Acute hot water immersion is protective against impaired vascular function following forearm ischemia-reperfusion in young healthy humans

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ABSTRACT

Ischemia-reperfusion (I/R) injury is a primary cause of poor outcomes following ischemic cardiovascular events. We tested whether acute hot water immersion protects against forearm vascular I/R. METHODS: Ten (5 male, 5 female) young (23±2 years), healthy subjects participated in two trials in random order 7-21 days apart, involving: 1) 60-min of seated rest (control), or 2) 60-min of immersion in 40.5°C water (peak rectal temperature: 38.9±0.2°C). I/R was achieved 70 min following each intervention by inflating an upper arm cuff to 250mmHg for 20-min followed by 20-min of reperfusion. Brachial artery flow-mediated dilation (FMD) and forearm post-occlusive reactive hyperemia (RH) were measured as markers of macro- and micro-vascular function at three time points: 1) pre-intervention, 2) 60-min post-intervention, and 3) post-I/R. RESULTS: Neither time control nor hot water immersion alone affected FMD (both $p>0.99$). I/R reduced FMD from 7.4±0.7 to 5.4±0.6% ($p=0.03$) and this reduction was prevented following hot water immersion (7.0±0.7 to 7.7±1.0%; $p>0.99$). I/R also impaired RH (peak vascular conductance: 2.6±0.5 to 2.0±0.4mL·min^{-1}·mmHg^{-1}, $p=0.003$), resulting in a reduced shear stimulus (SR_{AUC}·10^{-3}: 22.5±2.4 to 16.9±2.4, $p=0.04$). The post-I/R reduction in peak RH was prevented by hot water immersion (2.5±0.4 to 2.3±0.4mL·min^{-1}·mmHg^{-1}; $p=0.33$). CONCLUSIONS: We observed a decline in brachial artery dilator function post-I/R, which may be (partly) related to damage incurred downstream in the microvasculature, as indicated by impaired RH and shear stimulus. Hot water immersion was protective against reductions in FMD and RH post-I/R, suggesting heat stress induces vascular changes consistent with reducing I/R injury following ischemic events.

Keywords: heat therapy; endothelial function; microvascular function; reactive hyperemia; ischemia-reperfusion injury

INTRODUCTION

Cardiovascular disease, and its associated sequelae, continues to be the leading cause of morbidity and mortality in the developed world, despite significant advancements in available treatment modalities (41). Ischemic heart disease specifically represents a significant source of lethal and sub-lethal complications of acute and chronic cardiovascular related disease (41). The resulting tissue damage associated with ischemic cardiovascular events occurs due to a combination of ischemia and a paradoxical reperfusion following restoration of blood flow to ischemic tissue, commonly referred to as ischemia-reperfusion (I/R) injury. The molecular underpinnings of this I/R-induced cascade of accelerated tissue damage are still being elucidated (58). One contributing factor may stem from damage to the endothelial cells, which are particularly sensitive to I/R. Injury and subsequent swelling in endothelial cells can impede blood flow upon reperfusion, which is termed the "no-reflow phenomenon". This phenomenon has been shown to occur with I/R in both the myocardium (20) and brain (4) and is associated with worse clinical outcomes and increased mortality in patients who have undergone percutaneous coronary intervention (20).

In clinical settings, a paucity of intervention strategies exist for mitigating the effects of I/R injury, which animal models suggest may account for approximately 50% of the infarct size in myocardial events (29). Recently, Seeger et al. (48) demonstrated that a single bout of high-intensity interval exercise is protective against impaired vascular function following I/R in humans *in vivo* utilizing a brachial artery model, a commonly used model of I/R injury as brachial artery function has been shown to be correlated with coronary artery function (1). Exercise and passive heat stress have many common physiological effects, including increases in body core temperature and increases in blood flow and vascular shear stress (32). Increases in

body core and tissue temperature induce expression of heat shock proteins (HSPs), which in turn stabilize and/or upregulate a variety of proteins important to the cardiovascular system, including nitric oxide (NO) (46), which is important for endothelial function and protective against I/R injury (3). Furthermore, animal studies have demonstrated that both acute heat stress (3, 23, 40) and chronic heat exposure, possibly through upregulation of HSPs, are protective against I/R injury in cardiac (5, 25) and brain tissue (57). It is also possible some of this protection may be related to endothelial protection. Therefore, acute heat exposure may have protective effects against vascular I/R in humans.

