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**Impact of whole-body passive heat stress and arterial shear rate
modification on radial artery function in young men**

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Running Title: Heat stress and peripheral vascular function

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27 **ABSTRACT**

28 We sought to determine how whole-body heating acutely influences radial artery
29 function, characterized using flow mediated dilation (FMD) and low-flow mediated
30 constriction (L-FMC), and the mechanistic role of shear rate modification on radial artery
31 functional characteristics during heating. Eleven young healthy men underwent whole-body
32 heating (water-perfused suit) sufficient to raise core temperature +1°C. Trials were repeated
33 with (Heat+WC) and without (Heat) the application of a wrist cuff located distal to the radial
34 artery examined, known to prevent increases in mean and anterograde shear rate but increase
35 retrograde shear. Radial artery characteristics were assessed throughout each trial, with FMD
36 and L-FMC assessed prior to and upon reaching the target core temperature. Heat markedly
37 increased radial artery mean and anterograde shear rate, along with radial artery diameter and
38 blood flow ($P<0.05$). Heat+WC abolished the heat-induced increase mean and anterograde
39 shear rate ($P>0.05$), but markedly increased retrograde shear ($P<0.05$). Concomitantly,
40 increases in radial artery diameter and blood flow were decreased (Heat+WC vs Heat,
41 $P<0.05$). Heat attenuated FMD (8.6 ± 1.2 vs. $2.2\pm 1.4\%$, $P<0.05$), whereas no change in FMD
42 was observed in Heat+WC (7.8 ± 1.2 vs. $10.8\pm 1.2\%$, $P>0.05$). In contrast, L-FMC was not
43 different in either trial ($P>0.05$). In summary, acute whole-body heating markedly elevates
44 radial artery shear rate, diameter and blood flow, and diminishes FMD. However, marked
45 radial artery vasodilation and diminished FMD are absent when these shear rate changes are
46 prevented. Shear rate modifications underpin the radial artery response to acute whole-body
47 heat-stress, but further endothelial-dependent vasodilation (FMD) is attenuated likely as the
48 vasodilatory range limit is approached.

49

50 **New and Noteworthy:**

51 We observed that acute whole-body heating elevates radial artery shear rate, diameter
52 and blood flow. This results in a diminished flow-mediated dilatation (FMD) but does not
53 change low-flow mediated constriction (L-FMC). Preventing shear rate changes during
54 whole-body heating reduces radial artery vasodilation, reverses FMD reductions but has no
55 affect on L-FMC. These findings indicate that shear rate changes underpin conduit artery
56 responses to acute whole-body heat-stress, but further endothelial-dependent flow-mediated
57 vasodilation is attenuated as the vasodilatory range limit is approached.

58

59 **ABBREVIATIONS**

60 BP, blood pressure; ECG, electrocardiograph; EDHF, endothelium-derived
61 hyperpolarizing factors; eNOS, endothelial nitric oxide synthase; FMD, flow mediated
62 dilatation; Heat, whole body heat stress sufficient to raise core temperature by 1 °C; Heat +
63 WC, whole body heat stress sufficient to raise core temperature by 1 °C with concurrent
64 inflation of a cuff placed around the right wrist to 75 mmHg; HR, heart rate; LBNP, lower
65 body negative pressure; L-FMC, low-flow mediated constriction; MAP, mean arterial
66 pressure; NO, nitric oxide; SR_{AUC} , shear rate area under the curve; T_{pill} , temperature pill
67 telemetry system; T_{sk} , Mean skin temperature; TVR, Total vessel reactivity;

68

69 **INTRODUCTION**

70 Endothelial-dependent processes provide an important mechanism whereby arterial
71 diameter adapts in response to localized changes in blood flow (5, 20). Conversely,
72 endothelial dysfunction disrupts vascular homeostasis and is integral to the pathophysiology
73 of many cardiovascular diseases (22, 51). The flow mediated dilatation (FMD) technique
74 provides a widely-used, non-invasive method of assessing endothelial function in response to
75 an acute, marked increase in blood flow shear stress (10). However, it is less widely
76 recognized that the acute reductions in arterial blood flow shear stress can evoke a low-flow
77 mediated constriction (L-FMC) (16, 29). L-FMC has promising clinical utility and
78 compliments the information provided by FMD (17, 18). However, in contrast to FMD,
79 limited work has explored the mechanisms underlying L-FMC or considered how it is
80 affected by environmental factors, such as temperature.

81 Exposure to a hot environment results in pronounced cardiovascular autonomic
82 adjustments that includes an increase in sympathetic nervous system activity, heart rate, and
83 cardiac output, along with elevations in conduit artery and skin blood flow (11). Notably,
84 local forearm heating increases brachial artery diameter, anterograde shear rate and FMD
85 (44). While studies in animals and *in-vitro* studies of human endothelial cell cultures have
86 shown an increased anterograde shear rate upregulates the release of endothelial nitric oxide
87 synthase (eNOS) and cytochrome-related endothelium-derived hyperpolarizing factors
88 (EDHF) (4, 9, 19, 28), this fails to occur with increases in retrograde shear rate, and instead
89 there is an augmented release of endothelial derived vasoconstrictor molecules, such as
90 endothelin-1 (49, 50, 54). Experimental induction of an increase in retrograde arterial shear
91 rate in the human brachial artery can be achieved by inflation of pneumatic cuff (30-75
92 mmHg) placed distal to the site of investigation (8, 44, 47), and this maneuver prevents the
93 brachial artery vasodilation during local heating (36). Acute increases in sympathetic

94 vasoconstrictor activity can also increase retrograde shear rate and attenuate FMD (25, 35,
95 44). Unlike local forearm heating, acute whole-body passive heat stress evokes major
96 systemic cardiovascular effects along with sympatho-excitation, both of which have the
97 potential to modify artery blood flow pattern and functional characteristics. However, the
98 influence of whole-body passive heat stress on radial arterial shear rate and function is
99 incompletely understood.

100 In contrast to FMD, the influence of heat stress on L-FMC has not been considered,
101 and whether L-FMC is modulated by the manipulation of local shear rate either
102 independently or with concomitant heat stress is unknown. The L-FMC response to heating
103 cannot be assumed to track that of FMD. While FMD and L-FMC responses complement one
104 another in healthy and clinical populations, they are not significantly correlated (16, 17).
105 Like FMD, L-FMC is at least partly endothelium mediated (5), but unlike FMD, L-FMC is
106 not altered by pharmacological antagonism of nitric oxide synthase (16). Therefore, non-
107 endothelial factors, such as an increase in sympathetic nerve activity, cannot be discounted as
108 contributing to L-FMC (14). Thus, during whole-body passive heat stress, both increases in
109 sympathetic nerve activity and antegrade shear rate could potentially modify L-FMC.

