.6 ^{ab}	58.4	±	37.1 ^{b}	Time	**0.053**
SR Antero (s^{-1})																				Interaction	**<0.001**
Ankle	16	135.3	±	51.0 ^{a}	282.1	±	152.5 ^{b}	362.4	±	210.5 ^{b}	477.1	±	184.4 ^{c}	499.8	±	170.9 ^{c}	152.0	±	75.0 ^{a}	Condition	<0.001
Knee	16	121.2	±	38.3 ^{a}	412.0	±	147.6 ^{b*}	532.6	±	199.6 ^{c*}	678.3	±	202.3 ^{d*}	762.5	±	199.9 ^{d*}	194.3	±	116.2 ^{a}	Time	<0.001
Control	7	95.3	±	44.0	94.3	±	43.2	96.9	±	45.7	100.6	±	56.0	93.3	±	46.6	84.6	±	43.0	Time	0.305
SR Retro (s^{-1})																				Interaction	0.351
Ankle	16	-19.0	±	12.7	-13.6	±	17.4	-13.0	±	12.6	-6.5	±	6.6	-7.1	±	10.4	-15.0	±	13.0	Condition	0.751
Knee	16	-21.2	±	14.9	-5.7	±	6.8	-13.1	±	28.6	-11.5	±	25.9	-4.2	±	1.9	-12.6	±	9.0	Time	**0.013**
Control	7	-25.7	±	13.1	-21.2	±	7.9	-25.3	±	9.7	-20.3	±	5.7	-19.1	±	6.0	-26.2	±	14.9	Time	0.270

All data are expressed as mean ± standard deviation. A 2x6 (condition x time) repeated measures ANOVA was conducted to compare the flow and shear responses to heating in the Ankle and Knee conditions. A separate one-way repeated measures ANOVA with 6 timepoints was conducted to track the flow and shear changes over time with a Control resting condition, the results of which are highlighted in gray. Within a condition (Ankle/Knee), values with no common letters are different from each other; within a timepoint, a * indicates that Ankle and Knee conditions are different from each other. Significant main effects and interactions that apply for each variable are in **bold**. Abbreviations: BF = blood flow, Re = Reynolds number, SS = shear stress, SR = shear rate.

Supplemental Table 3. Superficial femoral artery blood flow and shear responses to heating

	N	Pre			10 min			15 min			30 min			45 min			Post				P
BF (cm^3/min)																				Interaction	**<0.001**
Ankle	16	130.9	±	54.1 ^{a}	392.9	±	109.8 ^{b}	475.2	±	147.2 ^{b}	609.8	±	145.2 ^{c}	663.8	±	172.3 ^{c}	187.7	±	73.4 ^{a}	Condition	<0.001
Knee	16	117.0	±	30.2 ^{a}	628.9	±	145.8 ^{b*}	748.6	±	170.9 ^{c*}	895.3	±	208.9 ^{d*}	973.2	±	223.5 ^{d*}	285.9	±	84.4 ^{e}	Time	<0.001
Control	7	107.8	±	55.9	78.0	±	26.3	79.0	±	28.1	76.0	±	16.9	73.1	±	21.4	83.0	±	26.0	Time	0.069
Re (a.u.)																				Interaction	**<0.001**
Ankle	14	1303	±	718 ^{a}	4055	±	1356 ^{b}	4758	±	1689 ^{b}	6087	±	1648 ^{c}	6554	±	1777 ^{c}	2086	±	981 ^{a}	Condition	<0.001
Knee	14	1061	±	461 ^{a}	6193	±	1357 ^{b*}	7331	±	1589 ^{c*}	8648	±	1697 ^{d*}	9327	±	1920 ^{d*}	3024	±	934 ^{e*}	Time	<0.001
Control	7	928	±	891	569	±	439	552	±	442	480	±	425	466	±	357	607	±	538	Time	0.093
SS (dyn/cm^2)																				Interaction	**<0.001**
Ankle	14	9.3	±	1.2 ^{a}	21.9	±	6.3 ^{b}	24.0	±	6.7 ^{b}	29.8	±	7.1 ^{c}	31.5	±	7.3 ^{c}	12.6	±	3.5 ^{a}	Condition	<0.001
Knee	14	9.2	±	0.8 ^{a}	32.9	±	7.9 ^{b*}	36.9	±	8.5 ^{b*}	42.3	±	8.8 ^{c*}	45.7	±	9.9 ^{c*}	18.4	±	6.3 ^{d*}	Time	<0.001
Control	7	9.1	±	2.5	8.2	±	0.7	8.2	±	0.7	8.2	±	0.7	8.1	±	0.7	8.4	±	0.8	Time	0.367
SR Mean (s^{-1})																				Interaction	**<0.001**
Ankle	16	93.7	±	29.4 ^{a}	260.9	±	80.3 ^{b}	282.3	±	84.7 ^{b}	349.9	±	87.8 ^{c}	367.4	±	90.5 ^{c}	143.2	±	52.1 ^{d}	Condition	<0.001
Knee	16	87.5	±	22.5 ^{a}	380.0	±	84.8 ^{b*}	418.4	±	93.2 ^{b*}	485.8	±	93.4 ^{c*}	517.5	±	111.2 ^{c*}	218.0	±	73.6 ^{d*}	Time	<0.001
Control	7	78.4	±	53.9	58.1	±	29.4	56.1	±	28.3	52.7	±	25.5	51.7	±	25.5	60.6	±	32.1	Time	0.124
SR Antero (s^{-1})																				Interaction	**<0.001**
Ankle	16	148.6	±	31.8 ^{a}	280.1	±	90.9 ^{b}	304.9	±	101.5 ^{b}	366.6	±	98.4 ^{c}	382.5	±	98.3 ^{c}	185.6	±	50.2 ^{a}	Condition	<0.001
Knee	16	150.6	±	33.4 ^{a}	386.7	±	83.1 ^{b*}	422.3	±	92.2 ^{b*}	489.8	±	92.3 ^{c*}	521.7	±	110.7 ^{c*}	249.5	±	64.7 ^{d*}	Time	<0.001
Control	7	136.0	±	55.8	132.6	±	39.3	122.4	±	40.8	116.6	±	38.9	116.8	±	36.8	118.0	±	40.7	Time	0.093
SR Retro (s^{-1})																				Interaction	**0.001**
Ankle	16	-54.9	±	21.6 ^{a}	-19.2	±	19.5 ^{b}	-22.6	±	24.9 ^{b}	-16.7	±	19.9 ^{b}	-15.1	±	17.9 ^{b}	-43.3	±	16.6 ^{a}	Condition	0.021
Knee	16	-63.1	±	23.5 ^{a}	-6.7	±	6.2 ^{b}	-3.8	±	2.9 ^{b*}	-4.0	±	4.6 ^{b}	-4.2	±	2.6 ^{b}	-31.5	±	16.6 ^{c}	Time	<0.001
Control	7	-57.7	±	16.9 ^{a}	-74.6	±	12.5 ^{b}	-66.3	±	16.8 ^{ab}	-63.9	±	16.0 ^{ab}	-65.1	±	17.4 ^{ab}	-57.4	±	14.8 ^{a}	Time	**0.005**

All data are expressed as mean ± standard deviation. A 2x6 (condition x time) repeated measures ANOVA was conducted to compare the flow and shear responses to heating in the Ankle and Knee conditions. A separate one-way repeated measures ANOVA with 6 timepoints was conducted to track the flow and shear changes over time with a Control resting condition, the results of which are highlighted in gray. Within a condition (Ankle/Knee), values with no common letters are different from each other; within a timepoint, a * indicates that Ankle and Knee conditions are different from each other. Significant main effects and interactions that apply for each variable are in **bold**. Abbreviations: BF = blood flow, Re = Reynolds number, SS = shear stress, SR = shear rate.

CHAPTER 3

STUDY 2

Effects of local heat therapy, aerobic exercise training, or combined training and therapy on vascular function in recreationally active young adults: a randomized controlled trial

Effects of local heat therapy, aerobic exercise training, or combined training and therapy on vascular function in recreationally active young adults: a randomized controlled trial

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RUNNING HEAD: Responses to heat therapy and exercise training

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Abstract

Heat therapy may be an alternative or adjunct intervention to exercise training in terms of vascular function, however, the evidence for heating protocols that are more feasible and accessible than whole-body methods is lacking. This randomized controlled trial compared the effects of 8 weeks of no intervention (CON), lower limb heat therapy (HEAT), moderate-intensity cycling training (EX), or combined training and therapy (HEATEX) on vascular function in young, healthy recreationally active adults. Vascular function was assessed through brachial artery flow-mediated dilation tests (BA FMD) and measures of central (carotid-femoral, cfPWV) and peripheral (femoral-foot, ffPWV) pulse wave velocity. Assessments were conducted for cardiorespiratory fitness through a ramp peak oxygen uptake (VO_2peak) test; body composition using air displacement plethysmography; and isometric quadriceps muscle strength on a dynamometer. Sixty participants (23±3 years, 30 females) were randomly allocated into either CON (n=15), HEAT (n=15), EX (n=14), or HEATEX (n=16). Absolute and relative BA FMD did not change with intervention in any of the groups (all $p>0.05$). Both interventions with a heating component were, however, associated with chronic within-group reductions in cfPWV, and increases in absolute and relative VO_2peak after 8 weeks (HEAT: Δ-0.27 [-0.53, -0.02] m/s, Δ0.18 [0.06, 0.29] L/min, Δ2.18 [0.60, 3.76] ml/kg/min, respectively; HEATEX: Δ-0.33 [-0.58, -0.09], Δ0.21 [0.11, 0.32] L/min, Δ2.59 [1.06, 4.12] ml/kg/min, respectively), despite a lack of between-group differences ($p=0.25$, $p=0.21$, and $p=0.55$, respectively). There was also a within-group decrease in body fat percentage with EX (Δ-1.37 [-2.45, -0.29] %), but no changes in leg strength in any of the groups ($p=0.79$). There

were no therapy or training-related changes to resting heart rate, whole-body sweat rate, resting core temperature, resting mean skin temperature, and thermal comfort (all $p>0.05$). In this young, recreationally active cohort, 8 weeks of training with any of the protocols was insufficient to improve vascular function. Lower limb heat therapy or combined training and therapy may elicit improvements in central arterial stiffness and cardiorespiratory fitness, but further work is needed to confirm whether these effects persist in replication studies and with other populations.

INTRODUCTION

As humans continue to strive to extend both life- and health span, so will the need to diversify the available options for efficacious health-promoting interventions to satisfy a broad range of needs, interests, and preferences. Regular exercise and a balanced diet remain the most well-documented methods to improve and maintain health (1), and efforts to increase engagement in these activities should be prioritized. However, the development of other interventions that can be used alongside these recommended lifestyle habits may provide options to address barriers.

Heat therapy has been gaining traction as a health intervention in recent years. Controlled bouts of passive heating can increase core temperature, muscle temperature, vascular shear stress, and the content and activity of heat shock proteins and other circulating factors, making it an exercise mimetic for many physiological regulators and mechanisms of action (2, 3). Some heat therapy protocols can activate similar signaling pathways as those canonically associated with exercise (e.g., PGC-1α and AMPK) to achieve similar health outcomes (3). The effects of heat therapy on vascular function are particularly well-studied because the processes of heat dissipation and atheroprotection appear to have overlapping mechanisms of action: increased arterial blood flow and, consequently, shear stress. Broadly speaking, in young, healthy adults, 8 weeks of chronic hot water immersion (40-42 °C) of either the whole-body or forearms, between 30-90 minutes per session, and 3-5 times a week has been observed to improve microvascular endothelial function quantified by cutaneous vascular conductance (4–6), macrovascular endothelial function assessed using brachial artery flow-mediated dilation (BA FMD) (7–

10), and local and systemic arterial stiffness assessed as common carotid artery compliance, β-stiffness index, and carotid-femoral pulse wave velocity (cfPWV) (9). The beneficial effects of heat therapy have also been found to extend to other physiological systems, with increases in angiogenesis, mitochondrial function, glucose metabolism, cardiorespiratory fitness, and muscle mass and strength observed in cells, isolated tissues, and humans (3).

When compared to moderate-intensity aerobic exercise training, heat therapy has been found to elicit comparable improvements in BA FMD and relative peak oxygen uptake (VO_2peak) in recreationally active females (10). Moreover, when used in conjunction with exercise training, heat therapy may intensify the training stimulus, allowing for further improvements in VO_2peak, systolic blood pressure (SBP), body mass, body fat, blood glucose, resting metabolic rate, and fat oxidation compared to exercise alone (11, 12). These findings have been observed in cohorts of sedentary, middle-aged adults with at least one traditional cardiovascular disease risk factor and overweight or obese men and women (11, 12). These physiological changes appear to translate into quantifiable benefits in various aspects of health, which is corroborated by longitudinal data that successfully demonstrates reductions in the risk of all-cause mortality, cardiovascular diseases, and cardiovascular events with habitual sauna use by the Finnish, for whom it is a part of regular cultural practice (13).

Despite these promising findings, widespread adoption of heat therapy is limited by the cost and accessibility issues associated with existing modes of whole-body heat therapy such as saunas, spas, and hot tubs, as well as the tolerability of whole-body

heating for which most of the existing evidence for physiological changes has been developed. Recently, our lab showed that a single session of localized lower limb heating up to the ankles or knees through hot water immersion (45 °C) elicited acute improvements in BA FMD and femoral-foot pulse-wave velocity (ffPWV) following heating (14). The accumulation of acute responses with repeated exposure to this type of intervention may be sufficient to improve vascular function, as well as other metrics of health, but this has yet to be examined. Determining the efficacy of local foot heat therapy alone, compared to exercise training, and in conjunction with exercise training is important to further refine heating prescription and provide information about this potential alternative or adjunct to current exercise and public health guidelines.

The overarching purpose of this study was to compare the effects of 8 weeks of either no intervention/control (CON), local heat therapy (HEAT), exercise training (EX), or combined training and therapy (HEATEX) on vascular function in young, healthy recreationally active adults. We hypothesized that there would be no change in CON, and improvements in each outcome measure in all intervention groups, with the changes in HEATEX exceeding that of HEAT or EX alone.

METHODS

All study visits took place in the Ivor Wynne Centre at McMaster University in Hamilton, Ontario, Canada between June 2021 to June 2022. This study was approved by the Hamilton Integrated Research Ethics Board (project no. 12723) and prospectively

registered with ClinicalTrials.gov (identifier no. NCT04588103). Participants provided informed written consent prior to completing any portion of the study.

Participants

Participants were eligible for the study if they were a male or female between 18-35 years old and recreationally active at the time of recruitment. Exclusion criteria included current smoking and/or drug use; a history of cardiovascular, metabolic, or musculoskeletal disease; the inability to participate in physical activity according to the Physical Activity Readiness Questionnaire (2020 PAR-Q+); and a relative VO_2peak $\geq$ 60 ml/kg/min at baseline. Through a medical screening questionnaire administered during the consent phase, females reported details of their menstrual cycle history, including whether they were taking any hormonal contraceptives. During study visits, females reported the current day of their menstrual cycle, with day 1 being the first day of their most recent period or withdrawal bleeding.

Trial Design

This study was a 10-week randomized controlled trial in which pre- and post-testing occurred the week before and after an 8-week intervention, respectively **(Figure 1)**. Participants came to the laboratory for five *chronic* and two *acute* testing visits to allow for the assessment of both resting and intervention session responses across the study period. After baseline assessment, participants were randomized to CON, HEAT, EX or HEATEX group conditions. The CON group did not receive any heat therapy or

exercise training intervention and was instructed to continue with their usual activities. Participants that were allocated to an intervention group (i.e., HEAT, EX, HEATEX) attended supervised intervention sessions 3 times per week for 8 weeks, with no more than two days between intervention sessions. All participants were asked to maintain their current physical activity and dietary habits for the 10-week study duration.

Training Interventions

Heat Therapy (HEAT)

Heat therapy involved 45-minute sessions of lower-limb foot warm water immersion using a commercially available foot bath (IVATION, Edison, NJ, USA). The foot bath was filled with water up to the "MAX" line, which resulted in foot submersion just proximal to the malleoli for most participants. The temperature of the water was set to 109 °F (42.8 °C), and the water was allowed to circulate using the foot bath "Bubble" function.

Exercise Training (EX)

Exercise training involved 45-minute sessions of moderate intensity exercise on a stationary cycle ergometer (Lode Corival; Gronigen, The Netherlands/Kettler Ergorace; Ense, Germany/SCIFIT upright bike; Tulsa, OK, USA/LifeFitness 95Ci, Toronto, ON, Canada). The cycling protocol involved 40 minutes of cycling at 70-75% of maximal heart rate (HR_{max}) determined from individual baseline VO_2peak tests, and a 3-minute

warm-up and 2-minute cool-down at 50 W at the start and end of the session, respectively.

Combined Therapy/Training (HEATEX)

Combined training and therapy involved 90-minute sessions of the 45-minute EX protocol immediately followed by the 45-minute HEAT protocol as described above.

For all intervention sessions, participants wore a HR monitor (Polar A300 watch and H10 sensor; Polar Electro; Lachine, QC, Canada) so that average intervention HRs throughout the 8-week training program could be calculated.

Testing Protocols

For every testing visit, participants were asked to come to the laboratory having abstained from moderate-vigorous physical activity for 24 hours, alcohol and caffeine for 12 hours, and food for 6 hours.

Chronic Visit

The five chronic testing visits took place before the start of training (week 0), every 2 weeks during training (weeks 2, 4, 6), and after the end of training (week 8). All outcomes were assessed at weeks 0, 4, and 8, while only the vascular measures were performed for weeks 2 and 6.

Upon arrival at each testing visit, participants filled out the long form International Physical Activity Questionnaire (IPAQ). During bi-weekly testing visits from weeks 2-8, they also answered a one-item, 5-point Global Rating of Change Questionnaire that asked, "With respect to your physical activity levels, how would you describe yourself now compared to your last chronic visit (~2 weeks ago)?" These questionnaires were incorporated to provide a quantitative metric of physical activity level maintenance throughout the study period. After the questionnaires, anthropometrics and body composition measurements were completed, and vascular function assessments were conducted in a quiet, temperature-controlled room. Vascular function assessments began with 10 minutes of supine rest during which participants were instrumented with two sets of single-lead ECG. After the rest period, participants had an antecubital vein blood draw and resting blood pressure measurements performed in triplicate before arterial stiffness and endothelial function assessments were conducted. The visit concluded with maximal muscle strength and cardiorespiratory fitness testing.

