LEG HEAT THERAPY TO IMPROVE WALKING TOLERANCE AND VASCULAR FUNCTION IN PATIENTS WITH SYMPTOMATIC PERIPHERAL ARTERY DISEASE

by

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Dedicated my wife, Rachel, and my children, Addy, Mila, and Hollis.

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ABSTRACT

Lower extremity peripheral artery disease (PAD) is an increasingly prevalent manifestation of atherosclerosis that substantially limits mobility and increases mortality. Few options currently exists for practical conservative treatment of individuals with PAD. We have previously demonstrated that lower limb heat therapy (HT) can improve leg blood flow and reduce systolic blood pressure in patient with lower extremity PAD. Using three unique clinical trials, we sought to test the hypothesis that repeated exposure to HT would improve walking tolerance and vascular function in patients with lower extremity PAD. In these trials, we have sought to examine the clinical efficacy of HT, the physiological mechanisms which may underpin changes in walking endurance in this population, and also the practicality of employing HT in a home-based setting. The primary finding from these trials was that daily application of leg HT improved walking endurance in patients with lower-extremity PAD. Furthermore, the treatment adherence rate was excellent (<96%) and was not associated with severe adverse events. The changes in walking tolerance were consistently not associated with positive changes in vascular function, suggesting an alternative mechanism should be examined in future studies.

CHAPTER 1. INTRODUCTION

Introduction

Human adaptation to heat stress involves a myriad of complex and synchronized physiological reactions (1). While traditionally viewed from a thermoregulatory paradigm, many of the human adaptations to heat stress, such as increased heart rate (HR), cardiac output ($\dot{Q}$), and cutaneous vascular conductance (CVC), and decreased cardiac afterload may confer health benefits both transiently and chronically (2-10). Indeed, an exciting area of research during the last two decades has been the application of heat stress as a means for improving health outcomes in both healthy and clinical populations. This, so termed, heat therapy (HT) involves thermal stress exposure through means such as sauna, hot tub, infrared heating, tube-lined water-circulating garments and short-wave diathermy among others, with the goal of improving health. Patients with peripheral artery disease (PAD) present with greatly reduced walking ability and increased cardiovascular and all-cause mortality risk compared to healthy, age-matched counterparts (11). The unique cardiovascular benefits conferred by HT make it a promising potential treatment for individuals with PAD. The goal of this dissertation is to a) describe the pathophysiology of PAD, b) provide a physiological rationale for the health-promoting potential of HT and how HT might be of particular importance in the treatment of PAD, and c) to describe the results of three clinical trials which have demonstrated the benefit of utilizing HT to improve exercise tolerance and markers of cardiovascular disease (CVD) in patients with PAD.

Lower extremity peripheral artery disease

Peripheral artery disease (PAD) is a manifestation of systemic atherosclerosis which affects over 200 million individuals worldwide, but the global burden may indeed be much larger given

the high rate of atypical presentation and lack of diagnosis (12, 13). A hallmark symptom of PAD is intermittent claudication (IC), defined as muscular pain during activity induced by a mismatch of metabolic need and delivery of O_2 Although not all patients with PAD experience IC, even those with an asymptomatic presentation of the disease display impaired walking ability when compared to age-matched controls (13). A cross-sectional study conducted by McDermott *et al.* determined that compared to age-matched controls, patients with PAD had lower six-minute walk test (6MWT) distances, poorer balance, and slower walking velocity. Further, those patients which exhibited IC symptoms upon exertion had a more severe deficit in 6MWT distances, walking velocity, and frequency of repetitions on the chair raise test as compared to patients with PAD and atypical (i.e. no exertional leg pain) symptoms (14). Patients with PAD display reduced activity levels (15, 16), risk increased cardiovascular disease (17-20), cancer (21), all-cause mortality (18, 22) and reduced quality of life (23). Furthermore, patients with PAD are at risk for progression to critical limb ischemia (CLI), which can result in the necessity for amputation of the limb. The current primary conservative treatment for IC is supervised clinical exercise training. Exercise is considered the gold-standard frontline treatment for the walking impairment seen in patients with PAD. Studies have consistently demonstrated significant improvements in walking performance and a reduction in claudication symptoms after prolonged supervised clinical exercise training (24, 25). Despite these encouraging findings, adherence to supervised walking programs has been traditionally low. Indeed, a review published by Harwood *et al.* suggests that only 24.2% of patients with symptomatic PAD are recruited into supervised exercise programs, and of that number ~25% dropped out of the program prior to completion of therapy. The most commonly cited reasons for dropout or non-enrollment in patients with PAD are lack of education, discomfort of pain during ambulation, and comorbid health concerns (26, 27).

A primary pharmaceutical intervention for PAD involves the administration of the novel phosphodiesterase-3 (PDE-3) inhibitor cilostazol. Cilostazol has been shown to improve walking performance and reduce symptoms of IC in patients with PAD, however, the effect on long-term health outcomes in this population is still unclear (28-30). Peripheral revascularization interventions, including endovascular and surgical approaches may also be employed to reintroduce flow through the native arteries of the leg. While these interventions clearly improve blood flow in the affected limb (31), there is a high rate of stenotic relapse within one year of the procedure (32) and the rate of symptom relief with these interventions may be as low as 50%. Some evidence suggests a lack of functional benefit to the patient after surgery (33). Lastly, patients with low baseline microvascular function may be resistant to positive post-interventional outcomes (34). Patients with PAD, but not CLI or rest pain, typically do not qualify for these surgeries and must bear the cardiovascular consequences of reduced mobility and sedentary lifestyle. It is clear that there exists a need for novel, convenient, effective therapies which will target the reduced peripheral circulation and walking tolerance in patients with PAD not presenting with indications of risk for eminent tissue loss.

In order to gain insight into the mechanisms by which any therapy may exert a positive effect, it is first important to examine the physiological basis for the symptoms themselves. Excellent reviews on the pathophysiology of PAD have been published by Hiatt and colleagues (35) and McDermott *et al.* (13). The genesis of atherosclerotic plaques in the arteries of the neck, arms and, most commonly, in the legs typifies the condition. The etiology of these plaques is multifaceted and varies between individuals. It is believed that cigarette smoking plays a role in the creating of a pro-inflammatory and vasoconstrictive state in which vessels are primed for plaque deposition, as smoking has consistently been identified as the most central risk factor for

the development of PAD. Indeed, smokers have an approximate two-fold increase risk for development of PAD over non-smoking cohorts (22). Other factors which influence risk and severity of PAD include BMI, diabetes, sedentary lifestyle, CVD, and genetic predisposition (12, 22, 36). Once the peripheral atherosclerosis is established, systemic hemodynamics are heavily disrupted, resulting in the transition for a laminar flow pattern to a turbulent flow pattern and a pressure drop distal to the stenosis. This drop in perfusion pressure is typically small enough to be overcome by a drop in distal resistance which maintains an adequate pressure gradient at rest, however, during exercise when flows to the limb must increase by as much as 40-fold, the reduction in flow is non-compensated (35). Patients with PAD are known to have an increased exercise pressor reflex (37), increased presentation of inflammatory markers (38-41) and increased levels of vasoconstrictive peptides such as endothelin-1 (42), a potent endogenous vasoconstrictor, which may only exacerbate the already altered hemodynamic response to exercise. Further, patient with PAD exhibit significantly higher blood pressure than age-matched cohorts. Hypertension is considered a major risk factor for cardiovascular disease and all-cause mortality, and represents a substantial health burden to patients with PAD (17, 43-45).

The reduction in O_2 delivery to the working muscles during exercise appears to play a major role in the observed exercise intolerance at the onset of diagnosis of PAD. Early studies by Pernow and Zetterquist demonstrated that bulk blood flow is markedly reduced in patients with PAD (46). While the understanding of the complicated sequelae of PAD has progressed markedly in the last 50 years since this research, it still remains unclear why the observed bulk flow decrement does not fully describe the functional impairment seen in PAD. The ankle-brachial index (ABI) is a diagnostic measure for PAD which provides a ratio of the systolic blood pressure (SBP) taken at both the dorsalis pedis and posterior tibial arteries in the ankle to the SBP taken at

the brachial arteries in the upper arms. In healthy individuals, ankle pressures are typically slightly higher than the pressure taken at the brachial artery. This is due to antegrade pulse amplification by retrograde wave reflection from the peripheral arterioles as blood travels through large arteries to the lower periphery, as well as changing artery wall thickness in the lower limbs due to chronic exposure to increased hydrostatic pressure (47). The ABI has been consistently demonstrated to be accurate and reliable in assessing the hemodynamic limitations observed in PAD (13). Despite these consistent findings, research is equivocal regarding the association between ABI and walking ability in this group. Indeed, a study by Amighi and colleagues showed that in a nine-month follow-up study of 181 patients with PAD and severe IC, an increase in walking performance was not associated with an increase in ABI (48). Gardner and group has also cast doubt upon ABI as a predictor of walking ability in this patient population by demonstrating a lack of correlation between post-interventional increase in ABI and both walking performance and measured daily activity (33). One attractive hypothesis is that although bulk flow through the major conduit arteries may be improved, this flow may not be adequately diverted to working skeletal muscle due to a variety of factors. The evidence for skeletal muscle microvascular dysfunction suggests that this is a likely player in reducing skeletal muscle perfusion during exercise in patients with PAD and other clinical conditions (49-51).

Based upon the Fick principle: $\dot{V}O_2 = \dot{Q} \times (C_a - C_v)_{O2}$ applied to the muscle as: $\dot{V}O_{2m} = \dot{Q}_m \times (C_{am} - C_{vm})_{O2}$ it can be ascertained that the limitations in muscle $\dot{V}O_2$ observed in PAD are due to either deficits in O_2 delivery or extraction. Patients with PAD may have deficits in O_2 extraction capabilities, but this research is inconsistent, with some reports demonstrating potentially increased O_2 extraction capabilities of the skeletal muscle in this population (52). On the other side of the equation, research is more unequivocal on the existence of skeletal muscle malperfusion

during exercise in patients with PAD. This malperfusion is strongly related to dysfunction of the microvasculature (34, 53). In general, microvascular dysfunction refers to any abnormal response or anatomy of the small distal arterioles, capillaries or venules. Often it is the reduced reactivity or failure of the transient vasoactive response by the small arterioles in systemic circulation. This has been consistently described in a variety of clinical populations (54, 55), including PAD (34, 42, 49). Microvascular dysfunction reduces $\dot{Q}_m$ which is sufficient to reduce the partial pressure of O_2 (pO_2). Based upon Fick's law, the amount of a gas diffused across a semi-permeable membrane, in this case, the capillary wall, is directly proportional to the diffusion gradient of the substance, and indirectly proportional to the thickness of the membrane, and as such, a drop in partial pressure of O_2 (pO_2) would reduce the pressure gradient for diffusion and therefore reduce the amount of O_2 diffused through the capillary to the skeletal muscle. Adding insult to injury, microvascular dysfunction is also implicated in the development of hypertension (44). The arterioles represent the major source of vascular resistance in human physiology; therefore, any reduction in vasodilator response or increase in vasoconstrictor response has the capability to markedly increase mean blood pressure. Hypertension is known to cause damage to systemic blood vessels (44) and in this way, hypertension begets greater vascular dysfunction. This vicious cycle of vascular damage is a central conundrum in the treatment of patients with PAD. It is apparent that in order to commence a dissolution of this cycle, blood pressure must be adequately controlled, but also the health of the microvasculature must be restored.

While measurement direct of skeletal muscle microvascular function *in vivo* in humans is challenging, some recent technological advancements have made use of indirect measures to quantify function of the microvasculature. One measure which has been utilized to quantify the degree of microvascular dysfunction in clinical populations is laser-Doppler flowmetry (LDF).

While this does not measure the skeletal muscle microvasculature directly, the reactivity of the vascular beds of the skin has been shown to be highly correlated to the function in other vascular beds, potentially including those of the skeletal muscle (56). This technique is able to closely measure the flow through the skin microvasculature at rest and in response to stimuli. The red blood cell flux measures are often divided by MAP in order to provide a measure of conductance, rather than simply flow. One attractive property of this measure is the variety of stimuli which can be implemented and assessed. Local heating can be employed as a means to increase skin blood flow during LDF (57, 58). This technique can be paired with local microdialysis in order to determine specific mechanisms which may be driving the response to the stimulus. One methodology for utilizing LDF to assess NO-mediated vasodilator function in the skin is to represent CVC in response to local 39℃ heating compared to heating at 43℃. The response at 43℃ is typically taken to represent maximal vasodilation, while the response at 39℃ is predominantly NO-mediated (58). The skin blood flow response to heating is characterized by a biphasic response involving an initial autonomic reflex peak followed by a gradual increase in cutaneous flow up to a steady-state plateau (57, 59). Healthy patients will reach ~40-60% of CVC_{max} after 20-30 minutes of local heating at 39℃ (3, 58). Several conditions are known to present with altered skin blood flow dynamics. Patients with diabetes display an abnormal thermal hyperemic response to skin heating (60). The initial reflexive peak flow is apparent in healthy individuals, but may be reduced or absent in conditions such as spinal cord injury and systemic sclerosis (61). Laser-Doppler flowmetry has been shown to be of prognostic value for PAD, and has been used as a measure of microvascular function in a variety of clinical conditions. In 1986 Cochrane and colleagues described the reduced skin blood flow in the lower limbs in patients with PAD using LDF (62). More recently, a study conducted by Ishii and group of 128 hemodialysis patients, skin blood flow

of the dorsal portion of the foot was assessed using LDF. Patients with PAD had significantly dorsal skin blood flows than non-PAD hemodialysis patient counterparts (63). Despite these interesting findings, there is an apparent paucity of studies analyzing changes in skin microvascular function in PAD after therapeutic interventions, and thus this represents an exciting avenue for research.

Another exciting *in vivo* technique for assessing some measures of skeletal muscle microvascular function involves the use of near-infrared spectroscopy (NIRS). This technique leverages the intensities of light absorbed in differing chromophores, here: oxygenated and deoxygenated hemoglobin and myoglobin, in order to provide a quantification of tissue oxygenation. The relationship of incoming and emergent light to and from a chromophore has commonly been represented using the Beer-Lambert equation: $[\log_{10}(I_o/I)] = \varepsilon lc$, where l represents the length of the solution the light passes through and c represents the concentration of the solution. This equation may not be sufficient to describe oxygenation during conditions of increasing cutaneous blood flow, such as during exercise and/or heat stress, and may be susceptible to interference from subcutaneous adipose tissue layers. To address this, the use of spatially resolved spectroscopy (SRS) can be employed, which relays the slope of optical density change as a function of multiple optode distances. This technique has been demonstrated to be more resistant to influence from cutaneous circulation during thermal hyperemia (64). This represents an exciting technique due to the ability to measure oxygenation kinetics during exercise.

Indeed, NIRS has been employed in several studies aimed at gaining a greater understanding of the muscle O_2 kinetics during exercise in patients with PAD. One initial study by Bauer and colleagues using NIRS demonstrated that patients with PAD exhibit slowed muscle O_2 kinetics during exercise compared to healthy controls (65). In a later study from the same group,

results were corroborated during submaximal plantarflexion exercise testing. Interestingly, the deficits seen in time constant (τ_1) for patients with PAD was not correlated to limb blood flow as measured by plethysmography. Unfortunately, this study was performed with isolated calf muscle exercise, and thus likely does not represent the hemodynamic response to exercise seen during walking (66). Perhaps in contrast to these findings, more recent work from Gardner et al. have reported a more rapid decline in tissue saturation at the onset of exercise, and that this decline is predictive of ambulatory function and quality of life in this patient population. The authors concluded that this increased rate of tissue desaturation is indicative of deficits in the skeletal muscle microcirculation (67). In another recent ingenious study by Hart et al. examined the mechanisms of exercise limitations in patients with early stage PAD. Using NIRS, data demonstrated that patients with PAD had altered calf muscle oxygenation during exercise and in response to brief arterial occlusion as compared to healthy controls. This difference was attenuated through the increase in fraction of inspired O_2, thus increasing blood pO_2, such that there were no differences between participants with PAD and healthy controls. There was no reportable evidence suggesting a mitochondrial deficit in patients with PAD (68).

Despite these findings, acutely improving microvascular function through administration of phosphodiesterase-5 (PDE-5) inhibitor sildenafil does not appear to positively affect walking performance in this population. Sildenafil is known to mediate vasodilation through increased effect of NO through expanding the stores of cyclic guanosine monophosphate (cGMP). Dysfunction of either production or biological activity of NO is heavily implicated in the skeletal muscle microvascular dysfunction seen in a several populations (49, 54). Roseguini and colleagues studied the effects of the sildenafil on calf muscle oxygenation during treadmill walking in patients with symptomatic PAD. Patients demonstrated lower SBP, DBP and calf muscle

deoxygenation during exercise after ingestion of sildenafil as compared to a placebo. Despite these improvements, no changes were detected in walking performance (69). Future research clearly defining the mechanism of exercise intolerance in this population is still needed.

Positive health effects of HT

Large-scale population studies have demonstrated that exposure to heat stress via sauna bathing significantly reduces cardiovascular and all-cause mortality (71, 72). Zaccardi *et al.* examined the relationship between frequency of sauna bathing in a populations of 1621 Finnish men between the ages of 42 and 60 years. Researchers noted a significant and progressive reduction in hazard ratio for development of hypertension (SBP greater than 140 mmHg, DBP greater than 90 mmHg) with increased reporting of sauna bathing. Those men reporting 4-5 bathing sessions per week had the lowest risk of hypertension development (72). Laukkanen and group conducted a thorough literature review of the potential health benefits of HT, concluding that higher doses of HT are unequivocally associated with lower incidence of cardiovascular disease and risk for sudden cardiac death. Further evidence suggests HT may exert a protective effect against stroke, heart attack and respiratory illness (71). With this promising data, it is important to understand the physiology of thermal stress, and how these changes might subsequently affect health outcomes.

Thermal stress represents a profound challenge to the human physiology. Increases in core temperature of only 3°C can induce serious complications such as heat exhaustion, heat stroke and death. In order to tightly regulate temperature during a heating event, a complex set of changes occur. These adaptations have been detailed thoroughly by Crandall and colleagues (1), but here we will focus primarily on those transient and chronic adaptation which have the greatest potential to improve human health.

A reliable hallmark response to increases in core temperature is a more than two-fold increase in $\dot{Q}$ due nearly entirely to an increase in heart rate. Heat stress is typified as a hyper-sympathetic event, in which sympathetic activity may increases substantially; however skin, and in certain conditions, muscle, are able to override sympathetically induced vasoconstriction (70). Increases HR and, to a lesser extent, inotropy, maintains perfusion pressure in the context of the decreased cardiac preload observed during prolonged heat stress. During thermal stress, HR will often elevate significantly, matching that of moderate-intensity exercise (71). At the onset of heat stress, $\dot{Q}$ increases from ~5 L/min to ~11 L/min with nearly all of this increase being driven through the cutaneous vessels (1). This greater CVC allows for convective cooling of blood, which in turn helps to maintain a stable core temperature. Greater skin blood flow is achieved through two primary mechanisms. Firstly, there is a great drop in vascular resistance through the skin induced by arteriolar vasodilation. This vasodilation occurs in response to heating and may be mediated in part by nitric oxide (NO).