In the present study, we repeated the experimental design used by Seeger et al. (48), but instead investigated whether a 60-min bout of hot water immersion followed by 60-min of recovery protects against vascular I/R. In addition to investigating the effects of I/R on endothelial function in the brachial artery diameter responses, we examined microvascular function following I/R. We included a 60-min recovery period in order to allow core temperature to return to normal prior to further measurements and because pilot work in our laboratory in cultured endothelial cells and primary peripheral blood mononuclear cells, as well as reports by other investigators (22, 60), have shown HSP levels to peak in the range of 1-3 h post-heat stress. As this is the first study to investigate whether heat stress is protective against I/R in humans, we chose to study a non-patient population, since certain disease states or elevated risk may alter vascular function responses to interventions and I/R (53).

We hypothesized that hot water immersion (plus a 60-min recovery period) would prevent the reduction in brachial artery flow-mediated dilation (FMD), a measure of conduit vessel endothelial function, and prevent the reduction in forearm post-occlusive reactive hyperemia (RH), a measure of microvascular function, following I/R.

METHODS

Ethical Approval

This study was approved by the Institutional Review Board at the University of Oregon. Prior to participation, all subjects provided oral and written informed consent as set forth by the Declaration of Helsinki.

Subjects

Ten young, healthy, recreationally active subjects participated in the study. Subject characteristics are provided in Table 1. All subjects were nonsmokers, were not taking any prescribed medications other than contraceptives, and underwent medical history screening to rule out presence of cardiovascular disease, diabetes mellitus, hypertension, hyperlipidemia, recent surgery, dermatological conditions, and history of heat-related illness. Subjects were required to abstain from all over-the-counter medications (including vitamins and supplements) for >24 hours, alcohol and caffeine for >12 hours, and heavy exercise for >24 hours prior to each session. Subjects were instructed to eat a light meal no less than 4 hours prior to each session. Female subjects were required to demonstrate a negative pregnancy test prior to each study session, measured using urine hCG.

Experimental Design & Protocol

Subjects participated in two experimental sessions 7-21 days apart in randomized counter-balanced order. Sessions were held in a climate-controlled room (21-24°C) at the same time of day for each subject. For each session, brachial artery endothelial function was assessed

under three conditions: resting, post-intervention, and post-I/R utilizing flow-mediated dilation (FMD) (Figure 1).

For each session, subjects arrived at the laboratory and height and weight were recorded. Subjects were then instructed to lay supine and were instrumented with a 3-lead electrocardiogram (CardioCap; Datex Ohmeda, Louisville, CO, USA) for continuous monitoring throughout the study, an automated blood pressure cuff on the left upper arm, and a small cuff on the middle finger for periodic beat-by-beat blood pressure monitoring by photoplethysmography (Nexfin; BMEye, Amsterdam, the Netherlands). Baseline hemodynamic measurements, including baseline FMD, were recorded following 20 minutes of supine rest. Following the rest period, subjects underwent one of two 60-minute interventions: (1) time-control or (2) hot water immersion. Following the intervention, subjects again lay supine and brachial artery FMD measurements were repeated following another 20 minutes of supine rest. Following the second FMD, an inflatable occlusion cuff (E20 Rapid Cuff Inflater, D. E. Hokanson, Bellevue, WA, USA) was applied to the upper right arm. To induce ischemia, the cuff was inflated to >250 mmHg for 20 minutes to occlude blood flow to the arm. Occlusive pressure was then released allowing for 20 minutes of reperfusion. A third FMD was measured following the 20 minutes of reperfusion, as shown in Figure 1. This model of forearm I/R is frequently used to induce vascular I/R injury in humans *in vivo* in previous studies (36, 48).

