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Acute lower leg hot water immersion protects macrovascular dilator function following ischaemia–reperfusion injury in humans

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Abstract

Reperfusion that follows a period of ischaemia paradoxically reduces vasodilator function in humans and contributes to the tissue damage associated with an ischaemic event. Acute whole-body hot water immersion protects against vascular ischaemia–reperfusion (I–R) injury in young healthy humans. However, the effect of acute lower leg heating on I–R injury is unclear. Therefore, the purpose of this study was to test the hypothesis that, compared with thermoneutral control immersion, acute lower leg hot water immersion would prevent the decrease in macro- and microvascular dilator functions following I–R injury in young healthy humans. Ten young healthy subjects (5 female) immersed their lower legs into a circulated water bath for 60 min under two randomized conditions: (1) thermoneutral control immersion (~33°C) and (2) hot water immersion (~42°C). Macrovascular (brachial artery flow-mediated dilatation) and microvascular (forearm reactive hyperaemia) dilator functions were assessed using Doppler ultrasound at three time points: (1) pre-immersion, (2) 60 min post-immersion, and (3) post-I/R (20 min of arm ischaemia followed by 20 min of reperfusion). Ischaemia-reperfusion injury reduced macrovascular dilator function following control immersion (pre-immersion $6.0 \pm 2.1\%$ vs. post-I/R $3.6 \pm 2.1\%$; $P < 0.05$), but was well-maintained with prior hot water immersion (pre-immersion $5.8 \pm 2.1\%$ vs. post-I/R $5.3 \pm 2.1\%$; $P = 0.8$). Microvascular dilator function did not differ between conditions or across time. Taken together, acute lower leg hot water immersion prevents the decrease in macrovascular dilator function that occurs following I–R injury in young healthy humans.

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AUTHOR CONTRIBUTIONS

R.E.E. and S.A.R. contributed to conception or design of the work. R.E.E., H.W.H., O.G.P., A.H.O.-Y. and S.A.R. performed acquisition, analysis or interpretation of data for the work. R.E.E. drafted the manuscript and H.W.H., O.G.P., A.H.O.-Y. and S.A.R. revised it critically for intellectual content. All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

COMPETING INTERESTS

The authors have no competing interests to declare.

Keywords

blood flow; flow-mediated dilatation; heat stress; reactive hyperaemia; shear stress

1 | INTRODUCTION

Ischaemic events are a major contributor to cardio- and cerebrovascular morbidity and mortality (Benjamin et al., 2019). Ischaemic events can also cause significant damage in other organs, such as the gastrointestinal system, and in severe cases can lead to multiple organ failure (Eltzschig & Collard, 2004). The tissue damage associated with an ischaemic event is due to local hypoxia that occurs secondarily to a reduction in blood flow (ischaemia) and subsequent reperfusion following successful restoration of flow, a clinical phenomenon known as ischaemia–reperfusion (I–R) injury (Carden & Granger, 2000; Seal & Gewertz, 2005). While the precise signalling cascade mediating I–R-induced tissue damage remains to be fully elucidated, a clear vascular mechanism has emerged as a strong contributor to the overall condition (Eltzschig & Collard, 2004; Seal & Gewertz, 2005).

A number of non-pharmacological interventions appear to protect against vascular I–R injury in humans. For example, several studies have demonstrated that remote ischaemic preconditioning protects macro- and microvascular dilator functions following I–R (Kharbanda et al., 2001, 2002; Loukogeorgakis et al., 2005). More recently, Brunt et al. (2016) demonstrated that acute (~1 h) whole-body hot water immersion was protective against vascular I–R injury in young healthy humans. While providing clear evidence of the beneficial effects on vascular I–R injury, the therapeutic potential of whole-body heating remains uncertain given the thermal risks (e.g. syncope) and level of tolerance associated with such an intervention, particularly in populations at risk for ischaemic events (e.g. elderly adults). Thus, targeted heating of the lower legs may be an alternative therapeutic approach, provided that the thermal and haemodynamic responses are sufficient to protect against vascular I–R injury. Indeed, we (Romero et al., 2017a) and others (Tinken et al., 2009) have demonstrated that localized heating of a limb acutely improves vasodilator function. However, it is unclear if targeted limb heating is equally beneficial to that of whole-body heating within the context of vascular I–R injury.