Importantly, exposure to HT is associated with improvements in conduit artery and microvascular function, which also may mediate the positive health benefits of HT. To establish a foundation for the understanding of endothelial adaptation to thermal stress, Green and colleagues devised an experimental design which elucidates the role of shear stress during heating on improvements in endothelial function. Ten healthy men were exposed to 30 minutes of lower arm heating, three times each week for eight weeks. During the treatment, one arm was randomly selected to be cuffed at the brachial after at 100 mmHg in order to reduce increases in brachial artery shear stress. Microvascular vasodilator function was assessed using LDF to measure red blood cell flux in response to local heating at either 41°C or 42°C. Results showed that eight weeks of exposure to heat in the forearms induced positive nitric oxide (NO) dependent vasodilator

function in the skin only in the uncuffed arm. The arm which was cuffed to prevent increases in shear stress did not demonstrate improvements in vascular function (74). Further research from this lab has shown that repeated increases in forearm blood flow induced by localized thermal stress applied to the skin via heated-water bath also improves conduit artery vasodilator function independent of exercise. In this study nine healthy male volunteers underwent localized heating at 42℃ of both forearms for 30 minutes, three times per week for eight weeks. One arm was cuffed at 100 mmHg to reduce increases in brachial artery shear during the heat exposure, while the other forearm was left uncuffed. Endothelium-dependent dilation was assessed through FMD after five minutes arterial occlusion. In order to discern between NO-mediated and non-NO-mediated changes in flow, measurements were taken during a handgrip exercise protocol before and after eight weeks of treatment. Results showed that eight weeks of forearm heating increased flow-mediated dilation (FMD) in brachial artery of the uncuffed, but not the cuffed arm, suggesting that improvements in vasodilator function may be related to increases in artery shear stress. These increases were mediated by both NO and non-NO factors. Interestingly, exercise-induced vasodilation was enhanced despite no chronic exercise training being incorporated into the study protocol (75). In order to make a pointed mechanistic investigation into the local alterations within the periphery after sustained chronic HT, Brunt and colleagues administered HT sufficient to raise rectal temperature to 38.5℃, or a thermoneutral control therapy four to five times per week for eight weeks in sedentary, healthy volunteers, and examined microvascular function in the lower arm. In order to assess microvascular function, the use of LDF was employed on the forearms. Briefly, skin was locally heated to 39℃. At the site of skin heating, microdialysis was set up for infusion of 1) Lactated Ringer (control), 2) Nw-nitro-L-arginine (selective blocker of endothelial nitric oxide synthase [eNOS]), and 3) 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl

(antioxidant, Tempol). After eight weeks treatment, the group exposed to HT had significantly higher CVC compared to the control group, as represented as a percentage relative to maximum heat-induced skin blood flow. The incorporation of the reactive oxygen species (ROS) scavenger, Tempol, had no effect on the response to skin heating. Further, these changes were found to be largely due to enhancements in the NO-mediated vasodilation (3). Brunt and group have also demonstrated that passive HT can reduce arterial stiffness and MAP. When sedentary adults were exposed to eight weeks of HT, four to five times each week, results showed significantly lowered MAP and increased arterial compliance (4). Romero and colleagues sought to determine if these promising findings using HT to improve vascular function in the upper limbs would also apply to the lower limbs. The vascular beds of the lower limbs are known to be more prone to development of atherosclerotic plaque, and as such it is important to understand if the microvasculature in these beds responds in a similar manner to that of the upper body. Nine young and nine aged adults were exposed to a single session of lower limb heating through heated water immersion at 42°C. In order to assess macrovascular function, FMD of the superficial femoral artery was assessed prior to and after exposure to thermal stress. Microvascular function was determined through post-occlusive reactive hyperemia. Results showed only the group of aged individuals demonstrated improvements in macro and microvascular function. Interestingly, the group of older individuals showed a blunted response to acute heating, with a lesser increase in intestinal temperature, shear rate, blood flow, CVC and heart rate but displayed a much larger decrease in MAP as compared to young healthy controls (55).

A study conducted by Carter and colleagues has suggested that positive changes in conduit artery function occur with exposure to heat stress, and that transient increases in core temperature can mediate these changes (76). In this study, ten healthy males were exposed to 30 minutes of

lower limb heating at 40°C, three times each week for eight weeks. This thermal exposure regimen was sufficient to increase core temperature by ~0.6°C. Conduit artery function was assessed through FMD, shear rate, and blood flow measured at the brachial artery. This design is important, as the upper body was not exposed to direct heating during the protocol. One arm was cuffed at 100mg during heating in order to reduce increases in brachial artery shear. Results demonstrated that FMD in the brachial artery significantly increased after four weeks of exposure to heating, but not after six or eight weeks of exposure to lower body heating. Interestingly, blood flow and shear rate continued to increase significantly through eight weeks of treatment. The authors have suggested that the biphasic alterations in FMD are indicative of changes in artery size or remodeling.

Taken together, these studies suggest that a) localized, direct whole body and indirect whole-body heating may induce positive macrovascular and microvascular adaptations b) these changes are mediated through repeated increases in arterial wall shear stress c) positive adaptations appear to be related to alterations in the biological activity of NO, but other factors clearly play a pivotal role. A crucial area of research when seeking to use HT as a therapeutic tool is whether or not the positive alterations may translate to improved endothelial function in the vascular beds of skeletal muscle. The work of Heinonen *et al.* demonstrates that only direct *localized* heating increases skeletal muscle blood flow, and that an increase in core temperature may not be necessary to induce positive adaptations to thermal stress (77). Eight healthy males were recruited to receive indirect whole-body heating, in which the right calf was not exposed directly to a heating source, and localized heating of the right calf, in which only the right calf was exposed to a heating source. Skin and muscle blood flow were assessed on the right calf in both conditions using positron emission tomography (PET). Local heating of the right calf was sufficient to significantly increase

skin blood flow from 0.7 ± 0.3 to 5.5 ± 1.5 mL/100g/min and muscle blood flow from 1.4 ± 0.5 to 2.3 ± 1.2 mL/100g/min. While whole body indirect heating resulted in a similar increase is skin blood flow in the calf not exposed to heating, muscle blood flows did not increase (1.6 ± 0.5 to 1.7 ± 0.3 ml/100g/min. These results are supported by the work of Chiesa and colleagues, who demonstrated that both localized and direct whole body heating resulted in increases in skeletal muscle perfusion (78). These results suggest that HT has the potential not only to improve hemodynamics through the major arteries and microvasculature of the skin but also to the skeletal muscle.

Heat therapy in peripheral artery disease

While these data have reported promising findings in healthy and sedentary adult populations, many clinical conditions present with marked endothelial dysfunction compared to healthy controls. Research has consistently described a persistent endothelial dysfunction in patients with PAD (34, 49, 53), in addition to altered hemodynamic, metabolic and mechanical properties in the legs and as such, it is important to understand if this damaged and defective endothelium and supplied musculature will respond in a similar manner as compared to healthy controls. Further, a majority of the aforementioned studies examining adaptations to chronic HT examined measures of macrovascular and microvascular function in the upper body. Patients with PAD most commonly present with stenotic legions of the lower body, so it is vital to understand if the vascular adaptations to thermal stress which occur in the arteries of the upper body, also occur in those of the lower body. Research regarding the effects of HT on the macro- and microvascular function of patients with PAD is surprisingly scarce considering the depth of the aforementioned data demonstrating positive adaptations in healthy and sedentary populations. Indeed, a recent 2021 review by Harwood and group concluded that much research is still needed

in order to determine the physiological and practical effects of HT in patients with PAD (79). One landmark study by Neff *et al.* enrolling sixteen patients with symptomatic PAD showed that a single 90-minute session of localized HT at 48°C applied to the lower limbs through heated water-circulating trousers is sufficient to improve leg blood flow by ~100%. This improvement in popliteal artery blood flow was accompanied by a blunting of circulating levels of ET-1 (80). Further research by Thomas and colleagues examined limb hemodynamics during hot-water immersion HT in patients with PAD. Eleven patients with symptomatic PAD and ten age-matched controls underwent a single 30 minute session of lower limb heating via heated water bath at ~42°C (81). Leg blood flow was significantly increased and MAP was greatly decreased by HT. Moreover, popliteal artery antegrade shear rate in PAD patients was increased during and 30 minutes after heating such that it matched that of healthy controls. Based upon these data, it is appears that episodic thermal stress has the potential to induce positive adaptations in the macro- and microvasculature in individuals with PAD.

Patients with PAD are known to have increased sympathetic nervous system (SNS) activity, which is implicated in not only hypertension, but an exaggerated pressor reflex at the onset of exercise (82). This exaggerated blood pressure response to exercise is highly correlated to disease severity and walking impairment in patients with PAD, and as such represents an important consideration for treatment (83-85). Qin and colleagues have demonstrated in a murine model of femoral artery ligation that HT is able to blunt this exaggerated blood pressure response to exercise through reduced expression of $P2X_3$ receptors. While there are currently no human clinical trials examining the sympathetic activity in PAD, other clinical populations which share similar cardiovascular disease risk profiles have benefitted in this domain. In a study from Ely and colleagues, 18 obese women with polycystic ovarian syndrome (PCOS) were recruited to receive

either hot-water immersion HT at 40.5℃ 30 times, or no treatment, over the course of eight to ten weeks. Researchers assessed measures of vascular function through FMD and arterial structure, as well as, muscle sympathetic nerve activity (MSNA) recordings. Women with obesity and PCOS are known to display endothelial dysfunction, insulin resistance, increased MSNA, and, interestingly, appear to be relatively resistant to the positive adaptations to exercise training (86). Exposure to HT was significantly associated with reduced MSNA activity, SBP, DBP, femoral and carotid artery wall thickness, C-reactive protein, and total cholesterol. Additionally, FMD was significantly improved compared to controls with HT.

In addition to positive improvements in macro and microvascular function in patients with PAD, HT may confer additional benefits upon the skeletal muscle. Patients with PAD exhibit a variety of confirmed skeletal muscle abnormalities, including but not limited to: myofiber myopathy, mitochondrial dysfunction, and reduction in nerve conduction velocities. A recent study from Kim *et al.* has demonstrated the potential positive effects of HT on skeletal muscle function and recovery. Twelve young, sedentary adults were recruited to receive daily lower body HT through a custom water-perfused garment (circulating water temp ~52℃) five times each week for eight weeks on one randomly selected leg while the other leg would serve as a control. Isokinetic torque was significantly higher after eight weeks in the leg exposed to HT compared to the control leg. These increases in quadriceps strength occurred despite no meaningful increases in thigh muscle cross-sectional area, suggesting that HT may modify alternative mechanisms within the skeletal muscle to enhance force production. Further, eNOS levels within the thigh exposed to HT were significantly higher at both four and eight week time points (~18% and ~35% higher, respectively (87). This finding is of particular interest when seeking to apply therapies to patients with PAD, as eNOS is known to be deficient in patients with PAD, and eNOS is

responsible for facilitating the generation of NO. A recent study by Brunt and colleagues has reported that serum from healthy individuals exposed to HT had higher levels of eNOS and induced greater angiogenesis when applied to human umbilical vein endothelial cells (88). Many of these data apply to healthy individuals, but there is a body of research beginning to report findings related individuals with peripheral artery disease. Kim and colleagues investigated this using a pre-clinical mouse-model of PAD with customized application of HT through rodent thermal baths. Male rats had the femoral artery ligated to create ischemia-induced muscle damage. Two weeks post-surgery, rats were exposed to thermal stress of the lower limbs for 30 minutes, six times each week, for three weeks. Results demonstrated that rats exposed to heated water temperatures of 37℃ and 39℃ displayed increased soleus muscle force compared to controls (89).

While HT may improve or counter a variety of the negative consequences of PAD, such as endothelial and skeletal muscle dysfunction, it is vital to understand if these findings will result in improvements in practical outcomes for this patient population. Early reports of the potential positive effects of HT on symptoms of PAD involved the utilization of Waon therapy, a treatment involving short duration whole body exposure to infrared sauna at temperatures of ~60℃. Two such studies demonstrated significant increases in walking tolerance in patients with PAD after receiving Waon therapy at 60℃ for 15 minutes, five times each week for six weeks (8, 9). Unfortunately, neither of these studies included a sham treatment to control for the potential placebo effect evident in therapeutic trials. An important contribution to the understanding of HT as a treatment in PAD was a 2019 study by Akerman and group in which 22 patients with symptomatic PAD underwent either five times weekly applications of HT via sauna bathing plus banded calisthenics or approximately two weekly sessions of supervised treadmill exercise for twelve weeks. Eleven patients were allocated to each group. Adherence to the supervised treadmill

exercise program was low, which is consistent with the literature in this population (26). Both groups improved six-minute walk test distance by ~41m and pain free walking distance by ~43m. There was no significant difference between the groups. This is notable, as the HT group did not participate in any structured walking program, and yet made clinically significant improvements in walking tolerance. Also notable was the significant ~4mmHg reduction in MAP from baseline observed in both groups. The authors concluded that this indicates that HT may be as promising for improving walking tolerance and cardiovascular health as exercise. While the results from this study are exciting to report, it is worth noting that this trial did not include a control condition. Both conditions received exercise training to some degree and the total dose of exercise may have been equivalent between groups, although the group undergoing supervised treadmill walking exercise with undoubtedly greater task specificity. Additionally, while the low adherence to supervised exercise has strong external validity because it matches findings from reports of adherence in these programs elsewhere, it makes it impossible to conclude that HT is as effective as supervised exercise per se, and more appropriate to state that it may be similarly as effective as programs currently available given the most likely expectation for attendance and adherence. For these reasons, it is important to confirm these findings with more rigorously controlled trials which employ HT in a standalone form and contain a sham treatment to control for the possibility of placebo effect (2).

Given the reports from both Neff *et al.* and Thomas *et al.* that HT transiently improves the hemodynamic profile in the lower limbs in patients with PAD, there appears to a strong basis for studies which employ HT as an ergogenic tool for this population prior to exercise. One such small pilot study from Pellinger and colleagues has demonstrated that as little as 15 minutes of hot-water bathing HT prior to exercise improves walking distances and popliteal artery blood flow in patients

with symptomatic PAD. In this trial six patients with symptomatic PAD were enrolled to receive three treatments: 15 minutes of supervised heated-water immersion at 42℃, 45 minutes of supervised heated-water immersion at 42℃ or a control treatment. Popliteal artery blood flow was increased significantly in both HT groups. The groups receiving HT improved 6MWT distances by 10% and 12%, respectively, versus the control treatment (90). A larger sample size will be important in order to validate these data.

The rationale for future research utilizing HT to treat the symptoms and pathology of PAD is strong. Despite this, there have been no placebo-controlled clinical trials assessing the validity of HT for treatment of PAD. It is with this as a backdrop that we undertook a series of randomized clinical trials designed to test the hypothesis that HT will a) improve walking performance and tolerance, b) improve vascular function, and c) reduce blood pressure in patients with symptomatic PAD. The goal of the following chapters is to characterize three such studies in detail which seek to address these hypotheses.

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CHAPTER 2. SUPERVISED LEG HEAT THERAPY IN PERIPHERAL ARTERY DISEASE

Supervised leg heat therapy improves perceived physical function but does not enhance walking tolerance or vascular function in patients with peripheral artery disease

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Running Title: Heat therapy in patients with peripheral artery disease

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Abstract

A single session of heat therapy (HT) applied to the legs of patients with symptomatic peripheral artery disease (PAD) increases popliteal artery blood flow and reduces blood pressure (BP) and the circulating levels of the endogenous vasoconstrictor endothelin-1 (ET-1). We assessed whether 6 weeks of supervised leg HT (3 times/wk) using water-circulating trousers perfused with water at 48ºC improved 6-minute walk distance in people with PAD, compared to a sham treatment. Secondary outcomes included the assessment of leg vascular function, BP, quality of life and the levels of ET-1 and nitrite plus nitrate (NOx). Of thirty-two PAD patients randomized, thirty (age: 68±8 yrs; ankle-brachial index (ABI): 0.6±0.1) completed the 3 and 6-week follow ups.

Participants completed 98.7% of the treatment sessions. When compared to the sham treatment, exposure to HT did not improve 6-minute walk distance, BP, popliteal artery reactive hyperemia, cutaneous microvascular reactivity, resting ABI or the serum NOx levels. The change from baseline to 6 weeks in scores of the physical functioning subscale of the 36-item Short Form Health Survey was significantly higher in the HT group (Control: -6.9±10 versus HT: 6.8±15; 95% confidence interval: 2.5-24.3, p=0.017). Similarly, the change in ET-1 levels after 6 weeks was different between groups, with the HT group experiencing a 13% decrease (95% confidence interval: -0.8,-0.0, p=0.03). These preliminary results indicate that leg HT may improve perceived physical function in symptomatic PAD patients. Additional, larger studies are needed to confirm these findings and determine the optimal treatment regimen for symptomatic PAD patients.

New and Noteworthy

This is the first sham-controlled study to investigate the effects of leg heat therapy (HT) on walking performance, vascular function and quality of life in patients with peripheral artery disease (PAD). Adherence to HT was high and the treatment was well tolerated. Our findings revealed that HT applied using water-circulating trousers evokes a clinically meaningful increase in perceived physical function and reduces the serum concentration of the potent vasoconstrictor endothelin-1 in patients with PAD.

Keywords

Peripheral artery disease, intermittent claudication, heat therapy, quality of life, endothelin-1

Introduction

Lower-extremity peripheral artery disease (PAD) is a manifestation of systemic atherosclerosis that affects more than 236 million individuals worldwide (50). Approximately 1 in 10 individuals aged 70 years and nearly 1 in 5 people older than 80 years have PAD (50). As the prevalence of the primary risk factors for this condition, including obesity-associated diabetes and dyslipidemia, are expected to grow globally, the burden of PAD will continue to rise (17). Patients with PAD have worse quality of life than their healthy counterparts, in part due to the marked decline in physical functioning (12). Walking-induced ischemic pain may reduce daily physical activity levels and as consequence accelerate mobility loss and functional decline in these patients (36). Effective management of leg symptoms in symptomatic patients is hampered largely by the lack of non-invasive, widely accessible treatment options. The cornerstone therapy to improve functional capacity in patients with PAD consists of supervised treadmill walking sessions (34). Unfortunately, the paucity of programs and other barriers including the inconvenience of travel to rehabilitation facilities makes this option inaccessible to the vast majority of patients with this condition (11, 24).

Heat therapy (HT) has emerged as a practical treatment to improve cardiovascular health in young individuals (4), as well in patients with overt cardiovascular disease (25, 30, 41, 48, 49) and endocrine disorders (13, 14). The effects of HT, in the form of dry sauna and hot-water immersion, have been previously reported in patients with PAD (2, 47, 51, 52). Tei and colleagues first reported that dry sauna at 60ºC for 10 weeks (5 days/week) improved leg pain, hemodynamics and walking performance in patients with moderate-to-severe PAD (52). Recently, Akerman and coworkers showed that HT via spa bathing for 12 weeks (3-5 days/week) improved walking distance and resting blood pressure in patients with PAD (2). Although encouraging, none of aforementioned studies in patients with PAD included a sham-treated control group and it is

therefore difficult to exclude the possibility that the observed positive adaptations derived from a placebo effect.

The goal of the present study was to contrast and compare the effects of leg HT with a sham intervention in patients with symptomatic PAD. Leg HT was applied for 90 min, three times weekly for 6 weeks using customized water-circulating trousers perfused with water heated to 48ºC. We have previously shown that exposure to a single HT session using this modality increases popliteal artery blood flow, lowers blood pressure, and reduces the levels of the potent endogenous vasoconstrictor endothelin-1 (ET-1) (40). In the sham group, water at 33ºC was circulated through the trousers. This regimen elevates skin temperature by approximately 2ºC, but does not evoke measurable changes in core body temperature, heart rate and blood pressure (40). The primary outcome was a change from baseline to 6 weeks in 6-minute walking distance. We hypothesized, based upon the findings of studies with other HT modalities (2, 47, 52), that leg HT using tube-lined trousers would improve exercise capacity in people with PAD. Secondary outcomes included the change in health-related quality of life, blood pressure, conduit artery function and systemic levels of ET-1 and nitrite plus nitrate (NOx).