On the week 8 visit (post-intervention), after all assessments, participants completed a questionnaire asking about their intentions to continue a similar exercise training and/or heat therapy program and a modified Exercise Benefits/Barriers scale.

Acute Visit

The two acute testing visits took place on the first day (session 1) and during the last week of an intervention (session 22, 23, or 24). Acute visit assessments were performed before and after an in-lab intervention session.

At these visits, participants wore a fitted sensor belt (EQ02+ LifeMonitor; Equivital by Hidalgo; Cambridge, UK) and were instrumented with a set of single-lead ECG and four skin temperature probes affixed to the left foot, calf, thigh, and arm (MLT422/A probes with ML309 thermistor pods; AD Instruments; Colorado Springs, CO, USA). Participants followed the protocol for the intervention they were assigned and those allocated to CON sat quietly for 45 minutes. Heart rate, core temperature (T_{core}), and skin temperatures (T_{skin}) were measured continuously throughout the testing session. Blood pressure, perception, and enjoyment were assessed before and during the intervention (approximately minutes 43-45 and 88-90, where applicable). Nude body weight (NBW) was taken before and immediately after the intervention (within 5 minutes) for the quantification of whole-body sweat rate (WBSR). Arterial stiffness and endothelial function were assessed before and after the intervention (within 30 minutes), but this data will not be presented in the current manuscript.

Outcome Measures

The primary outcome measure was BA FMD, while our secondary outcome measures included all other vascular function measures, central hemodynamics, cardiorespiratory fitness, skeletal muscle strength, body composition, core and skin temperatures, whole-body sweat rate, and perception and enjoyment.

Endothelial function

Endothelial function was assessed using a BA FMD test performed according to current guidelines using Duplex mode ultrasonography and a 12 MHz linear array probe at 7.7 fps to allow for simultaneous recording of artery diameter and blood velocity (Vivid q; GE Medical Systems; Horten, Norway) (15). The Doppler velocity gate was set to cover the entire arterial lumen and an insonation angle of 68° was used. This test involved having the participant abduct their right arm just under 90° away from midline of the body with a pneumatic cuff applied around their forearm. In the baseline phase, the brachial artery was imaged for 30 seconds to capture resting conditions. In the occlusion phase, the cuff was inflated to 200 mmHg for 5 minutes to generate an ischemic stimulus using a rapid cuff inflator (E20 Rapid Cuff Inflator and AG101 Air Source; Hokanson; Bellevue, WA, USA). In the reactive hyperemia phase, arterial imaging was conducted for 3 minutes following cuff deflation. Ultrasound images were exported and blinded prior to being analyzed using semi-automated edge-tracking software (Arterial Measurement System; Gothenburg, Sweden). Blood flow audio signals were passed through a spectral analyzer (Model Neurovision 500 M TCD; Multigon Industries; Yonkers, NY, USA) and the intensity weighted mean blood velocity signal was then analogue to digitally converted and analyzed beat-by-beat on data acquisition software (Labchart 8; AD Instruments; Colorado Springs, CO, USA) for the calculation of mean shear rate and shear rate area under the curve to peak artery diameter. For poor quality blood velocity signals (n=17 participants), the velocity traces were instead analyzed from the raw ultrasound images using pixel-based tracking software (Measurements from Arterial Ultrasound Imaging; Hedgehog Medical; Waterloo, ON, Canada). The same

blood velocity analysis method was used for all data from the same participant for consistency. FMD was expressed in absolute and relative terms using the equations below:

$$FMD\ (mm) = D_{peak} - D_{base} \quad (1)$$

$$FMD\ (\%) = (\frac{D_{peak} - D_{base}}{D_{base}}) \times 100 \quad (2)$$

where D_{base} is baseline artery diameter and D_{peak} is peak artery diameter. An experienced ultrasound sonographer (JLC) performed all FMD tests, and images were analyzed by three raters (JLC, CAP, GKB) in a 60/20/20% split. To allow for the calculation of inter-rater agreement, a random sample of 7 participants (56 tests) were analyzed by all three raters and were included in the agreement analysis. BA FMD was assessed at all timepoints during the study.

Arterial stiffness

Central and peripheral arterial stiffness was assessed by measuring pulse wave velocity between the carotid to femoral (cfPWV) and femoral to dorsalis pedis arteries (ffPWV), respectively, according to current guidelines (16, 17). If the dorsalis pedis pulse could not be detected, the tibialis posterior pulse was used instead. The same pulse sites were used for all visits within the same participant. For PWV measurements, two operators used applanation tonometers (SPT-301 handheld tonometer; Millar Instruments; Houston, TX, USA) to simultaneously collect at least 30 continuous, clean pressure waveforms from the artery sites of interest. Afterwards, a tape measure was used to measure the distance over the surface of the body between the pulse sites. Pulse transit

times were determined by foot-to-foot waveform analysis on data acquisition software (Labchart 8; AD Instruments; Colorado Springs, CO, USA). PWV was calculated using the equation below using two sets of 10 heart cycles of data. If the average of the two sets differed by more than 0.5 m/s, a third set of 10 heart cycles was included in the analysis. For cfPWV, distance in the equation was multiplied by a factor of 0.8 as is recommended by the most recent consensus statement (17). PWV was assessed at all timepoints during the study.

$$PWV\ (m/s) = \frac{distance}{pulse\ transit\ time} \quad (3)$$

Resting central hemodynamics

Resting blood pressure and heart rate were measured in triplicate using an automated sphygmomanometer (Dinamap Carescape V100; GE Healthcare; Mississauga, ON, Canada). Participants were instructed to remain quiet and at rest in the anatomical reference position for the entire assessment period. The first value was always eliminated; the second and third values were averaged. If there was >5 mmHg systolic blood pressure discrepancy between the second and third measurements, a fourth measurement was taken and included in the average. Resting central hemodynamics were assessed at all timepoints during the study.

Core and skin temperatures

Prior to each acute visit, participants were provided an activated, wireless T_{core} pill to swallow 2-3 hours before their scheduled testing session. Once they were in the

laboratory, the pill was synchronized with a sensor electronic module (SEM) (EQ02+ LifeMonitor; Equivital by Hidalgo; Cambridge, UK), the data from which wirelessly transmitted to the data acquisition unit (PowerLab PL3516; AD Instruments; Colorado Springs, CO, USA) for continuous collection. T_{skin} were measured through a combination of skin thermistors (foot, calf, thigh, arm) and a temperature sensor on the SEM (chest). Temperature data were analyzed as the average of 1-minute blocks at baseline and during the interventions. T_{core} and foot skin temperature (T_{foot}) were reported as proof of principle that our interventions had the intended effect. The remaining T_{skin} locations were used to calculate mean T_{skin} according to the Ramanathan equation (18):

$$T_{skin}(°C) = 0.3T_{chest} + 0.3T_{arm} + 0.2T_{thigh} + 0.2T_{calf} \quad (4)$$

where T_{chest}, T_{arm}, T_{thigh}, and T_{calf} represent the skin temperatures at each specified body location. Temperature measurements were captured at week 0 and week 8 during the acute visits.

Whole-body sweat rate

WBSR was estimated by measuring NBW before and immediately after each participant's 45 or 90-minute intervention. Participants did not use the bathroom for the duration of the visit, and water intake was measured to allow for the calculation of WBSR as follows:

$$WBSR = \frac{((NBW_{Pre} - NBW_{Post}) + water\ consumed) \times 60}{intervention\ duration\ (\text{min})} \quad (5)$$

WBSR was assessed at week 0 and week 8 during the acute visits.

Cardiorespiratory fitness

Cardiorespiratory fitness was assessed using a ramp VO_2peak test on a cycle ergometer (Lode Corival; Gronigen, The Netherlands/Kettler Ergorace; Ense, Germany). Participants wore a heart rate monitor and were fitted with a face mask that was completely sealed apart from one-way inflow and outflow tracks, which was then connected to a mixing chamber and metabolic cart (Quark CPET; COSMED; Chicago, IL, USA) for the measurement of gas exchange. Participants were asked to cycle at a cadence between 70-90 rpm and told that the test would stop once they could no longer maintain 60 rpm. The test protocol began with a 3-minute warm-up at 50 W followed by a ramp increase of 5W every 10 seconds until exhaustion. Achievement of VO_2peak was defined as either an observed plateau in VO_2 in the final 30 seconds of the test (i.e., 3 x 10-second bins that were within 0.1 L/min), or attainment of two out of the following three criteria: (1) rating of perceived exertion ≥17, (2) respiratory exchange ratio ≥ 1.13, (3) heart rate ≥ 93% of age-predicted maximum ((208-0.7*age)*0.93) (19). VO_2peak was calculated as the average of the highest 3 x 10-second bins, and was expressed in both absolute terms and relative to body mass. VO_2peak tests were completed at weeks 0, 4, and 8 of the study.

Body composition

Body composition was assessed using air displacement plethysmography (BodPod; COSMED; Chicago, IL, USA). Participants were instructed to wear minimal, form-fitting clothing and a swim cap, and removed any jewelry, glasses, and other metals

prior to entering the BodPod. Any piercings that could not be easily removed were noted on the data collection sheet for repeat assessments. Body composition was assessed at weeks 0, 4, and 8 of the study.

Skeletal muscle strength

Isometric quadriceps muscle strength was measured on the Biodex dynamometer (System 3; Biodex Medical Systems; Shirley, NY, USA). The protocol used placed the right leg at 60° of knee extension, and participants performed four maximal 5-second contractions each separated by 2 minutes of rest. The maximum values of each repetition in Newton-meters were recorded and then averaged. Muscle strength was assessed at weeks 0, 4, and 8 of the study.

Perception and psychosocial outcomes

Affect

General affective valence was assessed using the single-item, 11-point Feeling Scale (20). Participants were asked to indicate the number that most accurately represented how they were currently feeling on the scale ranging from -5 “Very Bad” to +5 “Very Good”. Arousal state was assessed using the 20-item, 4-point Activation Deactivation Adjective Check List (21). Participants were asked to indicate whether they “Definitely feel” (4), “Feel slightly” (3), “Cannot decide” (2), or “Definitely do not feel” (1) a list of adjectives that describe arousal. Scores for the adjectives “wakeful” and “wide-awake” were reversed before all scores were summed to produce an overall arousal

score out of 80, with a higher score indicating higher arousal levels. Affect was assessed at weeks 0 and 8 during the acute visits.

Thermal perception

Thermal comfort and sensation were both assessed with single-item, 9-point scales (22). Based on the whole-body temperature they were experiencing, participants were asked to indicate how comfortable they felt on a scale ranging from -4 “Very uncomfortable (cold)” to +4 “Very uncomfortable (hot)” with 0 being the neutral “Comfortable”, and how they would label the feeling on a scale ranging from 1 “Very Cold” to 9 “Very Hot”. Thermal perception was assessed at weeks 0 and 8 during the acute visits.

Effort perception

Effort perception was assessed using the single-item, 15-point Rating of Perceived Exertion Scale (23). Participants were asked to provide a rating of their physical effort on a scale ranging from 6 “No exertion; sitting and resting” to 20 “Maximal exertion”. Participants were instructed to provide ratings that were representative of the whole body rather than the lower limbs or feet only. Effort perception was assessed at weeks 0 and 8 during the acute visits.

Enjoyment

Enjoyment was assessed using the single-item, 7-point Exercise Enjoyment Scale, which asks participants to indicate how much they enjoyed the intervention session (24). They were also asked to complete the 18-item, 7-point bipolar Physical Activity Enjoyment Scale (PACES) (25). The ends of the scale were anchored by opposing statements, such as "I feel bored/I feel interested" or "I find it energizing/I find it tiring," and participants were asked to rate themselves on each item. Scores for 11 items were reversed, in line with previously validated guidelines (25). The scores of all 18 items were then summed to provide a complete enjoyment score out of 126, with a higher score indicating higher levels of enjoyment. Enjoyment was assessed at weeks 0 and 8 during the acute visits.

Intentions

Participants' intentions were assessed with a single-item, 7-point question ranging from 1 "Extremely unlikely" to 7 "Extremely likely" (24). The instructions provided were "Please rate the extent to which you agree with the following statement: 'I intend to engage in the type of activity I performed today at least 3 times per week over the next month (similar to the current activity program).'" This question was asked at week 8, after the training program had been completed.

Activity Benefits and Barriers

Participants completed a modified version of the 43-item, 4-point Exercise Benefits/Barriers scale (26). Response options ranged from 1 "Strongly disagree" to 4

"Strongly agree". All instances of the word "exercise" were replaced with "activity" so as to encompass all training and/or therapy protocols performed in the study. Barriers and benefits were scored together to produce an overall score. As such, all items considered to be barriers were reverse-scored before summing the scores of all 43 items to provide an overall score out of 172, with higher scores indicating more favourable views of the activity. This questionnaire was administered at week 8, after the training program had been completed.

Randomization

After baseline data was collected, participants were randomly allocated (1:1:1:1 allocation ratio) into either the control (CON), local heat therapy (HEAT), aerobic exercise training (EX), or combined training/therapy (HEATEX) group. Random allocation was achieved using a random number sequence, stratified by biological sex (2 levels: male and female) and baseline aerobic fitness (2 levels: VO_2peak, ≥42.0 and <42.0 ml/kg/min) in blocks of 4 for feasibility purposes. The randomization schedule was prepared by a statistician (KSN), who was blind to all baseline data except stratification variables (sex and aerobic fitness). Allocation concealment was attained in this study by ensuring that the password-protected randomization schedule was accessed only by the statistician, and only at the time of randomization for each participant.

Sample size calculation

Sample size was calculated *a priori* based on the primary research question and outcome measure of the expected changes in relative flow-mediated dilation (FMD%) in response to each 8-week intervention (i.e., one-way ANOVA on the change scores from week 0 to 8). Using a simulation-based R package (27), we entered the following information: (1) anticipated mean changes of 0% for CON, 2% for HEAT and EX, and 3.5% for HEATEX; (2) a common standard deviation of 1.2 calculated from all previous data from the ultrasound sonographer and image analyzer (JLC); (3) α level set at 0.05; and (4) N=15 per group. These parameters yielded ≥80% power to detect differences between groups.

Statistical Analysis

Participant demographic information and baseline data were quantified with means and standard deviations for continuous, normally distributed data. Data was presented as medians and interquartile ranges for continuous, non-normally distributed or ordinal data. Frequencies and percentages were used to describe categorical data. All analyses followed a modified intention-to-treat analysis. Multiple imputation was used in the case of missing data. However, if interpretation was not different, the complete-case analysis was presented for variables with <5% missing data. All analyses and randomization procedures were conducted using StataSE (Version 17.0, StataCorp, College Station, TX, USA). The accepted significance level was set *a priori* to $p<0.05$.

Characterization of intervention stimuli

Data from the first acute visit (intervention session #1) was used to characterize the intervention stimuli in terms of the temperature, hemodynamic, perceptual, and enjoyment responses. One-way ANOVAs were conducted, with the outcome of interest as the dependent variable and time as the independent variable. For temperature and hemodynamic outcomes, there were 3-4 levels for time (whichever apply): pre-intervention, during (2 levels for HEATEX group), and post-intervention; whereas for perceptual and enjoyment outcomes, there were 2 levels for time: pre-intervention and post-intervention. The ANOVAs were performed separately for each group for two reasons: 1) We were uninterested in comparing the acute change in each stimulus between groups, as this is outside the scope of the current investigation and has been previously established for many of our variables, and 2) the HEATEX group responses were measured at two timepoints during the intervention (during the exercise phase and during the heating phase), which would violate the assumption of independent observations in our models. Nonetheless, we thought it necessary to display the effects of our intervention stimuli. We followed the same procedures as the primary chronic analysis to test the assumptions of the ANOVAs and navigate data not meeting the assumptions.

Intervention responses

Data from the chronic visits was used to examine the chronic effects of the interventions on vascular function, central hemodynamics, cardiorespiratory fitness, body composition, and skeletal muscle strength; whereas data from the acute visits was used to

examine the chronic effects of the intervention on resting core and skin temperatures, whole-body sweat rate, perception and enjoyment.

For our primary analyses of interest, we conducted one-way analyses of variance (ANOVAs) for each variable, with group allocation (4 levels: CON, HEAT, EX, HEATEX) as the independent variable, and change in the tested outcome (pre- to post-intervention) as the dependent variable. Normality of the dependent variables and residuals were first assessed by visual inspection, followed by the Shapiro-Wilk and Cook-Weisberg test, respectively. If the assumptions of the ANOVAs were violated, non-parametric tests were used. Data are reported as means and 95% confidence intervals for parametric analyses, and as medians with interquartile ranges for nonparametric analyses.

Secondary, exploratory, analyses were conducted to characterize trajectories of change in each outcome measure across each timepoint of the intervention. First, we visually inspected the relationship between the outcomes and time (max 5 levels: weeks 0, 2, 4, 6, and 8). Mixed-model analyses were then used. Time by group allocation (4 levels: CON, HEAT, EX, HEATEX) interactions were included to examine differences in trajectories of change between groups. If the interactions were not significant, they were removed from the subsequent model. If they were significant, the lower-order independent variables (group and time) remained in the model, irrespective of statistical significance. Models were tested for fixed and random intercepts and slopes, and the Bayesian Information Criteria and log likelihood ratio tests were used to determine the best fitting model, while also considering model parsimony. Level 1 and 2 residuals were examined carefully, and any data point which had substantial impacts (i.e., >10%

difference in beta coefficients) on the analysis were considered for removal. For both primary and secondary analyses, Sidak-adjusted pairwise comparisons were performed when statistically significant effects were observed.

RESULTS

Participant characteristics

From the 84 individuals approached for this study, 60 participants were eligible, agreed to participate and were randomly allocated into either CON (n=15), HEAT (n=15), EX (n=14), or HEATEX (n=16) **(Figure 2)**. There was no loss to follow-up. Furthermore, adherence to the training programs was 99.7%, 97.3%, and 98.7% in the HEAT, EX, and HEATEX groups, respectively and was not different across the groups (p=0.33). Participant characteristics, including menstrual cycle information for females and racial and ethnic information, are outlined in **Table 1**. On average, the complete cohort was young (23±3 years old) and healthy as defined by normal BMI (24.0±3.6 kg/m^2) and resting blood pressure (SBP: 112±9 mmHg, DBP: 63±6 mmHg). Further, mean baseline VO_2peak was 42.5±8.7 ml/kg/min, with the male average being 46.2±9.3 ml/kg/min and the female average being 38.8±6.3 ml/kg/min. Our participants were in the 60th and 70th percentile for cardiorespiratory fitness for men and women aged 20-29 (ACSM Guidelines for Exercise Testing and Prescription, 6th edition), respectively, further suggesting that they were indeed generally healthy but not in the top fitness categories. In accordance with our randomization procedures, groups were balanced for sex and

baseline relative VO_2peak, such that there was an equal proportion of males and females and similar baseline cardiorespiratory fitness across all four groups.