Therefore, the purpose of this study was to determine if lower leg hot water immersion protects against vascular I–R injury induced in the arm of young healthy humans. We tested the hypothesis that, compared with control immersion, acute lower leg hot water immersion would prevent the decrease in macro- and microvascular dilator functions following I–R. Additionally, based on the findings from Neff et al. (2016) demonstrating that acute leg heating simultaneously reduces arterial blood pressure and circulating concentrations of endothelin-1, a potent vasoconstrictor substance, we tested the hypothesis that acute lower leg hot water immersion would reduce plasma endothelin-1 concentrations measured in the arm exposed to I–R.

2 | METHODS

2.1 | Subjects

Subject physical characteristics are shown in Table 1. Written informed consent was obtained from all subjects subsequent to a verbal and written briefing of all experimental procedures. This study was approved by the North Texas Regional Institutional Review Board (Project no. 2018-135) and was performed in accordance with the principles outlined in the *Declaration of Helsinki*, except for registration in a database. Ten young adults (5 women) participated in this study. All subjects were free from known cardio-metabolic disease. Subjects were deemed healthy following the completion of an in-depth medical history questionnaire and a resting 12-lead electrocardiogram. Subjects were required to abstain from caffeine, supplements, alcohol and exercise for 24 h before the study. Additionally, subjects reported to the laboratory after an overnight fast and were provided with a standardized breakfast prior to each experiment. Subjects were required to abstain from over-the-counter or prescription medications at the time of the study. Women were studied during the early follicular phase of their menstrual cycle.

2.2 | Experimental approach

An experimental schematic is shown in Figure 1. On two separate occasions, subjects were exposed to bilateral thermoneutral control immersion or hot water immersion. Thermal conditions were randomized, counterbalanced and separated by at least 7 days.

For each experiment, subjects reported to the laboratory at 08.30 h. Following instrumentation and a ~20 min rest period, vasodilator function was assessed in the arm while the subject was supine (Pre). Subjects were then moved to the seated position and immersed their lower legs ~33 cm into a circulated water bath maintained at a thermoneutral temperature of ~33°C. To minimize the influence of hydrostatic pressure changes, baseline haemodynamic measures were recorded after 10 min of thermoneutral leg immersion. Thereafter, water temperature was maintained at 33°C for control immersion or raised rapidly to 42°C for hot water immersion. Thermal and haemodynamic measures were recorded every 15 min during 60 min of leg immersion. Upon the completion of leg immersion, subjects immediately removed their legs from the water bath and returned to the supine position. Vasodilator function was reassessed subsequent to a 60 min recovery period (60 min Post) and immediately following the I-R protocol (Post-I/R). I-R was induced in the right arm by placing a pneumatic cuff (SC10, Hokanson, Bellevue, WA, USA) immediately distal to the axilla and rapidly inflating it to 250 mmHg (E20 Rapid Cuff Inflator, Hokanson, Bellevue, WA, USA). The cuff was always placed proximal to the ultrasonography site and complete circulatory arrest was checked manually via palpation of a radial pulse. After 20 min of ischaemia, the cuff was rapidly deflated and reperfusion was allowed for 20 min.

2.3 | Measurements

Subjects were asked to remain quiet and relaxed during all haemodynamic measurements. Laboratory temperature was maintained at ~21°C during experimentation.

2.4 | Central haemodynamics

Arterial blood pressure was measured periodically from the left arm using an automated sphygmomanometer (Tango M2, SunTech Medical, Morrisville, NC, USA). Beat-by-beat arterial blood pressure was measured non-invasively by finger photoplethysmography (Finometer, Finapres Medical Systems BV, Enschede, the Netherlands). Stroke volume was measured via Modelflow. Heart rate was monitored continuously via electrocardiogram (Solar 8000M, GE Healthcare, Chicago, IL, USA).

2.4.1 | Peripheral haemodynamics—Brachial artery diameter and blood velocity were measured via duplex ultrasonography, approximately 2–5 cm proximal to the antecubital fossa, using a linear-array transducer (11 MHz, Phillips iE33, Andover, MA, USA) and an insonation angle of 60 deg. The Doppler ultrasound was interfaced with a computer running custom software to capture blood velocity.

