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

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Running title: Hot water immersion and forearm ischemia-reperfusion

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1 ABSTRACT

2 Ischemia-reperfusion (I/R) injury is a primary cause of poor outcomes following ischemic
3 cardiovascular events. We tested whether acute hot water immersion protects against forearm
4 vascular I/R. **METHODS:** Ten (5 male, 5 female) young (23 ± 2 years), healthy subjects
5 participated in two trials in random order 7-21 days apart, involving: 1) 60-min of seated rest
6 (control), or 2) 60-min of immersion in 40.5°C water (peak rectal temperature: $38.9\pm 0.2^{\circ}\text{C}$). I/R
7 was achieved 70 min following each intervention by inflating an upper arm cuff to 250mmHg for
8 20-min followed by 20-min of reperfusion. Brachial artery flow-mediated dilation (FMD) and
9 forearm post-occlusive reactive hyperemia (RH) were measured as markers of macro- and micro-
10 vascular function at three time points: 1) pre-intervention, 2) 60-min post-intervention, and 3)
11 post-I/R. **RESULTS:** Neither time control nor hot water immersion alone affected FMD (both
12 $p>0.99$). I/R reduced FMD from 7.4 ± 0.7 to $5.4\pm 0.6\%$ ($p=0.03$) and this reduction was prevented
13 following hot water immersion (7.0 ± 0.7 to $7.7\pm 1.0\%$; $p>0.99$). I/R also impaired RH (peak
14 vascular conductance: 2.6 ± 0.5 to $2.0\pm 0.4\text{mL}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$, $p=0.003$), resulting in a reduced
15 shear stimulus ($\text{SR}_{\text{AUC}}\cdot 10^{-3}$: 22.5 ± 2.4 to 16.9 ± 2.4 , $p=0.04$). The post-I/R reduction in peak RH
16 was prevented by hot water immersion (2.5 ± 0.4 to $2.3\pm 0.4\text{mL}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}$; $p=0.33$).

17 **CONCLUSIONS:** We observed a decline in brachial artery dilator function post-I/R, which may
18 be (partly) related to damage incurred downstream in the microvasculature, as indicated by
19 impaired RH and shear stimulus. Hot water immersion was protective against reductions in FMD
20 and RH post-I/R, suggesting heat stress induces vascular changes consistent with reducing I/R
21 injury following ischemic events.

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

24 INTRODUCTION

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

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

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

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

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

70 **METHODS**

71 *Ethical Approval*

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

75

76 *Subjects*

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

87

88 *Experimental Design & Protocol*

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

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

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

110

111 ***Interventions***

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

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

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

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

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

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

145 Following 60 min of immersion, subjects exited the tub and transferred to a recovery
146 chair. We continued to monitor T_{re} and HR for at least 10 min, or until T_{re} had fallen below
147 38.5°C . After this time, nude body weight was measured a second time, subjects got dressed
148 (rectal thermistor remained in place) and rested seated until they had been out of the tub for 40
149 minutes. This time duration was selected so that the second FMD measurement would take place
150 exactly 60 min after exiting the hot tub, which would allow time for body core temperature to
151 return to baseline (confirmed by T_{re}) and for increased expression of heat shock proteins (22). If
152 subjects did not drink enough fluids to fully replace water lost during heating, they drank the
153 remaining fluid volume during this recovery time so that hydration status would be similar across
154 FMD measurements.

155

156 ***Measurements***

157 FMD measurements were made in accordance with established guidelines (34). Subjects
158 rested supine with the right arm extended $80\text{-}90^{\circ}$ away from the body at heart level. A high-

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

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

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

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

184

185 *Statistics*

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

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

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

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

211

212 **RESULTS**

213 *Temperature and heart rate during hot water immersion trial*

214 Hot water immersion resulted in an increase in rectal temperature from $37.1\pm 0.3^{\circ}\text{C}$ at rest
215 to a peak of $38.9\pm 0.2^{\circ}\text{C}$ ($p<0.001$) and an increase in heart rate from 81 ± 18 beats/min prior to
216 entering the hot tub to a peak of 127 ± 18 beats/min ($p<0.001$) (Figure 2). T_{re} had returned to
217 baseline by the time FMD measurements were taken at the post-intervention ($T_{\text{re}} = 37.2\pm 0.3$;
218 $p>0.99$ vs. resting T_{re}) and post-I/R ($T_{\text{re}} = 37.0\pm 0.3$; $p>0.99$ vs. resting T_{re}) time points.