Methods

Subjects

Eligible patients were identified and contacted by the Indiana Clinical and Translational Science Institute Research Network (ResNet) research assistants. After interest in participation was established, patients were contacted directly by the researchers. Additional study participants were obtained by direct physician referral from Department of Vascular Surgery at Methodist Hospital and the Roudebush Veterans Affairs Medical Center. Thirty-two participants were randomized into the study. All patients had ankle-brachial index (ABI) values below 0.90 and

claudication pain during exercise in one or both legs for greater than 6 months prior to enrolling in the study. Patients were excluded if their electronic medical record showed: 1) a hemoglobin A1C value above 8.5% within three months of screening, 2) exercise limiting comorbidities (arthritis, heart failure, chronic obstructive pulmonary disease, etc.), 3) tissue loss, 4) prior amputation, 5) non-healing wounds, 6) evidence of critical limb ischemia, 7) recent (<3 months) infrainguinal revascularization (surgery or endovascular revascularization) or revascularization planned during study period; 8) planned change in medical therapy during the duration of the study, 9) active cancer, 10) chronic kidney disease (eGFR <30), 11) HIV positive, active hepatitis B virus (HBV) or hepatitis C virus (HCV) disease, 12) peripheral neuropathy, numbness, or paresthesia in the legs, and 13) a body mass index (BMI) > 35. Patients with cardiovascular or other implants not compatible with magnetic resonance imaging (MRI) were allowed to participate in the study, but were excluded from undergoing the phase-contrast MRI measurements. The protocol was approved by the Institutional Review Board of Indiana University and the Roudebush Veterans Affairs Medical Center (no. 1601589496), and registered with the United States Library of Medicine on clinicaltrials.gov (NCT02770547). Written, informed consent was obtained, and all procedures adhere to the requirements of the U.S. Federal Policy for the Protection of Human Subjects (45 CFR, Part 46), and support the general ethical principles of the Declaration of Helsinki.

Experimental Design

A schematic of the experimental protocol is depicted in Figure 1. Participants were assigned, using a randomized, balanced design, to undergo either HT or a sham treatment 3 times per week, for a total of 18 sessions across the 6 weeks. Participants were informed that there were two different categories of HT: “low-heat” and “high-heat,” and that both might be beneficial for

claudication symptoms. Participants were asked to report to the laboratory in a fasted state (>8 hrs postprandial), refrain from exercise (24 hrs) and smoking (>4 hrs) and take their usual prescription medications prior to the experimental visits. All visits took place in the morning in a temperature-controlled room (22-24ºC). On visit 1, participants were asked about their health history and were asked to complete the 36-item Short-Form Health Survey version 2 (SF-36v2, Optum, Eden Prairie, Minnesota, United States) and the Vascular Quality of Life Questionnaire (VascuQoL). Participants were then familiarized with the 6-minute walk test. Visit 2 was conducted at least 72 hours after visit 1. Upon arrival to the imaging research facility, participants rested in the supine position for 15 min. Blood pressure was then measured using an automated device and the participant was transported to the scanning room for the assessment of post-occlusive reactive hyperemia in the popliteal artery using phase-contrast MRI. After completion of the MRI assessment, participants were escorted to the Clinical Research Center to undergo the remaining experimental tests. After 10 min of quiet rest in the supine position, blood pressure measurements were taken in duplicate in the arms and ankles for the calculation of the ABI. The time period between the post-occlusive hyperemia test and the resting ABI assessment was approximately 30 min. Next, blood samples were collected from a vein in the antecubital space. Participants were then instrumented with skin heaters and laser-Doppler flowmetry probes for the assessment of leg cutaneous microvascular reactivity. This test lasted 70 min and blood pressure was measured every 5 min throughout the test using an automated device. Lastly, patients were escorted to an adjacent hallway and completed the 6-minute walk test. Experimental visits 3 and 4, which were similar to visit 2, were conducted after 3 and 6 weeks of treatment, respectively. Treatment sessions were ceased at least 48 hours prior to the experimental sessions to ensure that the chronic, rather than the transient acute effects of HT, were being assessed.

Intervention

Participants were asked to put on the water-circulating trousers (Med-Eng, Ottawa, Ontario, Canada) and sit in a semi-recumbent reclining chair. Water at 48ºC (HT) or 33ºC (control) was circulated through the garment for 90 min using a stainless-steel heated bath circulator (SAHARA S21, ThermoFisher Scientific, USA). An automatic oscillometric sphygmomanometer (Carescape Dinamap V100, GE Healthcare, USA) was used to measure blood pressure every 15 minutes throughout the treatment. Tympanic temperature was also recorded in a subset of patients using a digital thermometer (Braun ThermoScan PRO 6000, Welch Allyn, USA). Thermal comfort scores were assessed using an 9-point ISO categorical scale every 15 minutes during the treatment. The scale ranges from -4 ("Very Cold") to +4 ("Very Hot) (16).

6-Minute Walk Test

The 6-minute walk test was performed on a 30 m long, flat, straight corridor following the American Thoracic Society guidelines (33). The length of the corridor was marked every 10 feet and the turnaround points were marked with a cone. Patients were instructed to walk as far as possible for 6 min and were encouraged with standardized phrases every minute throughout the test. Two tests were completed at baseline to confirm reproducibility of results. The best-of-two tests was defined as the baseline walk distance (5).

Quality of Life

The Medical Outcomes Study 36-Item Short Form Health Survey (SF-36v2) was used to assess health-related quality of life (HRQoL). Prior studies revealed that the physical function subscale of SF-36v2 Health Survey is the most impaired subscale in patients with symptomatic PAD (26). The SF-36v2 Health Survey was scored using proprietary software (Health Outcomes,

Optum, USA). Disease-specific health-related quality of life was assessed using the Vascular Quality of Life (VascuQoL) questionnaire, a PAD-specific HRQoL instrument (37). The total VASCUQOL score is the average score of questions answered and ranges from 1 (worst QOL) to 7 (best QOL) (38).

Reactive Hyperemia

Post-occlusive reactive hyperemia in the popliteal artery was assessed using phase-contrast MRI as described previously (40). MRI scanning was performed on a Siemens 3 Tesla (T) Magnetom Prisma scanner (Siemens AG Healthcare Sector, Erlangen, Germany). A transmit/receive knee coil was placed around the knee of the most symptomatic leg, defined as the leg in which the participant self-described as having the most severe claudication pain. A cuff (SC12L, Hokanson, USA) was snugly wrapped around the upper thigh and connected to a commercially available air source (Hokanson AG101, Hokanson, USA) coupled to a rapid cuff inflator (Hokanson E20, Hokanson, USA). After baseline data acquisition, the cuff was inflated to suprasystolic values (75 mmHg above brachial SBP, as assessed prior to scanning). Phase-contrast imaging was performed during cuff inflation to ensure that total arterial occlusion of the upper leg was achieved. After five minutes of occlusion, the cuff was deflated and post-occlusion reactive hyperemia was monitored for 10 min.

For quantitative flow measurements, a two-dimensional gradient-echo technique was employed using the following acquisition parameters: TR 36.4 ms, TE 3.59 ms, flip angle 20°, FOV 200 mm, matrix size 256, and reconstructed voxel dimensions of 0.8 x 0.8 x 10.0 mm. A single slice was prescribed perpendicular to a straight section of the popliteal artery. Fifty dynamic phases were acquired to obtain flow waveforms over the cardiac cycle with a maximum range of flow velocity encoding of 100 cm/s. A pulse oximeter on the index finger was used for peripheral

gating with the minimum trigger delay. A quantitative flow analysis package (QFlow, Medis, Leiden, The Netherlands) was used to analyze phase-contrast flow data. A region of interest covering the entire visible cross section of the artery was manually drawn every 10 frames and then software interpolation created arterial lumen tracings for the remaining frames. Each series was manually checked in duplicate to ensure consistency of interpolation. Flow parameters included velocities averaged across the respective vessel lumen and corresponding flow volumes (53, 54).

Ankle-brachial Index

Cuffs (SC10, SC12, Hokanson, USA) were wrapped around the ankles and the upper arms. Systolic pressures were measured sequentially in the right posterior tibial artery, right dorsalis pedis artery, right brachial artery, left posterior tibial artery, left dorsalis pedis artery, and left brachial artery using a handheld 5MHz Doppler ultrasound (Lumeon, McKesson, USA). Pressures were taken at each site in duplicate. The ABI of each leg was calculated by dividing the higher of the dorsalis pedis pressure or posterior tibial pressure by the higher of the right or left arm blood pressure (1).

Cutaneous Microvascular Reactivity

Leg cutaneous hyperemia in response to rapid local skin heating to 39ºC was assessed as described by Choi and colleagues (7). Briefly, two local skin heaters (SH02 Skin Heater/Temperature Monitor; Moor Instruments, Axminster, United Kingdom) were affixed with dual sided stickers to the proximal portion of the lower leg, approximately three inches below the patella on the dermal surface overlaying the tibialis anterior muscle. Single-point laser-Doppler flowmetry probes (Moor Instruments, Axminster, United Kingdom) were positioned in the center

of each local heater. Red blood cell flux, an index of skin blood flow, was recorded for 10 min with skin temperature held constant at 33°C. Next, local skin temperature at each site was raised to 39°C at a rate of 0.1°C/s and maintained for 40 min (7). Finally, local skin temperature was raised to 43.0°C at a rate of 0.1°C/s and maintained at this level for 20 min. Red blood cell flux and the temperature of the skin heaters were recorded at 40 Hz using a data acquisition system (Powerlab and LabChart, ADInstruments, USA), and the last 2 min of every 5-min bin was averaged for the entire protocol. An automated device (Tango+, Suntech Medical, USA) was used to measure SBP and DBP every 5 min. Mean arterial pressure (MAP) was calculated as DBP plus one-third pulse pressure (i.e., the difference between SBP and DBP). Cutaneous vascular conductance (CVC) was calculated as red blood cell flux divided by MAP and was normalized as a percentage of maximal vasodilation (%CVCmax). There were no observable or statistical differences in %CVCmax between the two laser-Doppler sites within subjects for a given trial so values from each site were averaged. At the end of the session, the heating probes were traced with permanent marker and covered with a protective film (Tegaderm, 3M, USA). Participants were asked to preserve the marks to facilitate consistent placement of the probes in subsequent visits.

Analysis of blood markers

Samples were drawn into tubes containing sodium heparin or serum separator tubes (SST) (BD Vacutainer, BD, Ontrario, Canada). One tube was transported to the Indiana University Health Pathology Laboratory for the assessment of a routine basic metabolic panel. Serum samples were allowed to clot at room temperature for 30 min before centrifugation at 1,100 G for 10 min (ST16, Thermo Scientific, USA). Samples were then aliquoted and immediately placed into a -80°C freezer. Serum ET-1 concentrations were measured in duplicate using a commercially available enzyme-linked immunosorbent assay kit (DET100, Endothelin-1 Quantikine ELISA Kit, R&D

Systems, USA). Total NOx was assessed via high performance liquid chromatography (HPLC) (ENO-30, Eicom USA, USA) by injecting 10μL of serum and then colorimetrically determining the concentration by comparison to a nitrite (NO_2)/nitrate (NO_3) standard injection series as described previously (8).

Sample size calculation

An a priori power calculation indicated that n=16 subjects per group would have 80% power to detect a clinically meaningful change of 50 meters in the 6-minute walk distance after 6 weeks of treatment using a two-sided, two-sample t-test (alpha=0.05) and assuming a standard deviation of change of 48.7, estimated from Table 3 in Gardner et. al (21).

Statistical Analysis

All analyses were performed using SAS v9.4 and results are expressed as mean ± standard deviation (SD). Two-sample t-tests, Wilcoxon Rank Sum test, Chi-square tests, or Fisher's Exact tests were used to compare demographic and clinical characteristics between the two groups. Analysis of covariance (ANCOVA) was used to test whether the 3- and 6-week means were different between the HT and control groups for all the outcomes with baseline as a covariate. When a significant interaction of time and intervention was detected, SIDAK adjustment was used to adjust the group effect p-value at each time point, and the SIDAK adjusted 95% CI was reported for the group effect at each time point. If the interaction was not significant, it was removed, and the main effects for time and intervention tested, and 95% CI for group effect was reported. The intraclass correlation coefficient estimates for the 6-min walk distance and ABI were calculated based on a single-measurement, absolute-agreement, 2-way mixed effects model. The measures from the two Laser Doppler flowmeter probes were compared using linear-mixed models.

Results

Subject characteristics

A total of 345 patients were contacted regarding the study. Fifty-eight participants were deemed ineligible and 255 refused to participate. The remaining 32 patients were randomly allocated to receive HT (n=16) or the control treatment (n=16). One participant in the control group had recurrent hypertensive episodes that required a change in the medication regimen. As a result, this individual was withdrawn from the study after 12 treatment sessions. One female patient in the HT group had minor skin irritation and some blistering in the back of the right thigh, which was apparent after completion of the fifth HT session. Although the patient recovered promptly after interruption of the treatment, this individual was also withdrawn from the study. All others completed the treatment sessions and both the 3- and 6-week follow-ups. Demographic and clinical characteristics for those participants that completed all follow-ups are presented in Table 1. There were no baseline differences between groups. All but two patients were current or former smokers and 10 patients were diabetic. The treatment was well tolerated, with participants in the HT group reporting, on average, perceived thermal comfort scores ranging between +2 ("Warm") and +3 ("Hot") on a 9-point bipolar feeling scale. Participants completed 98.7% (532/540 sessions) of the required sessions.

Changes in blood pressure and temperature during treatment sessions

Before the onset of treatment, the average tympanic temperature and MAP were 36.5±0.2ºC and 92.4±8 mmHg in the control group and 36.6±0.4ºC and 92.8±10.5 mmHg in the HT group. Figure 2 depicts the average changes from baseline (0 min) in tympanic temperature (panel A) and MAP (panel B) during the 18 supervised treatment sessions. The changes in both

temperature and MAP were greater ($p<0.05$) in the HT group when compared to control group starting at 45 min and persisting until the end of the treatment.

6-Minute Walk Test

Test-retest reliability of the 6-min walk test at baseline was excellent (ICC=0.96). Baseline walk distances were 386±85 m in the control group and 414±109 m in the HT group. Figure 3 depicts the change from baseline in 6-minute walk distances after 3 and 6 weeks of treatment. Small improvements in both groups were noted after 3 weeks (Control: 2.4±18.5 m, HT: 0.8±18.2 m, 95% confidence internal: -15.6-12.6) and 6 weeks (Control:17.9±23.4 m, HT: 5.6±29.1 m, 95% confidence internal: -32.3-7.8). There was no group-by-time interaction ($p=0.14$), and no main effects of group ($p=0.8$), but there was a significant effect of time ($p=0.009$).

Quality of Life

The responses to quality of life data questionnaires were obtained from 14 patients in control group and 14 patients in the HT group. One outlier in the control group was identified and removed owing the change in scores being ~2.5 standard deviations above the mean. The changes in the scores for the physical function subscale of SF-36v2 questionnaire are shown on Figure 4. The scores for the other subscales are presented on Supplemental Table S2. In the HT group, the average PF scores increased by 3.6 and 6.8 points after 3 and 6 weeks of treatment, respectively. Conversely, the scores declined by 2.2 points after 3 weeks and by 6.9 points at 6 weeks in the control group. The ANCOVA analysis comparing the 3- and 6-week means between the HT and control groups revealed a trend for a main effect of treatment ($p=0.0507$) but no group by time interaction ($p=0.11$). Subsequent testing for the main effects of treatment at 6 weeks revealed a statistically significant difference between groups (95% confidence interval: 2.5-24.3, $p=0.017$).

There were no group differences in any of the other subscales of the SF-36 survey (Supplemental Table S1) or the score from the VascuQol questionnaires (Supplemental Table S2).

Reactive hyperemia

A total of 25 participants completed the baseline phase-contrast MRI assessment and 23 participants completed all three assessments. Technical issues, particularly with peripheral gating, prevented the analysis of data from 11 participants. One representative example of changes in peak flow in the popliteal artery at baseline, during occlusion and following cuff release is shown in Supplemental Figure S1 (panels A). Peak blood flow (Supplemental Figure S1, panel B) as well as other indices of flow and velocity (Supplemental Table S3) were unaltered in both groups after the interventions.

Cutaneous Microvascular Reactivity

A representative response from one subject to rapid local heating to 39°C at a rate of 0.1°C/s is shown on Supplemental Figure S2 (panel A). Cutaneous vascular conductance rose progressively, reaching at plateau by ~30-40 min into heating. On average, CVC at the end of the 39ºC heating period was ~55% of the maximal value obtained following 20 min of heating at 43ºC. There was no group-by-time interaction ($p=0.55$), and no main effects of group ($p=0.31$) or time ($p=0.88$) for the plateau CVC (Supplemental Figure S2, panel B).

Blood pressure

BP was measured on the right arm every 5 min for 70 min during the assessment of cutaneous microvascular reactivity. Supplemental Figure S3 displays the changes from baseline to 3 and 6 weeks of treatment of both systolic and diastolic pressures across the 70-min assessment.

A significant group-by-time interaction was observed for the changes in SBP from baseline to 6 weeks (p=0.0042) with significant group differences at 20 min (Control: 6.2±15.5 vs. HT: -7.6±13.6, p=0.0178). Although a significant group-by-time interaction was also observed for the changes in DBP (p=0.0168), post-hoc testing did not detect statistically significant group differences throughout the 70-min assessment. The averages of all 15 repeated measurements of systolic and diastolic blood pressures at baseline were 149±12 mmHg and 80±10 mmHg, respectively, in the control group and 153±12 mmHg and 82±8 mmHg in the HT group. The changes in average systolic and diastolic blood pressures after exposure to 6 weeks of control and HT are shown on Supplemental Figure S4. There were no group differences in the change of either systolic or diastolic BP.

Ankle-brachial index

Test-retest reliability of ABI was excellent (ICC = 0.90 for right leg and 0.91 for left leg). At baseline, the ABI in the most affected leg was 0.6±0.1 in the control group and 0.7±0.1 in the HT group. There was no group-by-time interaction (p=0.83), and no main effects of group (p=0.74) or time (p=0.94) for the most affected leg. Results were similar for the least affected leg (Supplemental Table S4).

Blood biomarkers

Serum ET-1 concentrations were 2.6±0.7 pg/mL in the control group and 2.3±0.5 pg/mL in the HT group at baseline. As shown on Figure 5, serum ET-1 concentration rose by ~6.5% relative to baseline after 6 weeks of treatment in the control group, while a 13% reduction was noted in the HT group. The ANCOVA analysis comparing the 3- and 6-week means between the HT and control groups revealed a trend for a main effect of treatment (p=0.053), but no group by

time interaction (p=0.24). Subsequent testing for the main effects of treatment at 6 weeks revealed a statistically significant difference between groups (95% confidence interval: -0.8,-0.0, p=0.03). The serum NOx concentrations at baseline were 64.3±66.3 μmol/L in the control group and 42.2±25.7 μmol/L in the HT group. There were no differences between groups for the changes in NOx concentrations following 3 and 6 weeks of treatment (Supplemental Figure S5).

Discussion

The primary findings of the present study were that 6 weeks of supervised leg HT improved perceived physical functioning but had no measurable impact on 6-minute walk distance and vascular reactivity in patients with symptomatic PAD. These findings are incongruous with earlier reports of improved leg hemodynamics (52) and exercise capacity (2, 47) following sauna or hot tub therapy in PAD patients. Of importance, a sham-treated group was not included in these previous studies and it thus unclear whether the reported benefits derive from the treatment or due to a placebo effect. The present study is unique because we compared the effects of leg HT against a sham treatment that produces small increases in leg skin temperature. We observed that while perceived physical function tended to decline over time in the group exposed to the sham device, individuals treated with leg HT reported a progressive, clinically meaningful improvement in quality of life during the 6-week intervention. These novel findings imply that leg HT may be a useful adjunctive therapy to restore perceived well-being in symptomatic PAD patients.

