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Lower-limb hot-water immersion acutely induces beneficial hemodynamic and cardiovascular responses in peripheral arterial disease and healthy, elderly controls

Running Title: Lower-limb hot-water immersion in PAD

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31 **Abstract**

32 Passive heat induces beneficial perfusion profiles, provides substantive
33 cardiovascular strain and reduces blood pressure, thereby holding potential for
34 healthy and cardiovascular disease populations. The aim of this study was to assess
35 acute responses to passive heat via lower-limb hot-water immersion in patients with
36 peripheral arterial disease (PAD) and healthy, elderly controls. Eleven patients with
37 PAD (age 71 ± 6 y, 7 male) and ten Controls (age 72 ± 7 y, 8 male) underwent hot-water
38 immersion (30 min waist-level immersion in $42.1\pm 0.6^\circ\text{C}$ water). Before, during and
39 following immersion, brachial and popliteal artery diameter, blood flow and shear
40 stress were assessed using duplex ultrasound. Lower-limb perfusion was measured
41 also using venous occlusion plethysmography and near-infrared spectroscopy.
42 During immersion, shear rate increased ($p<0.0001$) comparably between groups in
43 the popliteal artery (Controls: $+183\pm 26\%$; PAD: $+258\pm 54\%$) and brachial artery
44 (Controls: $+117\pm 24\%$; PAD: $+107\pm 32\%$). Lower-limb blood flow increased significantly
45 in both groups, as measured from duplex ultrasound ($>200\%$), plethysmography
46 ($>100\%$) and spectroscopy, while central and peripheral pulse wave velocity
47 decreased in both groups. Mean arterial blood pressure was reduced by 22 ± 9 mmHg
48 (main effect $p<0.0001$, interaction $p=0.60$) during immersion, and remained 7 ± 7
49 mmHg lower 3 h afterward. In PAD, popliteal shear profiles and claudication both
50 compared favourably with those measured immediately following symptom-limited
51 walking. A 30-min hot-water immersion is a practical means of delivering heat
52 therapy to PAD patients and healthy, elderly individuals to induce appreciable

53 systemic (chronotropic and blood pressure lowering) and hemodynamic (upper and
54 lower-limb perfusion and shear rate increases) responses.

55

56 **Key Words:** shear stress, passive heat, peripheral arterial disease, peripheral

57 vascular disease, PVD, PAD, heat therapy, antegrade, shear rate, elderly

58

59 **Introduction**

60 Peripheral arterial disease (PAD) is a prevalent atherosclerotic disease,
61 increasingly so with age for both men and women (1). It commonly manifests as
62 intermittent claudication – walking-induced muscle pain that is the metabolic
63 consequence of insufficient muscle perfusion during exercise. PAD is associated with
64 faster functional decline (30) and increased cardiovascular morbidity and mortality
65 (15, 22) than in those without PAD. As an alternative to conservative exercise
66 therapy, heat therapy has been suggested to have potential in those with PAD (47),
67 who are limited in their ability to perform traditional exercise for cardiovascular
68 benefit. Indeed, heat has shown promise for its ability to reduce symptoms and
69 improve several measures of lower-limb perfusion in PAD patients (44, 47). These
70 results have not yet been replicated by others though, and the potential of heat as
71 an acute stressor has not been fully characterized in this group. The acute responses
72 will be important in understanding if the role of heat in providing clinical benefit is
73 via an improvement in arterial function, via a downstream effector of increased
74 perfusion or systemic hemodynamics, or via some other mechanism.

75 Recent evidence in healthy individuals has demonstrated that heat has
76 potential to induce beneficial hemodynamic responses. Specifically, passive heat
77 increases antegrade shear stress in the arteries of the upper (8, 51) and lower limb in
78 healthy, young (11, 49) and older individuals (42), and in addition, *repetitive* hot-
79 water immersion improves upper-limb artery function and structure (3, 5, 9, 34).
80 Increased antegrade shear rate is thought to be the principle mechanism for
81 exercise- (and likely heat-) induced improvements in arterial health (27, 36).

82 Most investigations on shear patterns and related adaptations have focused
83 on the brachial artery, which is simple to assess and usually assumed to reflect global
84 arterial responses and overall cardiovascular risk (10). Many interventions involve
85 greater stress on the lower limbs, so upper-limb hemodynamics may not adequately
86 represent those of the lower limbs, although are still of interest for characterizing
87 the remote effects of the stimulus. And, atherosclerotic disease is far more prevalent
88 in the lower limbs than upper limbs (32), so understanding the flow profiles in both
89 upper-limb and lower-limb arteries – especially in those with disease – is important.
90 Recently Romero et al. reported that lower-limb heating acutely improved macro-
91 and microvascular function in healthy, elderly adults (42). Significant PAD serially
92 narrows conduit artery cross-sectional area, or occludes an artery altogether,
93 thereby increasing resistance to flow. Atherosclerosis in the arterial walls
94 additionally reduces arterial compliance, so for these reasons the flow and shear
95 profiles at rest already differ from those in healthy vessels (7), and the differential
96 responses to heat in healthy and diseased vessels of elderly individuals have not
97 been described.

98 Heat stress also causes several other significant physiological responses in
99 humans, including increased core temperature, cutaneous blood flow, heart rate and
100 cardiac output (13, 43, 53); all of which occur during an acute exercise bout (26). The
101 acute hemodynamic and cardiovascular responses to lower-limb heating have not
102 been fully characterised in individuals with PAD. Recent work by Neff and colleagues
103 (35) demonstrated increased limb blood flow and reduced blood pressure in
104 response to lower-limb heating (via a water-perfusion suit) in PAD patients. While
105 they hypothesised that increased blood flow and arterial shear stress may mediate

106 improvements in vascular health in this group, they did not measure shear stress so
107 this remains unquantified in PAD. We have previously used hot-water immersion to
108 examine the hemodynamic responses (including shear stress) to heating in healthy,
109 young participants, and to compare the flow profiles to those induced in response to
110 exercise (49). Whether the responses observed in these healthy, young participants
111 also occur in PAD, how they compare with those in healthy, elderly individuals is
112 unknown. Also of importance, whether they translate into changes in function has
113 not been determined. Understanding the acute hemodynamic and cardiovascular
114 responses to heating in elderly individuals with and without arterial disease are of
115 interest to inform potential long-term adaptations and to warrant pursuit of heat as
116 a conditioning strategy in this patient population and others. To put the
117 hemodynamic responses to heat in PAD into context, a comparison with the
118 response to the current conservative therapy, walking, was included. A symptom-
119 limited bout of treadmill walking was chosen as an ecologically-valid stimulus to
120 characterize, as patients are seldom able to perform a traditional 30-min walk
121 without stopping and resting. Finally, it seems reasonable to suggest that maximizing
122 conductance of both major vascular beds within the leg (i.e., muscle and skin) may
123 maximize the pressure gradient for perfusion, which might be achieved with a
124 combined stimulus of local and whole-body heat stress applied in conjunction with
125 localised exercise.

126 The aims of this study were therefore to assess in PAD patients and healthy,
127 elderly controls: 1) The acute peripheral (upper- and lower-limb) hemodynamic
128 effects of lower-limb hot-water immersion; 2) The acute systemic cardiovascular and
129 thermal effects of lower-limb hot-water immersion; 3) Whether the responses differ

130 in PAD participants from those in healthy, elderly controls; and 4) if the responses
131 are augmented by the addition of localised mild exercise during immersion. The final
132 aim, 5) was to examine the responses to hot-water immersion relative to those from
133 a symptom-limited bout of walking in PAD participants. We hypothesized that lower-
134 limb hot-water immersion would induce significant increases in limb blood flow,
135 shear stress and muscle perfusion in PAD and to a greater extent in healthy, elderly
136 controls.

137

138 ***Materials and Methods***

139 **Experimental Design**

140 Two cohorts were studied: PAD patients (PAD) and healthy, elderly controls.
141 Each participant underwent two immersion sessions, as well as one exercise session
142 for PAD only. One immersion was passive and one included mild intermittent
143 exercise, to ascertain if the flow-increasing stimulus could be maximised by heat
144 alone (i.e., passive immersion) or if the increased metabolic demand of exercised
145 muscle would provide additional local and systemic effects (i.e., active immersion).
146 Active immersion consisted of 3-min bouts of plantar flexion, performed at 0.5 Hz, at
147 10-min intervals (i.e., three bouts during immersion; Figure 1). The passive and
148 active immersion sessions were administered in a randomised, cross-over fashion.
149 Ethical approval was obtained from the Health and Disability Ethics Committee
150 (14/STH/44), and the study conformed to the standards set by the Declaration of
151 Helsinki.

152 **Participant Characteristics**

153 Inclusion criteria for PAD were: PAD confirmed by ankle-brachial index (ABI)
154 of ≤ 0.7 at rest in at least one leg; mild to moderate claudication described
155 corresponding to Fontaine Stage IIa to IIb (37); duplex ultrasound had been
156 performed to confirm disease location and distribution; ≥ 50 years old, and; post-
157 menopausal if female. Exclusion criteria for PAD were: Functioning bypass graft in
158 situ; diabetes; previous occurrence of heat intolerance; unstable angina, or;
159 myocardial infarction in the past 3 months. Inclusion criteria for controls were: ≥ 50
160 years old, no known history of PAD or other cardiovascular disease, resting ABI of \geq
161 0.9 in both legs, no claudication, and post-menopausal if female. Exclusion criteria
162 for controls were: known diabetes, PAD or cardiovascular disease, previous
163 occurrence of heat intolerance. Participant demographics are shown in Table 1.
164 Written consent was obtained and a questionnaire regarding health, medications
165 and comorbidities was completed.