Interventions

The time-control intervention consisted of seated inactivity for 100 minutes following the initial pre-intervention FMD. Subjects remained in the climate-controlled laboratory for the

duration of the time-control intervention, but could wear additional clothing or were provided with blankets if desired in order to maintain thermal comfort.

The hot water immersion intervention consisted of 60 minutes immersion in 40.5°C water followed by 40 minutes of seated recovery. Prior to immersion, euhydration was ensured by a first morning urine specific gravity of 1.02, subjects drank 5 $mL \cdot kg^{-1}$ prior to entering the hot tub. Nude body weight was measured behind a screen before and after hot water immersion for calculation of mean whole body sweat rate, after correcting for water intake. Subjects were instrumented with a sterile rectal thermistor probe (YSI Series 400, Yellow Spring Instruments, Yellow Springs, OH, USA) inserted ~10 cm past the anal sphincter, and a chest strap heart rate monitor (Polar; Lake Success, NY, USA). Rectal thermistors were used only on the hot water immersion day as a safety precaution and to ensure the desired heat stimulus was induced. Thus, we were not able to compare rectal temperature responses between the hot water immersion and time control sessions. However, we do not expect rectal temperature deviated greater than 0.2°C from resting during the time control session, similar to what we have observed under thermoneutral conditions in other studies (15).

Subjects were immersed up to the shoulder until rectal temperature (T_{re}) reached a target temperature of 38.5°C, which took ~20-30min. After T_{re} reached 38.5°C, subjects sat upright, such that the water reached approximately waist level for the remainder of the 60 min. During this second part of the heating protocol, T_{re} was maintained between 38.5-39.0°C while sitting upright. An upper limit of 39.0°C was set in order to ensure subject safety. The arm in which FMD measurements were taken remained outside the water for the duration of the entire session so that we could investigate the systemic effects of hot water immersion on vascular function

rather than the local effects of elevations in skin and muscle temperature. Subjects were instructed to drink *ad libitum* while in the hot tub.

This heating protocol (temperature of ≥38.5°C for 60 min) was selected to match other hot water immersion protocols performed in our lab which we have used to demonstrate long-term cardiovascular adaptations to repeated hot water immersion (14, 15). We originally selected this protocol as it has been shown to be the most effective for inducing hallmark signs of heat acclimation when using passive hyperthermia (26) and because HSP expression is dependent upon time spent above a threshold core temperature, which in humans has most commonly been reported to be in the range of 38.0-38.5°C (50).

Following 60 min of immersion, subjects exited the tub and transferred to a recovery chair. We continued to monitor T_{re} and HR for at least 10 min, or until T_{re} had fallen below 38.5°C. After this time, nude body weight was measured a second time, subjects got dressed (rectal thermistor remained in place) and rested seated until they had been out of the tub for 40 minutes. This time duration was selected so that the second FMD measurement would take place exactly 60 min after exiting the hot tub, which would allow time for body core temperature to return to baseline (confirmed by T_{re}) and for increased expression of heat shock proteins (22). If subjects did not drink enough fluids to fully replace water lost during heating, they drank the remaining fluid volume during this recovery time so that hydration status would be similar across FMD measurements.

Measurements

FMD measurements were made in accordance with established guidelines (34). Subjects rested supine with the right arm extended 80-90° away from the body at heart level. A high-

resolution Doppler ultrasound (Terason t3000cv; Teratech, Burlington, MA) equipped with 10.0-MHz linear array ultrasound transducer probe was used to image the brachial artery in the lower third of the arm, 3-9 cm proximal to the antecubital fossa, using an insonation angle of 60°. Probe placement (distances and angles) and subject position (including limb-trunk angles) were recorded and repeated to ensure consistency between FMD and RH measurements. Images were optimized using ultrasound contrast controls which were consistent across experimental trials for each individual subject (45). A blood pressure cuff was placed 0.5-2.0 cm distal to the antecubital fossa and inflated to 250 mmHg for 5 min. Following release of the occlusion, blood flow and thus shear rate increase substantially, resulting in dilation that peaks after ~40-90 sec (10). Measurements of brachial artery diameter and velocity were recorded 1 min of baseline prior to cuff inflation and for 3 min following release of the cuff.