Characterization of stimuli

T_{core} increased acutely in all but the CON group (all $p<0.05$). Foot T_{skin} increased during HEAT, EX, and in both the exercise and heating components of HEATEX and decreased at the Post timepoint in CON (all $p<0.05$). Mean T_{skin} only increased during both components of the HEATEX intervention ($p<0.001$) **(Supplementary Table 1)**. Mean arterial pressure (MAP) increased only during exercise (i.e., EX and the exercise component of HEATEX) (all $p<0.05$), whereas heart rate increased in all groups ($p<0.05$) except CON ($p=0.28$) **(Supplementary Table 1)**. Participants' intervention HRs averaged from all HEAT, EX, and HEATEX (average across both the exercise and heating portions of the sessions) intervention sessions were equivalent to 47%, 71%, and 60% of HR_{max}, respectively.

Neither affective valence nor arousal state changed with any of the interventions ($p=0.81$ and $p=0.06$, respectively). Thermal comfort and sensation increased (participants went from feeling slightly uncomfortable (cold) and slightly cool to slightly uncomfortable (hot) and warm) in HEAT, EX, and HEATEX but not CON (both $p<0.001$). RPE increased only in the EX group ($p<0.001$). According to the single-item enjoyment scale, the four activities were equally enjoyable ($p=0.74$). However, when using the PACES, there was an interaction effect ($p=0.003$) such that EX (mean: 84 [95%

CI: 74, 93]; p=0.04) and HEATEX (mean: 90 [95% CI: 80, 99]; p=0.002) were more enjoyable compared to CON (mean: 65 [95% CI: 56, 74]) **(Supplementary Table 1)**.

Chronic intervention responses

There were no differences in the 8-week change scores or trajectories for absolute or relative BA FMD across all groups (all p>0.05) **(Figure 3 and Table 2)**. Intra-class correlation coefficients for FMD analysis across raters was 0.79 (95% CI: 0.69, 0.86) for absolute BA FMD and 0.80 (95% CI: 0.72, 0.87) for relative BA FMD, indicating good reliability for both measures. There was a within-group decrease in cfPWV from week 0 to 8 in the HEAT (mean: Δ-0.27 [95% CI: -0.53, -0.02] m/s) and HEATEX (mean: Δ-0.33 [95% CI: -0.58, -0.09] m/s) groups, and a main effect of time in the corresponding trajectory analysis (p=0.043) but no between-group differences (p=0.49) **(Figure 3)**. There were no pre- to post-intervention changes in ffPWV, SBP, DBP and MAP either by change score or trajectory (all p>0.05) **(Figure 3 and Table 2)**. There were no differences in the 8-week change scores for resting HR, but there was a training group by time interaction (p=0.02) **(Table 2)**. However, following Sidak adjustment for multiple comparisons, all significant comparisons were lost.

There were within-group increases in absolute and relative VO_2peak from week 0 to 8 in the HEAT (mean: Δ0.18 [95% CI: 0.06, 0.29] L/min and mean: Δ2.18 [95% CI: 0.60, 3.76] ml/kg/min, respectively) and HEATEX (mean: Δ0.21 [95% CI: 0.11, 0.32] L/min and mean: Δ2.59 [95% CI: 1.06, 4.12] ml/kg/min, respectively) groups, but this was not different between groups in the 8-week change scores or in the trajectory

analyses (absolute: p=0.21 and p=0.20, respectively; relative: p=0.55 and p=0.65, respectively) **(Figure 4)**. There was a significant effect of group in the 8-week change scores (p=0.02) and a significant interaction in the trajectory analysis (p=0.017) for peak power output on the VO_2peak test **(Figure 4)**. With respect to the change score analysis, the change in PPO after 8 weeks was greater in HEATEX vs. CON (mean: Δ23.4 [95% CI: 14.3, 32.5] vs. mean: Δ3.6 [95% CI: -6.2, 13.3] W; p=0.025). However, after multiple imputation to account for missing data of n=1 (group effect: p=0.04) and Sidak adjustment for multiple pairwise comparisons, significance was lost (all Sidak-adjusted p≥0.05). With respect to the trajectory analysis, PPO increased at week 8 of EX (mean: 259.4 [95% CI: 227.0, 291.7] W; p=0.013) and at weeks 4 (mean: 295.9 [95% CI: 264.7, 327.2] W; p=0.001) and 8 (mean: 312.5 [95% CI: 281.2, 343.8] W; p<0.001) of HEATEX compared to week 0 (mean: 256.0 [95% CI: 223.7, 288.3] W).

There were no differences in the 8-week change score or trajectory analysis for body mass across groups (both p>0.05) **(Supplementary Table 2)**. There was a within-group decrease in %BF observed in the EX group only (mean: Δ-1.37 [95% CI: -2.45, -0.29]), but no differences between groups in the 8-week change scores or trajectory analyses (p=0.66 and p=0.59, respectively) **(Supplementary Table 2)**. There were no differences in the 8-week change score or trajectory analyses for isometric quadriceps muscle strength, resting T_{core} and mean T_{skin} , and WBSR across groups (all p>0.05) **(Supplementary Table 2 and 3)**.

Eight weeks of training did not change how any of the interventions were perceived when assessed by measures of affect (p=0.77), arousal state (p=0.26), thermal

comfort (p=0.30), thermal sensation (p=0.42), RPE (p=0.15), and enjoyment on the single-item scale (p=0.51). There was an interaction effect (p<0.001) for enjoyment reported on the PACES, wherein there was less of a decrease in enjoyment in EX vs. HEAT (p=0.02), as well as in HEATEX vs. CON (p=0.01) and HEAT (p=0.001), suggesting that interventions with an exercise component were perceived as the most enjoyable after the 8 weeks. There was an interaction effect (p=0.03) for intentions to continue with the prescribed training program over the next month, such that it was greater in HEATEX compared to HEAT (median: 5.5 [IQR: 3.0] vs. median: 3.0 [IQR: 3.0]; p=0.04). Using the modified Exercise Benefits/Barriers scale, there was no difference in the favourability of all three training/therapy interventions (p=0.09).

DISCUSSION

This study sought to evaluate the efficacy of local foot hot water immersion as a chronic intervention for improving vascular health, and to examine how it compares to traditionally prescribed moderate-intensity exercise training. Based on our findings, in recreationally active young adults, 8 weeks of heat therapy (HEAT), exercise training (EX), or combined training and therapy (HEATEX) were insufficient to improve endothelial function assessed using BA FMD. However, there may be beneficial effects of HEAT and HEATEX for central arterial stiffness and cardiorespiratory fitness, and of EX on body composition. Furthermore, augmenting an exercise training program with local heat therapy may improve performance as suggested by our PPO data.

Vascular function

There are several ways in which changes in vascular function can be elicited. Most commonly, increased vascular shear stress against the endothelial cell layer of the arterial wall triggers mechanotransduction that initiates molecular signaling to increase the bioavailability, activation, and release of nitric oxide and other vasodilators and suppress similar pathways for vasoconstrictors and inflammatory or oxidative agents (28). These signaling cascades can have immediate functional impacts on dilatory capacity and vascular tone. However, with repeated exposure to shear over time, this healthy arterial milieu also encourages structural remodeling of the arterial wall through the maintenance of balanced proportions of elastin and collagen in the extracellular matrix, and the reversal of advanced glycation end-products and collagen cross linkages that work to stiffen the artery (29).

The null findings for most of the vascular function outcome measures emphasizes that intensity matters. In heat therapy prescription, the intensity of a protocol can be modified by changing the frequency, temperature, mode, localization, and duration of heating (30). Our 45-minute foot hot water immersion intervention increased T_{core} by 0.4 °C and HR by 23 bpm – the equivalent of light physical activity (ACSM) – and participants performed this protocol 3 days per week. In comparison, Brunt *et. al.* (2016) observed sizeable and clinically relevant improvements in BA FMD, superficial femoral artery compliance and β-stiffness index, cfPWV, resting DBP and MAP, and common carotid artery intima-media thickness when participants performed whole-body hot water immersion for up to 90 minutes, 5-6 times per week, with a target T_{core} of 38.5 °C (9).

The necessity for a greater thermal stimulus is further corroborated by Laukannen *et. al.* (2015) in a longitudinal study in which they demonstrated that the magnitude of risk reduction accrued for sudden cardiac death, fatal coronary heart disease, fatal cardiovascular disease, and all-cause mortality increased as both the frequency and duration of sauna bathing increased. They observed significant additional reductions in risk with progression from sauna bathing once to 2-3 times to 4-7 times per week; and most of the benefits of sauna bathing were captured only when individuals performed sessions >19 minutes (13).

Although we succeeded at creating a more accessible and feasible heat therapy protocol that may improve adherence and enjoyment in comparison to whole body heating, our local foot heating intervention was not sufficient to elicit changes in BA FMD over an 8-week time frame. It is possible that there is an intensity-to-intervention period trade off, wherein longer interventions (e.g., 12 weeks to 6 months) are required to observe vascular function changes. The within-group reductions in cfPWV for the HEAT and HEATEX groups – and associated main effect of time in the corresponding trajectory analysis – suggest that local heat therapy is worth exploring further as a vascular health intervention. With more participants, increased stimulus and/or a longer time frame of observation, we may have been able to discern between-group differences that were not observed in the current study.

Curiously, neither of the exercise interventions were associated with an improvement in vascular function in the current study. The beneficial effects of moderate-intensity continuous exercise training on vascular function have been quite well-

established in prior research, including a meta-analysis (31), however, we were unable to replicate this finding. Anecdotally, most participants allocated to an exercise training group (EX and HEATEX) expressed that the cycling did not feel challenging; therefore, we surmise that the combination of the lower intensity exercise training protocol and relatively high fitness level of our cohort may have contributed to the lack of vascular function changes in these groups.

Fitness, body composition, and muscle strength

We did not observe the anticipated improvements in VO_2peak in the EX group. While surprising to us initially, this observation may be more common than the literature would suggest, especially when considering the systemic bias against the publication of null findings (32, 33). Given the fitness level of our cohort (60^{th}-70^{th} percentile for relative VO_2peak), it is possible that a more potent exercise training stimulus was required to trigger the metabolic signaling pathways associated with improved oxygen delivery and utilization, both of which would increase cardiorespiratory fitness. Interestingly, within-group improvements in VO_2peak were observed in the HEAT and HEATEX groups, and although future work should confirm its replicability, this pattern – which is consistent with the cfPWV findings – suggests that there is a mechanism being stimulated specifically by the heating but not the exercise protocol. In the few studies on healthy human subjects that have examined the skeletal muscle adaptations to heating, results suggest that while the same endpoint is reached – that is, similar magnitude improvements in VO_2peak – heat therapy achieves this outcome mainly through skeletal

muscle angiogenesis, whereas exercise training elicits changes in both capillarization and myocyte structure and function (34, 35). With repeated heating, researchers have observed increases in capillary to fibre ratio, capillary to fibre perimeter exchange index, capillary density, and eNOS content; while concurrent increases in mitochondrial density, GLUT4 content, and intramuscular triglyceride content are seen only with exercise training (34, 35). Based on our understanding of these mechanisms, it is likely that the increased PPO during the VO_2peak test with EX and HEATEX is due to the participants training in the same mode in which the test was administered (i.e., cycling) rather than an improvement in quadriceps muscle strength. Indeed, apart from a within-group decrease in %BF in the EX group, we found no changes in body composition or isometric quadriceps muscle strength with the interventions. The signaling pathways that lead to structural and functional changes to skeletal muscle require direct stimulation of the tissue either through muscle contraction (with exercise) or elevated muscle temperature (with heating). Compared to prior work that used either whole-body heating in an environmental chamber or heating of the entire thigh and gluteal region with water-perfused tube-lined pants, our foot heating protocol covers an area of the body with limited musculature and, further to that, does not directly apply heat to the muscle groups (i.e., quadriceps, hamstrings, and gluteals) that we were seeking to observe changes in. As a result, the local release of heat shock proteins and other metabolically active substances may not be enough to yield changes in body composition and muscle strength, at least using the methods in which they were assessed in this study.

Thermoregulatory variables

There were no thermoregulatory adaptations to the 8 weeks of intervention for any group. Based on exercise-induced heat acclimation studies conducted in athletes, it is well understood that the typical response includes decreases in HR during exercise, T_{skin} during exercise, and T_{core} at rest and during exercise; and increases in plasma volume, sweat rate, thermal comfort, and exercise capacity (36). The net effect is less thermoregulatory strain for a given fixed workload, which would allow an individual to perform for longer before reaching critical endpoints that would limit performance (37). In the current study, we observed no changes in resting HR, resting mean T_{skin}, resting T_{core}, WBSR, and thermal comfort. These findings are similar to those from other chronic heat exposure studies (9, 34), and suggests that the threshold for changes to health outcomes is likely lower than that required for performance-related heat acclimation or acclimatization.

Perception, enjoyment, and adherence

Based on most psychosocial metrics assessed in the study, HEAT, EX, and HEATEX were comparable with respect to perception and enjoyment. With the PACES, we were able to further discern that the EX and HEATEX protocols were rated as more enjoyable compared to the CON, but were not different from HEAT. Adherence to the training/therapy programs was excellent across the board, regardless of group allocation. Overall, these data provide further support for the development of an interventional menu to cater to wide-ranging personal preferences. Speaking to participants, it was clear that

there were polarizing views on both heat therapy and exercise training, and the choice on which intervention was more suited for someone from a behavioural perspective seemed to come down to arbitrary factors that were difficult to account for, such as current lifestyle, cost/accessibility, other health habits, and preferred thermal setpoint.

Strengths, limitations, and considerations for future research

This randomized controlled trial was designed in alignment with CONSORT guidelines. Groups were stratified by sex and baseline fitness level to reduce confounding variables and preserve power during the statistical analysis phase. A statistician was enlisted to perform group random allocation, blinding during data analysis, and statistical analysis. We used reference standard methods for the assessment of vascular function, and data was obtained by trained and experienced personnel.

Despite conducting an informed sample size calculation *a priori* for the main outcome measure (BA FMD), it is likely that many of our statistical analyses were underpowered to detect the hypothesized effects. We speculate that the common standard deviation of 1.2, which was extracted entirely from *acute* interventional studies conducted by JLC in the Vascular Dynamics Lab (i.e., less variability between FMD measurements), was not conservative enough for the more expansive 8-week *chronic* study that was performed. Unfortunately, an N=60 was the maximum that our research team could achieve and feasibly collect considering logistical constraints imposed by time, personnel, resources, and the COVID-19 pandemic.

In this study, we also opted to recruit a young, recreationally active population instead of a sedentary group, which would likely have been more responsive to our interventions in terms of vascular function. The decision to study those who are habitually active was intentional as our goal was for our findings to be as generalizable as possible. We argue – as did Booth & Lees in 2006 – that the sedentary individual does not represent the average person today, and that most people perform some physical activity even though it is unstructured and more seamlessly incorporated into their day-to-day lives (e.g., active commuting, walking a pet, running errands, etc.) (38).

For those looking to further refine heat therapy prescription, it is important to consider the limitation of local protocols in that the ambient temperature of the environment in which the intervention is performed could greatly alter the long-term physiological responses observed. Since many of the adaptations are thought to derive from specific threshold changes in T_{core}, if local heat therapy is performed in a cold vs. temperate or warm environment, there may be variations in the expected increases in T_{core} compared to laboratory settings with tight temperature and humidity control.

We would also like to acknowledge the limitations of the scales and questionnaires used for the enjoyment and feasibility data collected. In particular, the Physical Activity Enjoyment Scale and Exercise Benefits/Barriers Scale are validated for use with respect to physical or exercise activities and not alternative activities such as heat therapy. Although some modifications were made to both scales to accommodate for their use by the HEAT and HEATEX groups, this information should be evaluated with a critical lens.

CONCLUSION

Eight weeks of local foot heat therapy, moderate-intensity cycling training, and combined exercise training and heat therapy were insufficient to improve vascular function assessed by BA FMD in young, healthy recreationally active adults. While there is potential for local heating, alone or combined with exercise training, to reduce arterial stiffness and improve cardiorespiratory fitness, more work is required to verify these effects.

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DISCLOSURES

No competing interests, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

The author contributions are outlined using the Consortia Advancing Standards in Research Administration Information (CASRAI) CRediT taxonomy (https://credit.niso.org/).

Conceptualization: JLC, MJM; **Data curation:** JLC, CAP, KCM, KSN, CMA, GKB; **Formal analysis:** JLC, CAP, KCM, KSN, CMA, GKB; **Funding acquisition:** MJM; **Investigation:** JLC, CAP, KCM, CMA, GKB; **Methodology:** JLC, KSN, MJM; **Project administration:** JLC, MJM; **Resources:** MJM; **Software:** N/A; **Validation:** KSN; **Visualization:** JLC; **Writing – original draft:** JLC, KSN (statistical analysis); **Writing – review and editing:** JLC, CAP, KCM, KSN, CMA, GKB, MJM.