110 The objectives of this investigation were twofold. First, to characterize the effect of
111 whole-body passive heat stress on radial artery blood flow pattern, FMD and L-FMC.
112 Secondly, to determine whether the influence of whole-body passive heat stress on FMD and
113 L-FMC is mediated by a change in local shear rate. To achieve this, the influence of whole-
114 body passive heat stress (sufficient to raise core temperature +1 °C) on radial artery blood
115 flow pattern, FMD and L-FMC was investigated. Heating trials were conducted both with
116 and without the addition of a cuff, inflated to 75 mmHg, placed around the wrist that was
117 distal to the radial artery being examined. We hypothesized that; 1) whole-body passive heat
118 stress would augment antegrade shear rate and subsequently increase FMD and L-FMC via

119 endothelium mediated mechanisms, and 2) such increases in FMD and L-FMC would be
120 prevented if increases in anterograde shear rate were prevented, and retrograde shear rate
121 augmented, during whole-body passive heat stress (i.e., with a wrist cuff).

122 **METHODS**

123 *Ethical Approval.*

124 Ethical Approval for this study was received from the University of Birmingham,
125 Science Technology Engineering and Mathematics Ethical Review (approval number
126 ERN_18-0523). All study procedures were undertaken in accordance with the ethical
127 standards outlined in the *Declaration of Helsinki*, except for registration in a database.
128 Written informed consent was obtained from all study participants following a verbal and
129 written explanation of the study objectives and procedures.

130

131 *Participant characteristics.*

132 Thirteen healthy men were recruited. All participants were normotensive,
133 normothermic (36.2 – 37.6 °C), non-smokers and medication free. Prior to experimental trials
134 participants were requested to adhere to the following instructions: no food or beverages ≥ 6
135 hours, no alcohol or caffeine for ≥ 12 hours, no polyphenol rich food/beverages for ≥ 18 hours,
136 no vigorous exercise for ≥ 48 hours and no vitamin supplements for ≥ 72 hours. Eleven
137 participants completed the experiment, with two participants withdrawing from the study
138 after first trial due to personal reasons.

139

140 *Experimental measures.*

141 Heart rate (HR) was measured using a standard lead II surface electrocardiogram, and
142 systolic and diastolic blood pressure (BP) obtained non-invasively from left brachial artery
143 by automated sphygmomanometer (Tango+, SunTech Medical Instruments, Raleigh, NC,
144 USA). Core (intestinal) temperature was measured using an ingestible temperature pill
145 telemetry system (T_{pill} ; Jonah™ Core Body Temperature, Respironics, Bend, OR, USA).
146 Data were transmitted wirelessly to monitoring device (EQ02+ LifeMonitor, Equivital,

147 Hidalgo, Cambridge, U.K) and then gathered with embedded application software (eqView
148 mobile, Equivital, Hidalgo, Cambridge, U.K). Skin temperature was measured by using
149 thermistors located at four sites (chest_{sk}, biceps_{sk}, thigh_{sk} and calf_{sk}) (Squirrel SQ2010 Data
150 Logger; Grant, Cambridge, UK).

151 Right radial artery diameter and blood flow velocity were obtained using duplex
152 Doppler ultrasound (Terason uSmart 3300, Teratech Corporation, Burlington, MA, USA)
153 with the arm supported at heart level. The radial artery was insonated 10 – 15 cm distal to the
154 medial epicondyle using a multi-frequency linear-array probe (Terason uSmart 15L4)
155 operating at 4-15 MHz and fixed on an adjustable holder throughout the experiment. B-mode
156 imaging was used to measure radial artery diameter and pulse-wave mode to obtain radial
157 artery peak blood velocity. Measurements were made in accordance with recent technical
158 recommendations (34, 45). FMD studio software was used to record Doppler images as video
159 files and offline analysis conducted using automated edge detection and wall tracking
160 algorithms (Cardiovascular Suite Version 3.4.1, FMD Studio, Pisa, Italy).

161

162 *Experimental Protocol.*

163 Prior to experimental trials, participants attended a familiarization session during
164 which study procedures were explained and methods demonstrated. Participants then returned
165 for three separate experimental trials to investigate the impact of whole-body passive heat
166 stress on radial artery endothelial function and blood flow pattern. Trials were conducted on
167 three days separated by at least 24 hours and completed within 14 days. The three
168 experimental trials were; 1) whole-body passive heat stress sufficient to raise core
169 temperature by 1 °C (Heat), 2) whole-body passive heat stress sufficient to raise core
170 temperature by 1 °C with concurrent inflation of a cuff placed around the right wrist to 75
171 mmHg in order to modify the blood flow pattern of the right radial artery (Heat + WC), and

172 3) a Time Control trial with neither whole body heat stress nor wrist cuff inflation. The order
173 of the Heat and Heat + WC trials was randomized by a coin toss. By necessity the Time
174 Control trial was always performed last; its duration determined by the average of the Heat
175 and Heat + WC trials.

176 All experimental sessions and data collection were conducted at the same time of day
177 for a given individual. For the Heat and Heat + WC trials, participants came to the laboratory
178 and swallowed the T_{pill} with water two hours prior to testing. The T_{pill} was not provided for
179 Time Control trial. Experimental sessions commenced with securing skin temperature
180 thermistors to the participants and then putting on a tube-lined water-perfused suit covering
181 the entire body surface with the exception of the head and right forearm. Participants then
182 rested in a supine position on a medical examination table and were instrumented for
183 collection of the experimental measures outlined above. An inflatable cuff was placed around
184 the right wrist to modify the blood flow pattern as described above (Heat + WC) and was also
185 used for the assessment of L-FMC and FMD (described below). The suit was perfused with
186 water at a thermo-neutral temperature (34°C) for 15 min and temperature and hemodynamics
187 recorded. An assessment of radial artery function (L-FMC and FMD) was then made,
188 consisting of a 1 min baseline, followed by 5 min wrist cuff inflation to ≥ 220 mmHg, and a 3
189 min post-cuff inflation recovery period (16). In the Heat trial, the temperature of the water
190 perfusing the suit was then adjusted to 48 °C and applied until core temperature increased by
191 1°C. In the Heat + WC trial, the wrist cuff was inflated to 75 mmHg to modify radial artery
192 flow pattern (47), and the whole body heat stress protocol was replicated as in the Heat trial.
193 Once core temperature was elevated by 1°C (the desired amount) in the Heat and Heat + WC
194 trials, radial artery function testing (L-FMC and FMD) was repeated. During the Time
195 Control trial, the temperature of water perfusing the suit was maintained at a thermo-neutral

196 temperature (34°C) and pre and post intervention recordings of L-FMC and FMD were made
197 as in other two trials (Heat and Heat + WC).