166 **Experimental Protocol (see Figure 1)**

167 *Water immersion* – Each participant completed the two immersion sessions,
168 one to three weeks apart. The measurements taken during each were identical.
169 Participants were asked to abstain from exercise on the day before each session, and
170 to refrain from alcohol and caffeine for 12 h prior to testing. They were instructed to
171 consume a standardised meal the evening prior to the session with ~ 10 mL water
172 per kg body mass, and a standardised breakfast with 250 – 375 mL water between 6
173 and 7 am. Sessions began at or after 10 am and at the same time for each
174 participant. Cessation of medications was not possible, but medication usage was
175 continued as prescribed and recorded (Table 1). Sessions were performed in a

176 temperature-controlled environment at 24.4 ± 1.5 °C and ~40% relative humidity.
177 Each immersion session began with a 20-min supine rest period, during which
178 monitoring equipment was applied. Baseline measurements were then obtained (~1
179 h). For immersion, participants sat in a bath of hot water (maintained at 42.1 ± 0.6
180 °C) to the waist level for 30 min. Water temperature was checked continually and
181 adjusted throughout the immersion. In the last 3 min of immersion, ultrasound
182 measures (blood flow and shear rate) and plethysmography measures were
183 repeated as described in each respective section below. Following immersion,
184 measurements were repeated, beginning immediately and spanning ~45 min.

185 *Treadmill exercise* – A subgroup of PAD participants re-presented on a further
186 occasion to perform a 3-min treadmill-walking test, at 3 km/h on a 10% incline. This
187 is the standard exercise test used at this laboratory in the clinical diagnosis of PAD by
188 exercise-induced reduction in ABI. Participants rated their claudication pain on a
189 scale of 1 – 4 (2).

190 **Measurements**

191 **Peripheral artery (brachial and popliteal) blood flow and shear rate** were
192 measured using ultrasound (Terason t3000, Teratech Corporation, Burlington, MA,
193 USA) with a 10 MHz linear array transducer (bandwidth 5 – 12 MHz), by
194 simultaneously recording a longitudinal section B-mode image and a spectral
195 Doppler trace of blood velocity. Participants were supine during the brachial artery
196 measurement, then adopted a lateral recumbent position (on the contralateral side
197 to the leg being assessed) with the knee bent to 20-30° for the popliteal
198 measurement. Measurements were made in the distal third of the upper arm and in
199 the popliteal fossa, respectively; exact locations were marked and measured for

200 repeat tests. Ultrasound settings were optimised for each participant and reused for
201 the repeat tests. The same certified and experienced vascular sonographer (KNT)
202 performed all scans. Screen recording software (Camtasia Studio 8, TechSmith
203 Corporation, Okemos, MI, USA) captured the screen in a video file for later offline
204 analysis. Wall-tracking software (Cardiovascular Suite UE v 2.5, Quipu, Pisa, Italy) was
205 used to determine diameter and velocity, and shear rate was calculated as: Shear
206 rate = $4 * \text{mean velocity} / \text{diameter}$ (39, 41).

207 Blood flow was calculated as:

208 Flow = mean velocity * cross-sectional area,

209 Where mean velocity = peak envelope velocity / 2,

210 and cross-sectional area = $\pi * (\text{diameter}/2)^2$ (18, 29).

211 Test-retest reliability (coefficient of variation) for this operator using this
212 software for measuring diameter and velocity was 0.4% and 2.1%, respectively (n =
213 10). The resting hemodynamics (D_{base} , velocity, shear rate and flow) of the brachial
214 and popliteal arteries were assessed before and ~ 30 min after the intervention,
215 from a 30 – 60 s recording period. In addition, popliteal and brachial hemodynamics
216 were video-recorded for 30 s within the last 3 min of each immersion protocol. To
217 obtain the popliteal artery measurements, the participant raised the knee to 20-30°
218 and the sonographer reached around to the popliteal from a lateral window. During
219 the treadmill exercise session the same variables were measured in the popliteal
220 artery before and after exercise (recording began within 30 s of completing walking).

221 **Venous occlusion plethysmography** was used to measure calf blood flow via
222 an indium-gallium strain gauge and plethysmograph (EC6, Hokanson, Bellevue WA,
223 USA). Participants were supine with their leg elevated slightly above heart level. A

224 strain gauge was placed around the widest part of the calf and a cuff around the
225 ipsilateral thigh was inflated to 50 mmHg to occlude venous outflow for 5 – 10 s. This
226 was repeated 3 – 6 times until 3 reproducible traces were obtained. Data were
227 transmitted to a computer via an analogue to digital converter (Powerlab/16SP,
228 ADInstruments, Dunedin, New Zealand), and analysed later using Chart software
229 (LabChart Pro v 7.2.5, ADInstruments). Limb volume changes were calculated from
230 the steep linear portion of the plethysmographic trace following the inflation
231 artifact, and the average of 3 measurements is presented. Calf inflow was assessed
232 before, in the last 3 min of immersion, and within 5 min of completing the
233 immersion.

234 **Muscle oxygenation** was measured using near-infrared spectroscopy (NIRS)
235 on the posterior-medial calf (medial gastrocnemius muscle) of the leg under
236 investigation, via probes housed in a light-shielding case attached to the skin with
237 tape (NIRO-200; Hamamatsu Photonics KK; Hamamatsu, Japan). The NIRO-200
238 device measures changes in chromophore concentrations of oxy- and
239 deoxyhemoglobin (ΔO_2Hb and ΔHHb) via the modified Beer-Lambert law, and
240 provides depth-resolved measures of tissue O_2 saturation (total oxygenation index
241 (TOI)) and tissue Hb content (i.e., relative value of the total hemoglobin normalised
242 to the initial value, nTHI) using the Spatially Resolved Spectroscopy (SRS) method.
243 The SRS-derived NIRS parameters limit contamination from superficial tissue via
244 depth-resolved algorithmic methods, providing an index of targeted local tissue
245 saturation (TOI) and perfusion (nTHI) (see Davies et al. (17) for recent review). A **3-**
246 **min exercise test** was performed at baseline and post-immersion to assess the effect
247 of thermal status on exercise-induced NIRS responses. Exercise consisted of supine

248 plantar flexion against a resistance band held at a standard length by a co-
249 investigator (paced at 0.5 Hz using a metronome) and participants were asked to
250 match the effort pre- and post-immersion. All NIRS data are presented as change
251 values as is common practice to minimise the influence of variation between days
252 and individuals, and in recognition of the limitations of the technique to accurately
253 quantify absolute values due to factors that affect the absorbance and attenuation
254 of input light (17).

255 **Blood pressure (BP)** was measured using finger photoplethysmography
256 (Finometer, Finapres Medical Systems, Amsterdam, The Netherlands). BP was
257 validated intermittently throughout sessions using a non-invasive intermittent BP
258 oscillometric measurement device, (BP+, Uscom, Sydney, Australia). **Heart rate** was
259 obtained continuously using detection of the R wave of a three-lead
260 electrocardiograph (lead II of ECG, ADInstruments). The LabChart ECG Analysis Add-
261 On was used for quantifying the frequency of arrhythmias across a 5-min period
262 before and within 10 min of completing immersion. **Arrhythmias** were defined by an
263 abnormally-long compensatory pause following the ectopic beat. Baseline BP and
264 heart rate data were collected over 5 min before each immersion, and end of
265 immersion data consist of a 2-min average during the last 3 min of immersion.

266 **Ambulatory BP** was measured using an ambulatory monitoring system (AMBP, Oscar
267 2, SunTech Medical Ltd, England) every 30 min for 3 h following each immersion
268 session (beginning at the end of the supine measurements, ~1 h post-immersion),
269 and for the same 3-h period on the day prior to testing, for comparison.

270 **Pulse wave velocity (PWV)** was measured as central (carotid-femoral) and
271 peripheral (carotid-radial) PWV using a hand-held tonometer (SPT-301, Millar

272 Instruments, USA), following recommended guidelines (52). Pulse transmit times
273 were calculated from the R-wave of the ECG to the foot of the pressure wave, and
274 PWV was calculated as the anatomical distance / time (54) for each of the central
275 and peripheral components, averaged over at least 20 cardiac cycles. The anatomical
276 distance was calculated by subtracting the distance from the carotid location to the
277 suprasternal notch and the radial or femoral site of measurement respectively.

278 **Core body temperature** was measured as the external auditory canal
279 temperature (aural temperature, T_{au}) using a thermistor in a moulded plug
280 (BetaTHERM 2.2K3A1B NTC thermistors, BetaTHERM, USA). The ear was covered
281 with cotton wool taped in place to reduce the effects of ambient temperature.
282 Participants' **ratings of body temperature and thermal discomfort** were noted using
283 a 13- and 5-point scale, respectively (extended from (20)), at 10-min intervals during
284 the immersion.

285 **Statistical Analysis**

286 Participant characteristics were compared across groups using an unpaired t-
287 test. A mixed-design two-way ANOVA was used where possible to examine effects of
288 (i) passive and active hot-water immersion, (ii) hot-water immersion in PAD
289 participants and Controls, and (iii) hot-water immersion and treadmill exercise in
290 PAD participants. Post-hoc tests were performed where appropriate using the Holm-
291 Šídák method with p -values corrected for multiple testing. However, some research
292 questions were examined using paired t-tests because of the specific question (e.g.,
293 NIRS data represented as a change from baseline), following consultation with a
294 biostatistician. For all analyses, $p \leq 0.05$ was considered statistically significant.