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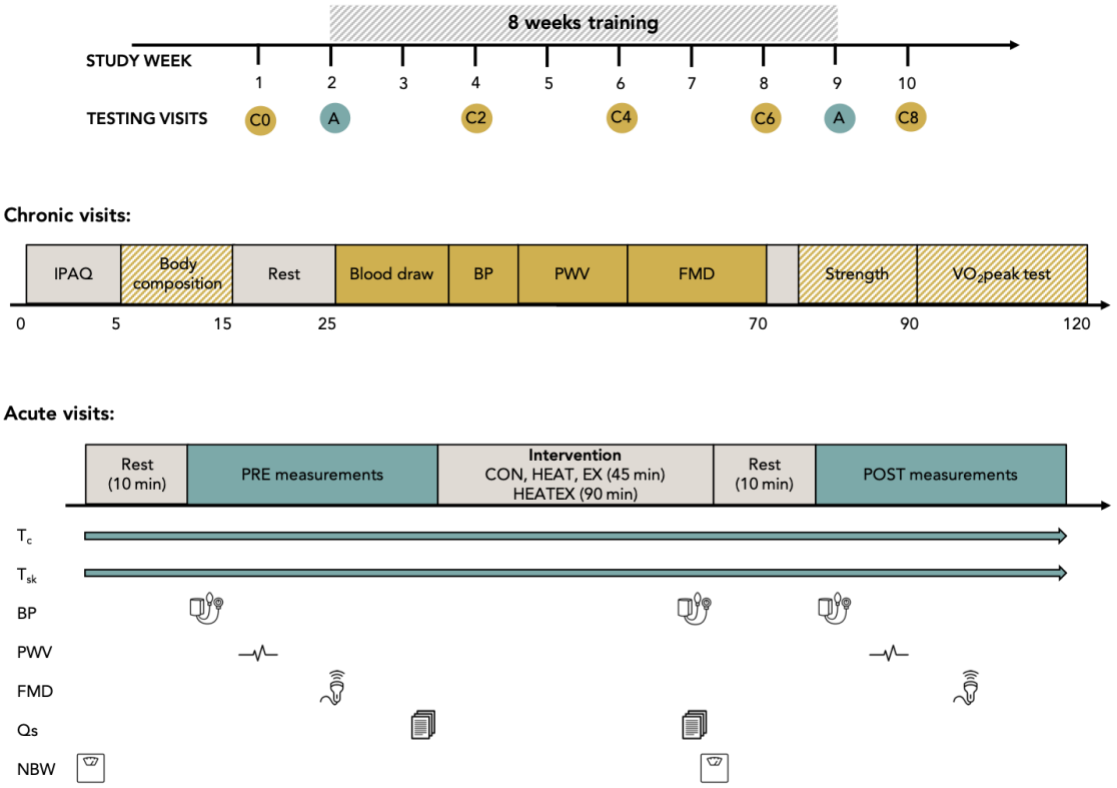

Figure 1. Study timeline and experimental protocols. This randomized controlled trial involved 8 weeks of training embedded within a 10-week time period. Participants came to the lab for both chronic visits (C0, C2, C4, C6, C8) to track responses to training over time, and acute visits (A) to examine immediate responses to each intervention prescription. Abbreviations: IPAQ = International Physical Activity Questionnaire, BP = blood pressure, PWV = pulse wave velocity, FMD = flow-mediated dilation test, CON = control, HEAT = heat therapy, EX = exercise training, HEATEX = combined training, T_c = core temperature, T_{sk} = skin temperature, Qs = perceptual, effort, and enjoyment questionnaires, NBW = nude body weight.

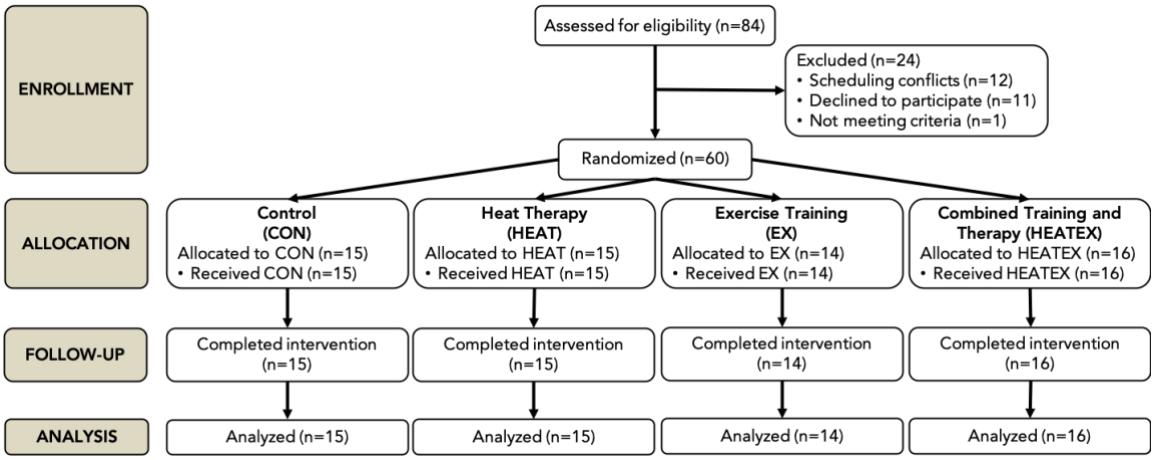

Figure 2. Participant recruitment flow diagram. Abbreviations: n, number of human participants

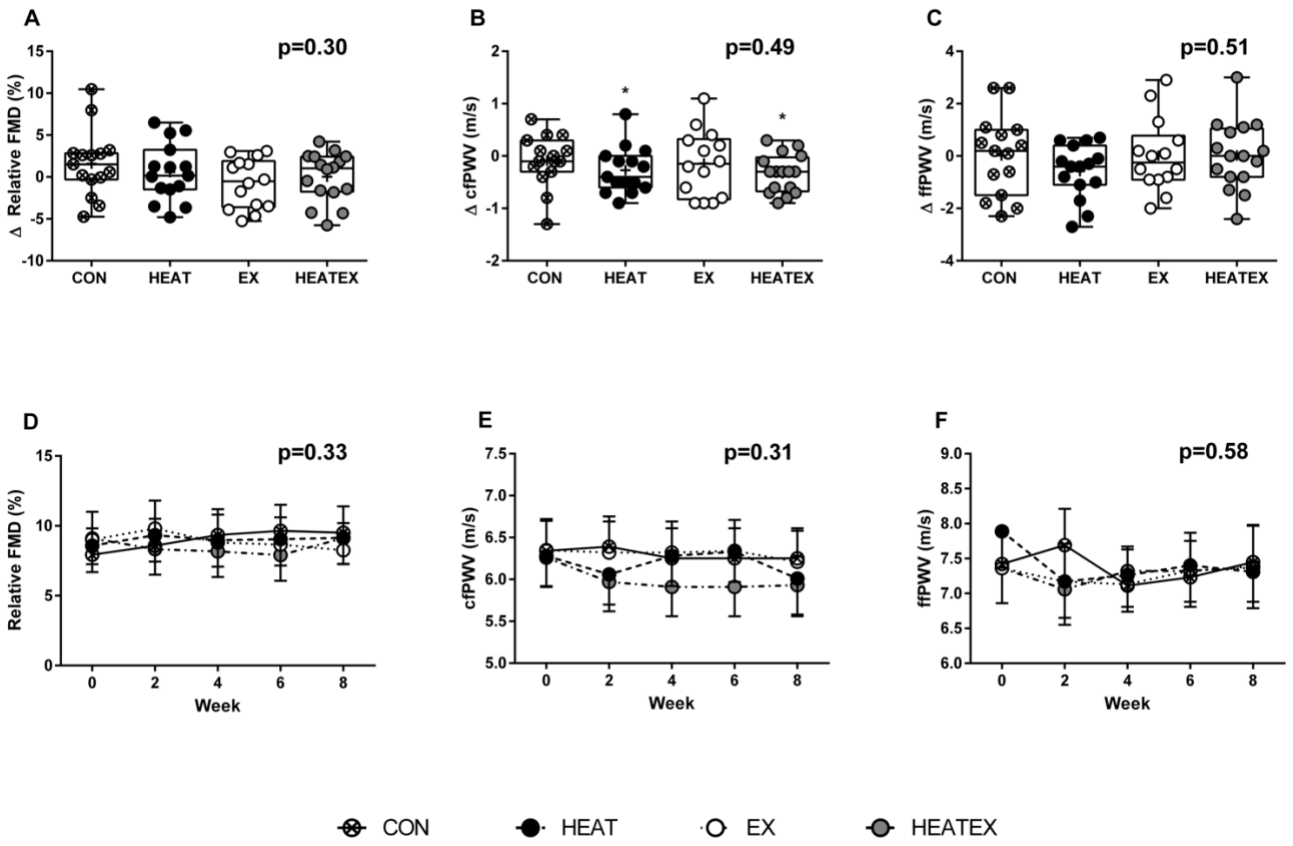

Figure 3. Vascular function responses to therapy and/or training. Panels A-C depict the one-way ANOVA on the week 0 to 8 change scores in standard box and whisker plots with individual data points overlaid. Panels D-F depict the trajectory analyses for each outcome variable with the error bars representing the 95% CI. Solid lines and circles with an "X" represent CON, dashed lines and black circles represent HEAT, dotted lines and white circles represent EX, and dotted-dashed lines and gray circles represent HEATEX. P values indicated are for the intervention group by time interaction effect. Abbreviations: FMD = flow-mediated dilation, cfPWV = carotid-femoral pulse wave velocity, ffPWV = femoral-foot pulse wave velocity. Significance indicators: * = Significant within-group change.

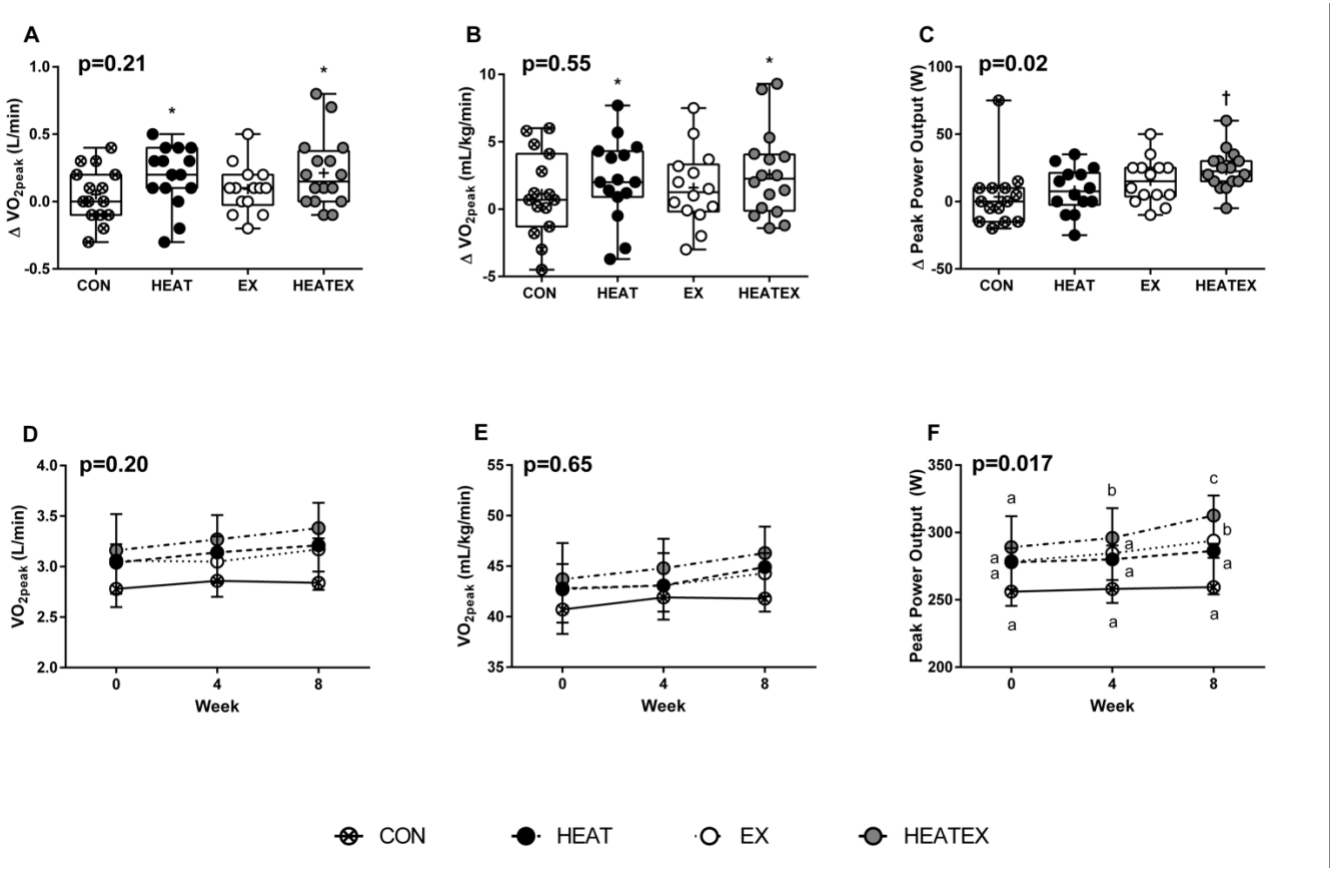

Figure 4. Cardiorespiratory fitness responses to therapy and/or training. Panels A-C depict the one-way ANOVA on the week 0 to 8 change scores in standard box and whisker plots with individual data points overlaid. Panels D-F depict the trajectory analyses for each outcome variable with the error bars representing the 95% CI. Solid lines and circles with an "X" represent CON, dashed lines and black circles represent HEAT, dotted lines and white circles represent EX, and dotted-dashed lines and gray circles represent HEATEX. P values indicated are for the training group by time interaction effect. Abbreviations: VO_2peak = peak oxygen uptake. Significance indicators: * = Significant within-group change, † = Different vs. CON.

Table 1. Participant characteristics

	All (N=60)	CON (N=15)	HEAT (N=15)	EX (N=14)	HEATEX (N=16)
Sex and gender, F and W (%)	30 (50.0%)	7 (46.7%)	8 (53.3%)	7 (50.0%)	8 (50.0%)
Age (years)	23 ± 3	24 ± 4	23 ± 3	22 ± 4	22 ± 2
Height (m)	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1
Weight (kg)	70.8 ± 14.3	68.0 ± 12.3	71.2 ± 13.7	71.2 ± 17.1	72.4 ± 15.0
BMI (kg/m^2)	24.0 ± 3.6	23.2 ± 3.4	24.4 ± 3.2	24.9 ± 5.0	23.6 ± 2.9
Resting SBP (mmHg)	112 ± 9	110 ± 9	109 ± 10	113 ± 7	115 ± 9
Resting DBP (mmHg)	63 ± 6	64 ± 5	63 ± 8	63 ± 4	63 ± 6
Resting MAP (mmHg)	81 ± 6	81 ± 6	80 ± 8	81 ± 4	82 ± 7
Resting HR (bpm)	63 ± 10	64 ± 8	61 ± 8	64 ± 12	64 ± 13
VO_2peak (ml/kg/min)	42.5 ± 8.7	40.7 ± 8.7	42.8 ± 6.1	42.7 ± 11.7	43.7 ± 8.2
Female menstrual cycle					
Type of contraceptive (%)					
Naturally cycling	14 (46.7%)	4	2	4	4
Oral contraceptive pill	14 (46.7%)	3	5	3	3
Vaginal ring	1 (0.03%)	0	0	0	1
Intrauterine device	1 (0.03%)	0	1	0	0
Phase of cycle at baseline (%)					
Early follicular	7 (23.3%)	3	1	3	0
Late follicular	6 (20.0%)	0	2	1	3
Early luteal	5 (16.7%)	2	1	1	1
Late luteal	11 (36.7%)	2	3	2	4
No period	1 (0.03%)	0	1	0	0
Race and ethnicity					
Race					
Aboriginal	0 (0.0%)	0	0	0	0
Asian	19 (31.7%)	7	3	5	4
Black or African American	0 (0.0%)	0	0	0	0
Hispanic, Latino, or Spanish	0 (0.0%)	0	0	0	0
Middle Eastern or North African	5 (8.3%)	1	2	1	1
Pacific Islander	0 (0.0%)	0	0	0	0
White or Caucasian	32 (53.3%)	6	10	6	10
Prefer to self-describe	4 (6.7%)	1	0	2	1
Ethnicity (origins)					
African	4 (6.7%)	1	1	1	1
Asian	21 (35.0%)	7	4	6	4
Caribbean	0 (0.0%)	0	0	0	0
European	20 (33.3%)	4	5	3	8
Latin, Central, and South American	0 (0.0%)	0	0	0	0
North American Aboriginal	0 (0.0%)	0	0	0	0
Other North American	3 (5.0%)	0	1	1	1
Oceania	0 (0.0%)	0	0	0	0
Pacific Islands	0 (0.0%)	0	0	0	0
Mixed	12 (20.0%)	3	4	3	2

Data are expressed as mean ± standard deviation. Abbreviations: F = female, W = women, BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, MAP = mean arterial pressure, HR = heart rate, VO_2peak = peak oxygen uptake.