198

199 *Data analysis*

200 Mean skin temperature (T_{sk}) was calculated as (38):

$$T_{sk}(C^{\circ}) = 0.3 \times (\text{Biceps}_{sk} C^{\circ} + \text{Chest}_{sk} C^{\circ}) + 0.2 \times (\text{Thigh}_{sk} C^{\circ} + \text{Calf}_{sk} C^{\circ})$$

201

202 Mean arterial pressure (MAP) was calculated as (39):

$$\text{MAP (mmHg)} = \text{Diastolic BP (mmHg)} + [0.33 + (\text{HR} \times 0.0012)] \times [\text{Systolic BP (mmHg)} \\ - \text{Diastolic BP (mmHg)}]$$

203

204 Radial artery blood flow was calculated as:

$$\text{Blood Flow (ml/min)} = \text{Mean Blood Velocity (cm/s)} \times \pi \times \text{radius (cm)}^2 \times 60 \text{ (s/min)}$$

206

207 Radial artery vascular conductance was determined by dividing arterial blood flow (ml/min)
208 by mean arterial pressure (mmHg).

209 Radial artery wall shear rate was defined as:

$$\text{Arterial Wall Shear Rate (SR, s}^{-1}\text{)} = \frac{4 \times \text{Mean Blood Velocity (cm/s)}}{\text{Diameter (cm)}}$$

210 Anterograde and retrograde shear rate were calculated using anterograde and retrograde
211 blood velocities, respectively.

212 Core temperature (Heat and Heat + WC only), skin temperature, HR, BP and radial
213 artery characteristics were obtained prior to the start of intervention, and then every 5 min
214 during the intervention (Heat, Heat + WC, Time Control trials). In order to make between
215 trial comparisons of the temporal response pattern for temperature and cardiovascular
216 variables, values were selected that corresponded to 25%, 50%, 75% and 100% of total trial

217 duration. A 20 s average was used to provide radial artery measure for a given participant
218 each time point.

219 For radial artery function testing, L-FMC was defined as the change from average
220 baseline diameter to the average diameter of the last 30 s of wrist cuff occlusion, while FMD
221 was taken as the change from the average baseline diameter to the maximal post cuff
222 occlusion diameter (16). L-FMC and FMD responses are presented as relative (%) and
223 absolute (mm) change (45). Total vessel reactivity (TVR) was calculated as the change from
224 the average diameter of the last 30 s of wrist cuff occlusion to the maximal diameter post cuff
225 deflation divided by the average baseline diameter (37) and is presented as a relative (%)
226 change. TVR was used to assess the vascular reactivity range (6). The time-to-peak diameter
227 and shear rate area under the curve (SR_{AUC}), calculated as an integral, were determined from
228 cuff deflation until maximum artery dilation. A ratio of L-FMC against change in mean shear
229 rate (difference between baseline shear rate and shear rate during last 30 s of cuff occlusion;
230 L-FMC-to- Δ mean SR ratio, au) and FMD against SR_{AUC} (FMD-to- SR_{AUC} ratio, au) were
231 calculated and the values multiplied by 1000 (26, 34). Recent guidelines suggest considering
232 whether allometric scaling is necessary when evaluating FMD (3). Accordingly, baseline and
233 nadir / peak diameters were natural log-transformed for slope and upper bound 95%
234 confidence intervals (CI). Further allometric scaling for baseline diameters was not
235 performed as the slope of the relationship between log(peak diameters) and log(baseline
236 diameters) did not deviate significantly from 1 (i.e., all slopes > 0.86 and all upper bound
237 95% CI < 1.42).

238

239 *Statistical Analysis.*

240 All statistical analyses were conducted using Statistical Package for Social Sciences
241 (SPSS, version 21.0). One-way repeated measures analysis of variance (ANOVA) was used

242 to test for differences in total trial durations (Heat, Heat +WC, Time Control). Two-way
243 repeated measures ANOVA was used to test for differences in core and skin temperature,
244 cardiovascular responses, radial artery characteristics and blood flow pattern with respect to
245 time (baseline, 25%, 50%, 75% and 100% of intervention duration), trial (Heat, Heat + WC,
246 Time Control), and their interaction. Two-way repeated measures ANOVA was also used to
247 investigate main effects of time (Pre vs. Post intervention), trial (Heat, Heat + WC, Time
248 Control) and their interaction, for radial artery function (e.g., L-FMC and FMD). An analysis
249 of covariance (ANCOVA), with SR_{AUC} as covariate, was used to statistically assess the FMD
250 response for the shear rate stimulus (SR_{AUC} -corrected-FMD%) (48). Significant main effects and
251 interactions were investigated *post hoc* using Students t-tests with Bonferroni adjustment. $P <$
252 0.05 was recognized as being statistically significant. Data are presented as mean (SD) unless
253 stated.
254

255 **RESULTS**

256 *Core and mean skin temperature.*

257 Total duration was not different between the Heat (78.6 ± 9.5 min) and Heat + WC
258 (76.3 ± 12.2 min) and Time Control (77.5 ± 9.1) trials ($P = 0.554$). Core temperature
259 increased progressively from baseline during Heat and Heat + WC trials, with both being
260 different from baseline after 50%, 75% and 100% of intervention duration, respectively (all P
261 < 0.05) and this increase in core temperature was similar between heat trials ($P = 0.922$;
262 Figure 1). Mean skin temperature also increased from baseline during the Heat (32.8 ± 0.68
263 $^{\circ}\text{C}$) and Heat + WC (32.7 ± 0.83 $^{\circ}\text{C}$) trials ($P < 0.05$ vs. baseline and Time Control trial after
264 25% of intervention duration and beyond), but was not different between the Heat and Heat +
265 WC trials (Heat vs. Heat + WC: baseline; after 25%, 50%, 75% and 100% of intervention
266 duration, $P = 1.00$; $P = 0.089$; $P = 0.620$; $P = 1.00$; $P = 0.166$, respectively). Mean skin
267 temperature during Time Control trial remained between 32 and 34 $^{\circ}\text{C}$ throughout the trial
268 (Time Control after 25%, 50%, 75% and 100% of intervention duration vs. baseline: $P =$
269 1.00 ; $P = 0.994$; $P = 0.955$; $P = 0.994$, respectively).

270

271 *HR and BP.*

272 HR progressively increased from baseline during both whole-body passive heat stress
273 trials (Heat and Heat + WC, $P < 0.05$ vs. baseline and Time Control at 25% intervention
274 duration and beyond; Figure 2). Systolic BP also increased during the Heat and Heat + WC
275 trials ($P < 0.05$), and diastolic BP fell slightly during the heating trials.

276

277 *Radial artery characteristics.*

278 Mean and anterograde shear rate increased progressively and robustly throughout the
279 Heat trial ($P < 0.05$ vs. baseline and Time Control at 25% intervention duration and beyond;

280 Figure 3), while retrograde shear rate decreased slightly from baseline values and was
281 significantly different to Time Control at 75% and 100% intervention duration ($P < 0.05$ vs.
282 Time Control). In the Heat + WC trial, increases in mean and anterograde shear rate were
283 abolished ($P < 0.05$ vs. Heat), while increases in retrograde shear rate were pronounced ($P <$
284 0.05 vs. baseline and Heat). In the Time Control trial, radial artery mean, anterograde and
285 retrograde shear rates remained unchanged from baseline ($P > 0.05$).