Ultrasound images were captured at 20Hz using video recording software (Camtasia®; TechSmith®, Okemos, MI, USA) and were later analyzed for changes in arterial diameter and peak blood velocity using a custom-designed edge-detection and wall-tracking software, which is largely independent of investigator bias (56). From these measurements, FMD was calculated as the percent change in brachial artery diameter from baseline to peak dilation post-occlusion. The shear stimulus responsible for eliciting dilation was calculated as area under the curve above baseline shear rate from the time of release to peak dilation (SR_{AUC}).

To characterize the RH response, blood velocity and diameter were averaged across cardiac cycles and used to calculate forearm vascular conductance (FVC) as (peak blood velocity/2) x vessel cross-sectional area (from diameter) / mean arterial pressure. Beat-by-beat FVC values were zero-hold interpolated to 5Hz. Peak RH was determined as the peak FVC following release of the occlusion (usually in the range of 3-10 sec post-cuff release). Area under

the curve (AUC) RH was calculated as the integral of FVC values above baseline FVC (average FVC across the 1-min baseline) until return to baseline (usually 120-180 sec post-cuff release).

Statistics

Statistical analyses were conducted using SigmaPlot 11.0 (Systat Software, Inc., San Jose, CA, USA) and SPSS (Version 22; IBM, Chicago, IL, USA). *A priori* sample size analysis for two-way repeated measures analysis of variance (ANOVA) performed using FMD% data from two pilot subjects and standard deviations reported by Seeger et al. showed that a sample size of N=15 subjects would be required to reach statistical significance with a power of >0.80 and two-tailed alpha level of 0.05. However, statistical significance was reached in all variables after studying N=10 subjects. Subsequent power analysis using actual data and standard deviations from the present study indicated we had achieved a power of 0.85 at an alpha level of 0.05 with this sample size. Data for all variables were normally distributed (Shapiro-Wilk test) and passed Levene's Test of Equality of Variances (p=0.89 for FMD%).

FMD%, FMD presented as absolute peak diameter, SR_{AUC}, baseline brachial artery diameter, peak RH, and AUC RH were all compared using two-way repeated measures ANOVA with factors of intervention (time control and hot water immersion) and time point (pre-intervention, post-intervention, and post-I/R). In order to evaluate the influence of SR_{AUC} and baseline brachial artery diameter on FMD%, we used a linear mixed model with a random factor of "subject" and fixed factors of intervention, time point, and the interaction of trial x time point, both with and without SR_{AUC} and baseline diameter added as covariates (6). T_{re} on the hot water immersion day was compared across time (resting, peak during immersion, at FMD2, and at FMD3) using one-way repeated measures analysis of variance. For all analyses, when significant

main effects were detected, pairwise comparisons were made between FMD# within trials and within FMD# across trials (9 total comparisons) using Bonferroni's posthoc test. Significance was set at α=0.05. P-values were two-tailed.

Demographic, temperature, and heart rate data are presented as mean±S.D. All other data are presented as mean±S.E. P-values given denote pairwise comparisons unless otherwise indicated.

RESULTS

Temperature and heart rate during hot water immersion trial

Hot water immersion resulted in an increase in rectal temperature from 37.1±0.3°C at rest to a peak of 38.9±0.2°C ($p<0.001$) and an increase in heart rate from 81±18 beats/min prior to entering the hot tub to a peak of 127±18 beats/min ($p<0.001$) (Figure 2). T_{re} had returned to baseline by the time FMD measurements were taken at the post-intervention (T_{re} = 37.2±0.3; $p>0.99$ vs. resting T_{re}) and post-I/R (T_{re} = 37.0±0.3; $p>0.99$ vs. resting T_{re}) time points.