2.4.2 | Skin and intestinal temperatures—Body core temperature was measured via a telemetric pill (HQ Inc., Palmetto, FL, USA) ingested ~1.5 h prior to data collection. Local skin temperature was measured by placing a thermocouple (IT-18, Physitemp Instruments LLC, Clifton, NJ, USA) on the anterior aspect of the lower leg.

2.4.3 | Vasodilator function—Macrovascular dilator function was assessed via endothelium-dependent flow-mediated dilatation of the right brachial artery in accordance with recent guidelines (Harris, Nishiyama, Wray, & Richardson, 2010; Thijssen et al., 2011). Microvascular dilator function was assessed simultaneously via forearm post-occlusive reactive hyperaemia (area under the curve and peak). Briefly, a pneumatic cuff was placed on the right arm immediately distal to the antecubital fossa. Arterial inflow to the lower arm was occluded by rapidly inflating the cuff to 220 mmHg for 5 min. Prior to cuff inflation, brachial artery diameter and blood velocity were recorded during a 1 min baseline period. Recording of these measures resumed 20 s prior to cuff deflation and continued for 3 min thereafter.

2.4.4 | Blood sampling and analysis—A 22-gauge I.V. catheter was placed in an antecubital vein of the subject's right arm and was used to sample blood from the arm exposed to I–R. Venous blood was collected into a Vacutainer® blood collection tube and centrifuged at 1300 *g* within 5 min. Blood plasma was then aliquoted into cryogenic vials, immediately frozen in liquid nitrogen, and stored at –80°C until analysis. Plasma concentrations of the vasoconstrictor substance endothelin-1 were analysed using a commercially available enzyme-linked immunosorbent assay (Quantikine ELISA, R&D Systems, Minneapolis, MN, USA) and performed in accordance with the manufacturer's instructions.

2.5 | Data and statistical analyses

Central haemodynamic and thermal measures were collected at 200 Hz and analysed using a commercially available data acquisition and analysis system (PowerLab, ADInstruments, Bella Vista, Australia). Data were averaged across 1 min bins at each indicated time point. Blood velocity measurements were determined from Doppler ultrasound audio recordings

using an intensity-weighted algorithm (custom software), subsequent to demodulation of forward and reverse Doppler frequencies (Buck, Sieck, & Halliwill, 2014; Romero et al., 2015, 2016, 2017b). Vessel diameter was determined using custom edge-detection and wall-tracking software (Black, Cable, Thijssen, & Green, 2008; Woodman et al., 2001). Blood flow was calculated by multiplying the cross-sectional area of the brachial artery by mean blood velocity and reported in ml min^{-1} . Vascular conductance was calculated by dividing blood flow by mean arterial pressure and expressed as $\text{ml min}^{-1} \text{mmHg}^{-1}$. Shear stress (i.e. the frictional drag of red blood cells along the interior surface of the blood vessel) was estimated using shear rate, which was calculated by multiplying 8 by the quotient of blood velocity and vessel diameter and expressed as s^{-1} .

Peak diameter measured during flow-mediated dilatation was determined using an algorithm previously described by Black et al. (2008). Reactive hyperaemia area under the curve was determined by averaging blood flow (determined via simultaneously acquired diameter and velocity) across 4 s bins for the initial 20 s subsequent to release of arterial occlusion and averaged across 10 s bins thereafter (Romero et al., 2017a, 2018). Vascular conductance was then determined for each bin to account for changes in perfusion pressure. Reactive hyperaemia area under the curve was calculated by summing the product of each bin multiplied by its duration in minutes and was normalized for baseline conductance.

Our primary outcome variables were analysed using a two-way (thermal condition $\times$ time) mixed model analysis of variance with repeated measures (JMP Pro 12; SAS Institute Inc., Cary, NC, USA). Follow-up tests were performed using Tukey's *post hoc* procedure. Flow-mediated dilatation was assessed using the allometric modelling solution proposed by Atkinson *et al.* (Atkinson & Batterham, 2013; Atkinson, Batterham, Thijssen, & Green, 2013), subsequent to verification of the presence of inadequate scaling by examining the slope of the relation between logarithmically transformed baseline and peak diameter. Shear rate area under the curve summed through peak diameter was also entered into the model as a covariate to account for changes in shear stimulus. Data are reported as means $\pm$ SD.