286 During the Heat trial, radial artery diameter, velocity, blood flow and vascular
287 conductance all increased progressively and markedly ($P < 0.05$ vs. baseline and Heat + WC
288 at all intervention durations; Table 1 and Figure 4). In contrast, these radial artery
289 characteristics remained close to baseline values throughout the Heat + WC and Time
290 Control trials; the exceptions being Heat + WC radial artery diameter which increased
291 slightly at 50% and 75% of intervention duration, and Time Control vascular conductance
292 which fell slightly at 100% of intervention duration (both $P < 0.05$ vs. Baseline). At baseline,
293 radial artery blood flow, velocity and vascular conductance were slightly but significantly
294 elevated in the Time Control trial compared to the Heat trial ($P < 0.05$).

295

296 *Radial artery function responses.*

297 Table 1 provides radial artery characteristics before and after intervention in the Time
298 Control, Heat and Heat + WC trials. At baseline, FMD, L-FMC and TVR % were not
299 different between the Heat, Heat + WC and Time Control trials (FMD % between trials at the
300 baseline, all $P=1.00$; L-FMC % between trials at the baseline, all $P=1.00$; TVR % at the
301 baseline, Heat vs. Heat +WC $P=0.511$, Heat vs. Time Control $P=1.00$, Heat+WC vs. Time
302 Control $P=0.764$, Figure 5.). Following whole body heating (Post) in the Heat trial, FMD %
303 was significantly decreased ($P < 0.05$ vs. Pre and Heat + WC, Figure 5), while FMD % was
304 unchanged in either the Heat + WC or the Time Control trials (Heat + WC vs. Pre, $P = 0.176$;

305 Time Control vs. Pre, $P = 0.464$). No between trial differences in L-FMC % were observed
306 either at baseline or following intervention in the Heat, Heat + WC and Time Control trials (P
307 > 0.05 , Figure 5.). Following intervention in the Heat + WC trial, TVR% was increased (Pre
308 vs Post, $P < 0.05$ vs. baseline and Time Control).

309 **DISCUSSION**

310 The objectives of this investigation were to characterize the effect of whole-body
311 passive heat stress on radial artery blood flow pattern and functional characteristics (i.e.,
312 FMD and L-FMC), and to determine whether the influence of whole-body passive heat stress
313 on FMD and L-FMC is mediated by a change in local shear rate (as induced via inflation of
314 pneumatic cuff (75 mmHg) placed distal to the site of investigation). We observed that
315 whole-body heating (i.e., Heat trial), sufficient to raise core temperature by +1 °C, markedly
316 and progressively increased radial artery mean and anterograde shear rate, along with radial
317 artery diameter, velocity and blood flow. Contrary to our hypothesis, whole-body passive
318 heat stress attenuated FMD, whereas L-FMC was unchanged. As expected, the addition of a
319 cuff, inflated to 75 mmHg around the wrist distal to the radial artery being examined (i.e.,
320 Heat + WC trial), abolished the heat-induced increase mean and anterograde shear rate, but
321 markedly increased retrograde shear. Associated with this, no changes in either radial artery
322 blood velocity, diameter, blood flow or vascular conductance were observed. Moreover,
323 neither FMD nor L-FMC were different following Heat + WC. Collectively, these findings
324 suggest that whole-body passive heat stress (+1 °C core temperature) acutely elevates radial
325 artery mean and anterograde shear rate, leading to radial artery vasodilatation and diminished
326 FMD, but unchanged L-FMC. However, when whole-body heating induced increases in
327 radial artery mean and anterograde shear rate are prevented, and instead retrograde shear is
328 increased, both radial artery vasodilation and the diminished FMD are prevented.

329 In healthy adults brachial artery FMD has been shown to be enhanced following
330 whole-body passive heat therapy (60 minutes sessions for 8 weeks) (7). Moreover, regular
331 whole-body heating for 3-4 weeks improves endothelial function, maximal O₂ uptake (33),
332 circulating NO metabolite concentrations and reduces oxidative stress markers in chronic
333 heart failure patients (15). While a single session of whole-body heating offers protection

334 from ischemia-reperfusion associated reductions in endothelial function (7). Local heating
335 (42 °C) is known to evoke cutaneous vasodilation, increase limb blood flow and shear stress
336 without producing major systemic cardiovascular effects (19). Moreover, local unilateral
337 limb heating prevents physical inactivity (43) and hyperglycemia (21) induced reductions in
338 FMD. Given this, we hypothesized that acutely applied whole-body passive heat stress would
339 cause an enhanced FMD secondary to an augmented anterograde shear rate and upregulated
340 release of endothelial NO synthase and EDHF (4, 12, 19, 28). This was only partly correct, in
341 that anterograde shear was increased during whole-body passive heat stress, but rather than
342 observing an increase in FMD, a decrease was found.

343 A poor FMD response under normothermic conditions is associated with increased
344 future cardiovascular risk (40, 41, 55) and indicative of endothelial dysfunction. Thus, the
345 reduced FMD during acute whole-body passive heat stress might be interpreted as a reduction
346 in endothelial function. However, it is more likely that the reduction in FMD during acute
347 whole-body passive heat stress was mediated by thermoregulatory-related radial artery
348 vasodilation, which reduced the capacity for subsequent vasodilation during the radial FMD
349 test. Indeed, the peak diameter observed during FMD prior to Heat (i.e., Pre, 2.79 mm) was
350 lower than that observed at baseline following heating (i.e., Post, 3.30 mm; Table 1).
351 Moreover, the SR_{AUC} was diminished during the FMD following whole body heating (Heat
352 trial; SR_{AUC} 16.4 vs. $7.50 \times 10^3 \text{ s}^{-1}$ for Pre vs. Post, respectively), and when FMD was
353 corrected for this attenuated SR_{AUC_2} , no difference in FMD was noted. An alternative
354 explanation is that an elevated sympathetic vasoconstrictor tone resulting from acute whole-
355 body heat stress reduced the FMD response in the current study. Some acutely applied
356 sympatho-excitatory maneuvers have been shown to attenuate FMD (25). Indeed, reductions
357 in FMD following strenuous dynamic exercise are reportedly prevented by alpha-adrenergic
358 blockade suggestive of a sympathetically mediated reduction in FMD (2). Although chronic

359 whole-body passive heat stress has been shown to decrease circulating norepinephrine
360 concentrations in heart failure patients (33), the extent and direction of any acute sympatho-
361 excitatory adaptive changes here remains unclear.