Table 2. Cardiovascular function variables with therapy and/or training

	CON		HEAT		EX		HEATEX			
	Mean	**95% CI**	**Mean**	**95% CI**	**Mean**	**95% CI**	**Mean**	**95% CI**	**p (Δ)**	**p (trajectory)**
Absolute FMD (mm)									0.52	0.34
Week 0	0.26	0.21, 0.32	0.29	0.23, 0.34	0.30	0.24, 0.35	0.31	0.25, 0.36		
Week 2	0.28	0.23, 0.34	0.32	0.26, 0.37	0.32	0.26, 0.38	0.29	0.24, 0.34		
Week 4	0.29	0.24, 0.35	0.31	0.26, 0.37	0.29	0.24, 0.35	0.28	0.22, 0.33		
Week 6	0.32	0.27, 0.38	0.31	0.26, 0.37	0.28	0.23, 0.34	0.27	0.22, 0.33		
Week 8	0.30	0.24, 0.35	0.31	0.26, 0.37	0.28	0.22, 0.33	0.32	0.26, 0.37		
BL arterial diameter (mm)									0.36	0.25
Week 0	3.43	3.10, 3.76	3.57	3.24, 3.90	3.54	3.19, 3.88	3.47	3.15, 3.79		
Week 2	3.38	3.05, 3.71	3.59	3.25, 3.92	3.55	3.21, 3.89	3.49	3.17, 3.81		
Week 4	3.31	2.98, 3.65	3.55	3.22, 3.89	3.53	3.18, 3.87	3.48	3.16, 3.80		
Week 6	3.42	3.09, 3.76	3.56	3.23, 3.89	3.52	3.18, 3.87	3.50	3.17, 3.82		
Week 8	3.36	3.03, 3.69	3.55	3.22, 3.88	3.58	3.23, 3.92	3.44	3.12, 3.76		
PK arterial diameter (mm)									0.89	0.49
Week 0	3.69	3.36, 4.02	3.86	3.53, 4.19	3.83	3.49, 4.18	3.78	3.46, 4.10		
Week 2	3.66	3.33, 3.99	3.90	3.57, 4.23	3.87	3.53, 4.21	3.78	3.46, 4.10		
Week 4	3.61	3.28, 3.94	3.86	3.53, 4.19	3.81	3.47, 4.15	3.75	3.43, 4.07		
Week 6	3.75	3.42, 4.08	3.88	3.54, 4.21	3.81	3.47, 4.15	3.77	3.45, 4.09		
Week 8	3.65	3.32, 3.98	3.86	3.53, 4.19	3.83	3.49, 4.17	3.75	3.43, 4.07		
AUC shear rate (10^3 s^{-1})									0.54	0.86
Week 0	16.0	9.1, 22.8	19.2	12.3, 26.0	23.3	16.2, 30.3	25.9	19.3, 32.5		
Week 2	23.3	16.5, 30.1	22.4	15.6, 29.2	23.0	16.0, 30.1	21.8	15.2, 28.4		
Week 4	23.7	16.8, 30.5	21.4	14.6, 28.3	21.2	14.1, 28.2	25.4	18.8, 32.0		
Week 6	19.4	12.6, 26.2	20.6	13.8, 27.4	21.0	13.9, 28.1	23.1	16.5, 29.7		
Week 8	20.6	13.8, 27.4	21.5	14.7, 28.3	26.1	19.0, 33.2	24.1	17.3, 30.9		
TTP (s)									0.18	0.35
Week 0	40.5	33.4, 47.7	38.4	31.3, 45.6	45.0	37.6, 52.4	44.6	37.6, 51.5		
Week 2	46.3	39.1, 53.4	41.6	34.5, 48.8	48.9	41.4, 56.3	44.8	37.9, 51.8		
Week 4	45.1	38.0, 52.3	42.9	35.8, 50.1	39.3	31.9, 46.8	49.1	42.2, 56.1		
Week 6	45.1	37.9, 52.2	42.3	35.2, 49.5	40.3	32.9, 47.8	43.1	36.2, 50.1		
Week 8	45.7	38.5, 52.9	46.7	39.5, 53.8	39.7	32.2, 47.1	48.2	41.2, 55.1		
Resting SBP (mmHg)									0.41	0.25
Week 0	110	105, 114	109	104, 113	113	108, 117	115	111, 120		
Week 2	111	107, 116	111	107, 115	113	109, 118	114	110, 118		
Week 4	111	107, 115	110	106, 115	111	107, 116	112	108, 116		
Week 6	110	106, 115	111	106, 115	112	108, 117	113	108, 117		

Week 8	111	106, 115	109	105, 114	111	106, 115	114	110, 118		
Resting DBP (mmHg)									0.50	0.68
Week 0	64	61, 67	63	61, 66	63	60, 66	63	60, 66		
Week 2	65	62, 68	63	60, 66	63	60, 66	62	59, 64		
Week 4	64	61, 67	62	60, 65	62	59, 65	60	57, 63		
Week 6	64	61, 67	64	62, 67	62	59, 65	60	58, 63		
Week 8	66	63, 69	63	60, 66	62	59, 65	62	59, 65		
Resting MAP (mmHg)									0.70	0.18
Week 0	81	78, 85	80	77, 84	81	78, 85	82	79, 86		
Week 2	83	80, 86	81	77, 84	82	78, 85	82	78, 85		
Week 4	82	79, 85	80	77, 84	80	77, 84	80	77, 83		
Week 6	81	78, 85	81	78, 85	81	78, 85	77	74, 80		
Week 8	83	79, 86	80	77, 84	81	77, 84	82	79, 85		
Resting HR (bpm)									0.25	**0.02**
Week 0	64	59, 69	61	56, 66	64	59, 69	64	59, 68		
Week 2	66	62, 71	64	59, 69	62	57, 67	63	59, 68		
Week 4	63	58, 67	63	58, 67	64	59, 68	60	56, 65		
Week 6	68	63, 72	63	58, 68	61	56, 66	60	56, 65		
Week 8	66	62, 71	60	55, 65	61	57, 66	62	58, 67		

Data are expressed as means and 95% confidence intervals (CI). Abbreviations: FMD = flow-mediated dilation, BL = baseline, PK = peak, AUC = area under the curve, TTP = time to peak, SBP = systolic blood pressure, DBP = diastolic blood pressure, MAP = mean arterial pressure, HR = heart rate. P values are indicated for both the one-way ANOVA on the week 0 to 8 change scores (p (Δ)) and the mixed-model trajectory analysis (p (trajectory)) for each outcome variable, and those that are bolded indicate statistical significance.

Supplementary Table 1. Characterization of intervention stimuli

	CON		p	HEAT		p	EX		p	HEATEX-EX		p
	M	**95% CI/IQR**		**M**	**95% CI/IQR**		**M**	**95% CI/IQR**		**M**	**95% CI/IQR**	
PHYSIOLOGICAL												
T_{core}			0.29			**0.004**			**<0.001**			**<0.001**
Pre	37.0	36.8, 37.2		37.1 [a]	36.9, 37.3		37.2 [a]	37.0, 37.4		37.1 [a]	0.4	
During (45)	36.9	36.7, 37.0		37.5 [b]	37.3, 37.7		37.9 [b]	37.7, 38.1		37.8 [b]	0.2	
During (90)										37.6 [b]	0.2	
Post	36.8	36.6, 37.0		37.3 [a]	36.9, 37.3		37.6 [ab]	37.3, 37.8		37.3 [a]	0.3	
T_{foot}			**0.03**			**<0.001**			**<0.001**			**<0.001**
Pre	28.8 [a]	27.9, 29.8		29.7 [a]	2.9		29.7 [a]	28.9, 30.5		30.2 [a]	1.5	
During (45)	27.3 [ab]	26.4, 28.3		41.1 [b]	0.8		32.1 [b]	31.4, 32.9		32.8 [b]	2.4	
During (90)										40.6 [b]	0.8	
Post	27.0 [b]	26.1, 28.0		31.9 [a]	2.0		30.3 [a]	29.5, 31.0		32.0 [a]	1.7	
Mean T_{skin}			0.17			0.14			0.09			**<0.001**
Pre	32.2	31.8, 32.6		32.4	0.7		32.1	31.6, 32.7		32.3 [a]	0.9	
During (45)	31.8	31.4, 32.1		32.8	0.8		32.9	32.4, 33.4		33.3 [bc]	1.8	
During (90)										33.2 [b]	1.1	
Post	32.2	31.8, 32.6		32.9	0.8		32.8	32.2, 33.3		32.6 [ac]	1.0	
MAP			0.17			0.054			**<0.001**			**<0.001**
Pre	81	78, 85		80	77, 84		81 [a]	77, 85		83 [a]	9	
During (45)	86	82, 89		87	83, 90		97 [b]	92, 101		104 [b]	16	
During (90)										87 [a]	9	
Post	84	80, 87		83	79, 87		83 [a]	79, 87		84 [a]	10	
HR			0.28			**<0.001**			**<0.001**			**<0.001**
Pre	64	60, 68		59 [a]	10		64 [a]	58, 70		67 [a]	20	
During (45)	67	62, 71		82 [b]	21		134 [b]	127, 140		140 [b]	14	
During (90)										92 [c]	15	
Post	62	58, 66		61 [a]	16		71 [a]	64, 77		70 [a]	14	
PSYCHOSOCIAL												
Affect												0.81
Pre	2.2	1.4, 3.1		2.7	1.4, 3.1		1.8	0.9, 2.7		2.9	2.1, 3.8	
Post	2.3	1.4, 3.1		2.7	1.8, 3.5		2.2	1.3, 3.1		3.0	2.2, 3.8	
Arousal												0.06
Pre	46	44, 49		45	43, 47		46	43, 48		46	44, 48	
Post	45	43, 47		39	37, 42		41	39, 43		43	41, 45	

RPE									**<0.001**
Pre	6.1 [a]	5.5, 6.6	6.1 [a]	5.6, 6.7	6.0 [a]	5.4, 6.6	6.0 [a]	5.5, 6.5	
Post	6.1 [a]	5.5, 6.6	6.9 [a]	6.4, 7.5	12.3 [b]	11.7, 12.8	6.7 [a]	6.2, 7.2	
TC									**<0.001**
Pre	-1.1 [a]	-1.6, -0.5	-1.0 [a]	-1.5, -0.5	-0.6 [a]	-1.1, -0.03	-0.6 [a]	-1.1, -0.1	
Post	-1.1 [a]	-1.7, -0.6	1.7 [b]	1.1, 2.2	1.6 [b]	1.1, 2.2	1.3 [b]	0.8, 1.8	
TS									**<0.001**
Pre	3.9 [a]	3.5, 4.4	3.7 [a]	3.3, 4.2	4.4 [a]	3.9, 4.8	4.4 [a]	4.0, 4.9	
Post	3.8 [a]	3.4, 4.3	7.4 [b]	7.0, 7.9	6.9 [b]	6.5, 7.4	6.9 [b]	6.5, 7.4	
Enjoyment									**0.003**
Post	65	56, 74	82	73, 91	84†	74, 93	90†	80, 99	

Data are expressed as means and 95% confidence intervals for parametric analyses and medians and interquartile ranges for nonparametric analyses. Physiological variables were analyzed using separate one-way ANOVAs for each group with time as the independent variable (3 levels for CON, HEAT, and EX; 4 levels for HEATEX). Psychosocial variables were analyzed using 4 x 2 group x time mixed ANOVAs (perception measures) and a one-way ANOVA with group as the independent variable (enjoyment). Abbreviations: T_{core} = core temperature, T_{foot} = foot skin temperature, T_{skin} = skin temperature, MAP = mean arterial pressure, HR = heart rate, RPE = rating of perceived exertion, TC = thermal comfort, TS = thermal sensation. Significance indicators: **P value** = Significant overall effect; abc = Within a condition, values with no common letters are different from each other; † = Different vs. CON.

Supplementary Table 2. Body composition and skeletal muscle strength with therapy and/or training

	CON		HEAT		EX		HEATEX			
	M	**95% CI**	**M**	**95% CI**	**M**	**95% CI**	**M**	**95% CI**	**p (Δ)**	**p (trajectory)**
Body mass (kg)									0.66	0.59
Week 0	23.4	18.9, 27.8	24.9	20.5, 29.3	28.2	23.6, 32.8	24.8	20.5, 29.1		
Week 4	22.7	18.2, 27.2	24.1	19.6, 28.6	27.1	22.4, 31.7	24.6	20.2, 28.9		
Week 8	22.7	18.1, 27.3	24.4	19.8, 29.0	26.8	22.1, 31.6	24.1	19.7, 28.6		
Body fat (%)									0.66	0.59
Week 0	23.4	18.9, 27.8	24.9	20.5, 29.3	28.2	23.6, 32.8	24.8	20.5, 29.1		
Week 4	22.7	18.2, 27.2	24.1	19.6, 28.6	27.1	22.4, 31.7	24.6	20.2, 28.9		
Week 8	22.7	18.1, 27.3	24.4	19.8, 29.0	26.8	22.1, 31.6	24.1	19.7, 28.6		
Peak torque (Nm)									0.79	0.45
Week 0	134	115, 153	138	119, 157	133	114, 153	128	110, 147		
Week 4	135	116, 154	132	113, 151	132	112, 152	127	108, 145		
Week 8	131	112, 150	139	120, 158	136	117, 156	130	111, 148		

Data are expressed as means (M) and 95% confidence intervals (CI). P values are indicated for both the one-way ANOVA on the week 0 to 8 change scores (p (Δ)) and the mixed-model trajectory analysis (p (trajectory)) for each outcome variable.

Supplementary Table 3. Thermoregulatory variables with therapy and/or training

	CON		HEAT		EX		HEATEX		p
	M	**95% CI/IQR**	**M**	**95% CI/IQR**	**M**	**95% CI/IQR**	**M**	**95% CI/IQR**	
Resting T_{core} (°C)									0.15
Δ Week 0 to 8	0.07	0.31	-0.01	0.41	-0.23	0.60	-0.13	0.31	
Resting mean T_{skin} (°C)									0.99
Δ Week 0 to 8	-0.02	-0.44, 0.40	0.05	-0.36, 0.45	0.01	-0.41, 0.43	0.08	-0.31, 0.47	
WBSR (L/h)									0.73
Δ Week 0 to 8	0.00	0.43	-0.10	0.27	-0.04	0.30	0.03	0.22	

Data are expressed as means (M) and 95% confidence intervals (CI) for parametric analyses and medians (M) and interquartile ranges (IQR) for nonparametric analyses. Multiple one-way ANOVAs on the week 0 to 8 change scores in each of the variables were conducted. Abbreviations: T_{core} = core temperature, T_{skin} = skin temperature, WBSR = whole-body sweat rate. The p value is listed for the group effect.

CHAPTER 4

STUDY 3

Relationships between acute and chronic vascular function responses to interventions in young, healthy recreationally active adults

Acute vascular function responses predict chronic vascular function responses to heating and exercise interventions in young, healthy recreationally active adults

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RUNNING HEAD: Relationships between acute and chronic vascular responses

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Abstract

Vascular physiologists frequently employ acute study designs, particularly in the preliminary assessment of potential health-promoting interventions, because they are more feasible to execute. In theory, the physiological signals generated during an acute intervention session can be extrapolated in chronic repeated interventions to generate either functional or structural changes in the artery. However, there is currently a lack of consensus for how acute responses should be interpreted as they relate to predicting chronic changes. The objectives of this study were to determine (1) whether acute changes in arterial endothelial function and stiffness in response to blood flow stimulating interventions predict chronic changes in the same variables following an 8-week repeated intervention, and (2) whether acute *functional* changes in the vasculature in response to blood flow stimulating interventions are associated with chronic *structural* artery changes. In this randomized controlled trial, young, healthy recreationally active adults underwent 8 weeks of either no intervention (CON), lower limb heat therapy (HEAT), moderate-intensity exercise training (EX), or combined training and therapy (HEATEX). Endothelial function was assessed via a brachial artery (BA) flow-mediated dilation test (FMD), and arterial stiffness was assessed via central (carotid-femoral, cfPWV) and peripheral (femoral-foot, ffPWV) pulse wave velocity. BA diameter was measured using ultrasonography. Vascular assessments were conducted before and after each participant's first intervention session as well as after the training and/or therapy period to determine acute and chronic responses respectively. Forty-five participants allocated into HEAT (n=15), EX (n=14), or HEATEX (n=16) were included in the analysis, while the control

group was not included as there was no blood flow increasing acute intervention in that condition. The acute responses were associated with chronic responses for absolute (β=0.56 [SE=0.18]; p=0.003) and relative BA FMD (β=0.54 [SE=0.21]; p=0.013) and ffPWV (β=0.73 [SE=0.17]; p<0.001), however there was no significant relationship between the acute BA FMD response (*functional*) and the chronic BA diameter (*structural*) response (p=0.36). Acute responses of BA FMD and ffPWV to a blood flow increasing session can predict chronic (post intervention) responses in the same indices, and may be used in the future as a way to individualize health interventions.

INTRODUCTION

In vascular function research, acute intervention experiments are conducted for a number of reasons. Acute responses to stimuli can provide insight into the mechanistic underpinnings of an observed effect; but they are also interpreted as an indicator of the chronic changes in variables of interest when those stimuli are applied repeatedly as a longitudinal intervention. Theoretically, with repeated application of a stressor, the physiological signals that are triggered accumulate until eventually a new set point is reached (1). When exploring an intervention for which evidence is limited, researchers often favour acute studies as they are logistically more feasible. Acute findings may then provide the justification to conduct longer-term training studies to determine whether such interventions are effective.

Unfortunately, there exists an incomplete understanding of how the structure and function of the vasculature is regulated, and therefore additional examinations of the direction, time course, and pattern of acute responses are required. In a review by Dawson *et. al.* (2013), the authors propose that the acute brachial artery flow-mediated dilation (BA FMD) response to exercise abides by the biological phenomenon of hormesis such that immediately after exercise, there is a biphasic response where vascular function is transiently impaired before it recovers to baseline or supra-baseline levels, and over time, these repeated physiological stimuli serve as the impetus for sustained vascular remodeling (2). Thus far in the literature, the acute phase has been broadly defined (anywhere from immediately to 24 hours after an intervention), and documented findings have included the full spectrum of possible responses including acute decreases, no

change, and increases in BA FMD (2). The papers in this synthesis focus specifically on the BA FMD responses to exercise, but similarly variable outcomes have been observed with other vascular function indices such as arterial stiffness (3, 4) and physiological stressors like passive heat stress (5, 6).

To our knowledge, only a 2018 paper by Dawson and colleagues has attempted to discern whether acute vascular function responses have the ability to predict training-induced responses (7). In this study, researchers examined the BA FMD responses in 21 young, healthy men who performed 2 weeks of moderate-vigorous intensity cycling. They observed a significant, positive correlation between the acute and chronic changes in BA FMD ($r=0.63$, $p=0.002$), but proposed that, "Further work is needed to determine if similar relationships are found with [a] longer training programme, more intense exercise, in subjects with different activity/fitness levels or if it is necessary for structural rather than functional adaptations. (7)" Additionally, it is important to explore other physiological stressors (e.g., passive heating) and vascular function outcome measures (e.g. arterial stiffness). The focus of this paper will be on endothelial function assessed via BA FMD and arterial stiffness assessed via pulse wave velocity (PWV) as both are non-invasive metrics of vascular function that have been shown to be causally linked with future cardiovascular disease risk (8, 9).

The objectives of this study were two-fold: (1) to determine whether acute responses in BA FMD, cfPWV, and ffPWV as a result of a physiological stressor predict chronic changes in the same variables following an 8-week intervention, and (2) to determine whether acute vascular *function* (BA FMD) changes are associated with

chronic vascular *structural* changes (BA diameter). We hypothesize that there will be a relationship between acute and chronic vascular responses; and that there will be a relationship between acute functional and chronic structural changes in vascular function.

METHODS

Participants

Interested individuals were eligible for the study if they were between the ages of 18-35 years old and recreationally active. They were excluded from participation if they were currently smoking and/or using any vasoactive or recreational drugs; had a history of cardiovascular, metabolic, or musculoskeletal disease; were unable to participate in physical activity according to the Physical Activity Readiness Questionnaire (2020 PAR-Q+); and/or had a relative peak oxygen uptake (VO_2peak) ≥ 60 ml/kg/min at baseline.