Vascular responses

We observed a significant interaction effect of intervention x time point on FMD%, both using ANOVA ($p=0.02$) and linear mixed model analyses ($p=0.04$) (Figure 3A). Using ANOVA posthoc analyses, we found no significant effect of hot water immersion or time control on FMD%. Post-I/R, we observed a significant reduction in FMD% on the time control day ($p=0.03$ vs. FMD1). In contrast, hot water immersion prevented the reduction in FMD% post-I/R ($p>0.99$ vs. FMD1). When FMD was presented as absolute peak diameter (Table 2), we observed a significant interaction effect of intervention x time ($p<0.001$), but only a trend towards a

decrease in peak diameter post-I/R on the time control day ($p=0.07$). In contrast, hot water immersion increased peak diameter following the intervention ($p<0.001$) and post-I/R ($p=0.01$; $p=0.04$ vs. FMD3 on time control session).

We observed no significant changes in baseline brachial artery diameter on the time control day, either after the intervention ($p>0.99$) or post-I/R ($p=0.40$). Baseline brachial artery diameter was increased following hot water immersion ($p<0.001$), and this persisted post-I/R ($p=0.047$). Furthermore, the shear stimulus was reduced following hot water immersion ($p=0.03$). SR_{AUC} was also reduced post-I/R on both the time control ($p=0.04$) and hot water immersion ($p=0.02$) days. Data are summarized in Table 2.

In linear mixed model analyses, SR_{AUC} was found to be a significant predictor of FMD% ($p=0.02$), with lower values of FMD% being associated with a lower SR_{AUC}. However, after statistically correcting for changes in SR_{AUC} and baseline diameter, the significant interaction effect of intervention x time point on FMD% persisted ($p=0.02$) (main effect of intervention: $p=0.06$, main effect of time point: $p=0.24$).

In the microvasculature, there was no significant effect of hot water immersion alone on either peak ($p=0.24$) or area under the curve RH ($p=0.65$). On the time control day, I/R resulted in significant reductions in both peak RH ($p=0.003$) and AUC RH ($p=0.01$). However, hot water immersion prevented the reduction in peak RH post-I/R ($p=0.33$ vs. FMD1). Area under the curve RH was still significantly reduced post-I/R relative to FMD2 ($p=0.004$), although it only tended to be reduced relative to FMD1 ($p=0.09$). Data are summarized in Figure 3.

DISCUSSION

The present study is the first investigation of the potential protective effects of hot water immersion against I/R-induced vascular dysfunction in humans. By performing multiple analyses, we were able to comprehensively characterize how I/R affects the vasculature and how hot water immersion may protect against the damaging effects of I/R. Specifically, we confirmed previous reports that forearm I/R results in a reduction in FMD%, and discovered that hot water immersion prevents this reduction in FMD% post-I/R. Furthermore, I/R reduced the shear stimulus responsible for inducing brachial artery vasodilation. However, despite the influence of SR_{AUC} on FMD%, statistically accounting for these changes confirmed the ability of hot water immersion to protect the brachial artery against impaired vascular function following I/R. Finally, and in agreement with forearm conduit arteries, I/R reduced forearm microvascular peak RH, whilst this reduction was prevented by hot water immersion. Taken together, these observations may have some future clinical relevance for adopting hot water immersion as a strategy to minimize I/R injury.

In humans, vascular function in the brachial artery is commonly studied as a surrogate for coronary function as FMD has been shown to be correlated in the two vessels (1). Accordingly, we utilized a model of I/R which has been shown in multiple previous studies to consistently impair brachial artery FMD% (36, 37, 53). Most recently, Seeger et al. (48) reported a ~40% reduction in brachial artery FMD following I/R using the exact procedures used in the present study. We observed a similar, albeit slightly smaller, reduction of ~27%, but FMD% was still consistently reduced across subjects.