362 The inflation of a cuff (to 75 mmHg) distal to the artery being examined is an
363 established method of manipulating shear rate (47). In the present study, wrist cuff inflation
364 abolished the heat-induced increase in mean and anterograde shear rate, but markedly
365 increased retrograde shear (i.e., Heat + WC trial). Associated with this, and in stark contrast
366 to the Heat trial, no increases in either radial artery diameter, velocity, blood flow or vascular
367 conductance were observed. It should be noted that the wrist cuff was positioned distal to the
368 portion of the radial artery being interrogated and therefore did not directly occlude flow to
369 where the vessel was being imaged. Further, it seems unlikely that the wrist cuff inflation to
370 75 mmHg, which is lower than mean BP and much lower than systolic BP, was sufficient to
371 reduce downstream radial artery blood flow into the hand; yet this possibility cannot be
372 completely excluded (27). Nonetheless, such an effect should not have severely compromised
373 hand circulation as no participants reported altered sensation in the hands. Notably, while
374 Heat diminished FMD, it was preserved during Heat + WC likely as a consequence of the
375 greater SR_{AUC} during the FMD. This provides further support for the contention that the
376 attenuation in FMD for the Heat trial was mediated by the reduction in shear stimulus and not a
377 true change in endothelial vasodilator function. Heat and Heat + WC trials were well matched
378 in so far as the evoked increases in core temperature, blood pressure and heart rate were not
379 different, suggesting that a non-specific systemic factor was not involved. The between trial
380 difference in FMD is likely explained by the wrist cuff preventing an increase in radial artery
381 mean and anterograde shear rate, and thus no radial artery vasodilatation occurring.
382 Therefore, with the radial artery at a baseline level in the Heat + WC trial, the FMD response
383 was normal, despite core temperature being raised.

384 We hypothesized that whole-body passive heat stress would augment L-FMC,
385 whereas it remained unchanged. Among the various suggested mechanisms underlying L-
386 FMC is an endothelial contribution (13). Indeed, L-FMC is attenuated by inhibition of
387 endothelial derived hyperpolarizing factors, prostaglandins (16) and the endothelin receptor
388 antagonist BQ-123 (40). Notably, FMD was diminished with whole-body passive heat stress
389 and it is well established that FMD is at least in part determined by endothelial dependent
390 mechanisms. Given this, one might have expected L-FMC to change similarly, but despite
391 the augmented baseline diameter this was not the case. A sympathetic mechanism has also
392 been postulated to contribute to L-FMC, and whole-body passive heat stress is well known to
393 increase sympathetic activity. Inflation of a wrist cuff (i.e., Heat + WC trial) had no influence
394 on L-FMC. This further supports the concept that manipulating shear rate, such that increases
395 in anterograde shear rate are prevented and retrograde shear rate augmented, has a minimal
396 effect on L-FMC. Elliott et al., (14) observed an augmented L-FMC following dynamic
397 exercise and among the potential mechanisms suggested was an increase in sympathetic
398 nerve activity. It might have been reasonable to expect that with the prevailing vasodilation,
399 meaning more scope for vasoconstriction, along the increase in retrograde shear to attenuate
400 endothelial function, a more pronounced L-FMC would have been exhibited. Further, it could
401 also have been expected that the increased retrograde shear during Heat + WC would have
402 worsened endothelial function (47) and attenuated L-FMC. However, this was also not
403 observed.

404

405 *Experimental Considerations*

406 Herein we assessed the radial artery and studies are required to verify these findings
407 in other conduit vessels (e.g., coronary arteries). While the relationship between the brachial
408 artery FMD and acetylcholine infusion responses with bradykinin, acetylcholine, adenosine

409 and dobutamine infusion responses of the coronary vessels has been determined (31, 32, 41,
410 42), to the best of our knowledge such an investigation has not been carried out for any other
411 peripheral conduit vessels. While some previously published investigations have examined
412 the radial artery (e.g., (13, 14, 16, 52)), human studies of peripheral vascular function more
413 commonly examine the brachial artery. Similar blood flow and shear patterns would be
414 expected in both the radial and brachial arteries during passive whole-body heat stress (46). A
415 notable difference between the brachial and radial arteries relates to the propensity to observe
416 an L-FMC response, with this being more commonly seen in the radial artery (52).

417 Radial artery function was only assessed at a single time point following the whole-
418 body passive heat stress intervention. As such we were unable to ascertain the time-course of
419 the vascular response, and specifically determine how long the whole-body heat stress related
420 decrement in FMD persisted for in the post-heat period, and if/when a conversion to an
421 augmented FMD response occurred. It is a limitation that only health young men were
422 studied. There are important sex-differences and ovarian hormone effects on vascular
423 function (24). Unfortunately, resource and logistical issues meant that we were unable to
424 study young women at a standard phase of their menstrual cycle (e.g., early follicular phase)
425 for the three separate experimental sessions that our study design necessitated, potentially
426 over several months. Additional studies are required to ascertain whether sex-differences are
427 present in our findings, and the extent to which they similarly manifest in patient populations
428 in whom underlying impairments in vascular function are reported (e.g., healthy ageing,
429 hypertension).

430 The use of a wrist cuff inflation to 75 mmHg is an established method to alter shear
431 rate patterns, particularly during experimental conditions in which shear stress is elevated
432 (e.g. (8)). Despite its utility, this model of shear rate manipulation simultaneously decreases
433 anterograde and increases retrograde shear rates, respectively. As such, we are unable to state

434 definitively whether the FMD response following Heat + WC is mediated by attenuated mean
435 and anterograde shear, or is it driven by the large increase in retrograde shear, or a
436 combination of both. The wrist cuff and associated changes in shear pattern lead to a
437 diminished blood flow response to whole-body heating. However, blood flow was not
438 significantly reduced below baseline or time control values. We assume that forearm
439 metabolic rate was not different between trials and as such do not expect differences in
440 downstream tissue oxygen to have occurred and contributed to the vascular responses
441 observed. We cannot discount the possibility that wrist cuff inflation may have evoked
442 venous distension and a reflex increase in vasoconstrictor sympathetic nerve activity (23, 30).
443 The inclusion of assessments of sympathetic nerve activity (1) or blood based biomarkers of
444 vascular function (53) would have provided additional mechanistic insight and strengthened
445 this study.

446

447 *Conclusions*

448 Collectively, these findings suggest that whole-body passive heat stress acutely
449 elevates radial artery mean and anterograde shear rate, leading to a vasodilatation of the
450 radial artery and a diminished FMD, but not L-FMC. Preventing these shear rate induced
451 changes reduces radial artery vasodilation and the acutely diminished FMD. Therefore, shear
452 rate modifications appear to underpin the conduit artery response to acute whole-body heat-
453 stress, but further endothelial-dependent flow-mediated vasodilation is attenuated as the
454 vasodilatory range limit is approached.