3 | RESULTS

3.1 | Skin and intestinal temperatures

Skin and intestinal temperatures measured during leg immersion are shown in Figure 2. On average, skin temperature increased to $41.2 \pm 0.4^\circ\text{C}$ during hot water immersion ($P < 0.05$ vs. control immersion), but was well maintained at $33.7 \pm 0.4^\circ\text{C}$ during control immersion. Sixty minutes of hot water immersion increased intestinal temperature by $0.7 \pm 0.2^\circ\text{C}$ ($P < 0.05$ vs. control immersion), whereas temperature was maintained within $0.1 \pm 0.1^\circ\text{C}$ of baseline for control immersion. Skin temperature did not differ between thermal conditions during the assessment of vasodilator function at Pre, 60 min Post and Post-I/R time points ($P = 0.7$, for interaction). Likewise, intestinal temperature did not differ between control immersion (Pre, 37.1 ± 0.2 ; 60 min Post, 37.2 ± 0.2 ; Post-I/R, 37.2 ± 0.2) and hot water immersion (Pre, 37.0 ± 0.3 ; 60 min Post, 37.2 ± 0.2 ; Post-I/R, 37.1 ± 0.2 ; $P = 0.7$, for interaction).

3.2 | Central haemodynamics

Central haemodynamics measured during leg immersion are shown in Table 2. Heart rate and cardiac output increased during hot water immersion (both $P < 0.05$ vs. control immersion), whereas mean arterial pressure was reduced ($P < 0.05$ vs. control immersion). Heart rate, cardiac output, and mean arterial pressure did not differ between thermal conditions during the assessment of vasodilator function at Pre, 60 min Post, and Post-I/R time points (all $P \geq 0.5$, for interaction).

3.3 | Brachial artery haemodynamics

Brachial artery haemodynamics measured during leg immersion are shown for all subjects in Figure 3. On average, brachial artery mean shear rate increased to $572 \pm 179 \text{ s}^{-1}$ during hot water immersion ($P < 0.05$ vs. control immersion). Blood flow increased to $154 \pm 68 \text{ ml min}^{-1}$ during hot water immersion, owing to an increase in conductance of $1.90 \pm 0.80 \text{ ml min}^{-1} \text{ mmHg}^{-1}$ (both $P < 0.05$ vs. control immersion). Brachial artery mean shear rate ($175 \pm 87 \text{ s}^{-1}$), blood flow ($41 \pm 24 \text{ ml min}^{-1}$) and conductance ($0.49 \pm 0.28 \text{ ml min}^{-1} \text{ mmHg}^{-1}$) were unchanged during control immersion.

3.4 | Vasodilator function

Macro- and microvascular dilator functions measured at Pre, 60 min Post and Post-I/R time points are shown in Figures 4 and 5, respectively. Additional measures relevant to the assessment of vasodilator function are presented in Table 3. Brachial artery flow-mediated dilatation did not differ between thermal conditions at Pre ($P = 0.9$) and 60 min Post time points ($P = 0.4$). I-R reduced flow-mediated dilatation following control immersion ($P < 0.05$ vs. Pre), but was prevented with prior hot water immersion ($P < 0.05$ vs. control immersion).

Microvascular dilator function, assessed via reactive hyperaemia AUC and peak, did not differ between thermal conditions at Pre and 60 min Post time points (all $P \geq 0.6$). Interestingly, I-R did not attenuate microvascular dilator function for either thermal condition (both $P = 0.9$ vs. Pre).

3.5 | Plasma endothelin-1

Plasma concentrations of the vasoconstrictor substance endothelin-1 are shown in Table 4. Plasma endothelin-1 did not differ between thermal conditions at Pre, 60 min Post and Post-I/R time points ($P = 0.9$).