In previous studies, others have attributed this reduction in FMD% post-I/R to damage to the brachial artery. However, in the present study, reductions in FMD% were accompanied by a

reduced shear stimulus for vasodilation, likely secondary to the reduction in microvascular peak RH. Given earlier reports highlighting the importance of shear for artery dilation (47), the reduction in FMD% may be at least partly related to the reduced shear stimulus. However, statistically correcting for changes in SR_{AUC} did not remove the significant impact of I/R on FMD%. Together, these findings suggest that the reduction in FMD% post-I/R is caused by a combination of both impairments in brachial artery endothelial function and a reduced shear stimulus. Although our findings may dispel conclusions made in previous studies specifically regarding brachial artery function post-I/R, we do not believe they necessarily diminish the utility of studying forearm I/R in future studies. With ischemic events, such as heart attack or stroke, the majority of damage occurs in the downstream tissue, rather than in the conduit vessels. Thus, given our findings, forearm I/R may actually be an ideal model for replicating ischemic events in humans. However, the damaging effects of I/R may be better captured by assessing damage in both the macro- (i.e., brachial artery dilator function) and microvasculature (i.e., using reactive hyperemia), rather than just using FMD alone.

Effects of hot water immersion on the brachial artery

Following hot water immersion, resting brachial artery diameter was increased, which resulted in a reduction in the shear stimulus following release of the arterial occlusion for the post-intervention FMD. Elevations in body core temperature during hot water immersion require redistribution of blood to the skin for thermoregulation, creating significant increases in shear rate on the brachial artery, resulting in shear-induced vasodilation (17). Although we waited an hour post-hot water immersion before making post-intervention measurements and T_{re} had returned to resting, the brachial artery still remained dilated. However, despite this slight dilation and reduction in shear stimulus following release of the arterial occlusion, FMD% was

297 unchanged after hot water immersion, suggesting that acute hot water immersion improved
298 brachial artery vasodilator function (i.e., greater dilation for a given shear stimulus). Repeated
299 elevations in core temperature via hot water immersion have also been shown to chronically
300 increase FMD (15, 18). As such, the acute improvements we observed in our study may
301 potentiate long-term effects.

302 Following I/R, the aforementioned effects of hot water immersion persisted, including an
303 increased brachial artery diameter, a reduction in the shear stimulus, and presumably an
304 increased responsiveness of the brachial artery for shear-induced dilation. As a result, we
305 observed no reduction in FMD% post-I/R.

306 We believe our findings related to the impact of hot water immersion on vascular
307 function are attributable to the effects of both elevations in body core temperature and shear
308 stress on NO bioavailability, since FMD is primarily dependent on NO (28, 31). Elevations in
309 body core temperature induce the expression of HSPs, which are detectibly elevated in human
310 cells by 1h post heat stress (22). Hsp90 associates with endothelial NO synthase (eNOS) and is
311 necessary for several steps leading up to activation of eNOS, including binding of calcium-
312 calmodulin (27) and Akt phosphorylation (13). Hsp90 is also an essential cofactor for eNOS
313 (46), regulating the balance between NO and superoxide production by eNOS. Therefore,
314 increases in Hsp90 expression can result in greater NO production for a given stimulus. In
315 animal work, both Hsp70 (23, 40) and NO (3) have been implicated in acute heat stress-induced
316 protection from I/R injury in cardiac myocytes. Additionally, Hsp70 has also been shown to
317 upregulate the antioxidative enzyme superoxide dismutase (21, 42), which scavenges superoxide,
318 therefore preventing superoxide from binding with NO. However, given that our subjects were

young and healthy, likely with minimal baseline oxidative stress, it is unknown whether this mechanism would have contributed to our results.

Shear stress increases considerably during hot water immersion, to an extent comparable to or greater than during aerobic exercise (52). Increases in shear stress can also increase both eNOS expression and eNOS activity (55). The latter occurs through activation of the receptor for vascular endothelial growth factor (30), activation of phosphoinositide-3-kinase which in turn activates protein kinase A (11), and increased expression of tetrahydrobiopterin (54), which is an essential cofactor for eNOS. In isolated arteries, these changes result in improved endothelium-dependent dilation (55). Conversely, in humans, acute reductions in shear rate impair FMD (51). Although these changes were observed while still in the presence of altered shear stress, elevations in shear stress are known to have longer-lasting effects. For example, elevations in shear stress are essential for chronic arterial adaptation to exercise training (9) and to repeated passive heat stress (18). As such, it remains possible that some of the acute changes in protein expression and phosphorylation persisted in our human subjects until the time when the second and third FMD measurements were made, even though baseline shear had returned to or below resting by this time.