Experimental Design & Setting

All study visits took place at the Vascular Dynamics Lab (Ivor Wynne Centre, Room E102) at McMaster University in Hamilton, Ontario, Canada and were collected between June 2021 to June 2022. Participants provided informed written consent prior to completing any portion of the study.

The present study was a secondary exploratory analysis of a randomized controlled trial (NCT04588103). The trial was approved by the Hamilton Integrated Research Ethics Board (project no. 12723). Details of the trial are reported elsewhere (Cheng *et. al.* 2023, unpublished). In brief, participants were randomly allocated into

either 8 weeks of a control (CON), heat therapy (HEAT), aerobic exercise training (EX), or combined training and therapy (HEATEX) intervention. The CON group continued with their usual lifestyle habits, while those participants that were allocated to an intervention group (i.e., HEAT, EX, HEATEX) attended supervised sessions 3 times per week for 8 weeks, with no more than two days between sessions. All participants were asked to maintain their current physical activity and dietary habits for the entire study duration. Only the training groups (i.e., HEAT, EX, HEATEX) were included in this study to address our specific research questions. Participants came to the laboratory for determinations of *acute* responses to the interventions on the day of their respective first intervention session, as well as for *chronic* testing visits before and after the 8-week intervention period for the assessment of the chronic responses to the repeated application of the interventions.

Interventions

Heat Therapy (HEAT)

Heat therapy involved 45-minute sessions of lower-limb foot warm water immersion using a commercially available foot bath (IVATION, Edison, NJ, USA) filled up to the "MAX" line. The temperature of the water was set to 109 °F (42.8 °C), and the water was allowed to circulate using the machine's "Bubble" function.

Exercise (EX)

Exercise training involved 45-minute sessions of moderate intensity cycling on a stationary cycle ergometer (Lode Corival; Gronigen, The Netherlands/Kettler Ergorace; Ense, Germany/SCIFIT upright bike; Tulsa, OK, USA/LifeFitness 95Ci; Toronto, ON, Canada). Participants warmed up for 3 minutes at 50 W, followed by 40 minutes of steady-state exercise between 70-75% of maximal heart rate (HR_{max}) achieved on a VO_2peak test performed at baseline, and cooled down for 2 minutes at 50 W.

Combined Heat Therapy and Exercise (HEATEX)

Combined heat therapy and exercise training involved 90-minute sessions of the 45-minute EX protocol immediately followed by the 45-minute HEAT protocol.

Testing Protocols

Prior to each testing visit, participants were reminded to come to the laboratory having abstained from moderate-vigorous physical activity for 24 hours, alcohol and caffeine for 12 hours, and food for 6 hours.

For the *acute testing visit*, participants came to the laboratory and were instrumented with our data acquisition devices while resting in the supine position for 10 minutes. Participants then followed the intervention protocol for the group they were assigned, and those allocated to CON performed quiet sitting for 45 minutes. Blood pressure and heart rate, blood flow, and shear rate were assessed before and during the intervention (approximately minutes 43-45 and 88-90, where applicable). Arterial

stiffness and endothelial function were assessed before and after the intervention (within 30 minutes).

For the *chronic testing visits*, participants came to the laboratory and began with 10 minutes of supine rest, during which participants were instrumented with two sets of single-lead ECG. Following the rest period, participants underwent assessments of resting heart rate and blood pressure (Dinamap Carescape V100, GE Healthcare; Mississauga, ON, Canada), arterial stiffness, and endothelial function in sequence.

Outcome Measures

Endothelial function

Endothelial function was assessed using a BA FMD test performed according to current guidelines using Duplex mode ultrasonography (Vivid q; GE Medical Systems; Horten, Norway) and a 12 MHz linear array probe at 7.7 fps to allow for simultaneous recording of artery diameter and blood velocity (10). The Doppler velocity gate was set to cover the entire arterial lumen and an insonation angle of 68° was used. For this test, participants abducted their right arm just under 90° away from midline of the body, and a pneumatic cuff was placed around their forearm. In the baseline phase, the brachial artery was imaged for 30 seconds to capture resting conditions. In the occlusion phase, the cuff was inflated to 200 mmHg for 5 minutes to generate an ischemic stimulus. In the reactive hyperemia phase, the artery was imaged for 3 minutes following cuff deflation. Ultrasound images were exported and blinded prior to being analyzed using semi-automated edge-tracking software (Arterial Measurement System; Gothenburg, Sweden).

Blood velocity audio signals from the ultrasound machine were passed through a spectral analyzer, which converts the analog intensity-weighted mean signal into a digital format for our data acquisition unit. The digital blood velocity data were then analyzed beat-by-beat on the associated data acquisition software (Labchart, AD Instruments) for the calculation of mean and peak shear rate and shear rate area under the curve to peak artery diameter. For poor quality blood velocity signals (n=17 participants), the velocity traces were instead analyzed from the raw ultrasound images using pixel-based tracking software (Measurements from Arterial Ultrasound Imaging (MAUI); Hedgehog Medical, Waterloo, ON, Canada). The same analysis tool was used for all files from the same participant for consistency. FMD was expressed in absolute and relative terms using the equations below:

$$(1)\ FMD\ (mm) = D_{peak} - D_{base}$$

$$(2)\ FMD\ (\%) = (\frac{D_{peak} - D_{base}}{D_{base}}) \times 100$$

where D_{base} is baseline artery diameter and D_{peak} is peak artery diameter. An experienced ultrasound sonographer (JLC) performed all FMD tests.

Arterial stiffness

Arterial stiffness was assessed both centrally and peripherally by measuring PWV between the carotid to femoral (cfPWV) and femoral to dorsalis pedis arteries (ffPWV), respectively. If the dorsalis pedis pulse could not be found, the tibialis posterior pulse was used instead. Pulse sites used remained consistent between visits for each participant. For PWV measurements, two operators used applanation tonometers to simultaneously collect

at least 30 continuous, clean pressure waveforms from the artery sites of interest. Afterwards, a tape measure was used to measure the distance over the surface of the body between the pulse sites. Pulse transit times were determined through foot-to-foot waveform analysis on data acquisition software (Labchart 8, AD Instruments; Colorado Springs, CO, USA). PWV was calculated using the equation below using two sets of 10 heart cycles of data. If the average of the two sets differed by more than 0.5 m/s, a third set of 10 heart cycles was included in the analysis. For cfPWV, the distance was multiplied by a factor of 0.8 as is recommended by the most recent consensus statement (11, 12).

$$(3)\ PWV\ (m/s) = \frac{distance}{pulse\ transit\ time}$$

Blood flow and shear rate

Blood flow and shear rate were calculated as the average of 30-second images collected using Duplex ultrasonography at rest and during each intervention (between 43-45 and 88-90 minutes, if applicable) using the formulas below:

$$(4)\ BF\ \left(\frac{ml}{\min}\right) = (\pi r^2 \times V) \times 60$$

$$(5)\ SR\ (s^{-1}) = \frac{8V}{D}$$

where r is the artery radius, V is mean blood velocity (MBV), and D is artery diameter. Mean SR was also divided into its component anterograde and retrograde parts to quantify the shear stimulus in both directions. A series of arithmetic functions was applied to the raw MBV channel on Labchart to extract anterograde and retrograde MBV

to use in equation 5. For files analyzed using MAUI, which automatically parses out anterograde and retrograde MBV, these variables were copied directly from the analysis output file. These variables were assessed during the acute visit.

Statistical Analysis

Participant demographic characteristics were summarized using means and standard deviations for continuous data, and frequencies and percentages for categorical data. To characterize the stimuli generated by each intervention, acute central hemodynamics, blood flow, and shear rate data were analyzed with separate one-way ANOVAs for each training group since the number of timepoints were not consistent across all groups (i.e., HEATEX had 4 timepoints due to the combination of both heating and exercise). The dependent variables were the vascular measure of interest, and the independent variable was time (3-4 levels: Pre, During (2 levels for HEATEX), Post). To characterize the acute and chronic training and/or therapy responses, one-way analyses of variance (ANOVAs) were conducted, with the change in the vascular function outcome (acute or chronic) as the dependent variable and group allocation (4 levels: CON, HEAT, EX, HEATEX) as the independent variable. If the assumptions for any of the ANOVAs were not met, non-parametric Kruskall-Wallis tests were used.

For both primary and secondary objectives, multiple regression analyses were conducted. For the primary objective (i.e., relationship between acute and chronic changes of the same variables), the dependent variable was the chronic change in the vascular measure and the independent variable of interest was the acute change in the

same measure at baseline. For the secondary objective (i.e., relationship between acute functional and chronic structural changes), the dependent variable was the chronic change in BA diameter and the independent variable of interest was acute change in BA FMD. Due to the use of different intervention stimuli, group allocation was included as an additional independent variable. To determine whether relationships might be intervention-specific, we added a group by acute change interaction term to all regression models. If the interaction was not statistically significant, it was removed from the model. All assumptions for multiple regression analyses were tested and criteria for statistical outliers were determined *a priori* as having high influence on the data, defined by a Cook's distance greater than 4 divided by the sample size (i.e., 45), as well as a >10% change in beta-coefficients and change in interpretation of the model after removal of the outlier. All analyses were conducted on StataSE (Version 17.0, StataCorp, College Station, TX, USA) and significance level was set *a priori* to $p<0.05$.

RESULTS

Participant characteristics

Out of 84 individuals screened for eligibility, 60 participants (30 female) were enrolled and randomized into either CON (n=15), HEAT (n=15), EX (n=14), or HEATEX (n=16) **(Figure 1)**. After removing the CON group, 45 participants were included in the analysis for our study objectives. Participants were young (age: 23±3 years), healthy (BMI: 24.0±3.6 kg/m^2, BP: 112±9/63±6 mmHg), and moderately fit

(VO_2peak: 42.5±8.7 ml/kg/min) **(Table 1)**. Intervention groups had similar sex distribution and baseline cardiorespiratory fitness levels.

Characterization of the acute and chronic responses to interventions

The acute cardiovascular responses to each intervention session are outlined in **Supplementary Table 1**. Blood pressure (MAP) increased acutely only in response to exercise (i.e., EX and the exercise component of HEATEX) (all $p<0.05$), whereas heart rate increased in all groups ($p<0.05$) except CON ($p=0.28$). Mean HR during all HEAT, EX, and HEATEX training sessions were equivalent to 47%, 71%, and 60% of HR_{max}, respectively. Mean and anterograde BF and SR increased in all but the CON group (all $p\leq0.001$), while retrograde BF and SR decreased with HEAT (median: -4.3 (IQR: 5.6) to -0.3 (1.2) ml^3/min; $p=0.002$ and median: -14.1 (IQR: 18.6) to -0.5 (4.3) s^{-1}; $p<0.001$ respectively) and increased with EX (median: -3.8 (IQR: 3.8) to -16.5 (18.6) ml^3/min; $p=0.002$ and median: -14.9 (IQR: 16.0) to -73.4 (47.8) s^{-1}; $p=0.001$ respectively) and during the exercise component of HEATEX (median: -3.1 (IQR: 7.0) to -15.1 (15.7) ml^3/min; $p=0.006$ and median: -10.1 (IQR: 18.8) to -48.6 (58.4) s^{-1}; $p<0.001$ respectively). There were no differences in the acute change in absolute FMD, relative FMD, and cfPWV ($p=0.76$) between groups following the first intervention session **(Supplementary Table 2)**. However, ffPWV ($p=0.044$), decreased acutely following HEAT vs. CON (median: Δ-0.60 (IQR: 1.10) vs. median: Δ0.20 (IQR: 1.70) m/s) **(Supplementary Table 2)**.

There were no differences in the chronic change in absolute FMD (p=0.52), relative FMD (p=0.30), ffPWV (p=0.51), and BA diameter (p=0.36) between groups with intervention **(Supplementary Table 3)**. While there were observed within-group reductions in cfPWV with HEAT (Δ-0.27 [-0.53, -0.02] m/s) and HEATEX (Δ-0.33 [-0.58, -0.09] m/s), between-group differences were not significant (p=0.49) **(Supplementary Table 3)**. A complete account of the training responses to each intervention is detailed in a companion paper (Cheng *et. al.* (2023), unpublished).

Relationships between acute and chronic vascular function responses

For all vascular function outcomes of interest, the group by acute change interaction was not significant (absolute FMD: p=0.17; relative FMD: p=0.36; cfPWV: p=0.05; ffPWV: p=0.35), suggesting that the relationship was not different between intervention groups. After removing the interaction term from each of the models, acute changes in absolute FMD (R^2=0.22; β=0.56 [SE=0.18]; p=0.003), relative FMD (R^2=0.16; β=0.54 [SE=0.21]; p=0.013), and ffPWV (R^2=0.36; β=0.73 [SE=0.17]; p<0.001) predicted corresponding chronic changes in these same variables following the 8-week interventions **(Figure 2A, B, and D and Table 2)**, but this was not the case for cfPWV (p=0.17) **(Figure 2C and Table 2)**.

Relationships between acute functional and chronic structural vascular outcomes

The group by acute change in relative FMD interaction was not significant (p=0.44), which suggests that the relationships were not different between intervention

groups. With the interaction term removed from the model, acute BA FMD (function) still did not predict chronic changes in BA diameter (structure) (p=0.36) **(Figure 2E and Table 2)**.

DISCUSSION

The aim of this study was to evaluate whether relationships exist between acute and chronic endothelial function and arterial stiffness responses to interventions that are associated with increases in blood flow and shear rate. We found that none of the interventions impacted BA FMD either acutely or chronically; but HEAT decreased ffPWV acutely, and both HEAT and HEATEX decreased cfPWV chronically. Regardless of intervention, acute responses in absolute FMD, relative FMD, and ffPWV were able to predict chronic responses in the same variables; whereas acute cfPWV changes held no predictive value for the same variable responses after the interventions. In the current study, acute changes in relative FMD (i.e., vascular function) were not related to chronic changes in artery diameter (i.e., vascular structure).

Acute responses to heat therapy and exercise training

Acute vascular function responses depend on a variety of factors. For exercise, these factors include the exercise intensity, duration, mode, and individual's training status. Generally, higher intensity, longer duration, resistance exercise, and lower fitness are associated with decreased FMD acutely post-exercise (2). In the current study, we did not see any acute changes in BA FMD, cfPWV, or ffPWV with EX which may be

indicative of insufficiently low exercise intensity and/or high baseline fitness. Some previous studies have also found no acute change in vascular function (13, 14). For heating, while less is known about how heat therapy parameters impact acute vascular responses, they seem to be dependent upon the timing of assessment, the proportion of the body heated, and the proximity of heating to the site of assessment (6, 15–23). The current results mostly replicate those found in a preceding experiment in our lab testing the efficacy of an acute bout of ankle- and knee-level heating (23). In the 2021 study, we observed that BA FMD (absolute, relative, and scaled) increased and ffPWV decreased acutely following 45 min of either ankle or knee-level heating. We attribute the transient reduction in lower limb arterial stiffness in both studies to a concomitant functional reduction in arterial tone to accommodate the need to dissipate heat. In the current study the acute decrease in ffPWV with accompanying chronic decreases in cfPWV in the HEAT group provides some support for the proposed temporality of the key mechanisms responsible for alterations in arterial stiffness (24). Specifically, acute changes in stiffness are functional and typically serve to maintain homeostasis in response to a stressor, which explains the localized nature of its effects; whereas chronic changes can be either functional or structural. If any structural adaptations take place, they may be detected more systemically and in association with the timeline for elastin and collagen deposition and breakdown in the adventitia (24). In contrast to our previous work, we did not see acute increases in BA FMD with HEAT. It's important to note that in our previous study, although there was a main effect of time, the finding appeared to be driven more by the larger magnitude acute changes in FMD with the knee rather than the ankle condition

(23). While we did consider using the knee condition for the current study, we ultimately opted for the ankle condition due to the commercial availability of foot baths and to assess a form of heat therapy that would be available to the general public. Heating rather than exercise may be responsible for this long-term response as the only notable effect of combined heat therapy and exercise training (HEATEX) was a chronic within-group decrease in cfPWV.

Relationships between acute and chronic changes in vascular function

We observed significant positive relationships between the acute responses and chronic responses in absolute FMD, relative FMD, and ffPWV in response to heating and exercise interventions. The model was not different based on intervention group allocation, indicating that at least for heat therapy, exercise training, and combined training, similar relationships exist. Although a negative relationship might have been expected based on the hormesis hypothesis, since we assessed vascular function approximately 30 minutes following each intervention, it is likely that we captured the normalized or supra-normalized phase of the acute response, which would yield a positive association. It is possible that the directionality of these relationships differs based on the timepoint of acute assessment, but further research is needed to confirm this hypothesis. Our results are aligned and build on the previous work by Dawson *et. al.* (2018) by exploring these relationships with longer duration training (8 vs. 2 weeks), more intervention types (heating and exercise vs. exercise only), more vascular function outcomes (PWV and FMD vs. FMD only) and in a moderately fit cohort of both males

and females (vs. males only) (7). Future research on this topic should expand on the design of the current study by including interventions that are known to acutely decrease vascular function (e.g., arterial compression, subsystolic cuff occlusion, Western diet, etc.) to see if these relationships persist in the negative quadrant (25–28). Indeed, a small number of participants in the current study had negative responses (e.g. decreased FMD or increased PWV) to their respective interventions both acutely and chronically which would support this idea, but given that these data points were scarce, more information is required in order to make such a conclusion.

We found no associations between the acute functional (BA FMD) and chronic structural (BA diameter) responses following the 8-week intervention period in the current study. That is, the acute relative BA FMD response to the first intervention session did not predict chronic changes in resting BA diameter. Our understanding of the time course of vascular adaptations comes from several papers that outline the chronic, exercise training-induced changes in endothelium-dependent and independent dilation in both animal and human models (29–31). Laughlin demonstrated that while short-term training (7-10 days) increased the endothelium-dependent dilation of the coronary arteries in dogs, swine, and rats, other data examining longer-term training did not show the same (32–34). He proposed that arterial remodeling in the form of increased arterial diameter results in a normalization of the shear stimulus experienced acutely during exercise, and therefore a reduction in the signaling stimulus for functional endothelial adaptation (30). Tinken *et. al.* (2008) then extended these findings to humans, and showed that BA FMD increased after 2-4 weeks of aerobic training before returning to baseline by 8 weeks,

while BA conduit artery dilator capacity progressively increased throughout the 8-week training period (31). In that study, conduit artery dilatory capacity in response to ischemic handgrip exercise was used as a surrogate of arterial remodeling as it has been shown to be less susceptible to fluctuations in sympathetic activity and local vasoactive agents. In contrast, our analysis involved the acute BA FMD responses (30 min post intervention) to the same interventions applied chronically (after 8 weeks of repeated interventions) in the training programs, which may have activated different regulatory pathways when compared to looking at changes in resting BA FMD week to week during a chronic intervention, as had been done previously. Furthermore, our index of vascular structure was resting BA diameter, which as mentioned above, has its limitations.