4 | DISCUSSION

The purpose of this study was to determine if acute lower leg hot water immersion protects against vascular I-R injury induced in the arm of young healthy humans. In partial support of our hypothesis, we found that I-R reduced macrovascular dilator function following control immersion, but was well-maintained with prior hot water immersion. Surprisingly, I-R failed to reduce microvascular dilator function following control immersion, which did not allow us to examine a potential protective effect of lower leg hot water immersion. Finally, the concentration of endothelin-1 measured in venous blood of the arm exposed to I-R was

unaltered by prior hot water immersion. Collectively, our results suggest that acute lower leg hot water immersion prevents the decrease in macrovascular dilator function that occurs following I–R injury in young healthy humans, a finding that does not appear to be mediated by endothelin-1.

4.1 | Heat stress and vascular ischaemia-reperfusion injury: does the magnitude of exposure matter?

Non-pharmacological therapies that prevent I–R injury continue to be an important area of investigation, given the ease of implementation and lack of contraindications and/or side effects typically associated with pharmacological therapies. Brunt and colleagues recently provided compelling evidence that acute (1 h) whole-body hot water immersion prevented the decrease in macro- and microvascular dilator functions induced by a standard arm I–R protocol (Brunt et al., 2016). We chose to adopt an identical experimental approach to that of Brunt and colleagues to maximize our ability to compare findings between studies. We found that macrovascular dilator function was reduced following the same I–R protocol, but was prevented with prior lower leg hot water immersion. Importantly, the protective effect induced by lower leg hot water immersion was similar in magnitude (~2% improvement in flow-mediated dilatation) to that observed by Brunt et al. (2016) when using whole-body hot water immersion. The similarity between heating modalities suggests that an exposure threshold may exist in which macrovascular dilator function is protected when exposed to I–R. On average, body core temperature increases by ~0.7°C when exposed to 1 h of lower leg hot water immersion and by ~1.8°C (Brunt et al., 2016) when exposed to whole-body hot water immersion for the same duration, suggesting that the temperature-dependent vascular benefits associated with acute heat exposure occur across a range of temperatures. However, it is possible that these benefits could occur at temperatures below 0.7°C, given the robust thermal and haemodynamic responses that occur prior to the attainment of peak temperature. While the precise temperature threshold remains unclear, our data would suggest that a significant thermal exposure is not a prerequisite to mediate a vasculoprotective effect.

4.2 | Possible mechanisms protecting against vascular ischaemia-reperfusion injury

Endothelin-1 can alter vascular function directly through its inherent vasoconstrictor properties and indirectly by serving as a physiological antagonist for nitric oxide-dependent vasodilatation (Bourque, Davidge, & Adams, 2011). Importantly, endothelin-1 has also been implicated in the pathophysiology of I–R injury (Pernow & Wang, 1997; Tamareille et al., 2013). Given the recent findings from Neff et al. (2016) demonstrating that acute leg heating (via water perfused trousers) simultaneously reduces arterial blood pressure and circulating endothelin-1 concentrations in individuals with peripheral arterial disease (presumably via vasodilation) and given recent evidence suggesting that endothelin-1 alters brachial artery flow-mediated dilatation (Nishiyama, Zhao, Wray, & Richardson, 2017), we reasoned that lower leg hot water immersion may protect against vascular I–R injury by attenuating the release of endothelin-1. However, we found that endothelin-1 was not augmented in the venous blood of the arm exposed to I–R and was unaltered by prior hot water immersion, suggesting that this vasoconstrictor substance does not play a mechanistic role in protecting against vascular I–R injury.