455

456 **TABLES**457 **Table 1.** Radial artery characteristics before (Pre) and after (Post) the Time Control, Heat and eat + WC trials.

	Time Control		Heat		Heat + WC		Trial	P values	
	Pre	Post	Pre	Post	Pre	Post		Time	Interaction
Baseline									
Diameter (mm)	2.66 (0.35)	2.63 (0.34)	2.57 (0.31)	3.30 (0.43)*†‡	2.53 (0.35)	2.79 (0.36)*	<0.001	<0.001	<0.001
Velocity (cm/s)	15.17 (6.9)	7.53 (6.6)*	8.07 (6.2)‡	39.48 (7.8)*†‡	9.48 (7.72)	11.59 (6.5)	<0.001	<0.05	<0.001
Blood flow (ml/min)	54.04 (33.0)	26.18 (23.7)*	25.6 (20.3)‡	204.17 (54.2)*†‡	31.32 (31.2)	45.60 (32.7)	<0.001	<0.001	<0.001
Mean shear rate (s ⁻¹)	227.2 (98.0)	113.3 (96.9)*	130.3 (102.1)‡	485.3 (105.2)*†‡	147.5 (112.4)	165.2 (88.4)	<0.001	0.022	<0.001
L-FMC									
Nadir Diameter (mm)	2.55 (0.34)	2.55 (0.34)	2.45 (0.27)	3.08 (0.41)*†‡	2.47 (0.36)	2.65 (0.31)	<0.001	<0.001	<0.001
Δ Diameter (mm)	-0.11 (0.06)	-0.08 (0.06)	-0.12 (0.09)	-0.22 (0.27)	-0.06 (0.08)	-0.15 (0.12)	0.174	0.195	0.162
Mean shear rate (s ⁻¹)	22.8 (6.2)	26.2(7.5)	21.4 (11.0)	74.0 (30.6)*†‡	23.5 (8.2)	109.2 (59.9)*†‡	<0.001	<0.001	0.001
Δ Mean shear rate (s ⁻¹)	204.4 (103.0)	87.1 (90.4)*	108.9 (104.5)‡	411.2 (93.6)*†‡	123.9 (111.1)	56.0 (57.2)	<0.001	0.229	<0.001
L-FMC-to-Δ mean SR ratio (au)	-0.029(0.038)	-0.064(0.082)	-0.110 (0.234)	-0.020 (0.025)	-0.004 (0.092)	-0.209 (0.348)	0.548	0.231	0.075
FMD									
Peak Diameter (mm)	2.88 (0.41)	2.79 (0.40)	2.79 (.35)	3.37 (0.46)*†‡	2.73 (0.40)‡	3.10 (0.40)*‡	<0.001	<0.001	<0.001
Δ Diameter (mm)	0.21 (0.14)	0.17 (0.12)	0.22 (0.11)	0.07 (0.15)*†	0.20 (0.14)	0.30 (0.11)	0.038	0.334	<0.05
Time to peak diameter (s)	80.72 (32.68)	77.18 (46.95)	90.36 (41.62)	97.72 (152.58)	76.54 (42.49)	118.6 (39.66)	0.326	0.097	0.265
SR _{AUC} (x10 ³ s ⁻¹)	19.33 (6.7)	16.58 (5.7)	16.4 (6.56)	7.50 (7.0)†‡	17.8 (8.5)	29.57 (11.6)*†‡	<0.001	0.507	<0.05
FMD-to-SR _{AUC} ratio (au)	0.40 (0.26)	0.62 (0.97)	0.63 (0.46)	0.66 (1.24)	0.46 (0.27)	0.42 (0.19)	0.641	0.689	0.819
SR _{AUC} -corrected-FMD (%)	7.696 (4.60)	6.526 (4.60)	8.73 (4.60)	3.156 (5.25)	7.856 (4.60)	9.712 (5.42)	0.224	0.154	0.066

458

459 Values are means (SD). L-FMC, low-flow mediated constriction; FMD, flow mediated dilatation; SR_{AUC}, shear rate area under curve. P values
460 represent 2-way repeated ANOVA results (Trial; Time Control, Heat and Heat + WC: Time; Pre and Post: Interaction, Trial x Time). P value for
461 SR_{AUC}-corrected-FMD (%) represent ANCOVA results. * P < 0.05 vs. Pre; † P < 0.05 vs. Heat + WC; ‡ P < 0.05 vs. Time Control.

462

463

464 **FIGURE LEGENDS**

465 **Figure 1. Core and skin temperatures.**

466 Whole-body passive heat stress (Heat) and whole-body heat stress with wrist cuff (Heat +
467 WC) evoked similar increases in core and skin temperature. Skin temperature was not
468 changed from baseline in the Time Control trial. Values are mean \pm SE. * $P < 0.05$ vs.
469 baseline (BL); ‡ $P < 0.05$ vs. Time Control.

470

471 **Figure 2. Cardiovascular responses.**

472 Heart rate (HR), systolic blood pressure (systolic BP), diastolic blood pressure (Diastolic
473 BP), mean arterial pressure (MAP) responses were similar in the whole-body passive heat
474 stress (Heat) and whole-body heat stress with wrist cuff (Heat + WC) trials. Values are mean
475 \pm SE. * $P < 0.05$ vs. baseline (BL); † $P < 0.05$ vs. Heat + WC; ‡ $P < 0.05$ vs. Time Control.

476

477 **Figure 3. Radial artery blood flow pattern.**

478 Mean, anterograde and retrograde shear rate during the whole-body passive heat stress
479 (Heat), whole-body heat stress with wrist cuff (Heat + WC) and Time Control trials. Values
480 are the mean \pm SE. * $P < 0.05$ vs. baseline (BL); † $P < 0.05$ vs. Heat + WC; ‡ $P < 0.05$ vs.
481 Time Control.

482

483 **Figure 4. Radial artery characteristics**

484 Radial artery blood flow, diameter, velocity and vascular conductance during whole-body
485 passive heat stress (Heat), whole-body heat stress with wrist cuff (Heat + WC) and Time
486 Control trials. Values are the mean \pm SE. * $P < 0.05$ vs. baseline (BL); † $P < 0.05$ vs. Heat +
487 WC; ‡ $P < 0.05$ vs. Time Control.

488

489 **Figure 5. Radial artery function**

490 Radial artery flow mediated dilatation (FMD), low-flow mediated constriction (L-FMC), and
491 total vascular range (TVR) during the whole-body passive heat stress (Heat), whole-body
492 heat stress with wrist cuff (Heat + WC) and Time Control trials. Values are the mean \pm SE. *
493 $P < 0.05$ vs. baseline (BL); † $P < 0.05$ vs. Heat + WC; ‡ $P < 0.05$ vs. Time Control.