219

220 *Vascular responses*

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

228 decrease in peak diameter post-I/R on the time control day ($p=0.07$). In contrast, hot water
229 immersion increased peak diameter following the intervention ($p<0.001$) and post-I/R ($p=0.01$;
230 $p=0.04$ vs. FMD3 on time control session).

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

237 In linear mixed model analyses, SR_{AUC} was found to be a significant predictor of FMD%
238 ($p=0.02$), with lower values of FMD% being associated with a lower SR_{AUC} . However, after
239 statistically correcting for changes in SR_{AUC} and baseline diameter, the significant interaction
240 effect of intervention x time point on FMD% persisted ($p=0.02$) (main effect of intervention:
241 $p=0.06$, main effect of time point: $p=0.24$).

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

248

249

250

DISCUSSION

251
252 The present study is the first investigation of the potential protective effects of hot water
253 immersion against I/R-induced vascular dysfunction in humans. By performing multiple
254 analyses, we were able to comprehensively characterize how I/R affects the vasculature and how
255 hot water immersion may protect against the damaging effects of I/R. Specifically, we confirmed
256 previous reports that forearm I/R results in a reduction in FMD%, and discovered that hot water
257 immersion prevents this reduction in FMD% post-I/R. Furthermore, I/R reduced the shear
258 stimulus responsible for inducing brachial artery vasodilation. However, despite the influence of
259 SR_{AUC} on FMD%, statistically accounting for these changes confirmed the ability of hot water
260 immersion to protect the brachial artery against impaired vascular function following I/R.
261 Finally, and in agreement with forearm conduit arteries, I/R reduced forearm microvascular peak
262 RH, whilst this reduction was prevented by hot water immersion. Taken together, these
263 observations may have some future clinical relevance for adopting hot water immersion as a
264 strategy to minimize I/R injury.

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

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

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

288 *Effects of hot water immersion on the brachial artery*

289 Following hot water immersion, resting brachial artery diameter was increased, which
290 resulted in a reduction in the shear stimulus following release of the arterial occlusion for the
291 post-intervention FMD. Elevations in body core temperature during hot water immersion require
292 redistribution of blood to the skin for thermoregulation, creating significant increases in shear
293 rate on the brachial artery, resulting in shear-induced vasodilation (17). Although we waited an
294 hour post-hot water immersion before making post-intervention measurements and T_{re} had
295 returned to resting, the brachial artery still remained dilated. However, despite this slight dilation
296 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

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

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

335

336 *Effects of hot water immersion on the forearm microvasculature*

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

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

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

356 *Limitations*

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

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

368

369 *Conclusions & Perspectives*

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

376 Based on these findings, it is plausible that hot water immersion could be used to protect
377 against I/R injury in patient populations, for example, those at high-risk for myocardial infarction
378 or stroke. However, given the unexpected nature of when myocardial infarctions and strokes
379 occur, chronic use of hot water immersion (i.e., heat therapy) may be preferable as it is currently
380 unknown how long the protective effects of a single bout of hot water immersion may last.

381 However, a single bout could be utilized pre-operatively by patients undergoing surgeries in
382 which blood flow will be occluded through an artery or to a limb for an extended period of time
383 (e.g., aneurysm repair or joint replacement surgeries). For example, extensive damage is known
384 to occur secondary to tourniquet use (43, 59) which could be mitigated by prior hot water
385 immersion, and typically these patients are not able to exercise prior to surgery due to pain.

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

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

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

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592 **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 \pm 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 \pm 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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609 **TABLES**610 **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·m ⁻²	22.8±1.7
Resting mean arterial blood pressure, mmHg	81±5
Resting heart rate, beats/min	63±10

611 Data are mean±S.D.

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613

614 **Table 2.** Brachial artery characteristics across interventions

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

615 Data are mean±S.E. *p<0.05 vs. pre-intervention within trial, ‡ p<0.05 vs. time control session

616 during the same FMD time point; determined with multiple pairwise comparison (9 total) post-

617 hoc testing using Bonferroni correction. FMD, flow-mediated dilation; I/R, ischemia-reperfusion

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