Repeated exposure to heat stress in the form of hot tub therapy or sauna bathing has been shown to evoke robust increases in both conduit artery and microvascular endothelial function in young individuals (4, 23, 39) as well as in patients with cardiovascular risk factors (25) and chronic heart failure (30). Serial assessments of vascular function throughout the HT interventions in these previous studies revealed that improvements in vascular function are evident after as early as 2

weeks of treatment (4, 39) and are fully manifested within 6-8 weeks (4). Based upon these previous findings, the length of the intervention herein was of 6 weeks, with vascular assessments performed after 3 and 6 weeks of treatment. We anticipated that palpable improvements in vascular reactivity would occur within 3 weeks, thereby enabling a greater oxygen delivery capacity to the calf muscles and a consequent increase in exercise capacity. Contrary to these predictions, HT had no measurable impact on conduit artery and cutaneous vascular function and in walking performance on the 6-minute walk test.

The discrepancies between our findings and the aforementioned studies may stem from several important differences in study design and methods. First, the vast majority of the previous studies (4, 23, 25, 30, 39) examined vascular function in the arm through the assessment of the flow-mediated dilation of the brachial artery. In contrast, we focused on the legs because: 1) the degree of vascular impairment is greater in the legs than in the arms in PAD patients (45) and 2) leg peak hyperemic blood flow relates to peak exercise performance in these patients (44). Intriguingly, contrary to brachial artery FMD (35), lower-extremity reactive hyperemic blood flow does not consistently improve following exercise training in PAD patients (42), underscoring the notion that the lower-extremity vasculature may be less responsive to treatment interventions in these individuals. Second, most previous studies employed HT modalities that produced marked increases in core body temperature (38ºC-38.5ºC) during the treatment sessions (4, 39). The tube-lined trousers used herein elicit local leg heating and small changes in core temperature (Figure 2). Conceivably, a greater thermal strain may be necessary to evoke appreciable changes in vascular function in patients with symptomatic PAD. Third, it is possible that the treatment frequency and length of the intervention used in the present study (3 sessions/week) were not optimal for patients with severe vascular disease. Along these lines, studies reporting clinical improvements in patients

with heart failure and cardiovascular risk following repeated sauna bathing required participants to undergo the treatment 5 days/week (25, 30). Further, the length of previous studies in PAD patients ranged from 10 to 12 weeks of treatment (2, 52).

This is the first sham-controlled study to examine the impact of HT on 6-minute walk distance in patients with PAD. It is noteworthy that walking distance improved by 17 m in the control group and by 5 m in the HT group after 6 weeks of treatment (Figure 2). Albeit not clinically meaningful (46), these subtle improvements may be placebo-related and attest to the critical importance of incorporating a sham group to account for psychosocial factors that promote placebo effects (18). This is particularly important for clinical trials involving medical devices because there is evidence of an enhanced placebo effect compared with placebo pills (29). Water at 33ºC was selected as the sham treatment in the present study because it is perceived as slightly warm but it is not sufficient to provoke changes in core body temperature and the consequent hemodynamic adjustments (40). Nonetheless, it is important to highlight that the sham thermal treatment does not induce the same sensation as the active treatment and may unblind patients and change their expectations toward the efficacy of the treatment.

One consistent finding among prior studies is a permanent reduction in BP, particularly SBP, following repeated exposure to HT. The magnitude of the reduction in SBP varies considerably (from -4 to -10mmHg), likely reflecting differences in the experimental protocol, HT modality and the clinical characteristics of the target population (2, 15, 25, 30). The BP measuring method in these previous studies consisted of averaging of 2 to 3 readings obtained manually or using an automated device. There is little consensus on the optimal number of readings for accurate BP measurement, but it is clear that averaging multiple measurements may reduce the inherent variability of BP over short periods (43). In a study of 444 hypertensive individuals taking

antihypertensive medication, at least 5 BP measurements were needed to be 80% certain whether SBP was <140 mm Hg or not (43). Along these lines, one strength of the current study is that we performed a total of 15 BP measurements during the three experimental visits. After 6 weeks of treatment, the average difference between groups in the change in SBP from baseline was ~4 mmHg, but the variability across the 70-min interval was high (Figure 4). For instance, at 20 min, the groups differed by nearly 14 mmHg (p=0.01), while no differences (0.07 mmHg) were noted at 35 min (p=0.96). These results suggest although HT may possibly lower SBP in patients with PAD, the results are not uniform across multiple measurements. Future studies should incorporate other BP measurement strategies, including home-based and ambulatory monitoring, to confirm whether the BP-lowering action of HT holds true in unsupervised settings.

The reduction in serum concentrations of ET-1 following 6 weeks of HT aligns closely with our previous observations (40). The importance of this finding is evident when considering that: 1) ET-1 promotes hypertension, vascular and cardiac hypertrophy, fibrosis and atherosclerosis (10); 2) the systemic ET-1 concentrations are markedly elevated in PAD (9), 3) ET-1 levels predict adverse outcomes in patients with chronic heart failure (22) and all-cause mortality in the general population (27, 56). Nonetheless, it is unknown if the observed 13% reduction in serum ET-1 is clinical meaningful for PAD patients. Also, serum levels of ET-1 do not necessarily reflect the overall ET-1 production because this factor is secreted abluminally, towards the vascular smooth muscle and is cleared by ET_B receptors (10). It remains be determined if the HT-induced reduction in serum ET-1 is the result of reduced production, accelerated clearance or a combination of both.

Despite the absence of changes in exercise capacity and vascular function, supervised HT for 6 weeks had a marked impact on health-related quality of life. The scores for the physical

function subscale (PF), the most significantly impaired among all subscales of the short-form SF-36 questionnaire in PAD patients (26), improved by 3.6 and 6.8 points after 3 and 6 weeks of treatment in the group exposed to HT, while a progressive decline was observed in the control group (-2.2 at week 3 and -6.9 at week 6). Gardner and colleagues recently reported that the minimal clinically important differences for small, moderate, and large changes in the PF score following 3 months of supervised exercise in symptomatic PAD patients were 3, 9 and 14 points (19). Based upon these estimates, it appears that as little as 3 weeks of HT evokes small, albeit clinically significant changes in quality of life in claudicants. Further, the improvements attained after 6 weeks of HT are nearly equivalent to the changes observed after 12 weeks of supervised exercise, the gold standard treatment regimen for symptomatic PAD (19). Nonetheless, as the assessment of quality of life was a secondary outcome, additional, adequately powered studies are warranted to confirm these seminal findings.

The improvement in health-related quality of life in the HT group occurred despite no changes in 6-minute walk distances. This is not surprising given the well-documented weak relationship between objective measures of physical function and quality of life in this patient population (3, 6, 26). In fact, the strongest predictors of the PF scores in PAD patients include the perceived ability to walk fast, climb stairs and perform certain activities of daily living, including bathing (20). The mechanisms by which HT improves self-reported quality of life are unclear, but it is worth noting that we previously reported increases in muscle strength following repeated heat stress in a preclinical model of PAD (32) as well as in young individuals (31). It is thus tempting to speculate that HT may enhance muscle strength in PAD patients and it turn restore the capacity to execute functional tasks and improve perceived physical function.

Limitations

Participants underwent the assessment of several outcomes during the experiential visits and it is possible that the order in which the tests were conducted influenced the results of those tests. For instance, it is conceivable that thigh occlusion and reactive hyperemia during phase-contrast imaging altered the responses of subsequent tests, including the measurement of cutaneous microvascular reactivity and the 6-minute walk test. Nonetheless, it is important to note that all participants underwent the exact same protocol and it is therefore expected that the ordering effect was similar between groups. Second, the circulating levels of nitric oxide metabolites is heavily influenced by diet (55) as well other factors, such as the use of mouthwash (28). The large variability in serum NOx levels reported herein may thus derive by the lack of control for these important factors. Third, we have previously shown that a single HT session reduces serum levels of ET-1 in claudicants (40) and it is unclear how long this beneficial effect persists. Although the experimental outcomes were assessed at least 48 hrs after the last treatment session, it is impossible to exclude the possibly that the observed reduction in ET-1 concentrations partially reflect a residual effect of treatment. Lastly, we did not measure BP during the assessment of leg reactive hyperemia. This is an important limitation because eventual changes in BP due to discomfort or pain during thigh cuff occlusion or other factors can directly alter leg blood flow.

Summary and clinical implications

In summary, we report that leg HT using water-circulating trousers was well-tolerated and elicited important improvements in perceived physical function in patients with symptomatic PAD. The excellent adherence to the prescribed regimen in the present study raises the prospect of favorable uptake and compliance to this new modality in unsupervised settings. Indeed, water-circulating garments combined with a portable pump might be an ideal tool for delivering HT in

patients with symptomatic PAD because this strategy is amenable for home-use, which eliminates the need for patients to travel to a clinical facility to receive the treatment. In addition, water-circulating garments are practical for patients with multiple comorbidities that cannot undergo standard exercise regimens, and is amenable for combination with other established approaches employed for PAD treatment. Thus, given its accessibility, tolerability and ease of use, HT via water-perfused garments has the potential for rapid translation and application in the clinical setting. Nonetheless, additional studies are necessary to determine the optimal leg HT regimen and to compare the effectiveness of local versus whole-body HT modalities in PAD patients.

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Disclosures

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

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Table 1: Demographic and clinical characteristics

	All subjects (n=30[b])	Control (n=15)	HT (n=15)	p-value[a]
Age (yrs)	68.8 (8.2)	69.0 (7.8)	68.7 (8.9)	0.93
Height(cm)	174.3 (6.8)	173.4 (7.0)	175.2 (6.7)	0.47
Weight(kg)	83.4 (15.6)	81.3 (15.8)	85.6 (15.7)	0.47
ABI-most affected leg	0.6 (0.1)	0.6 (0.1)	0.7 (0.1)	0.15
ABI-the other leg	0.84 (0.18)	0.78 (0.15)	0.90 (0.20)	0.09
BMI (kg/m^2)	27.36 (4.11)	26.93 (4.21)	27.81 (4.11)	0.57
Sex, n (%)				0.59
Male	26 (86.6)	12 (80.0)	14 (93.3)	
Female	4 (13.3)	3 (20.0)	1 (6.6)	
Most symptomatic leg, n (%)				0.45
Left	12 (40.0)	5 (33.3)	7 (46.6)	
Right	18 (60.0)	11 (66.6)	8 (53.3)	
Stents, n (%)				0.87
No	17 (58.6)	8 (57.1)	9 (60.0)	
Yes	12 (41.3)	6 (42.8)	6 (40.0)	
Smoking status, n (%)				0.40
Never smoked	2 (6.6)	2 (13.3)	0 (0.0)	
Current smoker	13 (43.3)	7 (46.6)	6 (40.0)	
Past smoker	15 (50.0)	6 (40.0)	10 (60.0)	
Diabetes, n (%)				0.12
No	20 (66.6)	12 (80.0)	8 (53.3)	
Yes	10 (33.3)	3 (20.0)	7 (46.6)	
Race, n (%)				0.65
Black/African American	6 (20.0)	2 (13.3)	4 (26.6)	
White	24 (80.0)	13 (86.6)	11 (73.3)	
Medications, n (%)				
Beta Blocker	10 (33.3)	5 (33.3)	5 (33.3)	
ACE Inhibitor	7 (23.3)	4 (26.6)	3 (20.0)	
Statin	23 (76.6)	13 (86.6)	10 (66.6)	

Table 1 continued

	All subjects (n=30[b])	Control (n=15)	HT (n=15)	p-value[a]
Cilostazol	6 (20.0)	4 (26.6)	2 (13.3)	
Calcium Channel Blocker	12 (40.0)	7 (46.6)	5 (33.3)	
Diuretic	6 (20.0)	2 (13.3)	4 (26.6)	
Insulin	4 (13.3)	1 (6.6)	3 (20.0)	
Anti-Platelet Agent	22 (73.3)	10 (66.6)	12 (80.0)	
Angiotensin II Receptor Antagonist	8 (26.6)	4 (26.6)	4 (26.6)	
Other	26 (86.6)	12 (80.0)	14 (93.3)	

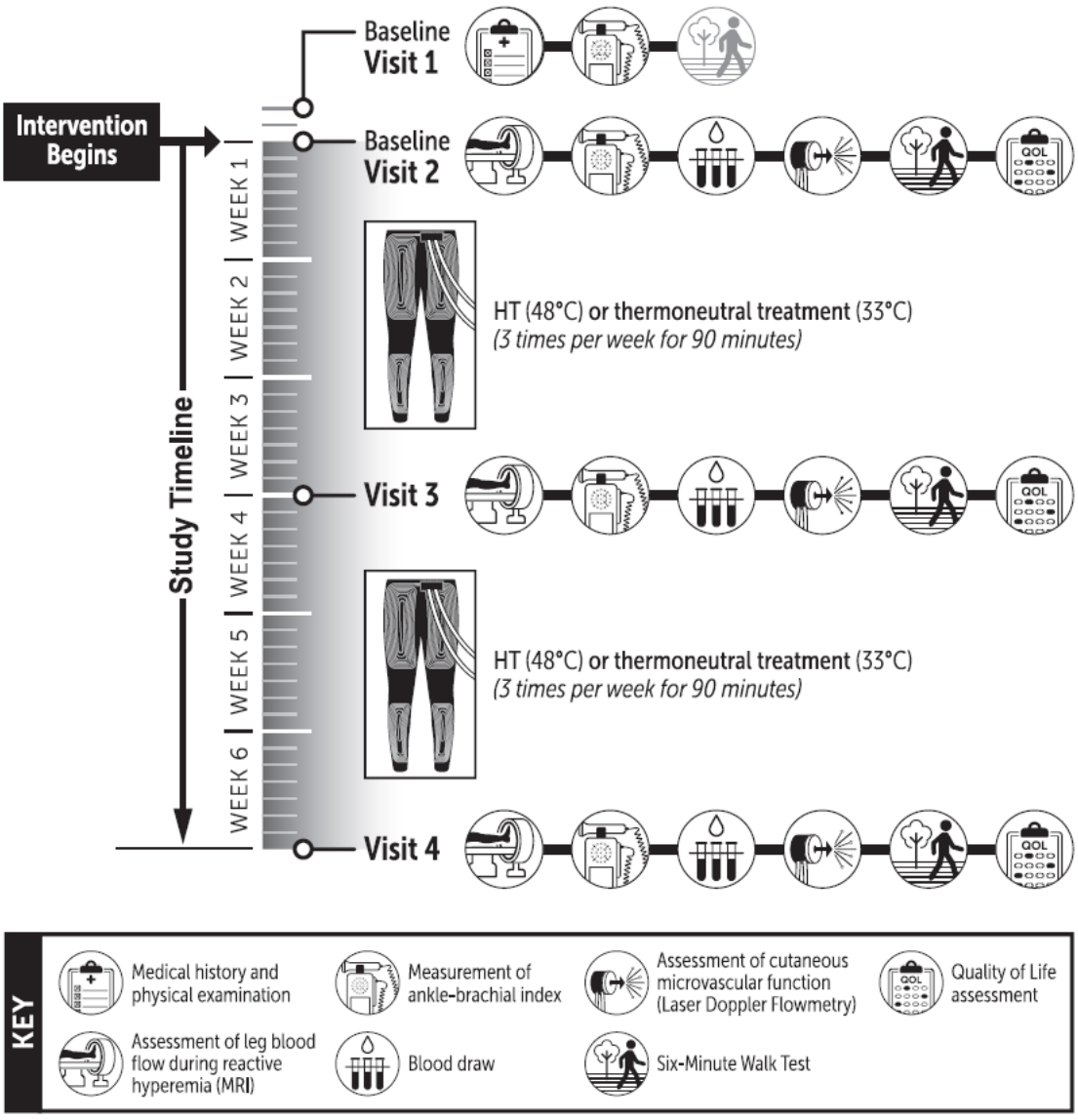

Figure 1: Schematic of the experimental protocol.

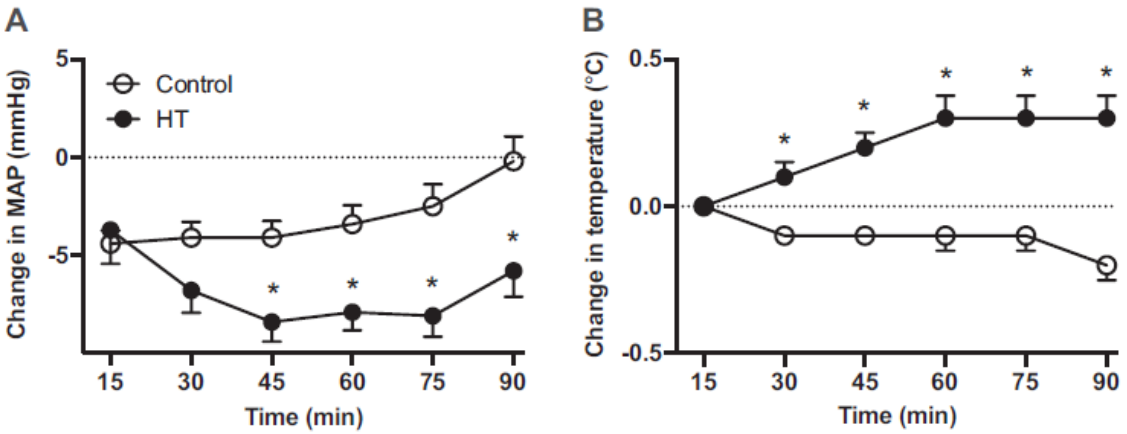

Figure 2: Average changes from baseline (time 0) in mean arterial pressure (MAP) (A) and tympanic temperature (C) during exposure to 90 min of HT (closed circles, n = 15) or the sham treatment (open circles, n = 15). Participants were asked to complete 18 sessions (3 times/week) over 6 weeks. The graphs display the average values across all 18 sessions. Data are mean ± SD. Data were analyzed using analysis of covariance (ANCOVA) with baseline as a covariate. The SIDAK adjustment was used to adjust the group effect p-value at each time point. *Difference between groups (P<0.05).

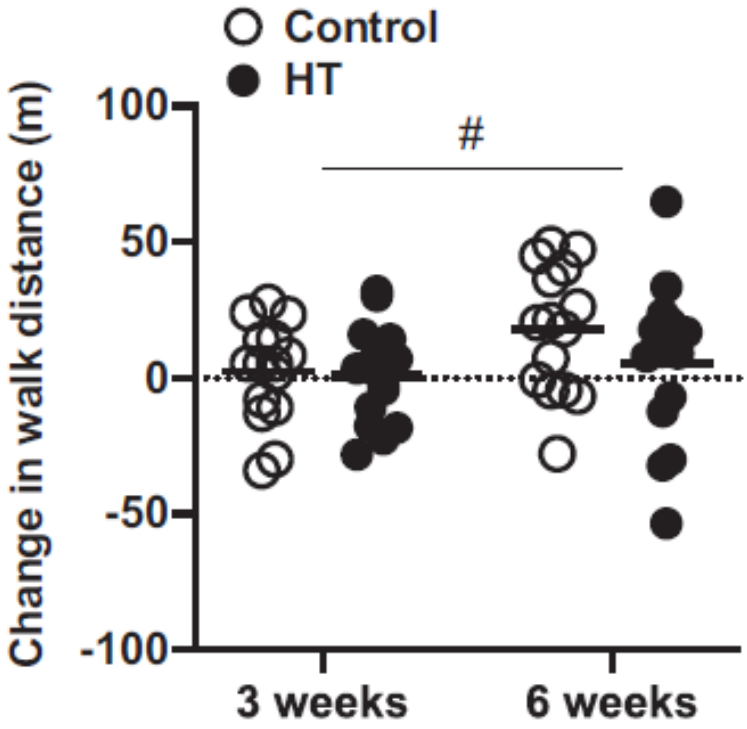

Figure 3: Individual and group mean changes from baseline in walking distances on the 6-minute walk test following 3 and 6 weeks of HT (closed symbols, n = 15) or the sham treatment (open symbols n = 15). Data were analyzed using analysis of covariance (ANCOVA) with baseline as a covariate.