494

495

496

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- 678

FIGURES

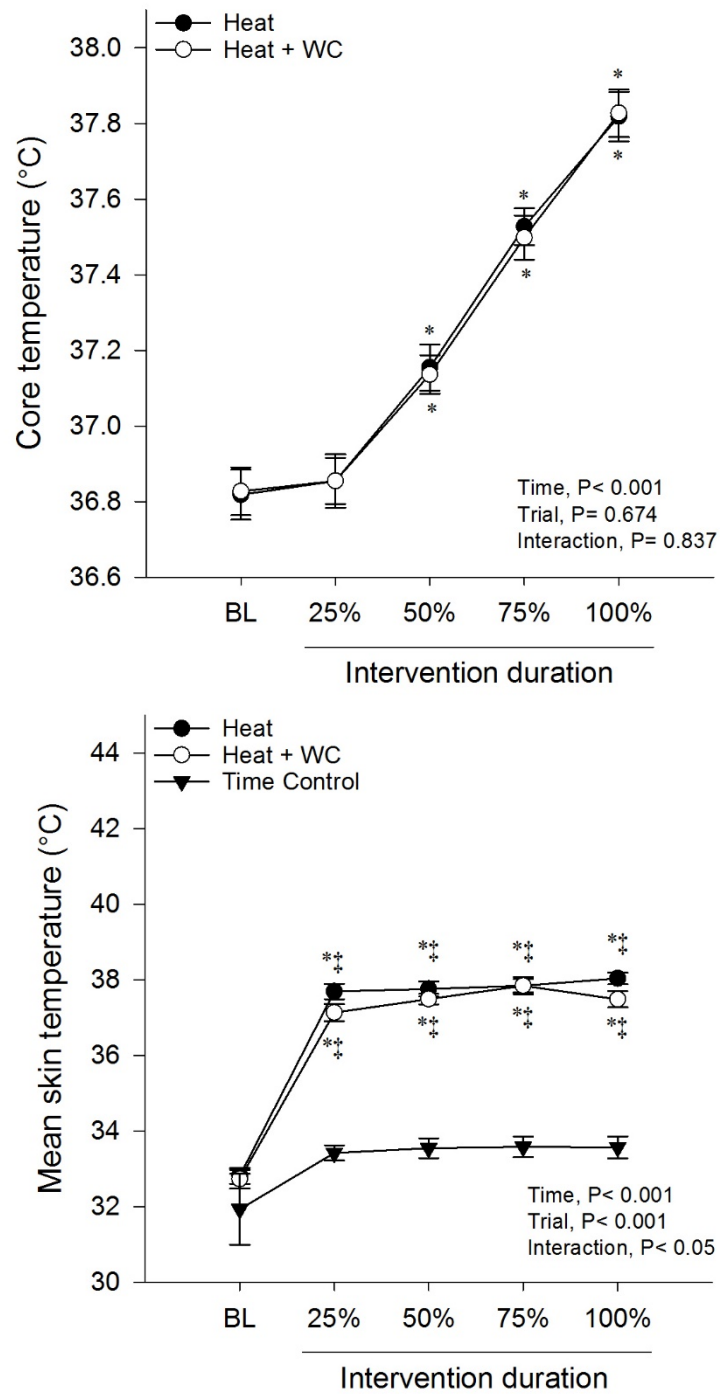


Figure 1