The vasculoprotective effects associated with exposure to heat stress are thought to be mediated in part by nitric oxide signalling (Cheng & MacDonald, 2019; Mccarty, Barroso-Aranda, & Contreras, 2009). Elevated body core temperature augments the expression and activity of endothelial nitric oxide synthase, secondary to the release of heat shock proteins (Harris, Blackstone, Ju, Venema, & Venema, 2003). Using an *in vitro* model of I–R, Brunt and colleagues (Brunt, Wiedenfeld-Needham, Comrada, & Minson, 2018) recently demonstrated that acutely exposing human umbilical vein endothelial cells to an elevated temperature or pretreatment with serum from subjects who underwent 8 weeks of chronic heat exposure reduces a number of cellular stressors (e.g. oxidative stress) that could suppress nitric oxide bioavailability. These findings suggest that heat stress may protect against I–R injury directly from an elevation in body core temperature and/or from blood-borne factors that may alter nitric oxide bioavailability. Importantly, the cell culture model used in the study by Brunt et al. (2018) does not account for the haemodynamic responses typically associated with elevated body core temperature, most notably increased shear stress. Endothelial cells express numerous mechanosensors that respond to alterations in shear stress (Baeyens, Bandyopadhyay, Coon, Yun, & Schwartz, 2016). In conditions of elevated shear stress, these mechanosensors activate signalling pathways in the endothelium and vascular smooth muscle that ultimately increase nitric oxide bioavailability. Additionally, the mechanotransduction of shear stress can indirectly augment nitric oxide bioavailability by reducing oxidative stress and inflammation (Laughlin, Newcomer, & Bender, 2008). Thus, the substantial increase in shear stress that occurs in response to acute heat exposure likely protects against vascular I–R injury by increasing nitric oxide bioavailability.

Taken together, because brachial artery flow-mediated dilatation is mediated, in large part, by nitric oxide-dependent signalling (Doshi et al., 2001; Green, Dawson, Groenewoud, Jones, & Thijssen, 2014), it is reasonable to hypothesize that acute lower leg hot water immersion prevents the decrease in macrovascular dilator function following I–R injury by increasing nitric oxide availability secondary to upregulating signalling pathways associated with elevations in body core temperature and/or shear stress. Future investigations could delineate these prospective heat-related mechanisms by isolating the increase in body core temperature from shear stress by clamping arterial blood flow at thermoneutral levels during hot water immersion (e.g. via brachial artery compression). With this experimental approach, a persistent reduction in vasodilator function following I–R injury despite an increase in body core temperature and in combination with the absence of increased arterial blood flow would suggest that shear stress is the primary mechanism by which hot water immersion protects against vascular I–R injury. Finally, endothelin-1 does not appear to contribute to the vasculoprotective effect associated with lower leg hot water immersion.

4.3 | Microvascular dilator function

In contrast to the findings of Brunt and colleagues (Brunt et al., 2016), we found that microvascular dilator function was well maintained following I–R injury. Indeed, peak reactive hyperaemia measured following I–R was nearly identical to that measured at the Pre time point. I–R is thought to reduce vasodilator function throughout the arterial tree. For example, Kharbanda et al. reported that the hyperaemic response to a graded intra-arterial

infusion of acetylcholine (a measure of microvascular dilator function) is blunted after arm I-R injury (Kharbanda et al., 2001). Interestingly, despite reporting a blunted acetylcholine-mediated hyperaemic response following I-R injury, Kharbanda et al. reported, in the same study, that post-occlusive reactive hyperaemia was unaffected, an outcome that has been reported by others (Gori et al., 2006; Lambert et al., 2016). Thus, it appears that the method used to assess vasodilator function may alter the ability to detect I-R injury, though it is possible that a subtle, unmeasurable change in vascular control also contributed to the response. Nevertheless, because we were unable to reduce microvascular dilator function, the efficacy of lower leg hot water immersion to prevent the decrease in microvascular dilator function following I-R remains equivocal.

4.4 | Experimental considerations

Several experimental considerations warrant discussion. First, we did not assess endothelium-independent vasodilator function. Thus, we cannot say with certainty that the protective effect mediated by lower leg hot water immersion is due primarily to improved endothelial function. Second, Modelflow estimates of stroke volume/cardiac output and other waveform derived indices (e.g. arterial pressure via P_{a}) are prone to inaccuracies when measured during heat stress (Ganio, Brothers, Lucas, Hastings, & Crandall, 2011; Shibasaki et al., 2011). Therefore, the absolute values reported herein should be interpreted judiciously. Third, only young healthy subjects were included in this study. Therefore, our results may not extend to clinical populations at risk for ischaemic events (e.g. elderly adults), an effect that has been reported previously with other non-pharmacological interventions targeting vascular I-R injury (van den Munckhof et al., 2013). Fourth, we did not measure circulating markers (i.e. nitrate and nitrite) of nitric oxide bioavailability. Finally, by not including a whole-body hot water immersion condition, we cannot say with certainty that lower leg hot water immersion is equally protective, despite reporting a similar improvement in macrovascular dilator function to that reported by Brunt and colleagues (Brunt et al., 2016).