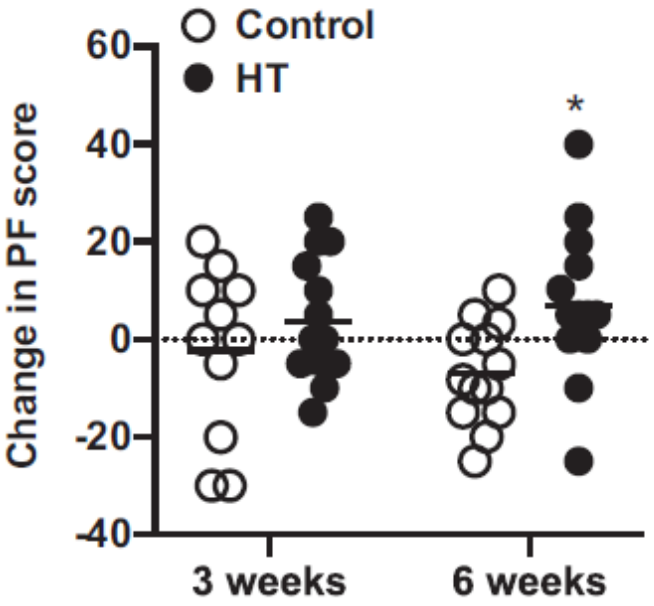

Figure 4**:** Individual and group mean changes from baseline in the scores for the physical functioning (PF) subscale of the Medical Outcomes Study 36-Item Short Form Health Survey (SF-36v2) following 3 and 6 weeks of HT (closed symbols, n = 14) or the sham treatment (open symbols n = 14). Data were analyzed using analysis of covariance (ANCOVA) with baseline as a covariate. *Difference between groups (P<0.05).

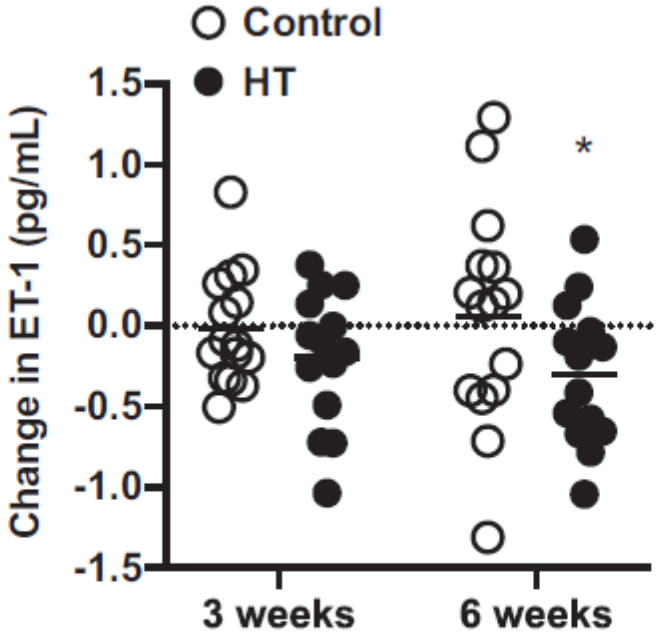

Figure 5: Individual and group means for the changes from baseline in the serum concentrations of ET-1 following 3 and 6 weeks of HT (closed symbols n = 14) or the sham treatment (open symbols, n =14). Data were analyzed using analysis of covariance (ANCOVA) with baseline as a covariate. *Difference between groups (P<0.05).

CHAPTER 3. ACUTE HEAT THERAPY IN PAD

Acute effects of leg heat therapy on walking performance and cardiovascular and inflammatory responses to exercise in patients with peripheral artery disease

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Running Title: Leg heat therapy in patients with peripheral artery disease

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Abstract

Lower-extremity peripheral artery disease (PAD) is associated with increased cardiovascular disease risk and impaired exercise tolerance. We have previously reported that leg heat therapy (HT) applied using liquid-circulating trousers perfused with warm water increases leg blood flow and reduces blood pressure (BP) and the circulating levels of endothelin-1 (ET-1) in patients with symptomatic PAD. In this sham-controlled, randomized, crossover study, sixteen patients with symptomatic PAD (age 65±5.7 years and ankle-brachial index: 0.69±0.1) underwent a single 90-min session of HT or a sham treatment prior to a symptom-limited, graded cardiopulmonary exercise test on the treadmill. The primary outcome was the peak walking time (PWT) during the exercise test. Secondary outcomes included the claudication onset time (COT), resting and

exercise BP, calf muscle oxygenation, pulmonary oxygen uptake ($\dot{V}O_2$), and plasma levels of ET-1, interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). Systolic, but not diastolic BP, was significantly lower (~7 mmHg, $p<0.05$) during HT when compared to the sham treatment. There was also a trend for lower SBP throughout exercise and the recovery period following HT ($p=0.057$). While COT did not differ between treatments ($p=0.77$), PWT tended to increase following HT (CON: 911±69 s, HT: 954±77 s, $p=0.059$). Post-exercise plasma levels of ET-1 were also lower in the HT session (CON: 2.0±0.1, HT: 1.7±0.1, $p=0.02$). Calf muscle oxygenation, $\dot{V}O_2$, COT, IL-6 and TNF-α did not differ between treatments. A single session of leg HT lowers BP and post-exercise circulating levels of ET-1 and may enhance treadmill walking performance in symptomatic PAD patients.

Keywords

Peripheral artery disease, intermittent claudication, heat therapy, endothelin-1, blood pressure

Introduction

Lower-extremity peripheral artery disease (PAD) affects an estimated 236 million individuals worldwide (50). Patients with PAD exhibit a marked reduction in exercise tolerance (24), lower habitual physical activity levels (48), and an accelerated functional decline when compared to their healthy counterparts (37). A hallmark symptom of PAD is intermittent claudication (IC), defined as exertional ischemic leg pain that subsides with rest. The prevalence of IC is estimated to be <1% in those aged <50 years, increasing to 6% in those aged >65 years (42). The genesis of the functional impairment in PAD is multifaceted and include abnormalities in the vasculature, peripheral nerves and skeletal muscle (25). Indeed, the walking impairment in PAD patients is associated, among other factors, with lowered muscle perfusion (3, 32, 46),

oxidative stress and inflammation (19, 43, 44), neuromuscular dysfunction (16, 17) and numerous pathological changes in skeletal muscle, including atrophy and increased fat accumulation (35). These patients also display abnormal responses to exercise, including an exaggerated pressor response (29, 38) and elevated circulatory levels of inflammatory mediators (5, 49) and the potent vasoconstrictor endothelin-1 (ET-1) (6, 33). The excessive inflammatory response, and in particular the overproduction of ET-1, is thought to antagonize muscle hyperemia during exercise, aggravate the endothelial dysfunction and thus contribute to the development of vascular and skeletal muscle sequelae (25).

Emerging evidence indicates that heat therapy (HT) may be a practical therapeutic option to alleviate the symptoms of PAD and improve the quality of life of symptomatic patients. We first demonstrated that a single session of leg HT using customized, liquid-circulating trousers perfused with warm water increases leg blood flow by ~100% and reduces blood pressure (BP) and the circulating levels of ET-1 in symptomatic PAD patients (41). Further, we recently reported that repeated exposure to leg HT for 6 weeks improved perceived physical functioning and reduced the levels of ET-1 by 13% when compared to a sham treatment. Other HT modalities, including sauna and water bath immersion, have also been shown to elicit beneficial adaptations in PAD patients, including an improvement in walking tolerance (1, 41, 45, 55). In a preclinical model of peripheral vascular insufficiency, repeated HT for as little as 3 days abrogated the pressor response to static muscle contraction (47).

The goal of the present study was to examine the effects of pre-treatment with leg HT or a sham intervention on the cardiovascular responses and tolerance to a symptom-limited exercise test on the treadmill in patients with symptomatic PAD. Building on our previous findings, we hypothesized that a single 90-min session of leg HT would: 1) improve calf muscle oxygenation,

2) enhance pain-free and maximal walking time, and 3) reduce blood pressure and the levels of ET-1 at rest and during exercise. Based upon the observations in cultured cells (8), rodents (9) and patients with ankylosing spondylitis (53) of reduced inflammation after exposure to heat stress, we further hypothesized that leg HT would reduce the plasma levels of interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF alpha).

Methods

Subjects

Participants were identified and contacted by the Indiana Clinical and Translational Science Institute Research Network (ResNet) research assistants. After interest in participation was established, patients were contacted directly by the investigators. Additional study participants were obtained by direct physician referral from the Division of Vascular Surgery at Methodist Hospital. All participants had an ABI of <0.90 and a history of exertional leg pain. Patients were excluded if they had uncontrolled diabetes (HbA1C > 8.5, <3 months prior to taking part in the study), chronic heart failure (stage C and D), evidence of non-healing wounds or tissue loss, recent vascular surgery or endovascular revascularization, were HIV, HBV or HCV positive, morbid obesity (BMI > 36), chronic kidney disease (eGFR < 30 mL/min/1.73m^2), current treatment for cancer, or if they were unable to safely and reliably complete a maximal graded treadmill walking test. The protocol was approved by the Institutional Review Board of Indiana University (no. 1708785351), and registered with the United States Library of Medicine on clinicaltrials.gov (NCT03435835). Written, informed consent was obtained, and all procedures adhere to the requirements of the U.S. Federal Policy for the Protection of Human Subjects (45 CFR, Part 46), and support the general ethical principles of the Declaration of Helsinki.

Experimental Design

A schematic of the experimental protocol is depicted in Figure 6. To familiarize participants with the symptom-limited cardiopulmonary treadmill exercise test and assess the test-retest reliability of walking tolerance, two tests were completed at baseline, at least 72 hours apart. On visit 1, participants underwent a lower extremity arterial examination to determine the ankle-brachial index (ABI). Next, participants were escorted to an adjacent room and underwent resting pulmonary function testing, followed by a graded cardiopulmonary treadmill exercise test. Pulmonary oxygen uptake ($\dot{V}O_2$) and calf muscle oxygenation were measured continuously throughout the test. Similar procedures were followed on visit 2, with the exception of the lower extremity arterial exam. If the difference in peak walking time (PWT) during the exercise tests between visits 1 and 2 was greater than 20%, participants were asked to complete a third exercise test, at least 72 hrs after visit 2. Once the variation in PWT was deemed acceptable (i.e. <20%), participants were assigned, using a randomized, crossover design, to undergo a single session of either HT or a sham treatment (CON) prior to a symptom-limited cardiopulmonary treadmill exercise test. The primary study outcome was the difference in peak walking time (PWT) between HT and the CON conditions. Secondary outcomes included the differences between visits in claudication onset time (COT), blood pressure and calf muscle oxygenation at rest and during exercise, pulmonary oxygen uptake ($\dot{V}O_2$) and plasma ET-1, IL-6 and TNF-a concentrations post-treatment and post-exercise. Prior to the experimental visits, participants were asked to report to the laboratory in a fasted state (>8 hrs postprandial), refrain from exercise (24 hrs), smoking (>4 hrs), and take their usual medications.

Pulmonary function tests

Pulmonary function tests were performed following the American Thoracic Society and European Respiratory Society guidelines (39). FVC and FEV1 were measured and reported as both raw and %predicted values (51).

Symptom-limited cardiopulmonary exercise test

Exercise testing was performed in a motorized treadmill (Pro 27, Woodway, St. Paul, Minnesota, United States) following the Gardner-Skinner protocol, which consists of walking at a constant speed (2 mph) with a 2%-grade increase every 2 min (21). A 12-lead electrocardiogram (ECG) was registered continuously. Blood pressure was measured in the left arm using a stethoscope and sphygmomanometer prior and during exercise and for 10 min during recovery. Expired respiratory gases were collected breath by breath via a facemask attached to a gas analyzer (MedGraphics, CardiO2, and CPX/D system using Breeze EX Software, 142090-001, ReVia; MGC Diagnostics, St. Paul, MN, United States). Calf muscle oxygenation was assessed using a near infrared spectroscopy device (NIRS; Portomon, Medis, the Netherlands), which was affixed on the skin over the medial portion of the gastrocnemius of the leg of which the participants self-described as having the more severe pain during exercise. Participants received standardized instructions and were asked to indicate when they first began to feel leg pain with a “thumbs up” signal (defined as COT), and then give a “thumbs down” signal when they could no longer continue with the test (defined as PWT). Participants were allowed to use the handrails for balance, but were not allowed to use them as an aid for walking (i.e. pulling themselves up).

Heat and sham treatments

Participants were instructed to ingest a core temperature sensor (CorTemp, HQ Inc., Palmetto, Florida, United States) the night before the experimental visits. Upon arrival, participants were allowed to rest for 15 min and were then instrumented with leg skin thermistors (MLT422; ADInstruments, Colorado Springs, CO, United States) and a blood pressure cuff on the upper arm that was connected to an automated monitor (Suntech CT40, Suntech Medical, Morrisville, North Carolina, United States). An intravenous catheter was placed in an antecubital vein of the left arm for blood sampling. After 30 min of resting in the semi-recumbent position, participants were fitted with liquid-circulating trousers as described previously (40). In the CON session, water at 33°C was circulated through the trousers using a water pump for 90 min (HTP-1500, Adroit Medical, Louden, Tennessee, United States). We have previously demonstrated that this regimen elevates skin temperature by approximately 2ºC, but does not evoke measurable changes in core body temperature, heart rate and blood pressure (40). In the HT session, water at 43°C was circulated through the tube-lined trousers using a heated bath circulator (HT; Aqua Relief Systems, Akron, Ohio, United States) with the goal of increasing leg skin temperature to 37-38ºC. The following parameters were assessed every 5 min during baseline and throughout the 90-min intervention: 1) core body temperature (HQ Inc., Palmetto, Florida, United States), leg skin temperature, blood pressure, and heart rate. Prior to the onset of the treatment and at 45 and 90 min, patients were asked to subjectively grade thermal comfort scores using on an 11-point feeling scale (23). After the completion of the treatment, participants were promptly escorted to an adjacent room and performed a symptom-limited exercise test as in visits 1 and 2. Blood samples were taken at baseline, at the end of the 90-min treatment and 10 min after the end of the exercise test.

Assessment of tissue oxygenation

The tissue saturation index (TSI%) of the most symptomatic leg was assessed with a commercially available NIRS system (Portamon, Artinis Medical Systems, The Netherlands). Initially, the skin over the medial gastrocnemius of the most symptomatic leg was cleaned and, if necessary, shaved. The device was affixed using medical tape and covered with a dark cotton sleeve. The location of the monitor was traced with permanent marker to ensure consistent placement in subsequent visits. The tracings were covered with and adhesive waterproof covering (Tegaderm, 3M, Maplewood, MN, United States) after the exercise test and re-drawn at each subsequent visit. Data were exported at 1Hz and the final 20-second average of each stage was utilized for analysis.

Analysis of blood markers

Samples were drawn into tubes containing EDTA (BD Vacutainer, BD, Ontario, Canada). One tube was transported to the Indiana University Health Pathology Laboratory for the assessment of a routine basic metabolic panel. All other plasma samples were centrifuged at 4ºC for 10 min at 1,100 g (ST16, Thermo Scientific, Waltham, MA, United States). Samples were then aliquoted and immediately placed into a -80°C freezer. Commercially available enzyme-linked immunosorbent assay kits were used to measure the plasma concentrations of ET-1 (DET100, Endothelin-1 Quantikine ELISA Kit, R&D Systems, Minneapolis, MN, United States), TNF-α (HSTA00E, Human TNF-alpha Quantikine HS ELISA kit, R&D Systems, Minneapolis, MN, United States) and IL-6 (HS600C, Human IL-6 HS ELISA Kit, R&D Systems, Minneapolis, MN, United States). Blood biomarker values were corrected for potential changes in plasma volume during heating and exercise as described elsewhere (28).

Statistical Analysis

All data were analyzed using SAS v9.4. Data are presented as mean ± SD. Variables assessed during exposure to HT or CON (blood pressure, core temperature, HR, and skin temperature) were compared using a two-way repeated measures analysis of variance (ANOVA). PWT and COT times were compared using a paired t-test. All measurements taken at COT and PWT were time-aligned (isotime) between visits 3 and 4 at the point of the earlier COT and PWT. Blood pressure during exercise was compared between groups using a two-way repeated measures ANOVA. Missing values during the exercise test were imputed by linear interpolation, using the mean of the values from the two adjacent stages. If the value for the final stage was missing, then it was recorded as a repeat of the previous stage. TSI% and $\dot{V}O_2$ are represented using the mean of the final 20 seconds for each stage. Staged averages were compared using a two-way repeated measures ANOVA. When a group-by-time interaction was detected, post-hoc analysis compared values at each time point. A Bonferroni adjustment was applied to correct for multiple comparisons when appropriate.

Results

Subject characteristics

Nineteen participants were consented, but three were withdrawn before receiving an experimental treatment due to inability to safely and consistently perform the treadmill exercise test. A total of 16 participants completed all study visits. Demographic and clinical characteristics are presented in Table 2. All but one of the participants was a current or past smoker. Eleven patients were taking one or more antihypertensive medication and five participants were currently taking the phosphodiesterase-3 inhibitor cilostazol.

Reliability of exercise parameters

Four patients were asked to complete a third baseline exercise test because the difference in PWT between tests 1 and 2 were greater than 20%. After the third test, all 4 patients met the criteria to continue in the study. As shown on Figure 7, there were no differences between tests for COT (test 1: 355±232s, test 2:367±224s, p=0.49) and PWT (test 1: 876±334s, test 2: 882±348s, p=0.84).

Physiological and perceptual responses to HT

The heat treatment was well-tolerated and no adverse reactions were reported. On average, participants scored comfort levels between "Good" and "Very Good" on an 11-point feeling scale (23). Figure 8 depicts the average responses of leg skin temperature, intestinal temperature, and systolic (SBP) and diastolic (DBP) blood pressures during exposure to HT and CON. During HT, leg skin temperature rose from a baseline of ~31ºC to approximately 37ºC (*Panel A,)* while core body temperature did not change significantly compared to CON (*Panel B)*. SBP (*Panel C*) and MAP, but not DPB (*Figure 3, Panel D*), was significantly reduced ($p<0.05$) during exposure to HT when compared to the sham treatment. This hypotensive effect was particularly evident after approximately 60 min of exposure to the treatment (*Figure 3, Panel C*). HR did not differ significantly between groups during treatment.

Effect of HT on walking performance

Analysis of walking times during the symptom-limited treadmill test after exposure to HT and CON revealed the presence of two outliers, which displayed differences in PWT between experimental visits of more than three standard deviations from the mean. One participant had a vasovagal episode due to trypanophobia after the blood draw during the HT session. On the

subsequent CON visit, the patient expressed fear in experiencing another such episode and displayed a declined of 304 seconds in PWT. The second outlier nearly doubled the PWT detected in the baseline tests after exposure to CON, which is indicative of a lack of maximal effort during the baseline assessments. With the outliers included, there were no differences between treatments for COT (CON: 452.6±53.2, HT: 442.9±65.1 seconds, p=0.77) and PWT (CON: 955.6±84.3, HT: 947.7±88.1 seconds, p=0.84). After removal of the outliers, there was a trend for improved PWT in the HT group (CON: 911.9±69.0, HT: 954.4±77.2 seconds, p=0.059) (Figure 9).