295

296 **Results**

297 Eleven PAD patients and ten Controls participated and completed both 30-
298 min immersions except that one PAD participant undertook one immersion only
299 (active). Seven PAD participants completed the exercise session. All PAD participants
300 had an occlusion of the superficial femoral artery > 5 cm in length (n = 6) or more
301 than one stenosis of > 75% (n = 5). No dependent measure showed a different
302 response between passive and active immersions except for a larger inflow by
303 plethysmography following active compared to passive immersion in PAD (+203% vs.
304 +114%, $p = 0.04$), and no order effects were evident, so for simplicity in reporting
305 and interpretation, all data are from the average of the two immersions for each
306 participant.

307 **Popliteal artery shear rate and blood flow**

308 **At baseline**, total shear rate (i.e., net) was higher in PAD than in Controls ($p =$
309 0.004), while retrograde shear was lower ($p < 0.001$) and popliteal diameter was
310 smaller ($p < 0.001$; Table 2). Popliteal antegrade shear rate increased ($p < 0.0001$)
311 two- to three-fold **during immersion** in both groups, with PAD showing higher levels
312 ($p = 0.01$) and a tendency for a larger increase (interaction: $p = 0.053$; Table 2 and
313 Figure 2). Retrograde shear was absent in PAD throughout but in Controls decreased
314 significantly during immersion (by 8 ± 5 /s, $p = 0.002$). Popliteal total shear rate
315 increased ($p < 0.0001$) during immersion by a similar proportion in each group
316 (interaction: $p = 0.11$), thereby remaining higher in PAD than Controls ($p = 0.003$).

317 **At 30 min after immersion**, antegrade shear rate remained above baseline
318 levels ($p = 0.0002$) in both groups (change from baseline: Controls: +65%, PAD:

319 +43%), and higher in PAD than Controls (group: $p = 0.003$, interaction: $p = 0.07$,
320 Figure 2).

321 Sample spectral Doppler traces obtained in the popliteal artery in a PAD and
322 Control participant are illustrated in Figure 3. The velocity profile in the popliteal
323 artery reflected lower resistance in response to immersion for both groups; the
324 characteristics associated with this are described in the legend for Figure 3.

325 Blood flow in the popliteal artery was not different **at baseline**, but
326 approximately tripled ($p < 0.0001$) **during immersion**, and tended to be higher in
327 Controls than in PAD (group: $p = 0.07$, interaction: $p = 0.12$). Popliteal flow remained
328 elevated ($p < 0.0001$) above baseline levels **at 30 min after immersion** (Controls:
329 +72%, PAD: +71%) with no difference between groups ($p = 0.14$, interaction: $p =$
330 0.51; Figure 4). The diameter of the popliteal artery appeared to reduce during
331 immersion in the Controls (by $9 \pm 3\%$, $p = 0.007$) but not in PAD ($-2 \pm 3\%$, $p = 0.38$).
332

333 **Brachial artery shear rate and blood flow**

334 The brachial artery showed no difference in any hemodynamic variable
335 between groups **at baseline**. Antegrade shear rate increased significantly **during**
336 **immersion** ($p < 0.0001$; Table 2), and by an equivalent extent between groups
337 (change from baseline: Controls: +107%, PAD: +117%; group: $p = 0.57$, interaction: p
338 = 0.68). Retrograde shear rate was attenuated significantly ($p < 0.0001$) in each
339 group during immersion, similarly so (by -12 ± 13 /s in Controls and -14 ± 8 /s in PAD;
340 group: $p = 0.28$, interaction: $p = 0.70$). Thus, total brachial shear rate increased
341 during water immersion in each group ($p < 0.0001$), and to a similar extent (group: p
342 = 0.50, interaction: $p = 0.72$). **At 30 min after immersion**, antegrade shear rate

343 remained elevated ($p = 0.0007$) and to a similar extent in each group (change from
344 baseline: Controls: +88%, PAD: +104%; group: $p = 0.53$, interaction: $p = 0.61$).

345 Blood flow in the brachial artery was also increased significantly ($p < 0.0001$)
346 **during the immersion** (Controls: +282%, PAD: +176%), but overall was higher in
347 Controls (group: $p = 0.03$, interaction: $p = 0.12$). Flow remained above baseline **at 30**
348 **min after immersion** ($p < 0.0001$), to a greater extent in Controls (Controls: 139 ± 51
349 mL/min, PAD: 100 ± 31 mL/min; group: $p = 0.04$, interaction: $p = 0.25$). Arterial
350 diameter increased in each group during immersion ($p = 0.0018$) with no differential
351 effect (group: $p = 0.26$, interaction: $p = 0.28$; Table 2) and remained larger at 30 min
352 after immersion.

353 **Arterial inflow via plethysmography**

354 Inflow was similar between groups **at baseline** (Controls: 7.9 ± 2.4 vs. PAD:
355 10.4 ± 5.1 mL blood / 100 mL tissue / min; $p = 0.18$). Inflow increased **across**
356 **immersion** in each group ($p < 0.0001$) but more so in Controls (Controls: $+380 \pm$
357 170% vs. PAD: $+152 \pm 104\%$; interaction: $p = 0.0004$). It remained ~ 234 and $\sim 85\%$
358 elevated above baseline at **10 min after immersion** (Controls and PAD respectively).
359 Correlation between antegrade shear rate and inflow via plethysmography was only
360 moderate in Controls ($R^2 = 0.49$, $p = 0.03$); for remaining variables and all
361 comparisons in the PAD group, there were no significant correlations ($R^2 \leq 0.02$).

362 **Muscle oxygenation via NIRS**

363 Figure 5 illustrates the changes in O₂Hb, HHb, nTHI and TOI in response to
364 immersion in each group. The key findings were:

365 **At rest:** O₂Hb volume increased to a greater extent in Controls than in PAD
366 across immersion (difference between means: $+151 \mu\text{M}\cdot\text{cm}$, 95% CI: 68 to 235

367 $\mu\text{M}\cdot\text{cm}$, $p = 0.002$). The change in HHb volume in response to immersion did not
368 differ significantly between groups (interaction: $p = 0.14$), nor did the increase in
369 nTHI and TOI (nTHI: Controls: +0.16 a.u., PAD: +0.17 a.u.; interaction: $p = 0.76$; TOI:
370 Controls: +6.7%, PAD: +5.8%; interaction: $p = 0.56$), indicating similar relative
371 increases in local perfusion between the two groups.

372 **In response to exercise:**

373 A supine, 3-min bout of plantar flexion exercise in PAD elicited a greater drop
374 in O_2Hb volume after immersion than before (by $98 \mu\text{M}\cdot\text{cm}$, 95% CI: -13 to -183
375 $\mu\text{M}\cdot\text{cm}$, $p = 0.03$) and TOI (by 7.6%, 95% CI: -1 to -15%, $p = 0.04$), and a larger rise in
376 HHb volume ($p = 0.02$), compared with before immersion. All parameters recovered
377 from exercise at similar rates whether performed before or after immersion ($p \geq$
378 0.08).

379 When comparing the response to exercise in PAD with Controls, not
380 surprisingly, 3 min of plantar flexion exercise before immersion produced greater
381 alterations from baseline in PAD than in Controls. O_2Hb volume dropped more in
382 PAD (vs. Controls: $-210 \mu\text{M}\cdot\text{cm}$, 95% CI: -357 to $-64 \mu\text{M}\cdot\text{cm}$, $p = 0.01$), and HHb
383 volume rose more (vs. Controls: $+217 \mu\text{M}\cdot\text{cm}$, 95% CI: 55 to $380 \mu\text{M}\cdot\text{cm}$, $p = 0.02$).
384 These changes and group differences were reflected in the TOI and nTHI derived
385 measures of tissue saturation and regional blood flow (Figure 5c and d). Recovery of
386 O_2Hb volume at 1 min post-exercise was heterogeneous in PAD and tended to be
387 impaired more than in Controls ($-137 \mu\text{M}\cdot\text{cm}$, 95% CI: -294 to $20 \mu\text{M}\cdot\text{cm}$, $p = 0.08$).
388 The different responses between groups to 3 min of repeated contractions remained
389 evident following immersion; O_2Hb and TOI declined despite increased nTHI in PAD,
390 and failed to show complete recovery in PAD by 1 min of rest.

391 **Systemic hemodynamics**

392 **At baseline**, SBP, DBP, MAP and heart rate were not different between
393 Controls and PAD (all $p \geq 0.1$). **During immersion**, heart rate increased ($p < 0.001$)
394 similarly in both groups (interaction: $p > 0.35$). SBP, DBP and MAP were reduced by
395 immersion (all $p \leq 0.001$) to a similar extent between PAD and Controls (Table 3;
396 interaction effects: all $p \geq 0.16$) although overall SBP and MAP were higher in PAD
397 (both $p = 0.04$). **At 30-min after immersion** SBP, DBP and MAP remained lower than
398 baseline in both groups (all $p \leq 0.03$).

399 Ambulatory BP was recorded on both a control day and an immersion day for
400 9 Controls and 8 PAD. SBP was significantly ($p = 0.001$) lower following immersion in
401 Controls and PAD (Controls: -14 ± 8 mmHg, PAD: -5 ± 12 mmHg), with a tendency for
402 more reduction in Controls (interaction: $p = 0.08$). DBP and MAP were also reduced
403 significantly ($p = 0.047$ and $p = 0.003$ respectively) following immersion (DBP:
404 Controls: -4 ± 7 mmHg, PAD: -6 ± 12 mmHg; MAP: Controls: -7 ± 7 mmHg, PAD: -6 ± 8
405 mmHg) with no differential effects between groups ($p \geq 0.64$ for all interaction and
406 group effects).