4.5 | Perspectives

In accordance with American Heart Association/American College of Sports Medicine position statements (Chodzko-Zajko et al., 2009; Nelson et al., 2007), exercise training remains a strong (American Heart Association Class I, American College of Sports Medicine evidence category A) therapeutic approach to improve cardiovascular health and reduce morbidity and mortality in populations at risk for ischaemic events, such as elderly adults. However, only 12% of those individuals report participating in any form of exercise training (Federal Interagency Forum on Aging-Related Statistics, 2016), an observation that is exacerbated by the fact that 50% of elderly adults have no intention of incorporating exercise into their daily routine (Allen & Morelli, 2011). Thus, given the ease of implementation (i.e. can be performed in-home), acceptability/tolerability and low cost, lower leg heating may be an alternative therapy to improve the health and wellbeing of those individuals who are unwilling or unable to exercise.

We induced I-R injury in the brachial artery using an experimental approach that has been extensively utilized in humans (Brunt et al., 2016; Kharbanda et al., 2001, 2002; Seeger et

al., 2015; van den Munckhof et al., 2013). Importantly, the brachial artery can be used as a surrogate for the coronary artery given the strong relationship between brachial and coronary vasodilator function (Anderson et al., 1995; Takase et al., 1998, 2005; Vallbracht-Israng, Morguet, & Schwimmbeck, 2007). Given that heat stress increases cardiac work (i.e. myocardial oxygen consumption) (Crandall & Wilson, 2015; Wilson et al., 2009), we can assume that blood flow, and thus shear stress, are increased in the circulatory system of the myocardium. Moreover, the myocardial circulatory system is likely exposed to the blood-borne factors released in response to an elevated body core temperature. Therefore, it is tempting to speculate that our findings of improved macrovascular dilator function may extend to vascular beds beyond that of the arm, particularly to the vasculature of the myocardium.

4.6 | Summary

Acute exposure to lower leg hot water immersion induces a number of thermal and haemodynamic changes that can alter vascular function. Our findings demonstrate that lower leg hot water immersion successfully protects macrovascular dilator function following I–R injury. This finding raises the possibility that targeted heating of the lower legs may be an alternative therapeutic approach to whole-body heating that is equally efficacious at protecting against vascular I–R injury.

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New Findings

- What is the central question of this study?

What is the effect of lower leg hot water immersion on vascular ischaemia–reperfusion injury induced in the arm of young healthy humans?

- What is the main finding and its importance?

Lower leg hot water immersion successfully protects against vascular ischaemia–reperfusion injury in humans. This raises the possibility that targeted heating of the lower legs may be an alternative therapeutic approach to whole-body heating that is equally efficacious at protecting against vascular ischaemia–reperfusion injury.

FIGURE 1.

Experimental schematic. Vertical arrow denotes thermal and haemodynamic measurements. Asterisk denotes assessment of vasodilator function. Blood collection tube denotes sampling of venous blood for quantification of plasma endothelin-1

FIGURE 2.

Leg skin (top panel) and intestinal (bottom panel) temperatures are shown at thermoneutral baseline and every 15min during leg immersion. Open circles, control immersion; filled circles, hot water immersion. * $P < 0.05$ vs. thermoneutral baseline; # $P < 0.05$ vs. control immersion

FIGURE 3.

Brachial artery mean shear rate (top panel), blood flow (middle panel) and vascular conductance (bottom panel) are shown at thermoneutral baseline and every 15 min during leg immersion. Open circles, control immersion; filled circles, hot water immersion. * $P < 0.05$ vs. thermoneutral baseline; # $P < 0.05$ vs. control immersion

FIGURE 4.