Blood pressure responses to exercise

Figure 10 depicts the changes in SBP and DBP during the graded treadmill test and during 10 min of recovery. On average, SBP was ~4 mmHg lower throughout exercise and recovery following exposure to HT when compared to the sham intervention. There was a trend for a main effect of treatment for SBP (Figure 5, Panel A; p=0.058), but DBP was similar after HT and CON (Panel B).

$\dot{V}O_2$ and skeletal muscle oxygenation

Figure 11 depicts the changes in $\dot{V}O_2$ (panel A) and calf muscle TSI (panel B) during the graded treadmill test and during recovery. There were no significant differences between treatments for either $\dot{V}O_2$ or TSI.

Plasma biomarkers

Figure 12 displays the individual and mean values for the plasma concentrations of IL-6, ET-1 and TNF-α at baseline, immediately after exposure to 90-min of HT or CON and 10 min after the maximal exercise bout. There was a significant group-by-time interaction for plasma ET-

1 (p=0.03) and subsequent post-hoc testing revealed a significant reduction in ET-1 levels after exercise following HT (CON: 2.04±0.16, HT: 1.73±0.14 pg/mL, p=0.02). IL-6 levels rose significantly with exercise (p=0.002), but to a similar degree between HT and CON. TNF-α levels did not differ between treatments at any time point.

Discussion

The primary goal of the present study was to test the hypothesis that leg heating prior to a maximal treadmill test would alleviate exertional leg pain and consequently improve walking tolerance in patients with symptomatic PAD. Based upon previous findings that leg heating enhances leg blood flow at rest (10, 40, 54) and during exercise (10), we reasoned that HT would improve calf muscle oxygenation during walking, thereby delaying the onset of ischemia leg pain and extending maximal walking time in claudicants. Contrary to this hypothesis, calf oxygenation during exercise and COT were unaffected by prior exposure to leg heating. However, the majority of patients experienced an increase in PWT following HT, thus revealing that a potential ergogenic effect of leg heating is unrelated to calf muscle oxygenation. We also report that HT lowered resting SBP and this hypotensive effect partially lingered throughout the exercise bout and recovery. Lastly, leg HT lowered the plasma concentration of the vasoconstrictor ET-1 after exercise by 13% relative to baseline, while an increase of ~11% was detected in the sham condition. Combined, these findings lend further support to the notion that HT may be a useful adjunctive therapy for symptomatic PAD.

We previously showed that perfusion of water at 48ºC through liquid-circulating trousers elevated leg skin temperature by ~7ºC and intestinal temperature by ~0.8ºC and caused a profound reduction in blood pressure in symptomatic PAD patients (40). Herein, we extend these previous observations by showing that a less strenuous thermal challenge induced by circulating water at

43ºC through the trousers also causes a marked reduction in SBP. During the last 30 of the 90-min leg HT treatment, SBP and mean arterial pressure were, on average, ~11 mmHg and ~6 mmHg lower when compared to the sham treatment. These reductions in BP occurred despite the absence of appreciable changes in intestinal temperature, thus implying that elevations in core body temperature are not a prerequisite for the hypotensive effects of HT in PAD patients. The mechanisms by which HT lowers BP remains poorly defined, but emerging evidence suggests that, contrary to young individuals, aged adults do not display the characteristic increase in muscle sympathetic nerve activity following leg heating (15). It has thus been proposed that a blunted neural compensatory response to HT-induced increases in leg vascular conductance partly underlies the hypotensive effects observed in elderly individuals (15).

The BP lowering effect of HT may be clinically meaningful for PAD patients, which have a heightened risk of cardiovascular morbidity and mortality (22). Hypertension is one of the most common comorbidities and a major contributor to the elevated risk of cardiovascular events in PAD patients (14). Although we report acute reductions in BP, it is reasonable to speculate that the cumulative effects of repeated bouts of HT would elicit a sustained reduction in BP. Along these lines, Akerman and colleagues reported that repeated spa bathing for 12 weeks reduced SBP to a greater extent than supervised exercise in PAD patients (2). It remains to be determined, nonetheless, if the acute hypotensive effect of a single session of HT predicts the chronic BP reduction following long-term HT treatment.

The reduction in resting BP after HT was partially sustained throughout the exercise bout and during the recovery period. This finding also holds potential clinical significance because elevated exercise BP is linked to ambulatory dysfunction (30) and is a strong independent risk factor for all-cause long-term mortality in people with PAD (12). However, it is important to note

that when expressed relative to pre-exercise levels, the increment in BP during exercise was similar following HT and CON. In other words, the modest reduction in BP during exercise after HT simply reflected the lower baseline level rather than an attenuated pressor response to exercise. These findings are incongruent with the recent observations of Qin and colleagues that repeated local leg heating for a little as three days abrogated the pressor response to static muscle contraction in rats subjected to femoral artery ligation (47). This discrepancy likely arises from important differences in the experimental model and protocol. First, the experiments of Qin and co-workers were performed in young Sprague-Dawley rats that had no exposure to the central risk factors for PAD (47). Second, the HT treatment protocol commenced only 3 days after the ligation procedure, when the natural compensation to the ischemic insult is still occurring (59). Third, synchronous, static muscle contractions in response to direct nerve stimulation do not mimic the mechanical, metabolic and pressor responses to voluntary exercise.

Endothelial dysfunction, caused in part by elevated inflammation and oxidative stress, is negatively associated with walking performance and calf muscle oxygenation in patients with symptomatic PAD (19, 20). Conversely, emerging evidence indicates that HT may impart resistance in endothelial cells against the deleterious effects of inflammation and oxidative stress. For instance, Brunt and colleagues recently showed that incubation of endothelial cells with serum from individuals treated with whole-body HT for 8 weeks reduced the cellular damage elicited by hypoxia-oxygenation (8). Along these lines, we reasoned that exposure to HT would reduce the circulating levels of inflammatory and vasoconstrictive agents, particularly after ischemia-reperfusion elicited by the maximal exercise bout. We found that although HT had no measurable impact on plasma TNF-□ and IL-6, the concentration of ET-1 after exercise was reduced to levels below baseline in the heating condition. As ET-1 is pro-inflammatory and a potent vasoconstrictor

(13), it is conceivable that a reduction in the post-exercise levels of this factor following leg heating may abrogate ischemia-reperfusion injury and possibly facilitate post-exercise hypotension (11), thereby augmenting the benefits of exercise in symptomatic PAD patients.

We chose to assess the effects of a single session of HT on walking performance during a graded, symptom-limited treadmill test, as opposed to other walk tests, because of the unique possibility to record ventilatory and gas exchange parameters, BP and calf muscle oxygenation. However, treadmill testing is associated with a significant learning effect in patients with PAD as revealed by the findings of increased PWT even in the absence of a therapeutic intervention (27). Patients with PAD randomized to the placebo group in drug trials also display increases in treadmill performance (26). To minimize this important limitation, patients in our study were asked to complete at least two baseline tests on separate days before commencing the experimental protocol. It is noteworthy that 25% of patients (4 out 16) exhibited a difference greater than 20% in PWT between the first two familiarization tests and had to complete a third test. This important finding underscores the critical importance of performing multiple baseline treadmill tests in some patients with PAD to ensure proper familiarization and consistency, and as a result, lower the potential for a placebo effect.

In a pilot study in 6 patients with PAD, Pellinger and colleagues observed that as little as 15 min of leg heating in a water bath heated to 42ºC improved 6-minute walk distance by nearly 10% (45). Our findings align with these earlier observations as 9 out of 14 patients displayed an increase in PWT following HT when compared to the sham regimen. Albeit not statistically significant ($p=0.059$), the average increase in PWT of ~42 s may be clinically meaningful for symptomatic PAD patients. Recent estimates of minimal clinically important differences (MCID) in walking performance by Gardner and colleagues reveal that an increase of 38 s is a small MCID

after 3 months of supervised exercise training (18). Thus, a single session of leg HT may increase PWT as much as supervised treadmill walking, the primary treatment modality for PAD. These improvements occurred without any significant alterations in calf muscle oxygenation, which indicates that alternative mechanisms underlie the improvements in walking tolerance. One possibility is that HT-induced analgesia (56) evoked an increase in pain tolerance, thereby enabling an increase in PWT.

Limitations

Several limitations of our study should also be acknowledged. Our study focused solely on patients that displayed the classical symptoms of IC, which are present in only a small fraction of the PAD population. It is estimated that ~10% of people with PAD report exertional leg pain that resolves with rest (34). The vast majority of patients report either no symptoms or leg symptoms that are not consistent with IC (34). Of note, asymptomatic patients have a significant functional impairment when compared to people without PAD (36). It is thus important for future studies to examine whether the benefits of leg HT described herein extend to people with asymptomatic PAD. Another important limitation of the current study that is intrinsic to experiments examining the therapeutic value of thermal therapies is that it is not possible to blind participants to the treatment. In other words, since the thermal strain is markedly different between the sham and HT regimens, it is possible for patients to become unblinded to the treatment assignment. One possible strategy to circumvent this problem and minimize the potential positive expectancy effects in future investigations is to compare HT to an effectively administered placebo intervention, rather than a sham/control (7, 57, 58).

Summary and clinical implications

The pillars of medical management of PAD are to reduce cardiovascular morbidity and mortality and to reduce limb morbidity (4). Our current findings, as well as other recent studies (2, 31, 45), indicate that HT may be a practical adjunctive therapy that fulfill both therapeutic goals. First, by reducing resting and exercise BP as well as the circulating levels of ET-1, HT may improve cardiovascular health and consequently diminish the risk of MI, stroke and death in patients with PAD. Second, by enhancing leg blood flow (40, 54) and improving muscle mass and strength (31), among other factors, HT may alleviate the leg symptoms and enhance the functional capacity of PAD patients. Third, by improving walking tolerance in some patients, leg HT may boost the adaptations to regular exercise. In mice, whole-body heat stress augments endurance training-induced mitochondrial adaptations (52). Whether similar benefits occur in people with PAD remains to be determined.

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Disclosures

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

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Table 2: Demographic and clinical characteristics

Age (yrs)	65.7 (5.7)
Height (cm)	177.1 (7.2)
Weight (kg)	81.6 (15.7)
ABI-most affected leg	0.69 (0.1)
ABI-other leg	0.92 (0.2)
BMI (kg/m2)	25.8 (3.8)
Systolic blood pressure (mmHg)	136.2 (13.5)
Diastolic blood pressure (mmHg)	74.7 (9.8)
FVC (L)	3.85 (0.85)
$\%FVC_p$	88.43 (10.50)
FEV1 (L)	2.84 (0.64)
$\%FEV1_p$	87.25 (13.45)
%FEV1/FVC	73.88 (7.04)
Sex, n (%)	
Male	14 (87.5)
Female	2 (12.5)
Most symptomatic leg, n (%)	
Left	8 (50.0)
Right	8 (50.0)
Stents, n (%)	
No	11 (68.7)

Table 2: Values are as means ± SD or n (%) when indicated. ABI, ankle-brachial Index; BMI, body mass index; ACE, angiotensin-converting enzyme; FVC, forced vital capacity; $\%FVC_{p/c}$, % predicted forced vital capacity, FEV1, forced expiratory volume 1 second; $\%FEV1_p$, % predicted forced expiratory volume 1 second.

No	11 (68.7)
Yes	5 (31.2)
Smoking status, n (%)	
Never smoked	1 (6.2)
Current smoker	9 (56.2)
Past smoker	6 (37.5)
Diabetes, n (%)	
No	15 (93.7)
Yes	1 (6.2)
Race, n (%)	
Black/African American	4 (25.0)
White	12 (75.0)
Medications, n (%)	
Beta Blocker	5 (31.2)
ACE Inhibitor	4 (25.0)
Statin	12 (75.0)
Cilostazol	5 (31.2)
Calcium Channel Blocker	6 (37.5)
Diuretic	3 (18.7)
Anti-Platelet Agent	3 (18.7)
Angiotensin II Receptor Antagonist	2 (12.5)
Opioid	4 (25.0)
Other	15 (93.7)

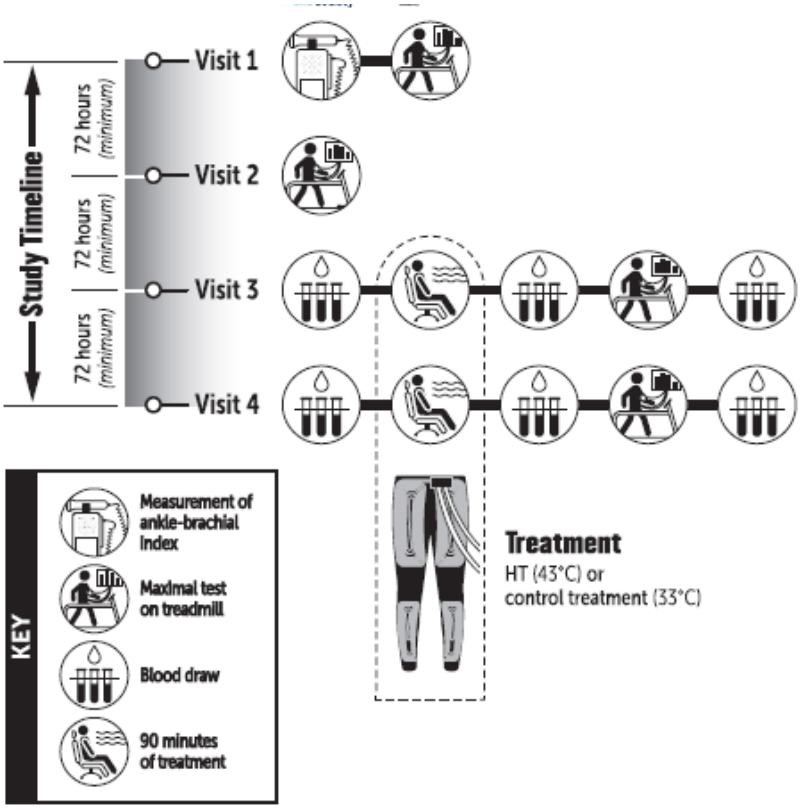

Figure 6: Schematic of the experimental protocol.

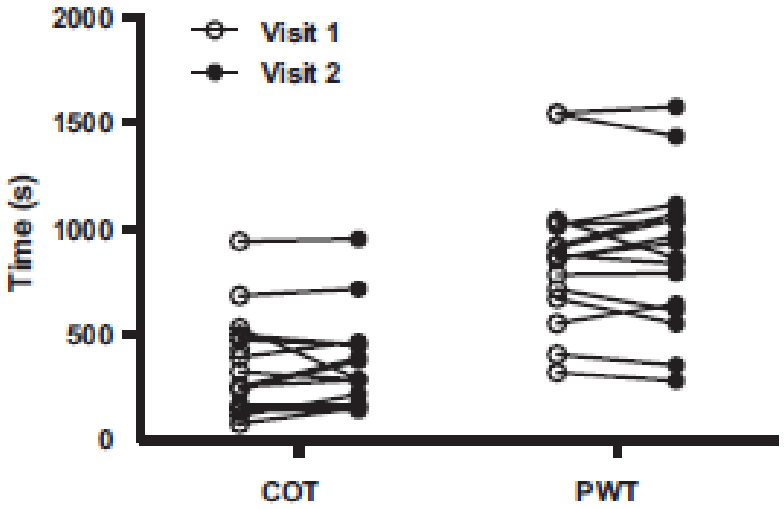

Figure 7**:** Individual values for COT and PWT at visit 1 (open squares) and visit 2 (open circles). Data were analyzed using a paired t-test. COT, claudication onset time; PWT, peak walking time.

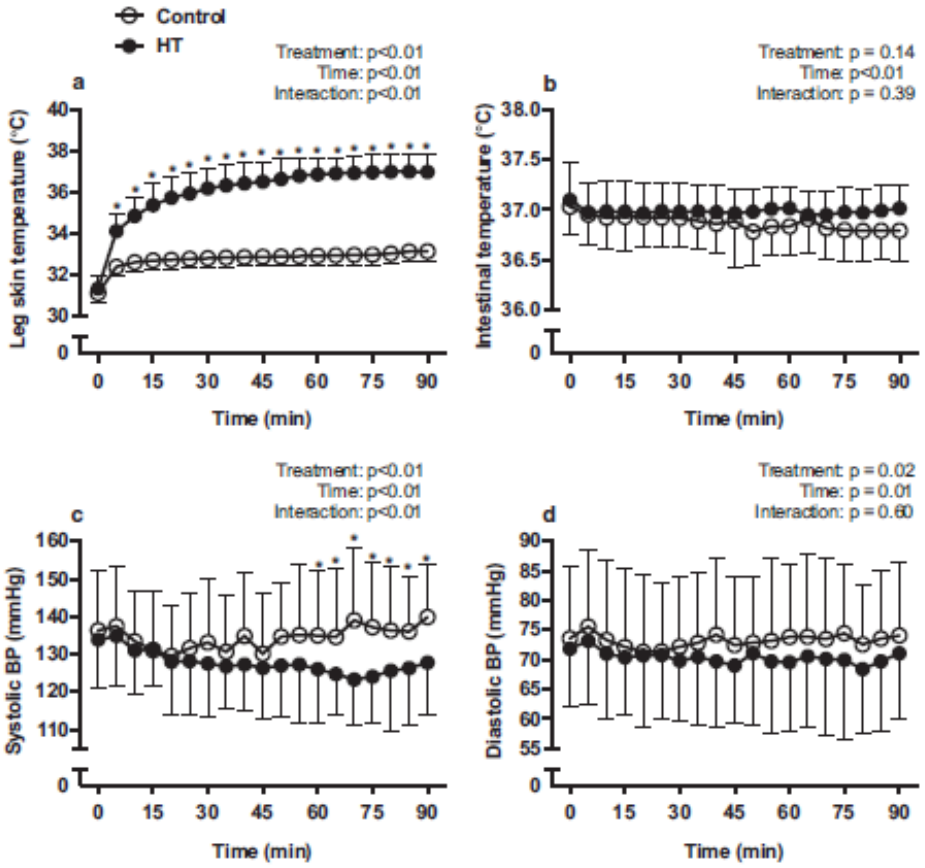

Figure 8**:** Mean leg skin temperature (panel A), intestinal temperature (panel B), systolic blood pressure (panel C), diastolic blood pressure (panel D) during exposure to 90 min of HT (closed circles) or CON (open circles). Data are presented as means ± SD. Data were analyzed using a two-way repeated measures analysis of variance (ANOVA). *Difference between groups (P<0.05).

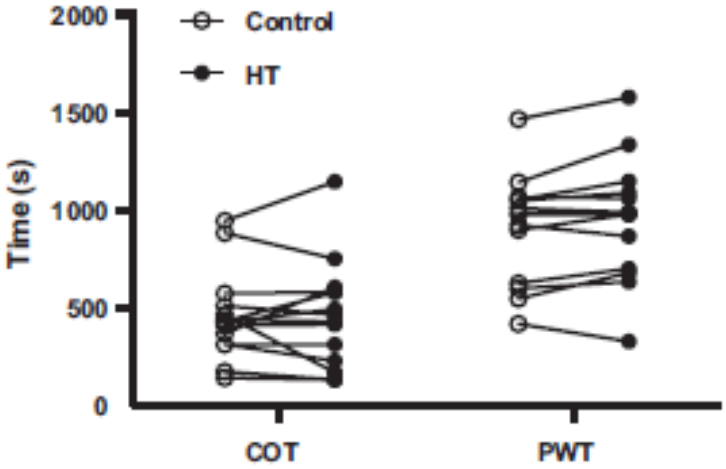

Figure 9**:** Individual values for COT and PWT after exposure to 90 min of HT (open squares) or CON (open circles). Data were analyzed using a paired t-test. COT, claudication onset time; PWT, peak walking time.