Effects of hot water immersion on the forearm microvasculature

In the microvasculature, we observed no effects of hot water immersion alone on RH. However, RH is much less dependent on NO than brachial artery FMD. Indeed, adenosine, adenosine diphosphate, prostaglandins, and myogenic responses appear important contributors to the RH response (7, 16). Therefore, even though improvements in NO bioavailability may have also been present in the microvasculature, we found no significant impact on RH. Furthermore,

hot water immersion was protective against the reduction in peak RH following I/R; however, the area under the curve RH response was still impaired. As such, we conclude that hot water immersion mitigates microvascular impairment, but does not fully prevent it. Regardless, given that I/R primarily affects the microvasculature, any protection may be beneficial, although studies utilizing repeated hot water immersion are necessary to determine whether protective effects can be obtained chronically.

In animals, acute sub-lethal heat stress has been shown to confer short-term protection from I/R injury (3, 23, 40), while long-term heat acclimation has been shown to result in a phenotype that is anti-oxidative (8) and anti-apoptotic (5), thus providing more lasting protection from I/R injury. Additionally, during ischemia, heat-acclimated cells are better able to shift towards a greater reliance on anaerobic metabolism and become more metabolically efficient so that the rate of glycogen depletion is reduced (25). In general, longer term heat exposure is required to attain these cytoprotective effects (5); however, it is possible we observed protective effects of acute hot water immersion through some of these mechanisms in the present study.

Limitations

We utilized a time control rather than a thermoneutral water immersion sham and therefore cannot distinguish effects of hydrostatic pressure from heat. Increased hydrostatic pressure during acute thermoneutral water immersion has been previously shown to alter cardiovascular hemodynamics, including increased cardiac output and mean arterial pressure (2), increased conduit vessel diameter (19), and increased arterial compliance (12), all of which could have contributed to the protective vascular effects of hot water immersion on I/R. In a previous study, we demonstrated that 8 weeks of repeated thermoneutral water immersion had no chronic effects on macro- or micro-vascular function (14, 15). However, it remains possible that the acute

effects of increased hydrostatic pressure could have lasted for the duration of experimental testing in the present study. In future studies, it would be interesting to see if acute sauna exposure offers equal protection against vascular I/R.

Conclusions & Perspectives

In the present study, we have demonstrated that one bout of hot water immersion prevents the reduction in brachial artery FMD% caused by forearm I/R. It appears that this protection occurs due to a combination of protection against the drop in shear-induced brachial artery vasodilation and protection against microvascular damage, as measured by RH. Our findings are supported by animal work and are in line with recent findings of Seeger et al. (48), who showed that one bout of interval exercise was protective against reductions in FMD% following forearm I/R.

Based on these findings, it is plausible that hot water immersion could be used to protect against I/R injury in patient populations, for example, those at high-risk for myocardial infarction or stroke. However, given the unexpected nature of when myocardial infarctions and strokes occur, chronic use of hot water immersion (i.e., heat therapy) may be preferable as it is currently unknown how long the protective effects of a single bout of hot water immersion may last. However, a single bout could be utilized pre-operatively by patients undergoing surgeries in which blood flow will be occluded through an artery or to a limb for an extended period of time (e.g., aneurysm repair or joint replacement surgeries). For example, extensive damage is known to occur secondary to tourniquet use (43, 59) which could be mitigated by prior hot water immersion, and typically these patients are not able to exercise prior to surgery due to pain.