Macrovascular dilator function, assessed via ANCOVA corrected flow-mediated dilatation, is shown at Pre, 60 min Post and Post-I/R time points. Open bars, control immersion; filled bars, hot water immersion. ‡ $P < 0.05$ vs. Pre and 60 min Post within thermal condition; † $P < 0.05$ vs. control immersion at the indicated time point

FIGURE 5. Microvascular dilator function, assessed via reactive hyperaemia area under the curve (AUC; left panel) and peak (right panel), is shown at Pre, 60 min Post and Post-I/R time points. Open bars, control immersion; filled bars, hot water immersion

TABLE 1

Subject characteristics

Characteristic	Value
Male/female	5/5
Age (years)	26 ± 4
Height (cm)	170 ± 7
Weight (kg)	70 ± 14
Body mass index (kg m ⁻²)	23.8 ± 3.2
Heart rate (beats min ⁻¹)	63 ± 7
Mean arterial pressure (mmHg)	80 ± 5

Values are means ± SD.

TABLE 2

Central haemodynamics during leg immersion

	Thermoneutral baseline				15 min		30 min		45 min		60 min	
	Control immersion	Hot water immersion	Control immersion	Hot water immersion	Control immersion	Hot water immersion	Control immersion	Hot water immersion	Control immersion	Hot water immersion	Control immersion	Hot water immersion
Heart rate (beats min ⁻¹)	70 ± 7	68 ± 6	70 ± 7	79 ± 8 [*]	72 ± 6	84 ± 8 [*]	72 ± 7	89 ± 8 [*]	73 ± 8	88 ± 9 [*]		
Cardiac output (l min ⁻¹)	5.8 ± 1.4	5.9 ± 1.1	6.0 ± 1.1	6.3 ± 1.3	6.0 ± 1.3	6.4 ± 1.3	5.7 ± 1.4	6.5 ± 1.2 [*]	5.9 ± 1.0	6.6 ± 1.1 [*]		
Mean arterial pressure (mmHg)	84 ± 9	82 ± 9	82 ± 10	80 ± 8	85 ± 9	81 ± 11	84 ± 10	80 ± 8	82 ± 9	79 ± 7		

Values are means ± SD.

^{*} *P* 0.05 vs. control immersion at indicated time point.

TABLE 3

Haemodynamics during the assessment of vasodilator function

	Control immersion			Hot water immersion		
	Pre	60 min Post	Post-1/R	Pre	60 min Post	Post-1/R
Mean arterial pressure (mmHg)	81 ± 6	84 ± 8	84 ± 9	83 ± 10	84 ± 6	84 ± 7
Baseline blood flow (ml min ⁻¹)	37 ± 16	30 ± 12	30 ± 13	41 ± 30	42 ± 37	36 ± 24
Baseline conductance (ml min ⁻¹ mmHg ⁻¹)	0.45 ± 0.17	0.35 ± 0.14	0.35 ± 0.12	0.49 ± 0.36	0.50 ± 0.47	0.42 ± 0.28
Baseline diameter (cm)	0.347 ± 0.052	0.339 ± 0.046	0.347 ± 0.044	0.342 ± 0.056	0.341 ± 0.058	0.335 ± 0.052
Peak diameter (cm)	0.366 ± 0.052	0.356 ± 0.045	0.358 ± 0.046	0.361 ± 0.054	0.361 ± 0.054	0.354 ± 0.053
Diameter (cm)	0.019 ± 0.007	0.017 ± 0.006	0.011 ± 0.008 ^{‡,†}	0.019 ± 0.004	0.020 ± 0.005	0.019 ± 0.006
Time to peak diameter (s)	46.1 ± 15.7	48.6 ± 15.6	55.2 ± 37.8	42.6 ± 13.6	49.1 ± 22.9	43.4 ± 19.7
Shear rate AUC	46828 ± 14643	47259 ± 17484	44332 ± 16345	44472 ± 22357	54636 ± 34304	44744 ± 23332

Values are means ± SD. Shear rate AUC, shear rate area under the curve through peak diameter.

[‡] *P* 0.05 vs. Pre and 60 min Post within condition.[†] *P* < 0.05 vs. hot water immersion at indicated time point.

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TABLE 4

Plasma endothelin-1

	Control immersion			Hot water immersion		
	Pre	60 min	Post-I/R	Pre	60 min	Post-I/R
Endothelin-1 (pg ml ⁻¹)	1.54 ± 0.40	1.68 ± 0.49	1.42 ± 0.26	1.60 ± 0.37	1.65 ± 0.34	1.39 ± 0.25

Values are means ± SD.