407 **At baseline**, arrhythmias were present in 13 participants (6 Controls, 7 PAD).
408 **During and following immersion**, one PAD participant showed an increase in
409 arrhythmias on both occasions (9% to 38% and 6% to 79%), while one Control
410 showed an increase following their first immersion (1% to 19%). A cardiologist
411 reviewed the ECGs and diagnosed the arrhythmias as benign premature ventricular
412 contractions. The remaining Controls and PAD all showed no obvious or consistent
413 change in frequency of arrhythmias following immersion. One Control participant

414 experienced a vasovagal episode at the completion of the session on getting up from
415 supine rest. There were no adverse effects associated with this.

416 **Pulse wave velocity**

417 **At baseline**, neither central nor peripheral PWV differed between Controls
418 and PAD (central: Controls: 9.5 ± 1.7 m/s, PAD: 9.4 ± 2.1 m/s, $p = 0.87$; peripheral:
419 Controls: 8.0 ± 1.1 m/s, PAD: 7.2 ± 0.9 m/s, $p = 0.11$). **In response to immersion**,
420 central PWV decreased in each group ($p < 0.03$) by 1.0 ± 1.5 m/s in Controls and $0.5 \pm$
421 1.3 in PAD, and peripheral PWV decreased by 0.9 ± 1.2 m/s in Controls and 0.3 ± 0.7
422 m/s in PAD ($p = 0.01$). There was no differential effect evident between groups
423 (interaction: both $p \geq 0.15$). There were no significant correlations between baseline
424 PWV and change with immersion in either group for central or peripheral PWV (all p
425 ≥ 0.10 , all $R^2 \leq 0.25$).

426 **Temperature**

427 The T_{au} increased $+1.8$ °C **with immersion** in both groups ($p < 0.01$, Table 3),
428 and recovered to lesser extent in PAD than in Controls ($p = 0.02$; remaining 1.4 ± 0.3
429 vs 1.0 ± 0.3 °C above baseline at 45 min after immersion). Perceived body
430 temperature was “hot” (i.e., 10 on the 13-point sensation scale) at the completion of
431 immersion for each group, and this was rated as comfortable to slightly
432 uncomfortable (Controls 1.6, PAD: 1.7 on the 5-point discomfort scale).

433 **Popliteal artery shear rate following 3-min treadmill exercise in PAD**

434 The 3-min treadmill-walking test was completed at the designated speed and
435 incline (distance ~ 150 m). All seven PAD patients reported claudication in the leg
436 studied, with an onset between 50 and 120 m, reaching moderate to intense pain (2-
437 3/4) at completion. Antegrade shear rate in the popliteal artery within 1 min of

438 completing exercise was elevated significantly from baseline, to 169 ± 81 /s ($p =$
439 0.02), with absent retrograde shear rate before and after exercise. The elevation in
440 antegrade shear rate ($p < 0.0001$) caused by exercise or immersion was comparable
441 (+112 /s and +102 /s respectively, condition: $p = 0.08$, interaction: $p = 0.79$).
442 Similarly, average blood flow increased ($p = 0.0002$), to 91 ± 32 mL/min, a
443 comparable increase to that seen during immersion (102 ± 46 mL/min, condition: $p =$
444 0.52, interaction: $p = 0.40$).
445
446

447 **Discussion**

448 A single bout of hot-water immersion induced shear stress patterns in the
449 popliteal and brachial arteries of PAD participants and healthy, elderly controls that
450 have been associated with beneficial adaptations (9, 34). This heat stress also
451 induced positive chronotropy, increased lower-limb perfusion, and a marked
452 lowering of blood pressure across at least the next 3 h. The regular repetition of this
453 stress has potential to provide cardiovascular conditioning for PAD patients and
454 other groups with limited access to exercise.

455 **Lower-limb hemodynamics**

456 *At baseline*

457 Despite no statistical differences in popliteal blood flow or arterial inflow
458 between groups at rest, total shear rate was higher in PAD. This is likely explained by
459 the smaller popliteal artery diameter in the PAD group, although may also reflect the
460 different antegrade / retrograde shear components between groups. Unsurprisingly,
461 hemodynamic differences between groups were revealed more obviously in
462 response to 3 min of plantar flexion exercise (supine, before and after immersion),
463 during which PAD participants showed a greater drop in tissue saturation, and
464 impaired recovery of NIRS parameters at 1 min after exercise.

465 *In response to immersion*

466 Blood flow in the popliteal artery was increased by > 200% in PAD and
467 Controls at the end of immersion, and remained elevated but to a lesser extent at 30
468 min post-immersion. Similarly, popliteal artery antegrade and total shear rate
469 increased two- to three-fold during immersion in PAD. In healthy controls, popliteal

470 antegrade and total shear was also significantly elevated during and following
471 immersion, but this tended to be to a lesser extent than in PAD (+180% during
472 immersion); this relative disparity is again likely due to differences in vessel diameter
473 between groups. The spectral Doppler blood velocity profiles changed during
474 immersion in most PAD participants to exhibit continuous antegrade flow through
475 the cardiac cycle (e.g., Figure 3b), which is interpreted clinically as an indicator of
476 peripheral vasodilation and a lower resistance vascular bed downstream (38). Also
477 reflecting lower resistance, a similar reduction in the retrograde component of the
478 spectral waveform was demonstrated in most controls during immersion. The
479 increased shear rates seen here is consistent with previous studies demonstrating
480 increased antegrade shear in response to heating, but in the brachial artery in
481 healthy individuals (8, 51), and also in the superficial femoral artery and common
482 femoral artery in young individuals in response to mild (+0.5 °C) and moderate (+1.0
483 °C) passive heat stress respectively (11), although greater shear rates were seen at
484 higher levels of passive heat. The lower-limb vessels are seldom studied, despite
485 being more prone to disease than upper-limb arteries (32); to our knowledge this is
486 the first study to describe this response of increased antegrade shear stress in
487 diseased arteries. A doubling of popliteal blood flow in PAD has been reported from
488 phase-contrast magnetic resonance imaging after 90 minutes of passive heat via a
489 water-perfused suit (35); while this is encouraging that other methods of passive
490 heating may also increase perfusion, the high conductive capacity of water
491 immersion may be a more time-efficient and readily accessible method; at 30 min it
492 had tripled blood flow in the present study. An unexpected finding was the reduction
493 of the popliteal artery diameter in Controls. There is no obvious physiological

494 explanation for this; random or systematic error is possible, so further study is
495 needed to confirm or refute this finding.

496 In PAD participants, the antegrade shear rate elevation with immersion was
497 comparable to that achieved during a 3-min bout of treadmill walking, but
498 importantly, the immersion achieved this with no claudication, and is a stimulus that
499 can be applied for substantially longer than an exercise stimulus, for reasons of
500 tolerance. What remains unknown is whether the magnitude of the increased
501 antegrade shear stress demonstrated here is sufficient to induce beneficial vascular
502 adaptation in atherosclerotic arteries following repetition, or if they respond to a
503 shear stimulus in the same way at all. The relationship of transiently increased shear
504 stress to improvements in functional capacity in diseased vessels is yet to be
505 ascertained. There was considerable variation in the magnitude of the response
506 between individuals, which is understandable given the heterogeneity of the
507 disease, i.e., the variability in its location, distribution, severity and duration.

508 Two other measures of lower-limb perfusion demonstrated an increase in
509 both groups: NIRS-derived measures of tissue hemoglobin volume and saturation,
510 and plethysmography. The increased lower-limb perfusion likely comprises both
511 increased cutaneous and muscle blood flow, as local heating induces vasodilation of
512 both skin and muscle vasculature (23, 40). In PAD, the increased perfusion due to
513 immersion appeared to better support the metabolic demands of exercise,
514 evidenced by higher absolute post-exercise O_2Hb even in the face of a greater
515 exercise-induced drop. In Controls, the exercise bout before and after immersion
516 both produced much smaller perturbations in the NIRS-derived parameters of
517 oxygen extraction (i.e., HHb and TOI), which may indicate that perfusion of the

518 exercising muscles was better matched to the corresponding metabolic need,
519 although a between group comparison is difficult with an exercise test of this nature.
520 Overall a larger increase in both inflow and NIRS-derived measures of perfusion was
521 seen in the muscle in Controls after immersion, indicative of a greater ability to
522 respond with conduit and microvessel vasodilation to the heat stimulus. A limitation
523 of using NIRS in this setting is baseline differences in tissue oxygenation were unable
524 to be distinguished. Nevertheless, PAD participants demonstrated significant,
525 relevant increases in all three measures of lower-limb perfusion.

526 In PAD, the adaptations to chronic obstruction and impaired flow result in
527 anatomical and functional changes beyond commonly-used measures of vasodilatory
528 function, such as beneficial modifications to muscle fibre characteristics (31),
529 metabolism (25) as well as formation of collateral vessels (12). These or other
530 responses may be adaptations to transiently increased blood flow e.g., angiogenesis
531 and collateral vessel formation stimulated by hemodynamic forces (24). In patients
532 with PAD in whom exercise to induce increased perfusion is not an appropriate
533 option, the increased blood flow during and persisting after immersion is potentially
534 the most clinically important finding of this study. However, whether this can
535 translate to the long-term benefits and functional effects need to be examined.
536 Overall, apart from understandable differences in the exercise response between
537 groups, hot-water immersion functioned to a similar extent as a hemodynamic
538 stressor in both groups. There were no significant differences between passive and
539 active immersion for all but one dependent variable (inflow via plethysmography).
540 On balance it appears that the addition of mild lower-limb exercise did not add to
541 the hemodynamic strain induced by the immersion itself. The 3-min bouts were

542 perhaps of insufficient intensity or duration, or could have been different relative
543 intensities between individuals, to reveal a group effect.