Strengths, Limitations, and Future Directions

Although this secondary analysis was part of a larger study that was a tightly-controlled randomized control trial that followed CONSORT guidelines and used reference standard methods and trained personnel for the assessment of vascular function, many elements of the study design were not chosen for the specific research questions addressed in this paper. Our data show that there is potential for indices of vascular function, such as FMD and PWV, to be used as tools to assist with informing individualized health prescription, but further research involving more heterogeneity in the types of interventions included and characteristics of the population studied (demographics, sex, ethnicity) will be necessary to move this field forward. Larger

samples should also be tested to allow for more complex regression analyses to determine the factors that contribute to positive vascular responsiveness.

CONCLUSION

Acute responses in BA FMD and ffPWV measured 30 minutes after an intervention session predict chronic responses in these outcome variables following 8 weeks of either heat therapy, exercise training, or combined exercise training and heat therapy. With further supporting evidence, these positive relationships may have utility in personalized lifestyle prescription to determine which health-promoting interventions would be well-suited to an individual with respect to maximizing vascular health outcomes.

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DISCLOSURES

No competing interests, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

The author contributions are outlined using the Consortia Advancing Standards in Research Administration Information (CASRAI) CRediT taxonomy (https://credit.niso.org/).

Conceptualization: JLC, MJM; **Data curation:** JLC, CAP, KCM, CMA, GKB, KSN; **Formal analysis:** JLC, CAP, KCM, CMA, GKB, KSN; **Funding acquisition:** MJM; **Investigation:** JLC, CAP, KCM, CMA, GKB; **Methodology:** JLC, KSN, MJM; **Project administration:** JLC, MJM; **Resources:** MJM; **Software:** N/A; **Validation:** KSN; **Visualization:** JLC; **Writing – original draft:** JLC, KSN (statistical analysis); **Writing – review and editing:** JLC, CAP, KCM, CMA, GKB, KSN, MJM.

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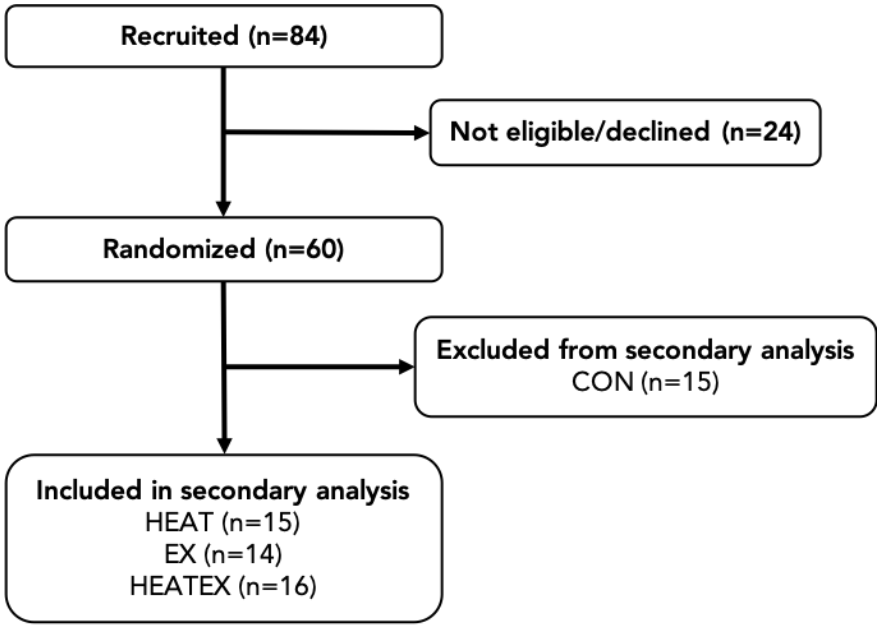

Figure 1. Participant recruitment flow diagram. While 60 participants completed the main trial, only 45 (HEAT, EX, and HEATEX groups) were included in the analysis for this study. n = number of participants.

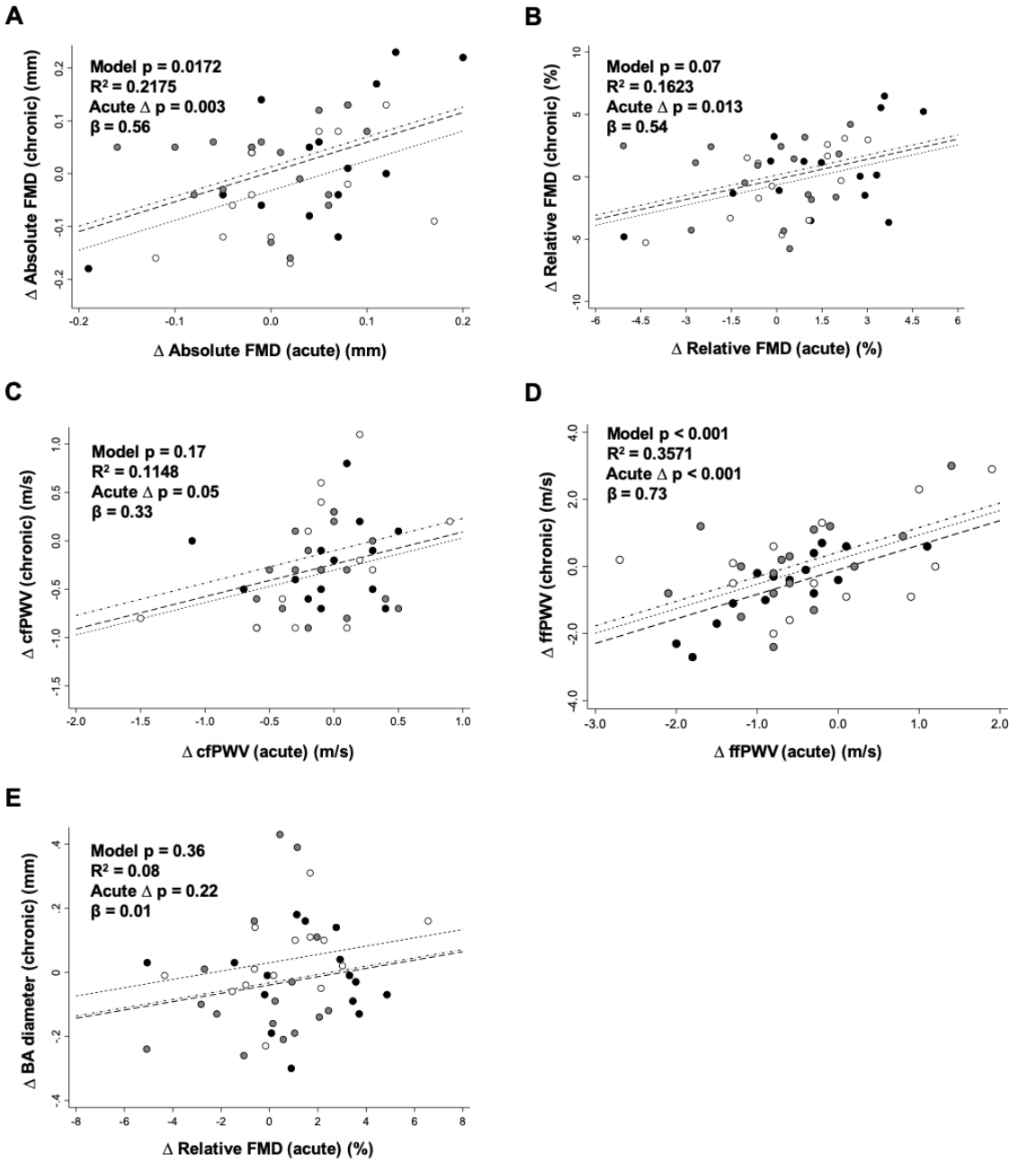

Figure 2. Regression models for vascular function predictive analyses. Panels A-D depict the relationships between the acute (x-axis, independent variable) and chronic (y-axis, dependent variable) intervention vascular function responses for absolute flow-mediated dilation, relative FMD, cfPWV, and ffPWV, respectively. Panel E depicts the relationship between the acute relative FMD response *(function)* and the chronic BA diameter response *(structure)*. Dashed lines and black circles represent HEAT, dotted lines and white circles represent EX, and dotted-dashed lines and gray circles represent HEATEX. Abbreviations: FMD = flow-mediated dilation, cfPWV = carotid-femoral pulse wave velocity, ffPWV = femoral-foot pulse wave velocity, BA = brachial artery.

Table 1. Participant characteristics

	All (N=45)	HEAT (N=15)	EX (N=14)	HEATEX (N=16)
Sex and gender, females and women (%)	23 (51.1%)	8 (53.3%)	7 (50.0%)	8 (50.0%)
Age (years)	22 ± 3	23 ± 3	22 ± 4	22 ± 2
BMI (kg/m^2)	24.2 ± 3.7	24.4 ± 3.2	24.9 ± 5.0	23.6 ± 2.9
Resting SBP (mmHg)	112 ± 9	109 ± 10	113 ± 7	115 ± 9
Resting DBP (mmHg)	63 ± 6	63 ± 8	63 ± 4	63 ± 6
Resting MAP (mmHg)	81 ± 7	80 ± 8	81 ± 4	82 ± 7
Resting HR (bpm)	63 ± 11	61 ± 8	64 ± 12	64 ± 13
VO_2peak (ml/kg/min)	43.1 ± 8.7	42.8 ± 6.1	42.7 ± 11.7	43.7 ± 8.2

Data are expressed as mean ± standard deviation. Abbreviations: BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, MAP = mean arterial pressure, HR = heart rate, VO_2peak = peak oxygen uptake.

Table 2. Regression analyses

Predicting chronic Δ absolute FMD					
Model statistics					
R^2	**Adjusted R^2**	**F**		**Model p**	
0.218	0.160	F(3,41) = 3.80		0.017	
Variable	**Beta coefficient**	**SE**	**t**	**95% CI**	**p**
Acute Δ absolute FMD	0.56	0.18	3.10	0.20, 0.93	**0.003**
Group (ref: HEAT)	-	-	-	-	0.41
EX	-0.04	0.04	-1.00	-0.11, 0.04	0.32
HEATEX	0.01	0.03	0.29	-0.06, 0.08	0.77
Predicting chronic Δ relative FMD					
Model statistics					
R^2	**Adjusted R^2**	**F**		**Model p**	
0.162	0.100	F(3,40) = 2.58		0.070	
Variable	**Beta coefficient**	**SE**	**t**	**95% CI**	**p**
Acute Δ relative FMD	0.54	0.21	2.61	0.12, 0.95	**0.013**
Group (ref: HEAT)	-	-	-	-	0.41
EX	-0.46	1.13	-0.40	-2.75, 1.83	0.69
HEATEX	0.34	1.10	0.31	-1.89, 2.57	0.76
Predicting chronic Δ cfPWV					
Model statistics					
R^2	**Adjusted R^2**	**F**		**Model p**	
0.115	0.050	F(3,41) = 1.77		0.170	
Variable	**Beta coefficient**	**SE**	**t**	**95% CI**	**p**
Acute Δ cfPWV	0.33	0.17	2.02	-0.00, 0.67	0.05
Group (ref: HEAT)	-	-	-	-	0.49
EX	0.14	0.17	0.81	-0.21, 0.49	0.52
HEATEX	-0.06	0.17	-0.37	-0.40, 0.28	0.71
Predicting chronic Δ ffPWV					
Model statistics					
R^2	**Adjusted R^2**	**F**		**Model p**	
0.357	0.310	F(3,41) = 7.59		<0.001	
Variable	**Beta coefficient**	**SE**	**t**	**95% CI**	**p**
Acute Δ ffPWV	0.73	0.17	4.41	0.40, 1.07	**<0.001**
Group (ref: HEAT)	-	-	-	-	0.38
EX	0.30	0.39	0.78	-0.49, 1.10	0.44
HEATEX	0.52	0.37	1.40	-0.23, 1.28	0.17
Predicting chronic Δ brachial artery diameter					
Model statistics					
R^2	**Adjusted R^2**	**F**		**Model p**	

0.080	0.007	F(3,41) = 1.11		0.360	
Variable	**Beta coefficient**	**SE**	**t**	**95% CI**	**p**
Acute Δ relative FMD	0.01	0.01	1.25	-0.01, 0.03	0.22
Group (ref: HEAT)	-	-	-	-	0.45
EX	0.07	0.06	1.15	-0.05, 0.19	0.26
HEATEX	0.01	0.06	0.12	-0.11, 0.13	0.91

Abbreviations: FMD = flow-mediated dilation, cfPWV = carotid-femoral pulse wave velocity, ffPWV = femoral-foot pulse wave velocity, SE = standard error, CI = confidence interval.

Supplementary Table 1. Characterization of intervention stimuli

	CON		p	HEAT		p	EX		p	HEATEX-EX		p
	M	**95% CI/IQR**		**M**	**95% CI/IQR**		**M**	**95% CI/IQR**		**M**	**95% CI/IQR**	
MAP (mmHg)			0.17			0.054			**<0.001**			**<0.001**
Pre	81	78, 85		80	77, 84		81 [a]	77, 85		83 [a]	9	
During (45)	86	82, 89		87	83, 90		97 [b]	92, 101		104 [b]	16	
During (90)										87 [a]	9	
Post	84	80, 87		83	79, 87		83 [a]	79, 87		84 [a]	10	
HR (bpm)			0.28			**<0.001**			**<0.001**			**<0.001**
Pre	64	60, 68		59 [a]	10		64 [a]	58, 70		67 [a]	20	
During (45)	67	62, 71		82 [b]	21		134 [b]	127, 140		140 [b]	14	
During (90)										92 [c]	15	
Post	62	58, 66		61 [a]	16		71 [a]	64, 77		70 [a]	14	
Mean SR (s^{-1})			0.52			**<0.001**			**<0.001**			**0.001**
Pre	129.5	96.7		142.2 [a]	97.9		173.7 [a]	92.9		169.0 [a]	187.0	
During (45)	124.3	80.9		465.2 [b]	410.1		389.5 [b]	308.8		419.8 [b]	232.6	
During (90)										373.6 [b]	369.8	
Anterograde SR (s^{-1})			0.27			**0.001**			**<0.001**			**<0.001**
Pre	151.4	72.8		162.0 [a]	98.3		197.5 [a]	63.0		199.1 [a]	169.0	
During (45)	135.4	87.8		469.0 [b]	414.8		468.2 [b]	365.8		457.1 [b]	274.8	
During (90)										377.6 [b]	350.7	
Retrograde SR (s^{-1})			0.49			**<0.001**			**0.001**			**<0.001**
Pre	-13.2	19.4		-14.1 [a]	18.6		-14.9 [a]	16.0		-10.1 [a]	18.8	
During (45)	-8.1	9.2		-0.5 [b]	4.3		-73.4 [b]	47.8		-48.6 [b]	58.4	
During (90)										-5.1 [a]	11.2	

Data are expressed as means (M) and 95% confidence intervals (CI) for parametric analyses and medians (M) and interquartile ranges (IQR) for nonparametric analyses. Outcomes were analyzed using separate one-way ANOVAs for each group with time as the independent variable. Abbreviations: SBP = systolic blood pressure, DBP = diastolic blood pressure, MAP = mean arterial pressure, HR = heart rate, SR = shear rate. Significance indicators: **P value** = Significant overall effect; abc = Within a condition, values with no common letters are different from each other.

Supplementary Table 2. Acute vascular function responses

	CON		HEAT		EX		HEATEX		
	M	**95% CI/IQR**	**M**	**95% CI/IQR**	**M**	**95% CI/IQR**	**M**	**95% CI/IQR**	**P (Δ)**
Absolute FMD (mm)	-0.004	-0.06, 0.05	0.04	-0.01, 0.09	0.02	-0.03, 0.08	-0.004	-0.05, 0.05	0.52
Relative FMD (%)	0.13	-1.51, 1.77	1.42	-0.22, 3.07	0.74	-0.96, 2.43	-0.22	-1.81, 1.37	0.50
BL AD (mm)	-0.06	-0.13, -0.001	-0.07	-0.13, -0.001	-0.04	-0.11, 0.02	0.02	-0.05, 0.08	0.20
PK AD (mm)	-0.07	-0.14, -0.003	-0.03	-0.09, 0.04	-0.02	-0.09, 0.05	0.01	-0.05, 0.08	0.36
AUC SR (10^3 s^{-1})	0.00	10.7	0.20	9.10	3.45	7.50	-3.10	22.3	0.67
TTP (s)	-3.73	-10.5, 3.04	3.42	-3.35, 10.2	1.41	-5.61, 8.42	2.21	-4.35, 8.77	0.46
cfPWV (m/s)	0.00	0.30	-0.10	0.60	-0.10	0.50	-0.10	0.40	0.76
ffPWV (m/s)	0.20	1.70	-0.60†	1.10	-0.45	1.70	-0.65	0.80	**0.04**

Data are expressed as means (M) and 95% confidence intervals (CI) for parametric analyses and medians (M) and interquartile ranges (IQR) for nonparametric analyses on the change scores pre to post intervention session. Abbreviations: FMD = flow-mediated dilation, BL = baseline, AD = artery diameter, PK = peak, AUC SR = shear rate area under the curve to peak diameter, TTP = time to peak, cfPWV = carotid-femoral pulse wave velocity, ffPWV = femoral-foot pulse wave velocity. Significance indicators: **P value** = Significant overall group effect, † = Different vs. CON.