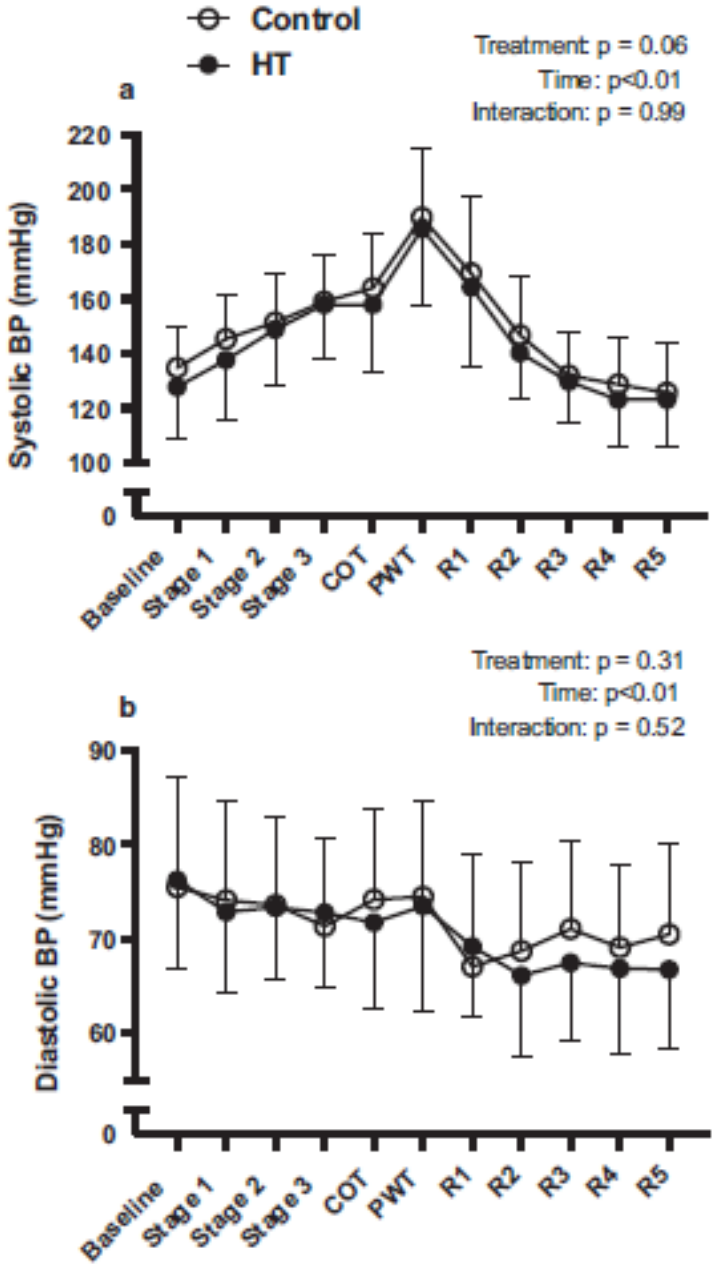

Figure 10**:** Mean systolic blood pressure (panel A) and diastolic blood pressure (panel B) during exercise after exposure to 90 min of HT (closed circles) or CON (open circles). Values are as means ± SD. Data were analyzed using a two-way repeated measures analysis of variance (ANOVA). COT, claudication onset time; PWT, peak walking time; R1, recovery stage 1; R2, recovery stage 2; R3, recovery stage 3; R4, recovery stage 4; R5, recovery stage 5.

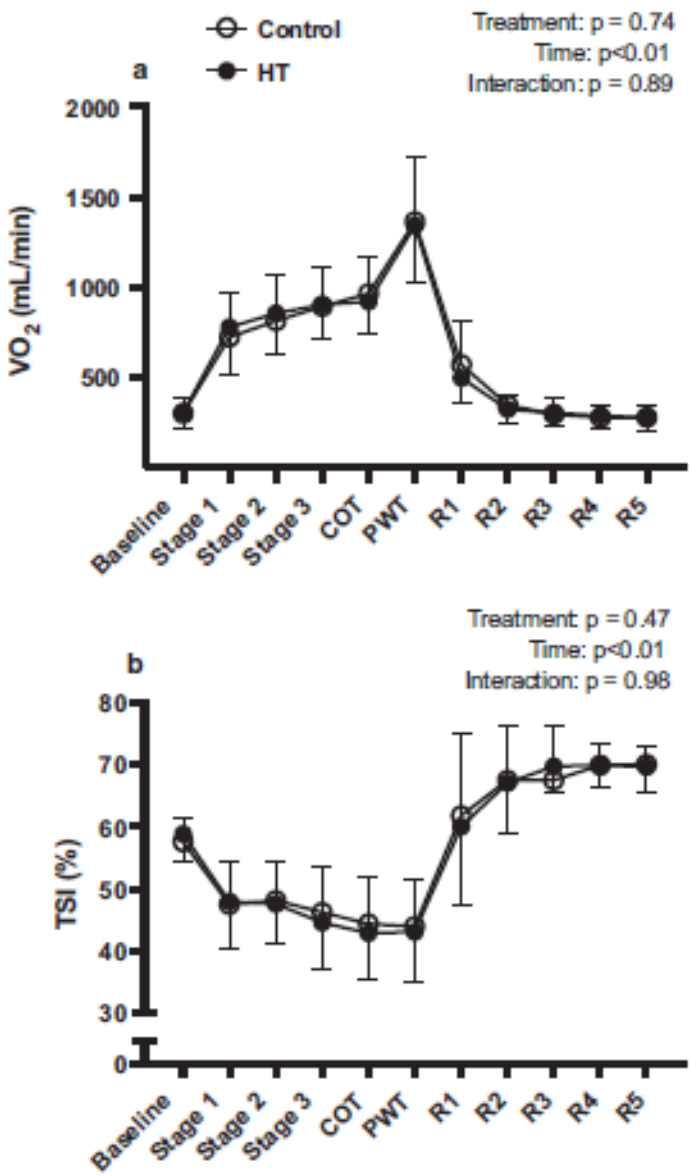

Figure 11**:** Mean responses of pulmonary $\dot{V}O_2$ (A) and TSI% (B) during exercise after exposure to 90 min of HT (closed circles, n=14) or CON (open circles, n=16). Data are mean ± SD. Data were analyzed using a two-way repeated measures analysis of variance (ANOVA). HT, heat therapy; CON, control; TSI%, tissue saturation index %; R1, recovery stage 1; R2, recovery stage 2; R3, recovery stage 3; R4, recovery stage 4; R5, recovery stage 5.

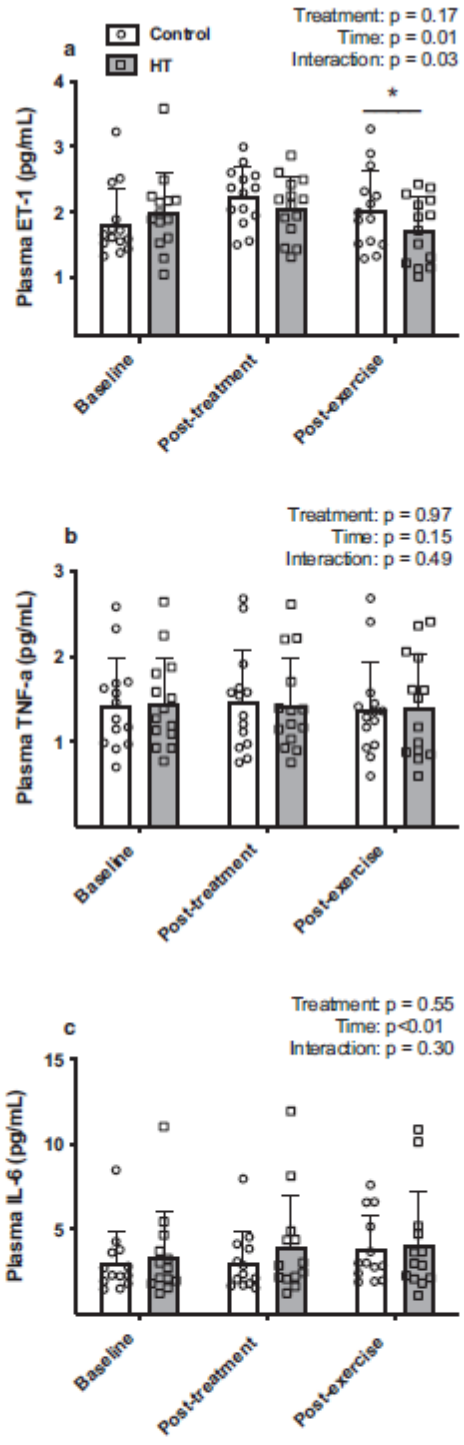

Figure 12**:** Individual and mean values of plasma ET-1 (panel A, n=14), TNF-a (panel B, n=14) and IL-6 (panel C, n=13) at rest, prior to and 10 min after exercise following HT (gray bars, open squares) or CON (white bars, open circles). Data are as means ± SD. Data were analyzed using a two-way repeated measures analysis of variance (ANOVA). *Difference between groups (P<0.05).

CHAPTER 4. HOME BASED HEAT THERAPY IN PAD

Pilot: Home-based leg heat therapy improves six-minute walk test distances in patients with peripheral artery disease and claudication

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Abstract

Peripheral artery disease (PAD) is an increasingly prevalent manifestation of atherosclerosis that substantially limits mobility and increases mortality. Few options currently exists for the practical, conservative treatment of individuals with PAD. We have previously reported that lower limb heat therapy (HT) can improve leg blood flow and reduce systolic blood pressure in patient with lower extremity PAD. We now assessed whether 8 weeks of daily home-based leg HT using water-circulating trousers perfused with water at 43ºC improved 6-minute walk distance in individuals with PAD, compared to a sham treatment. Secondary outcomes included the assessment of leg vascular function and resting blood pressure. Of thirty-four PAD patients randomized, 30 (age: 66±8 yrs; ankle-brachial index (ABI): 0.67±0.11) completed the 8 weeks of treatment. Participants completed 96.6% of the treatment sessions. The change in 6-minute walk distance was significantly greater (p=0.029) in the group exposed to HT (n=15; median: 21.3; 25%,75% percentiles: 10.0,42.3) as compared to the control group (n=14; median: -0.91; 25%,75% percentiles: -5.7,14.3). No difference between groups were detected for blood pressure, calf muscle reactive hyperemia, cutaneous microvascular reactivity, peak walking time, or claudication onset time. These results suggest that home-based HT is well-tolerated, associated with high levels of patient compliance, and improves walking endurance in individuals with PAD.

New and Noteworthy

This is the first sham-controlled randomized clinical trial to demonstrate improvements in walking endurance after repeated exposure to leg heat therapy in patients with symptomatic PAD. Furthermore, these findings demonstrate the safety and practicality of HT employed in a home-based, unsupervised setting.

Introduction

Peripheral artery disease (PAD) is a manifestation of systemic atherosclerosis affecting over 200 million individuals worldwide [1, 2]. It is estimated that PAD affects at least 10% of individuals over the age of 60 years and 20% of those over the age of 80 years globally [3]. This number is projected to increase as primary risk factors such as type 2 diabetes mellitus (T2DM) and advanced age continue to become more prevalent [4]. Patients with PAD exhibit an approximate 50% reduction in exercise tolerance which is accompanied by lower habitual physical activity levels, lower health-related quality of life (HRQoL) and an accelerated functional decline when compared to non-PAD, age-matched controls [5-8]. These consequences of PAD lead to increased risk for hospitalization, loss of independence, and all-cause mortality [3, 4, 9]. While initial reductions in walking endurance are tied to hemodynamic limitations [10-12], the functional impairment in PAD is multifaceted and include abnormalities in the vasculature, peripheral nerves and skeletal muscle [10]. Indeed, the walking impairment in PAD patients is associated, among other factors, with lowered muscle perfusion, elevated oxidative stress and inflammation, neuromuscular dysfunction, and numerous pathological changes in skeletal muscle, including atrophy and increased fat accumulation [13-18].

Supervised exercise training (SET) represents the current conservative treatment for PAD. While consistently effective for improving walking performance in this population [19, 20], SET is

associated with low levels of enrollment and adherence. As few as 24.2% of PAD patients are enrolled in a clinical supervised exercise training program and adherence is traditionally low[21]. Indeed, recent reports of adherence rates for SET programs in patients with PAD have ranged from ~50-70% [22-24]. Pain associated with ambulation, poor walking ability and co-morbid conditions are among the most frequently cited patient-reported reasons for a lack of exercise program completion [25]. As such, it is evident that there is a need for treatments which alleviate the symptoms of PAD, but are associated with lesser overall discomfort and greater ease-of-use.

Emerging evidence indicates that heat therapy (HT) may be a safe and practical therapeutic option to alleviate the symptoms of PAD and improve the quality of life of symptomatic patients [22, 26, 27]. We first demonstrated that a single session of leg HT using customized, liquid-circulating trousers perfused with warm water acutely increased leg blood flow by ~100% and reduces blood pressure (BP) and the circulating levels of ET-1 in symptomatic PAD patients [28]. Further, we have reported that repeated exposure to leg HT for 6 weeks improved perceived physical functioning and reduced the levels of ET-1 by 13% when compared to a sham treatment [27]. Current findings have suggested that chronic HT may improve walking endurance [29], perhaps to a similar degree as SET [22]. Importantly, HT is associated with high levels of compliance [22, 30]. We have reported that with 6 weeks of supervised HT in patient with symptomatic PAD, overall rates of compliance exceeded 97% [27].

The goal of the present study was to examine the effects 8 weeks of daily home-based HT on walking endurance in individuals with intermittent claudication. Building on our previous findings, we hypothesized that 8 weeks of daily 90-minute sessions of 43°C leg HT using tube-lined heated-water circulating pants would improve walking endurance and vascular function.

Methods

Subjects

Eligible patients were identified and referred from the Department of Vascular Surgery at Methodist Hospital, Indianapolis, Indiana. Thirty-four participants were randomized into the study. All patients had ankle-brachial index (ABI) values below 0.90 and claudication pain during exercise in one or both legs for greater than 6 months prior to enrolling in the study. Patients were excluded if their electronic medical record showed: 1) a hemoglobin A1C value above 8.5% within three months of screening, 2) exercise limiting comorbidities (arthritis, heart failure, chronic obstructive pulmonary disease, etc.), 3) tissue loss, 4) prior amputation, 5) non-healing wounds, 6) evidence of critical limb ischemia, 7) recent (<3 months) infrainguinal revascularization (surgery or endovascular revascularization) or revascularization planned during study period; 8) planned change in medical therapy during the duration of the study, 9) active cancer, 10) chronic kidney disease (eGFR <30), 11) HIV positive, active hepatitis B virus (HBV) or hepatitis C virus (HCV) disease, 12) peripheral neuropathy, numbness, or paresthesia in the legs, and 13) a body mass index (BMI) > 35. Patients with cardiovascular or other implants not compatible with magnetic resonance imaging (MRI) were allowed to participate in the study, but were excluded from undergoing the MRI measurements. The protocol was approved by the Institutional Review Board of Indiana University (no. 1801755556A009), and registered with the United States Library of Medicine on clinicaltrials.gov (NCT03763331). Written, informed consent was obtained, and all procedures adhere to the requirements of the U.S. Federal Policy for the Protection of Human Subjects (45 CFR, Part 46), and support the general ethical principles of the Declaration of Helsinki.

Experimental Design

Participants were assigned, using a randomized, balanced design, to undergo either home-based HT or a sham treatment daily for 8 weeks, amounting to approximately 56 total therapeutic sessions. Participants were informed that there were two different categories of HT: "low-heat" and "high-heat," and that both might be beneficial for claudication symptoms. Participants were asked to report to the laboratory in a fasted state (>8 hrs postprandial), refrain from exercise (24 hrs) and smoking (>4 hrs) and take their usual prescription medications prior to each experimental visit. On visit 1, participants were consented, asked about their health history, and then had ABI assessed after 15 minutes of quiet supine rest. Immediately following the ABI assessment, participants were escorted to the cardiovascular testing laboratory for a symptom-limited maximal Gardner-Skinner graded treadmill walking test. After completion of the maximal graded treadmill walking test, participants were familiarized with the performance of the 6-minute walk test. At least 72 hours later, on visit 2, participants underwent a second baseline maximal graded treadmill exercise test. At least 48 hours after visit 2, participants reported to the Indiana University Health Neuroscience Center for the final baseline assessment. Upon arrival to the imaging research facility, participants rested in the supine position for 15 min. Blood pressure was then measured using an automated device and the participant was transported to the scanning room for the assessment of post-occlusive reactive hyperemia of the lower leg using calf arterial spin labelling (ASL). After completion of the imaging assessment, participants were escorted to the Indiana University Clinical Research Center to undergo the remaining experimental tests. Participants were then instrumented with skin heaters and laser-Doppler flowmetry probes for the assessment of leg cutaneous microvascular reactivity. This test lasted 70 minutes and blood pressure was measured every 5 minutes throughout the test using an automated device. Patients were then escorted to an adjacent hallway and completed the 6-minute walk test. Lastly, patients underwent

a single 90-minute session of either HT or a sham (CON) treatment to record the acute physiological responses the treatment. Blood pressure, heart rate, skin temperature, and intestinal temperature were recorded every 5 min for the entirety of the 90-minute treatment. This session served not only as a confirmation of the physiological efficacy of the heating stimulus, but also as a training session to ensure that the participants clearly understood how to use and care for the equipment. Experimental visit 4, 6 and 8 were similar to visit 2, the only difference being the addition of an ABI assessment prior to the maximal graded treadmill test. Experimental visits 5, 7 and 9 were similar to visit 3, but did not include an assessment of the acute effects of the experimental treatment. These session were conducted after 4 and 8 weeks of treatment and 4 weeks after the cessation of the treatment, respectively. Treatment sessions were ceased at least 48 hours prior to the experimental sessions to ensure that the chronic, rather than the transient acute effects of HT, were being assessed.

Intervention

Participants were asked to wear water-circulating trousers (Med-Eng, Ottawa, Ontario, Canada) while water at 43ºC (HT) or 33ºC (control) was circulated through the garment for 90 minutes using a commercially available heated water pump (Aqua Relief Systems, Akron, OH, USA). In order to ensure the correct usage of the trousers and pump, a training checklist was employed by researchers during the visit 3 experimental assessment. Participants applied either HT or CON to the legs 90 minutes each day for ~8 weeks. To confirm compliance with the treatment, participants were instructed to keep a usage log. Researchers contacted the participants 1-2x/week to confirm that the treatment was being correctly applied and that the equipment was performing optimally. Participants were deemed non-compliant and withdrawn from the study if more than 8 sessions were missed during the 8-week treatment period.

6-Minute Walk Test

The 6-minute walk test was performed on a 30 m long, flat, straight corridor following the American Thoracic Society guidelines [31]. The length of the corridor was marked every 10 feet and the turnaround points were marked with a cone. Participants were instructed to walk as far as possible for 6 minutes and were encouraged with standardized phrases every minute throughout the test. Participants were instructed to indicate the point at which claudication pain became readily apparent, which was recorded as the claudication onset time (COT).

Symptom-limited cardiopulmonary exercise test

Exercise testing was performed on a motorized treadmill following the Gardner-Skinner protocol, which consists of walking at a constant speed (2 mph) with a 2%-grade increase every 2 min [32]. A 12-lead electrocardiogram (ECG) was registered continuously. Blood pressure was measured in the left arm using a stethoscope and sphygmomanometer prior and during exercise and for 10 minutes during recovery. Expired respiratory gases were collected breath by breath via a facemask attached to a gas analyzer (MedGraphics, CardiO2, and CPX/D system using Breeze EX Software, 142090-001, ReVia; MGC Diagnostics, St. Paul, MN, United States). Participants received standardized instructions and were asked to indicate when they first began to feel leg pain with a "thumbs up" signal (claudication onset time; COT), and then give a "thumbs down" signal when they could no longer continue with the test (peak walking time; PWT). Participants were allowed to use the handrails for balance, but were not allowed to use them as an aid for walking (i.e. pulling themselves up).