Not only could repeated bouts of hot water immersion counteract the unanticipated timing of myocardial infarction and stroke, but it may also impart greater protection against I/R

injury. Studies in animals have demonstrated longer-lasting and more extensive cytoprotective effects of heat acclimation in myocardial and brain tissue following I/R injury (5, 25, 57). In humans, heat acclimation has been well established to induce extensive cardiovascular adaptations (44). Studies in heart failure and coronary artery disease patients have demonstrated improvements in vascular function and clinical outcomes following short- and long-term infrared sauna therapy, including a reduced incidence of cardiac events (33) and improved myocardial perfusion (49). Laukkanen et al. (35) also recently published data demonstrating that lifelong sauna use greatly reduced the risk of cardiovascular-related (and all cause) mortality, including from ischemic events. Protection against vascular I/R may be in part responsible for these improved outcomes. Together, these and our data provide a strong basis for future studies to investigate the clinical utility of using hot water immersion to protect against ischemic cardiovascular events in at-risk patient populations.

Of note, sauna and hot water immersion have been shown to be safe for the majority of patient populations (34). For example, one study showed that the incidence of arrhythmias in acute myocardial infarction patients was significantly lower during sauna bathing compared to sub-maximal exercise (8% vs. 18% with exercise) (38). As shown in extensive studies demonstrating safety of Finnish sauna, heat stress is generally only contraindicated in patients with unstable cardiovascular and cerebrovascular diseases (e.g., conditions with potentially unstable plaques or where a blood clot could be dislodged), for which exercise would also be contraindicated, or in elderly individuals prone to orthostatic hypotension (24, 39).

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FIGURES LEGENDS

593 **Figure 1.** Timeline of the protocol. FMD, flow-mediated dilation.

594

595 **Figure 2. A)** Rectal temperature (T_{re}) and **B)** heart rate during 60 min of hot water immersion, 10
596 min of seated recovery, and supine at the time of the post-intervention and post-I/R flow-
597 mediated dilation (FMD) measurements. Data are mean ± S.D. Data were compared across time
598 using one-way repeated measures analysis of variance (main effects: T_{re}, $p<0.01$; heart rate,
599 $p<0.001$). *$p<0.05$ vs. 0 min on pairwise Bonferroni post-hoc comparisons.

600

601 **Figure 3. A)** Flow-mediated dilation (FMD), **B)** peak reactive hyperemia, and **C)** area under the
602 curve reactive hyperemia measured pre-intervention, post-intervention, and post-ischemia-
603 reperfusion (I/R) during the time control (white bars) and hot water immersion sessions (gray
604 bars). Data are mean±S.E. Nine pairwise comparisons were compared within each variable using
605 Bonferroni's post-hoc test. *$p<0.05$ vs. pre-intervention within session, † $p<0.05$ vs. post-
606 intervention with trial; ‡ $p<0.05$ vs. time control session during the same FMD time point.

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TABLES

Table 1. Subject characteristics

Subject Characteristics	
Male/female	5/5
Age, yrs	23±6
Height, cm	172±9
Body mass, kg	68±12
Body mass index, $kg \cdot m^{-2}$	22.8±1.7
Resting mean arterial blood pressure, mmHg	81±5
Resting heart rate, beats/min	63±10

Data are mean±S.D.

Table 2. Brachial artery characteristics across interventions

	Pre-intervention FMD1	Post-intervention FMD2	Post-I/R FMD3
Baseline brachial artery diameter, mm			
Time control	3.37±0.25	3.34±0.24	3.29±0.23
Hot water immersion	3.24±0.24	3.46±0.25*	3.37±0.26*
Area under the curve shear rate, $SR_{AUC} \cdot 10^{-3}$			
Time control	22.5±2.4	19.2±2.0	16.9±2.4*
Hot water immersion	21.1±3.0	15.5±1.7*	14.9±1.6*
FMD peak diameter, mm			
Time control	3.58±0.25	3.56±0.25	3.45±0.24
Hot water immersion	3.45±0.25	3.71±0.26*	3.62±0.26*‡

Data are mean±S.E. *$p<0.05$ vs. pre-intervention within trial, ‡ $p<0.05$ vs. time control session during the same FMD time point; determined with multiple pairwise comparison (9 total) post-hoc testing using Bonferroni correction. FMD, flow-mediated dilation; I/R, ischemia-reperfusion