544 **Upper-limb hemodynamics**

545 The upper-limb responses were qualitatively very similar to those of the
546 lower-limb: antegrade and total shear rate were increased and retrograde shear rate
547 was attenuated by immersion, and by similar extents between groups. These
548 findings are consistent with the data from Carter et al. (9), in which lower-limb
549 heating acutely increased shear rate in the brachial artery, and this resulted in
550 functional adaptations after repetition. The absolute shear rate and flow induced in
551 the study by Carter et al. were approximately twice those presented here, but their
552 participant demographic – healthy, young, active participants – may explain these
553 differences. Furthermore, it is not currently known if a threshold exists for shear-
554 mediated adaptations. The inclusion of the upper-limb hemodynamics provided
555 insight into the general arterial responsiveness in non-atherosclerotic vessels (and
556 those usually studied) to this form of stress, relative to diseased vessels within the
557 same individual, as well as compared with healthy upper-limb vessels in Controls
558 free from PAD. The upper-limb hemodynamic alterations seen with lower-limb hot-
559 water immersion were similar between groups (and between upper and lower
560 limbs), and thus highlight the systemic nature and wide-ranging applicability of the
561 stressor.

562 **Cardiovascular strain**

563 Heart rate increased by > 30% during immersion in each group, i.e., within
564 the range recommended for cardiac benefits from exercise training for an average-
565 aged participant in this study (50-75% max heart rate (19) for a 72 year-old: 74 – 111

566 beats/min). Passive heat stress also has a beneficial inotropic effect on the heart (13)
567 (by virtue of a decreased MAP, reduced central blood volume and therefore
568 ventricular filling pressure), but preserved or elevated cardiac output. These effects
569 have been demonstrated in healthy individuals (4, 14) and in patients with CHF (46,
570 48). The lower-limb immersion protocol may therefore be useful for inducing an
571 increase in cardiac work by virtue of chronotropic and inotropic changes without the
572 concomitant increases in afterload usually experienced during exercise. It may
573 therefore be a gentler, more appropriate stressor for those with a high
574 cardiovascular disease burden. Accordingly, studies of repeated heat stress in
575 congestive heart failure patients have shown improvements in multiple parameters
576 of cardiac function (33, 46, 48). Of note, the induced thermal stress used in this
577 study had little effect on the prevalence arrhythmias.

578 Furthermore, the temperature of heart tissue, not work per se, provokes
579 upregulation of heat shock proteins (45), and this may be the case for other
580 adaptations as well, such as the induction of protection from ischemic reperfusion
581 injury (16, 24). Therefore, the significant aural temperature elevation, which
582 occurred to a similar extent in both elderly groups (almost 2 °C above baseline), is
583 important for the provision of strain for the cardiovascular system, and the local
584 effects on tissue and organs (e.g., heart). Measurement of temperature via auditory
585 canal thermistor provides an indirect index of core temperature; nevertheless, waist-
586 level hot-water immersion has been shown to increase oesophageal measurement
587 of core temperature to a comparable extent (6).

588 Alongside the chronotropic effect was a significant reduction in BP in both
589 groups. This hypotensive effect was greatest at the end of the immersion, with an

590 average reduction in MAP of ~22 mmHg. Hypotension persisted for several hours,
591 albeit measured in a subsample of participants, and was most consistent in controls.
592 A major portion of the health-related benefits of regular bouts of stress is
593 attributable to the recovery period itself, e.g., post-exercise hypotension is likely
594 more important in cardiovascular risk reduction than the small reduction in resting
595 blood pressure induced by exercise training (50). The full duration of the post-
596 immersion hypotensive effect has yet to be determined, but the implications of this
597 are particularly valuable in a PAD population, who are commonly hypertensive yet
598 commonly unable to exploit a post-exercise hypotensive effect. A hypotensive effect
599 of similar magnitude and duration has been demonstrated in PAD participants
600 undergoing passive heating via a water-perfused suit (35).

601 Passive heat stress has been shown to reduce PWV acutely, with the largest
602 effect seen in those with highest PWV at normothermic baseline (21). The effect of
603 immersion in this study was to reduce central and peripheral PWV in both groups,
604 with no obvious relationship to normothermic baseline PWV. A reduction in PWV
605 may afford a reduction in myocardial work and an increase in coronary perfusion
606 (28), again potentially beneficial in a high cardiovascular risk group.

607

608 ***Perspectives and Significance***

609 This study has demonstrated acute hemodynamic, thermal and cardiovascular
610 responses to relatively brief immersion of the lower limbs in hot water; responses
611 that would be promising for cardiovascular conditioning in those less able to achieve
612 this by exercising. In particular, sustained increases in popliteal and brachial

613 antegrade shear rate were demonstrated, in elderly individuals with and without
614 PAD. At least in healthy vessels, these shear stress profiles are known to stimulate
615 functional then structural adaptation. Blood flow in the lower limbs of both groups
616 was also increased by immersion. Qualitatively, the hemodynamic responses to
617 immersion in those with and without arterial disease were similar, despite measures
618 of perfusion increasing to a greater magnitude in non-diseased participants. The
619 presence of PAD also did not appear to significantly alter the acute systemic
620 cardiovascular response. The results of this study complement those from Neff et al.
621 (35), together endorsing the further examination of lower-limb heating as a
622 therapeutic approach for PAD patients, as has previously been suggested (44, 47),
623 and for elderly individuals who cannot exercise for whatever reason. A natural
624 progression of this work is to explore the clinical and functional outcomes of the
625 repetition of this stressor as a therapeutic tool in PAD.

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808

809

810 **Table and Figure Legends**

811 **Table 1**

812 Participant demographics.

813

814 n, number; SD, standard deviation; BMI, body mass index; ABI, ankle-brachial index;

815 PVR, pulse volume recording; IQR, interquartile range. * different from Controls ($p <$

816 0.05).

817 **Table 2**

818 Popliteal and brachial artery hemodynamic measures at baseline and in the last 3

819 min of immersion. SR, shear rate.

820

821 Data are mean \pm SD for baseline and post values. Change scores are mean \pm SE. †

822 different from baseline ($p < 0.05$); * different from Controls ($p < 0.05$).

823 **Table 3**

824 Whole-body thermal and cardiovascular strain in response to 30-min hot-water

825 immersions in PAD and Control participants.

826

827 Data are mean \pm SD. Change scores (Δ) shown are percentages, apart from T_{au} which

828 is shown in $^{\circ}\text{C}$ as indicated above. Baseline data are the average of the two baselines

829 as there were no differences between sessions. Baseline data were averaged over 5

830 min, end-immersion data were averaged over 2 min within the last 3 min of

831 immersion. Tau, aural temperature; MAP, mean arterial pressure; SBP, systolic blood

832 pressure; DBP, diastolic blood pressure; HR, heart rate. † different from baseline ($p <$
833 0.05). No significant difference between groups.

834 **Figure 1**

835 Schematic of experimental protocol for a) an immersion session (PAD and Control
836 each performed two immersion sessions, one active and one passive), and b) the
837 exercise session (for PAD only). PWV, pulse wave velocity; VOP, venous occlusion
838 plethysmography; NIRS, near-infrared spectroscopy; BP, blood pressure.

839 Hemodynamic assessments included diameter, blood flow and shear rate.

840 **Figure 2**

841 Popliteal artery total, antegrade and retrograde shear rate at baseline, during the
842 last 3 min of immersion and 30-min post-immersion. † different from baseline ($p <$
843 0.05).

844 **Figure 3**

845 Sample spectral Doppler traces from the popliteal artery obtained from one control
846 participant (left) and one PAD participant (right) at a) baseline, b) during the last 3
847 min of immersion and c) 30-min post-immersion. The x-axis represents time, the y-
848 axis represents velocity, in cm/s. Note the different velocity scales. For the control
849 participant, in a) this is a typical triphasic waveform of a normal, healthy peripheral
850 artery, demonstrating moderate resistance, with a portion of the cardiac cycle
851 demonstrating retrograde flow followed by a further antegrade component
852 associated with good compliance. In b) the waveform is still triphasic, but a smaller
853 proportion of flow is retrograde, and the peak systolic velocity has increased.

854 Similarly in c), there is a smaller retrograde component than in a). For the PAD
855 participant, in a) this is a monophasic waveform demonstrating high resistance, with
856 no flow seen for a significant portion of the cardiac cycle. In b) and c) the waveforms
857 have become lower resistance with higher peak systolic velocity and antegrade flow
858 throughout the cardiac cycle.

859

860 **Figure 4**

861 Popliteal artery blood flow at baseline, during the last 3 min of immersion and 30-
862 min post-immersion in Controls and PAD. † different from baseline ($p < 0.05$).

863

864 **Figure 5**

865 Changes in muscle hemoglobin variables in response to lower-limb hot water
866 immersion in Controls and PAD. a) O₂Hb, oxyhemoglobin response to immersion; b)
867 HHb, deoxyhemoglobin response to immersion; c) nTHI, normalised tissue
868 hemoglobin index; d) TOI, total oxygenation index. In each panel, Controls are shown
869 on the left and PAD on the right. The grey background indicates pre-immersion and
870 the white background indicates post-immersion. End of ex, end of 3-min plantar
871 flexion exercise. † different from baseline ($p < 0.05$); ‡ different from pre-immersion
872 exercise response ($p < 0.05$); * different from Controls ($p < 0.05$).