Reactive Hyperemia

Post-occlusive reactive hyperemia in the calf was assessed using pulsed arterial spin labelling (PASL). MRI scanning was performed on a Siemens 3 Tesla (T) Magnetom Prisma scanner (Siemens AG Healthcare Sector, Erlangen, Germany). A transmit/receive knee coil was placed around the knee of the most symptomatic leg, defined as the leg in which the participant self-described as having the most severe claudication pain. A single 8.0 mm slice was prescribed perpendicular to a straight section of the widest portion of the lower leg to establish a reference anatomical image. Readout parameters for acquisition were: TR 4000 ms, TE 14.0 ms, flip angle 90°, FOV 160 mm, and reconstructed voxel dimensions of 2.5 x 2.5 x 10.0 mm. A pulse oximeter on the index finger was used for peripheral gating with the minimum trigger delay. A cuff (SC12L, Hokanson, USA) was snugly wrapped around the upper thigh and connected to a commercially available air source (Hokanson AG101, Hokanson, USA) coupled to a rapid cuff inflator (Hokanson E20, Hokanson, USA). After baseline data acquisition, the cuff was inflated to suprasystolic values (75 mmHg above brachial SBP, as assessed prior to scanning). Imaging was performed during cuff inflation to ensure that total arterial occlusion of the upper leg was achieved. After five minutes of occlusion, the cuff was deflated and post-occlusion reactive hyperemia was monitored for 10 min. Perfusion of the gastrocnemius, soleus, and whole calf was analyzed using MatLab (MathWorks, Natick, MA, USA). A region of interest covering the entire visible cross section of the individual skeletal muscle was manually drawn for each experimental visit on a single time-of-flight image. Time-of-flight images were fitted over perfusion images and the control image was subtracted from the tagged image to quantify skeletal muscle perfusion.

Ankle-brachial Index

Cuffs (SC10, SC12, Hokanson, USA) were wrapped around the ankles and the upper arms. Systolic pressures were measured sequentially in the right posterior tibial artery, right dorsalis pedis artery, right brachial artery, left posterior tibial artery, left dorsalis pedis artery, and left brachial artery using a handheld 5MHz Doppler ultrasound (Lumeon, McKesson, USA). Pressures were taken at each site in duplicate. The ABI of each leg was calculated by dividing the higher of the dorsalis pedis pressure or posterior tibial pressure by the higher of the right or left arm blood pressure [33].

Cutaneous Microvascular Reactivity

Leg cutaneous hyperemia in response to rapid local skin heating to 39ºC was assessed as described by Choi and colleagues [34]. Briefly, two local skin heaters (SH02 Skin Heater/Temperature Monitor; Moor Instruments, Axminster, United Kingdom) were affixed with dual sided stickers to the proximal portion of the lower leg, approximately three inches below the patella on the dermal surface overlaying the tibialis anterior muscle. Single-point laser-Doppler flowmetry probes (Moor Instruments, Axminster, United Kingdom) were positioned in the center of each local heater. Red blood cell flux, an index of skin blood flow, was recorded for 10 min with skin temperature held constant at 33°C. Next, local skin temperature at each site was raised to 39°C at a rate of 0.1°C/s and maintained for 40 min [34]. Finally, local skin temperature was raised to 43.0°C at a rate of 0.1°C/s and maintained at this level for 20 min. Red blood cell flux and the temperature of the skin heaters were recorded at 40 Hz using a data acquisition system (Powerlab and LabChart, ADInstruments, USA), and the last 2 min of every 5-min bin was averaged for the entire protocol. An automated device (Tango+, Suntech Medical, USA) was used to measure SBP and DBP every 5 min. Mean arterial pressure (MAP) was calculated as DBP plus one-third pulse

pressure (i.e., the difference between SBP and DBP). Cutaneous vascular conductance (CVC) was calculated as red blood cell flux divided by MAP and was normalized as a percentage of maximal vasodilation (%CVCmax). There were no observable or statistical differences in %CVCmax between the two laser-Doppler sites within subjects for a given trial so values from each site were averaged. At the end of the session, the heating probes were traced with permanent marker and covered with a protective film (Tegaderm, 3M, USA). Participants were asked to preserve the marks to facilitate consistent placement of the probes in subsequent visits.

Statistical Analysis

All analyses were performed using SAS v9.4 and R Studio v1.2.5019-6 and results are expressed as mean ± standard deviation or in cases of non-parametric testing, results are represented as median with 1st and 3rd quartiles. Normality was assessed using a Shapiro-Wilk test with α =0.05. Two-sample t-tests, and, when appropriate a Wilcoxon Ranked Sum Tests were used to compare changes in primary variables from baseline to 8- wks. Data is reported as a change from baseline to 8wks.

Results

Subject characteristics

A total of 518 patients were referred and screened for the study. Three hundred and ninety-seven participants were deemed ineligible and 87 refused to participate. The remaining 34 patients were randomly allocated to receive HT (n=18) or the CON treatment (n=16). Three participants from the HT group were deemed non-compliant due to missing more than 8 treatments. One participant in the control group voluntarily withdrew from the study after ~4wks of treatment. Another participant in the control group completed the 8wk treatment protocol,

however, was unable complete the wk8 experimental assessments due to ongoing COVID-19 safety concerns. All others (n=29) completed the treatment sessions and wk 8 experimental assessment.

Treatment safety and compliance

Participants included in final analysis completed 96.6% of all treatments. Three participants were withdrawn due to non-compliance and 1 voluntarily withdrew from the study. After factoring in withdrawn patients, total estimated completion of treatments was 88%. One participant in the HT group experienced mild skin irritation during HT after approximately 4 weeks of treatment. The participant was advised to cover the affected area with medical gauze. The irritation resolved by the next day and did not interfere with the continuation of the HT. There were no instances of acute hypotensive episodes, nor were there any further treatment related adverse events reported.

Six-minute walk test

Changes in 6MWD are represented in Figure 13. The change in 6-minute walk distance (6MWD) was significantly greater ($p=0.029$) in the group exposed to HT (n=15; median: 21.3; 25%,75% percentiles: 10.0,42.3 m) as compared to the control group (n=14; median: -0.91; 25%,75% percentiles: -5.7,14.3 m). There was no difference for COT between groups (HT: 9.7 ± 10.9, CON: 5.9 ± 20.0 m, $p=0.56$).

Effect of HT on treadmill walking performance

Changes in PWT are represented in Figure 14. The change in PWT on a maximal graded treadmill walking test was not different ($p=0.21$) between groups exposed to HT (n=15; median:

96.25; 25%,75% percentiles: 55.8,149.0 sec) as compared to the control group (n=14; median: 60.25; 25%,75% percentiles: -13.00,108.0 sec). There was no difference for change in COT between groups (HT: 63.4 ± 105.5, CON: 49.2 ± 133.6 sec; p=0.75)

Resting blood pressure

There were no differences between groups after 8 weeks for resting SBP (HT: 1.2 ± 14.3, CON: -2.8 ± 12.1 mmHg, p=0.42), DBP (HT: 0.7 ± 6.3, CON: -1.1 ± 6.8 mmHg, p=0.47) or MAP (HT: 0.7 ± 8.6, CON: -1.8 ± 8.5 mmHg, p=0.44).

Post occlusive reactive hyperemia

Three patients from the control group and four from the HT group were unable to undergo MRI scanning. There was no significant difference in the change in whole calf perfusion from baseline to 8 weeks (p=0.94) between those exposed to HT (n=11; -6.5 ± 14.3 mL/100g/min) and CON (n=11; -4.8 ± 27.8 ml/100g/min).

Skin microvascular function

There were no significant difference in the change in %CVCmax achieved in response to 39°C heating from baseline to 8wks (p=0.74) between those exposed to HT (n=15; -0.7 ± 16.0%) and CON (n=14; -2.6 ± 13.9%).

Discussion

The findings from this pilot reveal a significant improvement in 6MWD with daily application of home-based HT as compared to a sham treatment in patients with symptomatic lower-extremity PAD. The six-minute walk test is considered a robust, standardized assessment of

walking endurance for patients with PAD [35], and has been heavily studied and correlated to health outcomes and daily physical activity levels [36, 37]. The observed mean improvement in 6MWD of 23.7 m after 8 weeks of treatment likely represents a clinically meaningful improvement in walking endurance. Until recently the minimal clinically important difference (MCID) for 6MWD in PAD had not been established. Prior data on aging, community-dwelling adults were extrapolated to this population[38]. However, several recent studies have described the MCID for 6MWD as it specifically applies to adults with PAD. Gardner *et al.* have reported that result from a study of 156 patients with lower-extremity PAD undergoing 12 weeks of supervised treadmill walking, home-based walking, or a control, suggest that an increase in 6MWD as small as 12 m is clinically significant in patients with PAD [39]. Indeed, data shows that 12 weeks of supervised treadmill walking may increase 6MWD by just 18m in this population. A "large" meaningful improvement was reported to be 32 m. This study used an "anchor-based" approach, which tied the changes in 6MWD to changes in total score on the walking impairment questionnaire (WIQ). Changes in WIQ total score are strongly related to health outcomes in this patient population [40]. More recently, in an observational study examining walking endurance of 777 patients with PAD at baseline and 1-year follow-up, McDermott *et al.* have reported that a 20 m or greater improvement in 6MWD should be considered a "large" improvement in walking ability within this group [41]. These findings suggest a lower MCID than those previously posited by Gardner *et al.*[39], however, It is important to note key methodological differences between the studies. The anchor-based approach from Gardner *et al.* tied changes in 6MWD to changes in total WIQ score, while the anchor-based approach utilized by McDermott *et al.* tied changes in 6MWD to the average change of each question (ranging from -2 to +2). It is also important to note that the participants from McDermott *et al.* did not receive any intervention during the 12-month observational period, while those from

the sample of Gardner *et al.* utilized 2 treatments and an attention-control. The methodology of the present study is perhaps more appropriately compared to the findings from Gardner *et al.*, given the similar time-frames of treatment (8 weeks v. 12 weeks) and the use of a therapeutic intervention. Based upon this available data, the change in 6MWD we are here reporting represents a clinically meaningful difference for patients with lower-extremity PAD.

It has been reported that the six-minute walk test is more resilient to the effect of repeated testing than maximal graded treadmill walking tests [37], a claim which these data support. The group receiving a sham treatment displayed a mean 6MWD of 391.9 m at baseline and 395.8 m after 8 weeks of treatment. This was in contrast to the measurable improvements for PWT on a maximal graded treadmill walking test recorded by those receiving both HT and CON. Treadmill walking test PWT was improved with HT (+105 sec), but this was confounded by a tendency for the CON group to also make improvements (+55 sec) over the course of 5 treadmill tests. This is consistent with the literature which describes a robust learning effect of repeated treadmill testing [42]. Gardner and colleagues have reported that improvements in PWT on a maximal, symptom-limited treadmill walking test as little as 95 seconds represents a clinically significant improvement for patients with symptomatic PAD [39]. While we here report an increase in excess to this threshold, it cannot be ruled out that the changes were due in part to the training effect of repeated treadmill testing, rather than repeated application of lower limb HT.

This pilot study represents the first sham-controlled randomized clinical trial to expose patients with lower extremity PAD to HT in an unsupervised setting. These preliminary data demonstrate that a home-based HT regimen using heated-water circulating pants and a circulator pump can be employed safely in patients with PAD. Only one patient experienced minor skin irritation during the course of treatment which quickly resolved and did not affect further

participation in daily HT. Preliminary data from this pilot did not demonstrate any cases of acute hypotensive episodes. Furthermore, adherence and completion rates were excellent, with participants completing >96% of all prescribed HT sessions and 88% of patients randomized into the study finishing the post-treatment assessment. These data are congruent with what we have previously reported, finding adherence rates >97% during 6 weeks of supervised lower limb HT in patients with PAD [27]. Other studies using HT in PAD, such as that of Akerman et al., have reported adherence rates of 82% when administering sauna bathing HT combined with calisthenics exercise. The high rate of adherence to HT is a pivotal finding, given the current data on adherence to SET in this populations. It is estimated that typical adherence rates to clinical SET range from 50-75%, well below what we are reporting in this pilot. Future research should use a more rigorous design to determine if home-based HT would engender higher rates of enrollment than those typically seen with SET[21].

Blood pressure was not altered by the daily application of HT in this patient population. This finding is consistent with a previous findings we have reported regarding the use of HT for symptoms of PAD [27, 43], however, it is in contrast to the findings reported elsewhere [22]. It should be noted that we are reporting an average of 12 blood pressure readings taken over the course of 70 minutes during each experimental time point. The intra-subject and intra-test variabilities were high, suggesting that studies using a single measure to assess blood pressure at rest may not be truly representing resting BP. Future studies should focus on 24-hour measurement of BP in order to understand the sustained effect that HT may or may not have on resting BP.

Improvements in walking endurance did not coincide with positive changes in any marker of vascular function in the present study. Although it is apparent that the endothelium of patients with PAD can be acutely responsive to thermal stress [28, 44] it appears that repeated HT may not

elicit positive lower limb vascular adaptation in patients with symptomatic PAD. This is consistent with findings we have previously reported using 6 weeks of supervised HT in patients with IC. In accordance with this, Akerman and colleagues have reported similar findings using 12 weeks of sauna bathing in patients with PAD. In this study, patients significantly improved 6MWD at the end of treatment, however, this was not accompanied with improvements in vascular function. Further, there is some evidence of a vascular "resilience" to adaptations in this population. A study of 85 patients with symptomatic lower extremity PAD, Calanca et al. found that 3 months of supervised exercise training significantly improved walking performance, but this was not associated with an improvement in ABI or transcutaneous oxygen pressure [45]. Systematic reviews of the available literature on adaptations to exercise in PAD have come to similar conclusions, with a review published by Parmenter et al. suggesting that SET improves walking performance without concomitant improvements in markers of vascular function or hemodynamics [20]. Thus, if positive vascular adaptations do not occur with HT, the questions must be asked as to what mechanism might be driving the observed improvements in walking performance in this clinical population.

One attractive possibility is that HT may induce changes in neuromuscular function. It is well documented that patients with PAD exhibit deficits in nerve transduction with reduced skeletal muscle cross-sectional area and altered structural characteristics, and that the size and strength of the plantar flexors is correlated to walking performance [14, 15, 46, 47]. Several studies have demonstrated changes in skeletal muscle size and strength following application of HT in a variety of populations [48, 49]. No studies to date have performed an in-depth analysis of muscle function or cross-sectional area after application of HT in patients with PAD. Our lab has previously used a pre-clinical model of rodent femoral artery ligation to examine the effects of HT on skeletal muscle

morphology and function. Of note, rats exposed to repeated heating of the lower limbs showed increased soleus muscle twitch force compared to those rats undergoing a sham treatment [50]. Thus, an important area of future research using HT in PAD will be to examine the specific changes which may or may not occur in the skeletal muscle and compare these to parameters of walking ability.

Limitations

A limitation which may affect internal validity of the current study was the unsupervised nature of the treatment. This component was essential to the study methodology because it established the feasibility and safety of the treatment as a home-based therapy. In order to mitigate potential non-compliance and safety concerns, participants were carefully screened and then called twice each week to ensure the correct usage and performance of the HT pump and heated-water circulating trousers. Regardless, while patients reported excellent compliance to treatment and equipment performance, it cannot be ruled out that patients were less compliant or that the equipment performed to a lower standard than was reported.

Conclusion

Eight weeks of unsupervised, home-based leg HT in patients with symptomatic PAD improves walking endurance as demonstrated by improvements in 6MWD and was associated with excellent safety and compliance profiles. These improvements were not accompanied by concurrent improvements in vascular function. Future, larger-scale, randomized clinical trials should seek to validate the safety and compliance rates of home-based HT. Further it will be important to gain a clearer understanding of the mechanistic underpinnings that may induce positive effects on walking endurance with regular application of HT.

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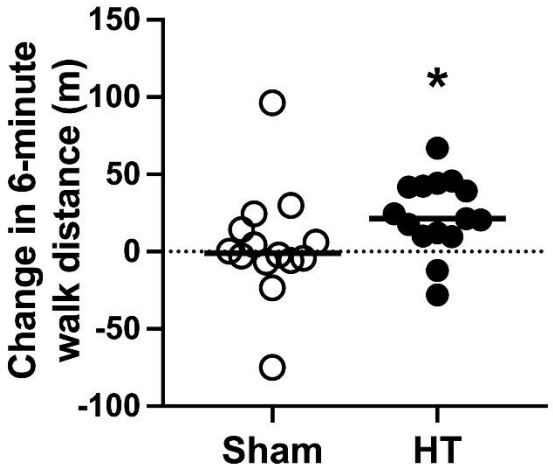

Figure 13: Individual and group mean changes from baseline to 8 weeks in 6MWD (HT, n =15) or the sham treatment (control, n=14). Data were analyzed using Wilcoxon Rank Sum Test. *Difference between groups ($P < 0.05$).

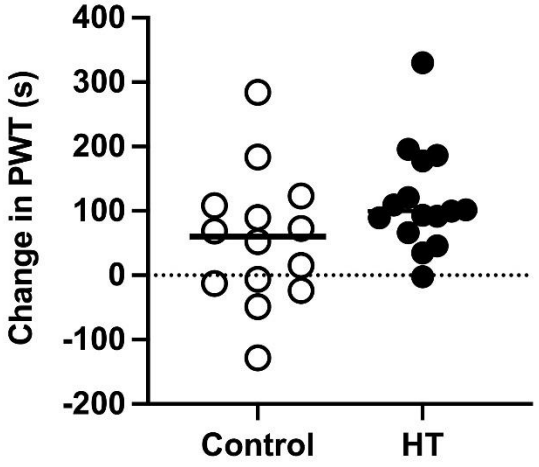

Figure 14: Individual and group mean changes from baseline to 8 weeks in treadmill PWT (HT, n=15) or the sham treatment (control, n=14). Data were analyzed using Wilcoxon Rank Sum Test. *Difference between groups ($P < 0.05$).

CHAPTER 5. CONCLUSION

These data support HT as a safe, convenient and practical treatment for the walking tolerance observed in patients with lower extremity PAD. Eight weeks of home based daily HT using a commercially available heated-water circulating pump was associated with a ~23 m increase in 6MWD. Importantly, adherence to the treatment was >88%, suggesting that it is a practical solution which is amenable to unsupervised use in this patient population. Further, the treatment was well-tolerated, with no serious adverse events reports during application. It appears that the dose of HT is important for producing improvements in walking endurance, as 3x/week application of HT for 6 weeks (18 total sessions) in a supervised setting did not improve walking endurance, however, 8 weeks of daily HT (56 total sessions) was associated with significant improvements. A lower dose of HT was capable of improving patient perceptions of physical functioning and lowering circulating levels of ET-1. The data presented here also suggest that acutely applied HT lowers SBP, but does not improve walking performance in patients with lower-extremity PAD. Thus it appears that HT using customized water-circulating pants and a commercially available water-circulating pump, when applied daily for 8 weeks at 43°C, but not 3x/week for 6 weeks is a potent stimulus for improving walking endurance in patients with symptomatic lower-extremity PAD.

All three of the clinical trials mentioned completed by Monroe *et al.* failed to demonstrate changes in vascular function with application of lower limb HT. This was contrary to the hypothesis that improvements in vascular function, and subsequent enhancements in O_2 delivery would drive improvements in exercise endurance. It is conceivable that improvements in neuromuscular control or mitochondrial function of the lower limb skeletal muscles could be driving the observed changes in walking endurance, and thus, these represent a promising target

for future research regarding HT as a therapeutic strategy to treat symptoms of lower extremity PAD.