Supplementary Table 3. Chronic vascular function responses

	CON		HEAT		EX		HEATEX		
	M	**95% CI**	**M**	**95% CI**	**M**	**95% CI**	**M**	**95% CI**	**P (Δ)**
Absolute FMD (mm)	0.04	-0.02, 0.09	0.03	-0.03, 0.08	-0.02	-0.08, 0.04	0.01	-0.04, 0.06	0.52
Relative FMD (%)	1.58	-0.15, 3.32	0.57	-1.17, 2.30	-0.74	-2.54, 1.05	0.02	-1.65, 1.70	0.30
BL AD (mm)	-0.07	-0.16, 0.02	0.02	-0.11, 0.07	0.04	-0.05, 0.13	-0.04	-0.12, 0.05	0.36
PK AD (mm)	0.04	-0.13, 0.05	0.01	-0.09, 0.10	-0.003	-0.10, 0.09	-0.03	-0.12, 0.06	0.89
AUC SR (10^3 s^{-1})	4.6	-2.2, 11.5	2.4	-4.5, 9.2	2.8	-4.3, 10.0	-2.2	-9.1, 4.7	0.54
TTP (s)	5.2	-3.6, 14.0	8.3	-0.6, 17.1	-5.3	-14.5, 3.8	3.6	-4.9, 12.1	0.18
cfPWV (m/s)	-0.09	-0.34, 0.17	-0.27	-0.53, -0.02	-0.14	-0.40, 0.12	-0.33	-0.58, -0.09	0.49
ffPWV (m/s)	0.03	-0.65, 0.72	-0.58	-1.26, 0.10	-0.01	-0.69, 0.72	-0.02	-0.64, 0.69	0.51

Data are expressed as means (M) and 95% confidence intervals (CI) on the change scores pre to post 8-week intervention. Abbreviations: FMD = flow-mediated dilation, BL = baseline, AD = artery diameter, PK = peak, AUC SR = shear rate area under the curve to peak diameter, TTP = time to peak, cfPWV = carotid-femoral pulse wave velocity, ffPWV = femoral-foot pulse wave velocity.

CHAPTER 5

DISCUSSION

5.1 Research Questions

Heat therapy is a health-promoting intervention that has the potential to make a substantial impact on the global community. Until recently, work in this area focused on cultures where many individuals already incorporate heat therapy into their everyday lives. Through these studies, researchers have found that regular sauna bathing (1–6), *onsen* bathing (7–9), Waon therapy (10–20), and Bikram yoga (21, 22) confer a multitude of benefits to physiological processes, clinical prognoses, and morbidity and mortality risk. Since the publication of these findings, most research has been focused on reverse-engineering heat therapy: uncovering the mechanisms underlying the positive effects of heat exposure so that more effective, feasible, and scalable passive heating prescriptions for traditional and novel health outcomes can be developed. Many experimental studies that have been conducted to date clearly demonstrate that heating protocols that use a "sledgehammer" approach in which heating is administered at relatively higher temperatures (40-42 °C), more frequently (4-5x/week), for relatively longer durations (45-90 min), and with a mode that involves heating most of, or the whole body, results in beneficial effects to almost every index of cardiovascular function (23–32), as well as some metrics of muscular and metabolic health (33–39). However, the volume and intensity of such protocols seem to be a deterrent to habitual use for a lot of individuals; especially when competing against the existing evidence about the health benefits of exercise training (40).

In **Chapter 1 (PhD Study 0)**, I was able to synthesize the findings from literature that existed at the time on the effects of passive heat therapy on vascular function in the

form of a narrative review as a means of identifying current gaps in knowledge from which I could develop a novel line of investigation. Through this paper, which was published in the Journal of Applied Physiology in 2019, I was able to deduce that despite having the evidence to support regular participation in heat therapy, its use was limited to those for whom facilities such as saunas, spas, and hot tubs were feasible, accessible, and tolerable (32). At this stage, it remained undetermined whether clinically relevant improvements in cardiovascular health could occur with a lower physiological stimulus delivered using a localized passive heating mode that would be far more scalable if found to be effective.

Therefore, the overarching goal of this PhD dissertation was to explore the utility of local heating to improve vascular function in young, healthy recreationally active adults. Young, healthy recreationally active adults were selected as a "proof of concept" population, recognizing that if local heating was effective and feasible, it could be applied to other populations in whom other health interventions such as exercise might be less available or adopted. As such, the broad aims of this thesis were as follows:

1. **Chapter 2 (PhD Study 1):** To establish, in the acute time frame, whether local ankle- or knee-level heating would be sufficient to alter upper and lower limb endothelial function and local and systemic arterial stiffness.
2. **Chapter 3 (PhD Study 2):** To evaluate the endothelial function and arterial stiffness responses to 8 weeks of ankle-level heat therapy alone, compared to, and in conjunction with moderate-intensity exercise training.

3. **Chapter 4 (PhD Study 3):** To determine whether relationships exist between the acute and chronic vascular function responses to heat therapy and exercise training as a means of understanding whether acute responses can inform personalized health and lifestyle prescriptions for the general public.

5.2 Contributions to Existing Knowledge

This section summarizes the main findings and research contributions from each study.

5.2.1 Development of a local heating protocol

In a cohort of 16 young, healthy adults, we found that 45-minute sessions of either ankle- or knee-level hot water immersion at 45 °C were equally effective at decreasing lower limb arterial stiffness (i.e., femoral-foot pulse wave velocity (ffPWV)) and increasing upper limb macrovascular endothelial function (i.e., brachial artery flow-mediated dilation (BA FMD)). Only knee-level heating additionally improved upper limb microvascular endothelial function (i.e., brachial artery peak vascular conductance) and plasma concentrations of extracellular heat shock protein-72. There were no changes in superficial femoral artery endothelial function (SFA FMD), carotid-femoral pulse wave velocity (cfPWV), common carotid artery distensibility, or plasma levels of interleukin-6. Using these data as evidence, we proceeded with exploring the chronic changes to vascular function, opting specifically for the implementation of ankle-level hot water immersion.

5.2.2 Evaluating the effectiveness of local heat therapy and exercise training for chronic vascular function changes

We employed an 8-week intervention involving heat therapy, exercise training, or combined exercise training and heat therapy 3 times per week between 45-90 minutes per session in 60 young, healthy recreationally active adults. The local foot heating protocol was modified from the initial acute study such that it was performed in the seated position and with the water temperature at 42.8 °C for ecological validity and based on previous participant feedback. None of the interventions resulted in training or therapy associated changes in BA FMD. For those interventions that involved heating (i.e., HEAT and HEATEX), there were observed within-group improvements in cfPWV and absolute and relative peak oxygen uptake (VO_2peak). For EX only, there was a within-group reduction in body fat percentage. There were no changes to leg strength or any thermoregulatory variables, including resting HR, whole-body sweat rate, core temperature, skin temperature, and thermal comfort. Overall, participants that were allocated to an intervention with an exercise component rated their activity to be more enjoyable when using the validated Physical Activity Enjoyment Scale (PACES) (41). We suspect that more definitive results may have been observed with a longer time frame of observation (e.g., 12 weeks to 6 months), a larger sample size (e.g., n=80-100), and/or a higher intensity of exercise.

5.2.3 Determining the predictive ability of acute to chronic vascular function responses

To further explore whether acute responses in vascular function following a single application of a heating and/or exercise intervention could predict chronic responses over time in an intervention period, we examined data from the first intervention session (acute visit) and data from the pre- and post-intervention chronic visits of our training and/or therapy groups (n=45). Our regression analyses showed that the acute responses in absolute FMD, relative FMD, and ffPWV following one session of heating and/or exercise can predict the chronic responses following 8 weeks of heat therapy and/or exercise training, with the model being the same regardless of intervention group allocation. There was no association between acute functional (i.e., BA FMD) and chronic structural (i.e., artery diameter) vascular outcomes. These findings suggest that assessing individual acute FMD and ffPWV responses to local heat therapy, aerobic exercise training, or combined exercise training and heat therapy may be useful for predicting if an individual will be responsive to a chronic intervention.

5.3 Limitations and Considerations

5.3.1 Intervention Protocols

When selecting the prescription parameters for our heating protocol, it was our goal to prioritize feasibility and scalability, such that if improvements in vascular function were observed, the general public could readily incorporate these modes of passive heat therapy into their daily lives. Although **Chapter 2** showed that knee-level heating was objectively more effective specifically when defined by the number of vascular function outcome measures acutely improved, we ultimately chose to use ankle-level warm water

immersion for the subsequent studies because of the ease and feasibility of the cost-effective and commercially available foot spas. Knee-level heating would have necessitated that we construct multiple of the custom-made apparatus we built for the study in **Chapter 2**. The duration and frequency of 45 minutes, 3 days per week was also chosen as a reasonable amount of time for an intervention, especially given that some individuals may already have an existing physical activity routine that we would encourage them to continue. Since lack of time is already a highly cited barrier to participating in exercise (42), we wanted to select a time commitment that again, would be feasible if the results proved heat therapy to be effective. Of note, an additional benefit to the use of local foot heating as it relates to the time commitment is that it can be performed alongside many other tasks such as using a computer, reading, or completing schoolwork. These types of activities would either be impossible or more challenging with exercise, whole-body heating, and even forearm heating which has been shown in some studies to be effective for improving BA vascular function long-term (23, 26). We set the temperature of the water to 42.8 °C (109 °F) as the generally agreed upon tolerable maximum. In Cheng *et. al.* (2021), we attempted to use a similar protocol with 45 °C water and anecdotally, participants reported that while it was tolerable for a single session, they would be highly unlikely to volunteer to continue if it was incorporated in a training regimen (43).

As for the exercise training protocol used in **Chapters 3 and 4**, we decided to have participants exercise on a stationary ergometer between 70-75% HR_{max} for 45 minutes. There are many methods that can be used for the prescription of exercise

intensity, such as %HR_{max}, %HR reserve, %VO_{2peak}, %VO_2 reserve, or % of lactate threshold. While we acknowledge using %HR_{max} is arguably one of the least physiologically controlled methods of exercise intensity prescription, it was selected so that exercise training would be similarly feasible to administer as heat therapy. Indeed, all that was required for participants to self-monitor training was a HR monitor and watch to ensure that they were working within the prescribed HR range for each training session. A prescription based on VO_2 or lactate threshold would have required the measurement of gas exchange at each training session. Moderate intensity was chosen for the extensive evidence base indicating that this intensity is associated with improvements in vascular function, and to provide a comparable stimulus to the "light-to-moderate" intensity of the heat therapy protocol. In addition, the specific intensity chosen was, in part, informed by previously published exercise training studies that have successfully demonstrated improvements in BA FMD with similar training prescriptions (44–47).

5.3.2 Population

For this series of studies, we examined young, healthy recreationally active males and females. These individuals are not likely to be the most responsive to our interventions, and in fact, the term "ceiling effect" typically comes into play when studying this population. The notion of the "ceiling effect" is that there is an optimal range or value of an outcome measure above which there is limited capacity for further gains. That is, if someone already has high vascular function at baseline, for instance, it is possible that there is limited further potential for improvements with an intervention. In

this case, the benefits of heat therapy and/or exercise training would likely be in facilitating vascular function maintenance rather increases. If we were designing our study specifically to maximize responsiveness to our interventions, a sedentary or clinical population would have been a more suitable choice. Both groups would possess some degree of vascular dysfunction, on average, leaving room for improvements with heat therapy and/or exercise training. Many previous studies have focused specifically on the responses to heat therapy in a range of clinical populations, including chronic heart failure (48), peripheral arterial disease (49–51), spinal cord injury (52), obesity (37), Alzheimer's and Parkinson's disease (53, 54), and type 2 diabetes (55). The effects of heat therapy have also been explored in older adults, with reduced physical activity, and in those who possess cardiovascular disease risk factors (6, 16, 56). In contrast, the main focus of this thesis was on intervention protocol development and the refinement of passive heat therapy prescription. Therefore, we wanted to select a population for which the results would be most generalizable. In recent history, most studies have used sedentary or physically inactive individuals as the "base" condition or control group. However, we would argue – as Booth & Lees did in their 2006 invited commentary in Medicine & Science in Sports & Exercise (57) – that sedentary individuals do not actually represent the normal, healthy state. Before the advent of modern technology and transportation, sedentarism was rare. Furthermore, given that physical inactivity contributes to the development of chronic disease, it should not be viewed as the normal human state.

5.3.3. Study Design and Methodological Controls

A limitation of **Chapter 2** is that the time-matched control condition was performed in only a subset of the participants. While there is no reason that vascular function should change with a 45-minute period of supine rest, the inclusion of a complete dataset for this third condition would have allowed us to determine whether the improvements in BA FMD and ffPWV observed were beyond the range of variability that would be established by the control condition.

As for **Chapters 3 and 4**, factors that could have affected our findings include: (1) study duration, (2) lack of a control intervention, and (3) difficulty with the maintenance of existing physical activity throughout the 8 week intervention period. Our randomized controlled trial involved an 8-week intervention period, which is the most common study duration used in heat therapy and exercise training studies (28, 34, 44, 58–60). Based on our understanding of the time course of arterial adaptations (58, 61), 8 weeks should have been sufficient to observe improvements in vascular function and structure. However, when considering the relatively low-moderate intensity of our intervention stimuli, it is possible that a longer duration intervention was required for any physiological signals to accumulate and elicit changes. For this project, limitations to personnel hours prevented us from administering a sham intervention for the control group, which would have strengthened the study design. Though all participants in this group were asked to maintain their physical activity and dietary habits, there may have been psychological and behavioural factors that impacted post-testing results. For example, participants may have subconsciously put more effort into their workout routines or eat slightly healthier knowing that their cardiorespiratory fitness, body

composition, and muscle strength would be re-evaluated at a future visit. Finally, given that we chose to recruit recreationally active adults, it is important to acknowledge the challenge of performing therapy and/or training in addition to an existing physical activity routine. As mentioned earlier, lack of time is a commonly cited barrier to performing physical activity (42). For the average person meeting the Canadian physical activity guidelines and therefore committing ~200-250 minutes per week to aerobic and resistance exercise (62), a therapy and/or training group allocation meant that they had to then incorporate an additional 135-270 minutes of activity/week into their lives. This volume of activity can be difficult to maintain on top of other personal responsibilities and commitments, such as schoolwork, jobs, chores, or other hobbies unrelated to exercise.

5.4 Recommendations for Future Work

5.4.1 Study design to support large sample sizes

Despite conducting *a priori* sample size calculations and attempting to adequately power our studies, it is still likely that **Chapters 3 and 4** were underpowered to detect the anticipated effects. Although sample size calculations seem straightforward, they often involve educated guesses, especially if there is no prior data from which to extract an effect size and if the variability around the effects depend on methodological issues including technical skill. Ideally, researchers are being conservative yet reasonable in these decisions, but striking that balance can be challenging. We recommend that researchers consider multi-site trials more frequently if the study design is more ambitious and large-scale so that common barriers such as limitations to resources,

personnel, and time become less of an issue. Reflecting on the HEATEX study, from which the data for **Chapters 3 and 4** derives, while n=60 is already large compared to similar physiology studies, an n=80 would have been a more conservative estimate based on our anticipated effects and the known variability in FMD assessments. As we conducted this study at a single site, an additional 20 participants would have equated to an extension of data collection alone by 5 months, with further downstream effects on data analysis and management, which was not feasible for the study team to complete.

5.4.2 Sex, gender, race, and ethnicity-based differences

Given the known differences between males and females in resting vascular function and the predisposition of certain races for the development of cardiovascular diseases (63–66), it is important that future work delineate whether there are sex-, gender-, race-, and ethnicity-based differences in the vascular function responsiveness to heat therapy. In **Chapter 2**, we scheduled female participant visits to occur during the early follicular and/or placebo pill phase of their menstrual cycles; and in **Chapters 3 and 4**, we recorded self-reported menstrual cycle information and race and ethnicity information. However, as the main research questions were focused on determining the effectiveness of the interventions for vascular function change, we did not have a large enough sample size or sufficient heterogeneity in our participant cohorts to explore the impacts of these variables and therefore did not conduct this analysis.

5.4.3 Further refinements in heating prescription

As might have been expected, local foot heating was a much weaker thermophysiological stimulus compared to whole-body heating. It is also more difficult to control as a stimulus because localization of the heating leaves more room for the ambient environment to compete with the strength of the stimulus. Further alterations in heating prescription could consider a multimodal approach to heating, such as incorporating the use of metalized polyethylene blankets together with local foot heating to bolster and maintain an elevated core temperature regardless of the environment in which heat therapy is performed. Alternatively, these data may provide the evidence necessary to advocate for increased accessibility of saunas and hot tubs in local community and recreation centres.

5.4.4 Mechanistic focus

When it comes to heat therapy, we have yet to find the prescriptive equivalent of the *One-Minute Workout* (67). This dissertation demonstrates that the answer to the question of the minimum required stimulus to elicit changes in vascular function likely lies somewhere in between the extremes set forth by our studies (i.e., local foot heating, 45 minutes, 42.8 °C, 3x/week) and others that have shown beneficial effects (i.e., whole-body heating, 30-90 minutes, T_c Δ1.5 °C, 4-5x/week). With the wide variety of options for each of the factors that can influence heating prescription, it would be more fruitful for future studies to focus on identifying the mechanisms responsible for vascular function change rather than testing various iterations of fixed heating protocols. Recent work by Coombs *et. al.* (2021) exploring the acute vascular function response to heating

has shown that changes core temperature, forearm skin temperature, and heart rate are the main predictors of the acute change in BA FMD, explaining 33.6%, 31.1% and 20.9% of the variance in Δ BA FMD, respectively (68). Follow-up studies should identify similar mechanistic links with respect to chronic exposure to heat therapy.

5.4 Conclusions

In young, healthy recreationally active males and females, (1) single bouts of 45-min of ankle- and knee-level hot water immersion at 45 °C similarly acutely improve BA FMD and ffPWV; (2) chronic exposure to ankle-level warm water immersion at 42.8 °C, moderate-intensity cycling between 70-75% HR_{max}, or the combination of exercise training and heat therapy 3x/week for 8 weeks is insufficient to change BA FMD, but may be effective for improving cfPWV and VO_2peak; and (3) acute responses in absolute BA FMD, relative BA FMD, and ffPWV following exercise or foot heating can predict chronic responses with 8 weeks of therapy and/or training, regardless of intervention group allocation. Overall, these findings suggest that while the optimal heating prescription, combining effectiveness and feasibility, remains out of grasp, it is very possible that only a small increase in intensity (e.g., longer intervention duration, higher temperature, more muscle mass) will be required to meet the threshold for change. Future work may use acute study designs and the assessment of BA FMD and ffPWV to test this theory.

5.6 References

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