873

874 **Table 1**

875

	Control	PAD
Sample size, n	10	11
Male, n	8	7
Age, years, mean (SD)	72 (7)	71 (6)
BMI, kg/m ² , mean (SD)	26 (3)	24 (5)
ABI for leg studied, mean (SD)	1.17 (0.11)	0.61 (0.11) *
PVR for leg studied, median (IQR)	Not performed	2 (2-3)
<i>Medications</i>		
Blood pressure control	1	8
Nitrates	0	1
Statins	1	7
Antiplatelet	1	6

876

877

878

879 **Table 2**

880

Variable	Controls			PAD		
	Baseline	Immersion	Δ , (%)	Baseline	Immersion	Δ , (%)
Popliteal Artery						
Total SR (/s)	21 ± 9	89 ± 42 [†]	+366 ± 69	51 ± 28 [*]	152 ± 57 ^{*†}	+260 ± 54
Antegrade SR (/s)	32 ± 9	92 ± 40 [†]	+183 ± 26	51 ± 28 [*]	152 ± 57 ^{*†}	+258 ± 54
Retrograde SR (/s)	-12 ± 6	-4 ± 4 [†]	-67 ± 9	0 ± 0 [*]	0 ± 0	0 ± 0
Diameter (mm)	7.5 ± 1.2	6.8 ± 1.0 [†]	-9 ± 3	5.0 ± 0.6 [*]	4.8 ± 0.7 [*]	-2 ± 3
Flow (mL/min)	47 ± 14	150 ± 61 [†]	+229 ± 44	36 ± 23	102 ± 54 [†]	+226 ± 48
Brachial Artery						
Total SR (/s)	81 ± 49	205 ± 123 [†]	+189 ± 24	68 ± 28	177 ± 100 [†]	+166 ± 50
Antegrade SR (/s)	95 ± 40	207 ± 122 [†]	+117 ± 24	87 ± 26	181 ± 96 [†]	+107 ± 32
Retrograde SR (/s)	-14 ± 16	-2 ± 6 [†]	-125 ± 52	-19 ± 7	-5 ± 6 [†]	-74 ± 11
Diameter (mm)	4.9 ± 0.8	5.2 ± 0.7 [†]	+7 ± 3	4.6 ± 0.5	4.8 ± 0.6 [†]	+4 ± 2
Flow (mL/min)	57 ± 38	149 ± 43 [†]	+282 ± 79	37 ± 12	102 ± 46 ^{*†}	+176 ± 35

881

882 **Table 3**

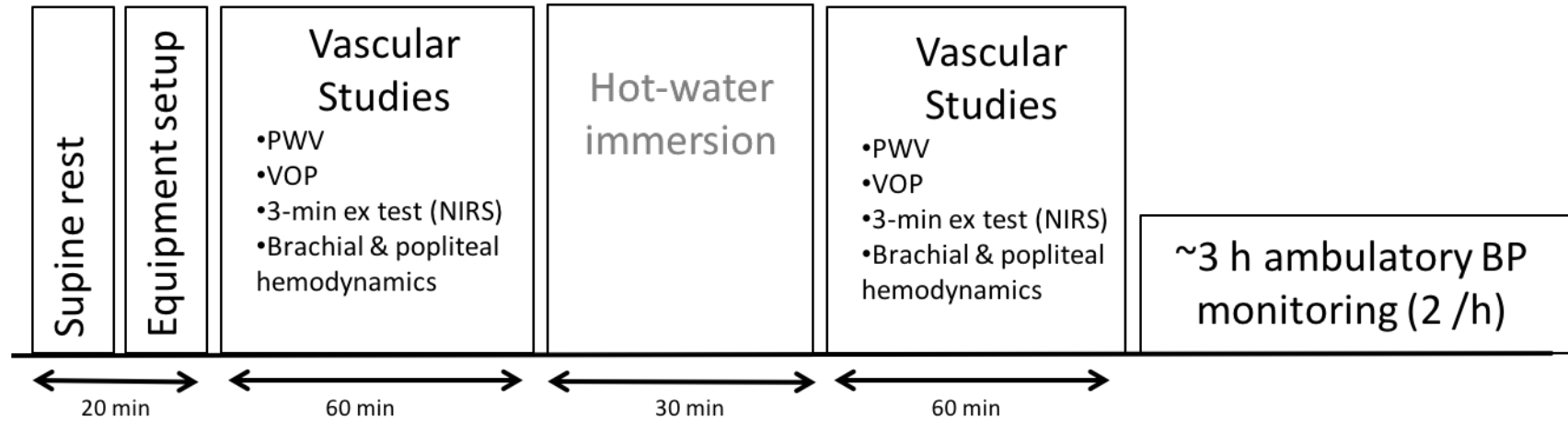
883

Variable	Controls			PAD		
	Baseline	Immersion	Δ (%)	Baseline	Immersion	Δ (%)
T _{au} (°C)	35.4 ± 0.4	37.2 ± 0.5 [†]	+1.8 ± 0.4 °C	35.1 ± 0.6	36.9 ± 0.4 [†]	+1.8 ± 0.3 °C
MAP (mmHg)	96 ± 7	73 ± 5 [†]	-23 ± 7	104 ± 15 [*]	83 ± 12 ^{*†}	-20 ± 8
SBP (mmHg)	144 ± 15	104 ± 7 [†]	-26 ± 8	158 ± 23 [*]	121 ± 20 ^{*†}	-23 ± 10
DBP (mmHg)	71 ± 5	57 ± 5 [†]	-20 ± 7	77 ± 13	64 ± 10 [†]	-16 ± 8
HR (beats/min)	62 ± 9	89 ± 17 [†]	+43 ± 21	59 ± 9	81 ± 13 [†]	+37 ± 16

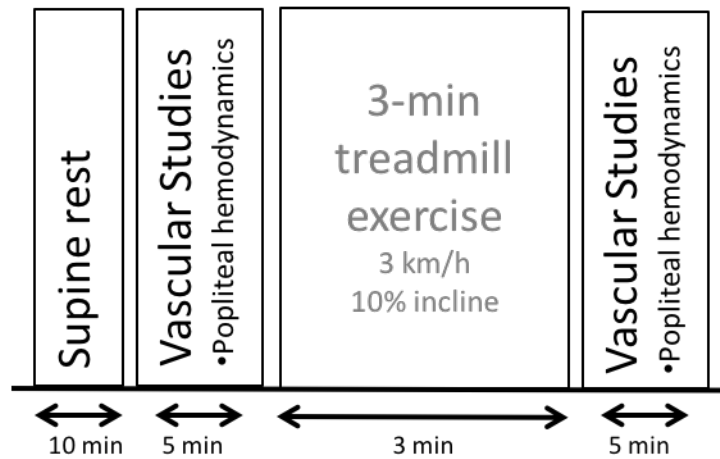
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885

a) Water immersion

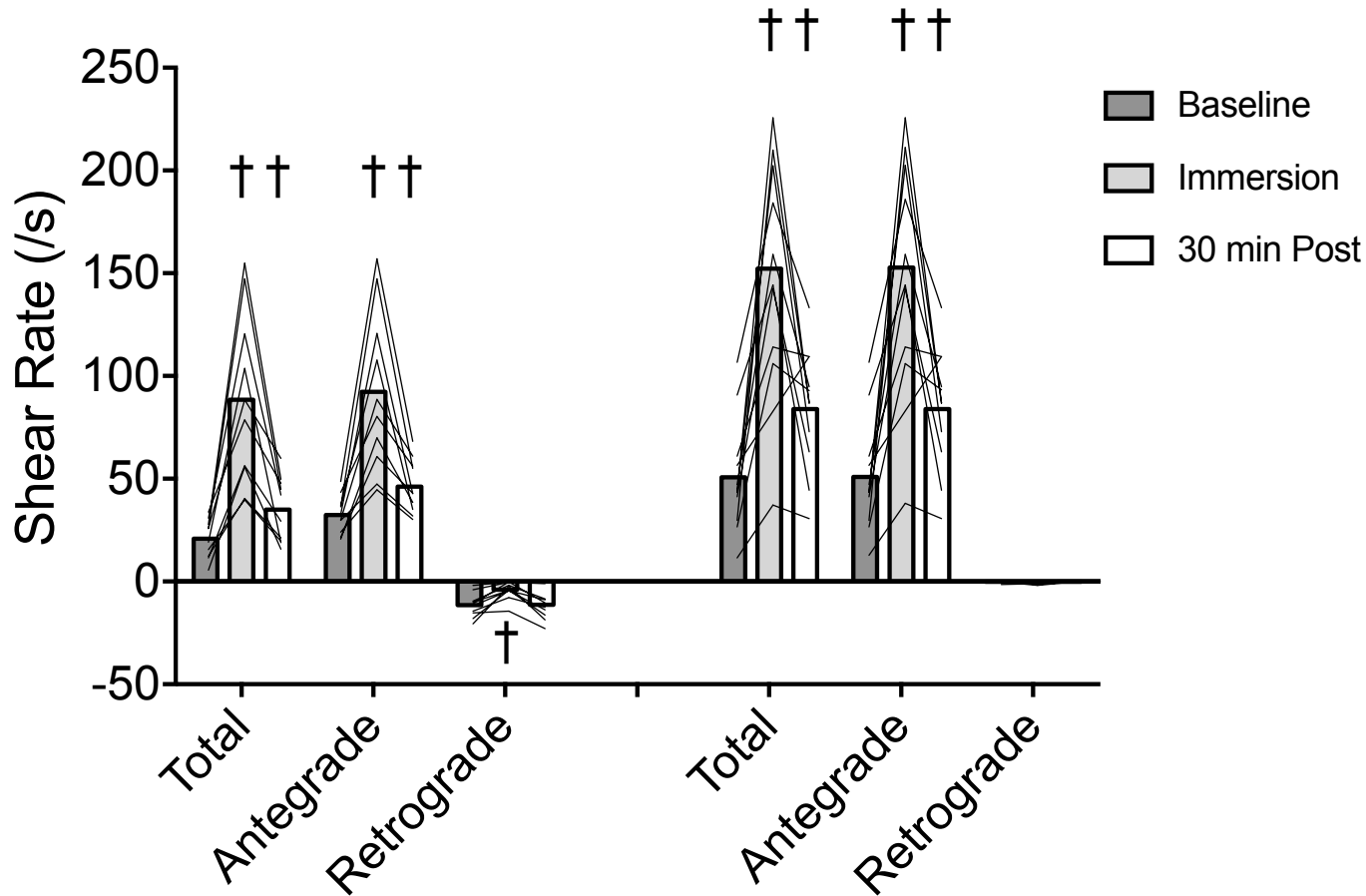


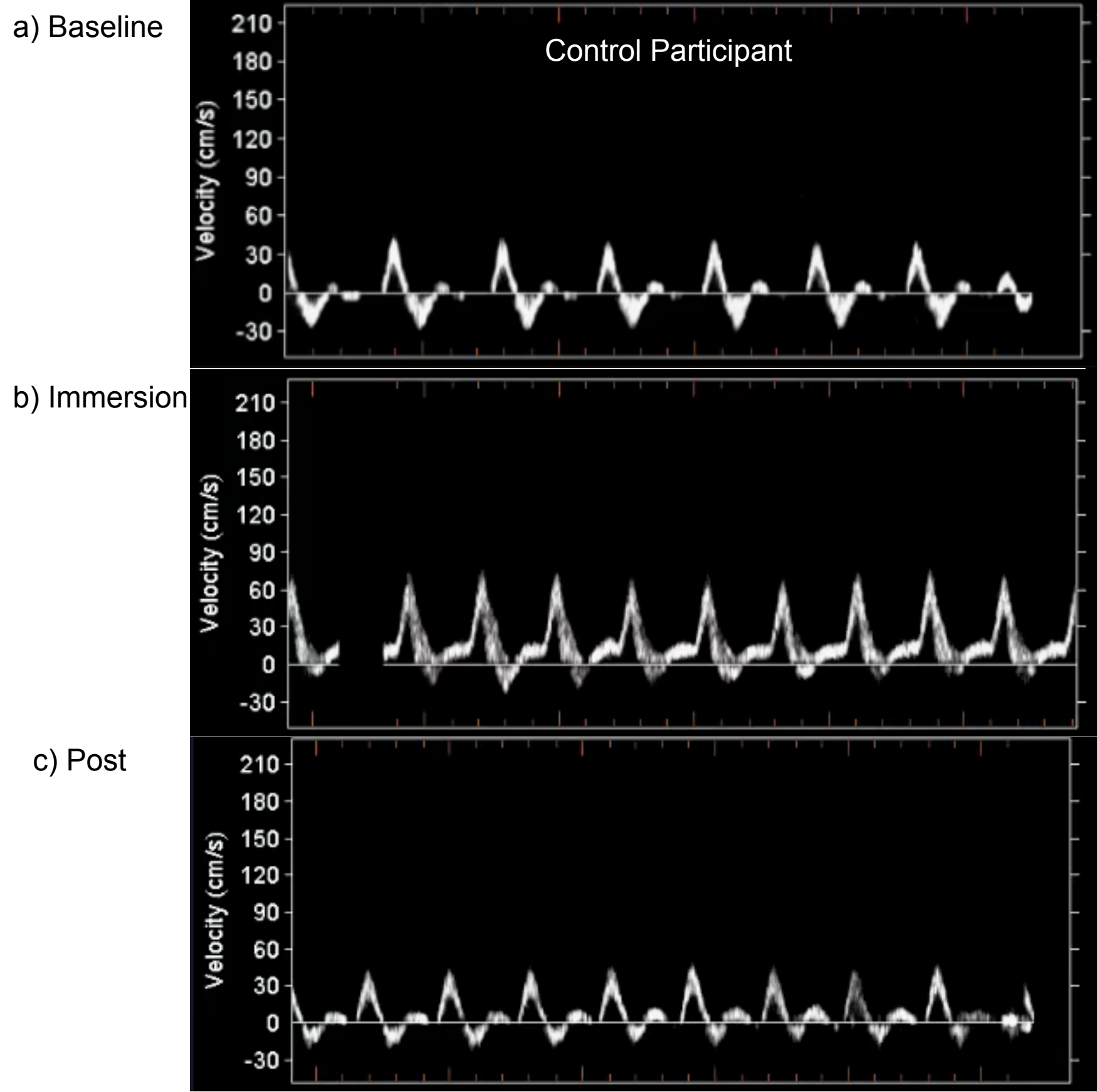
b) Treadmill exercise



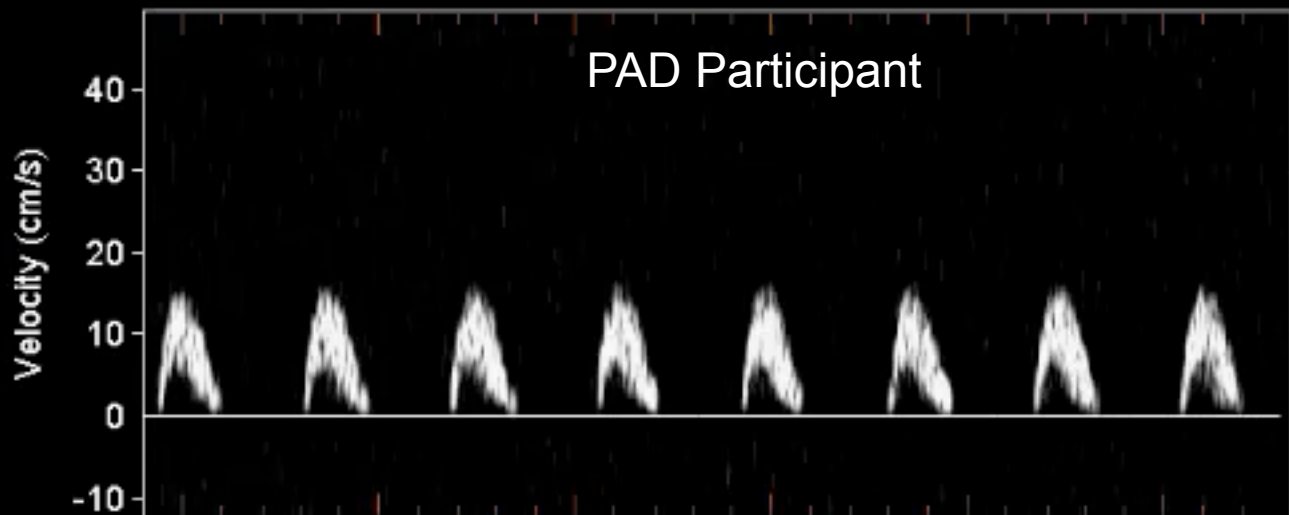
Controls

PAD

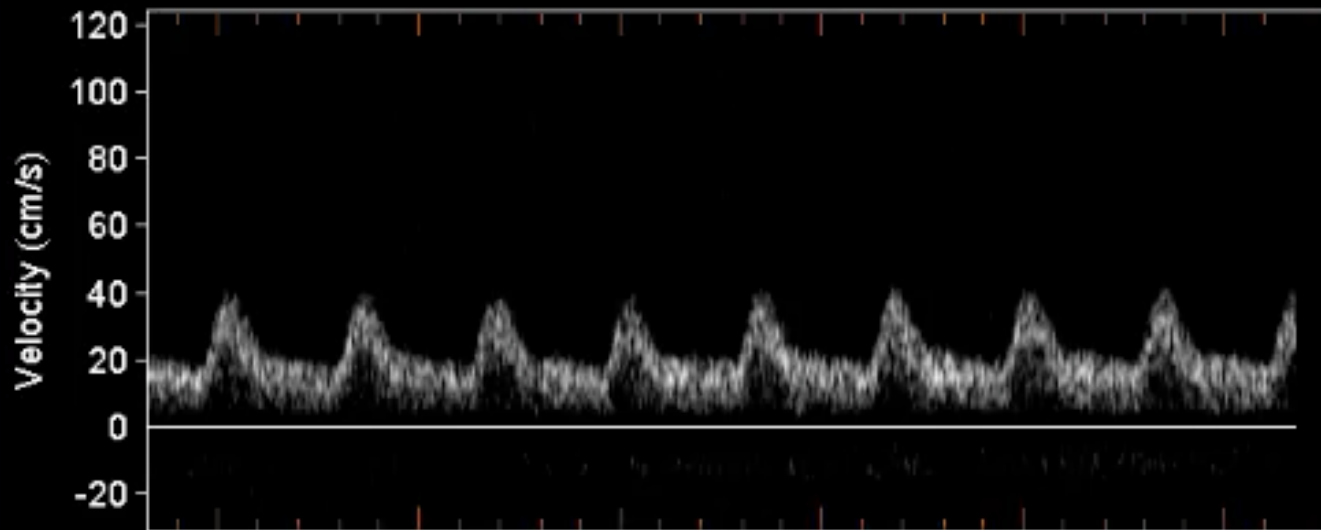




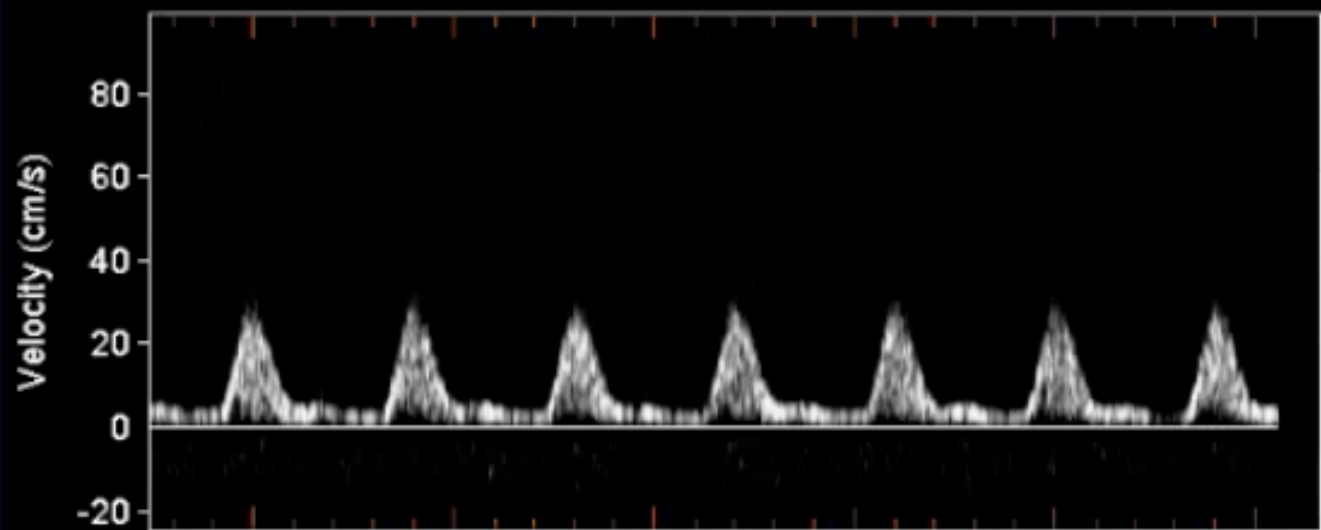
a) Baseline



b) Immersion

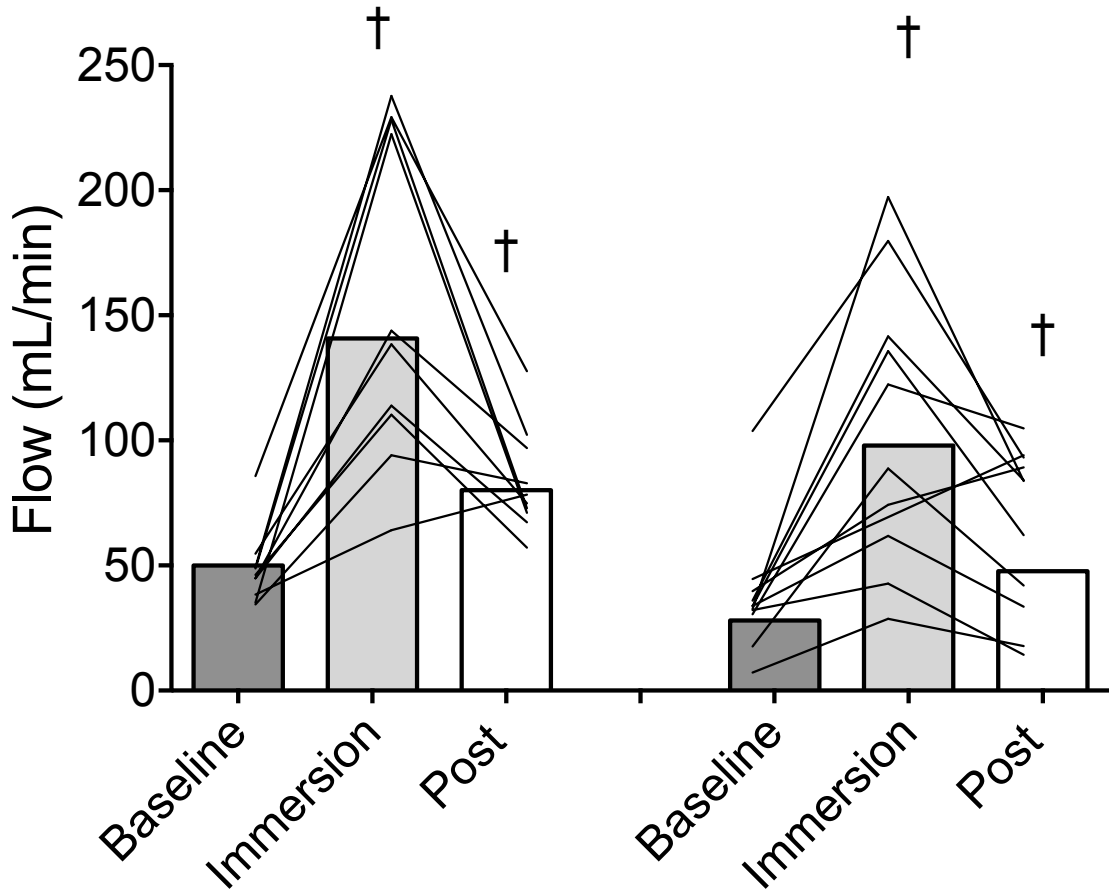


c) Post

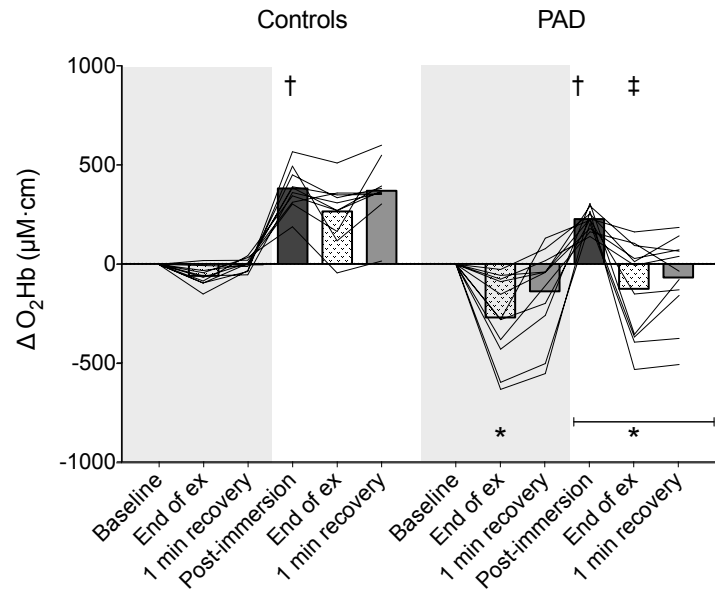


Controls

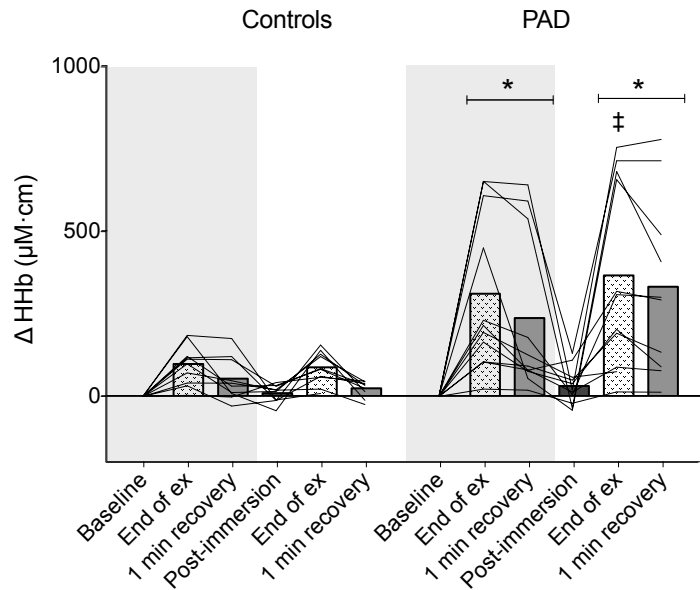
PAD



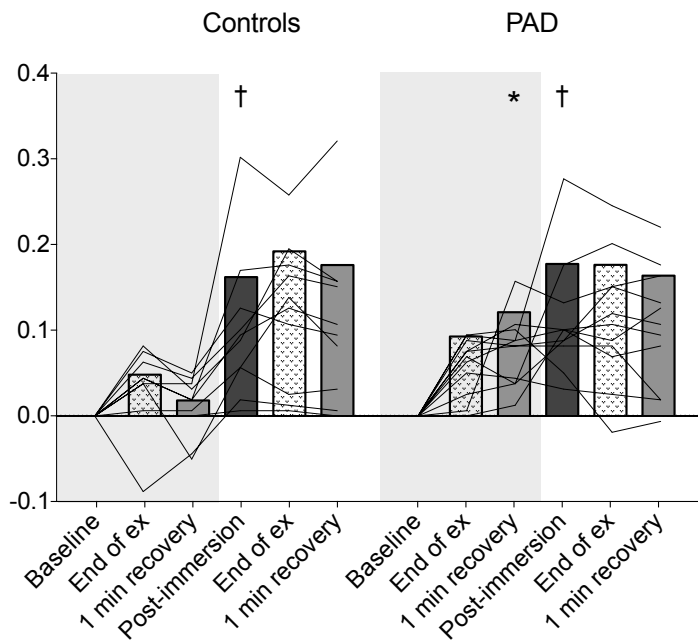
a)



b)



c)



d)