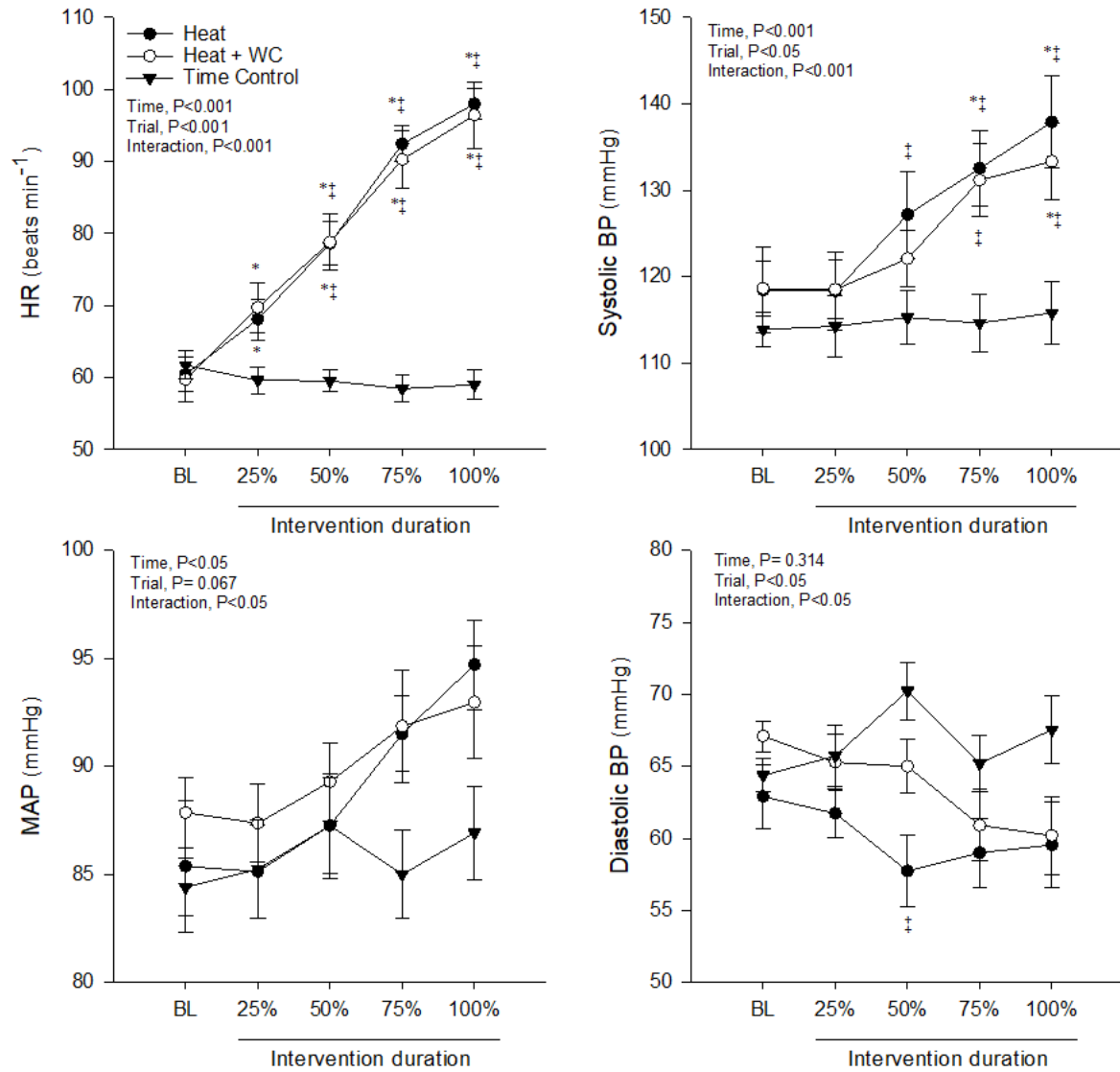


Figure 2

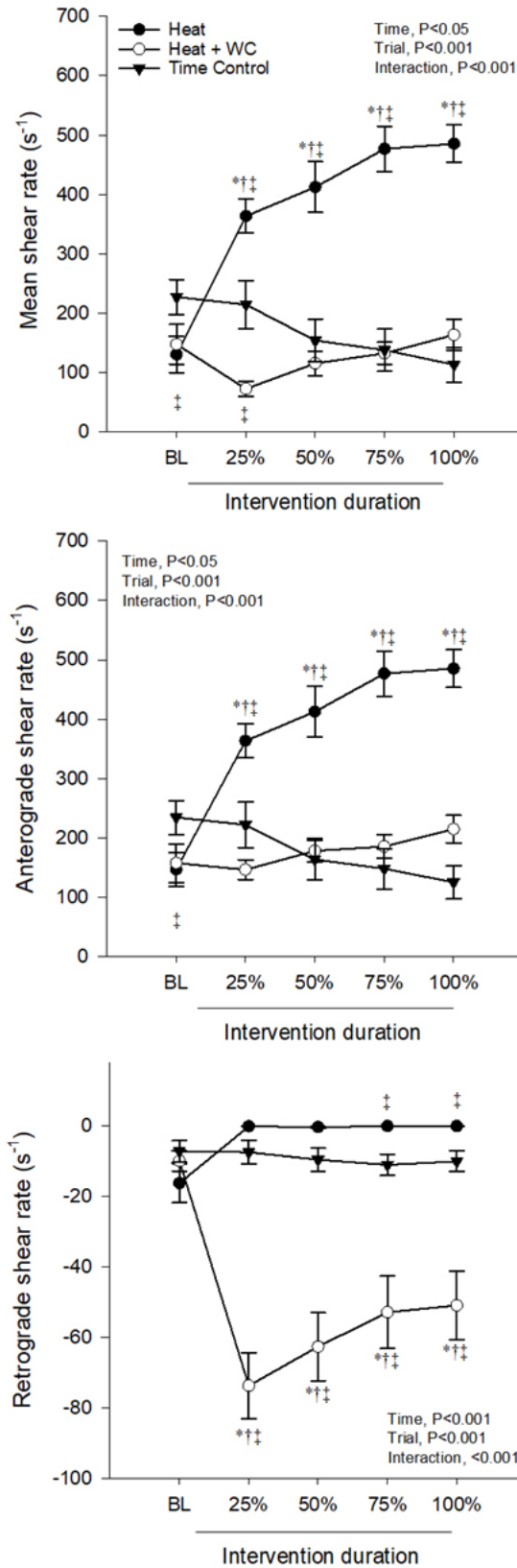


Figure 3.

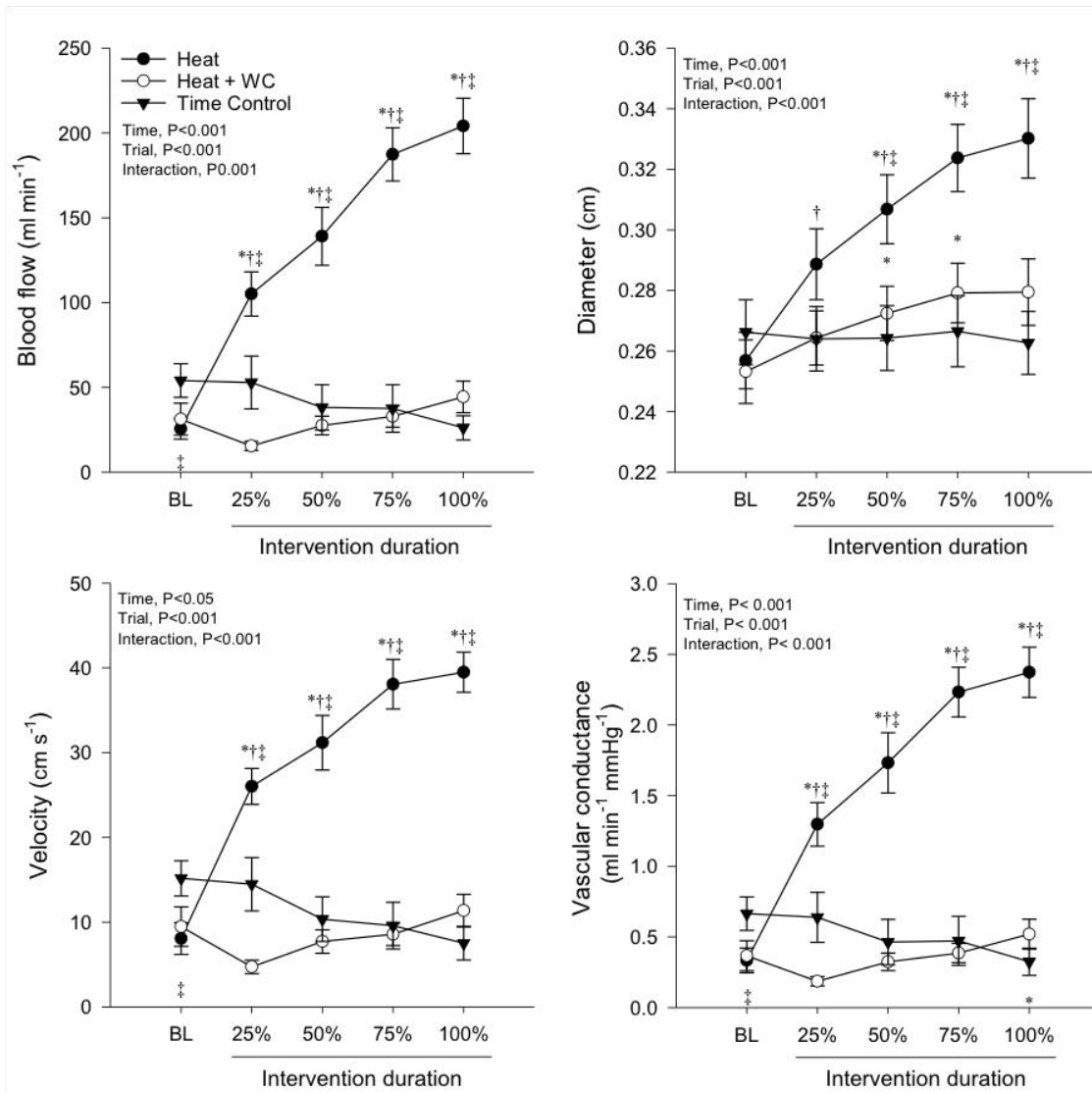


Figure 4.

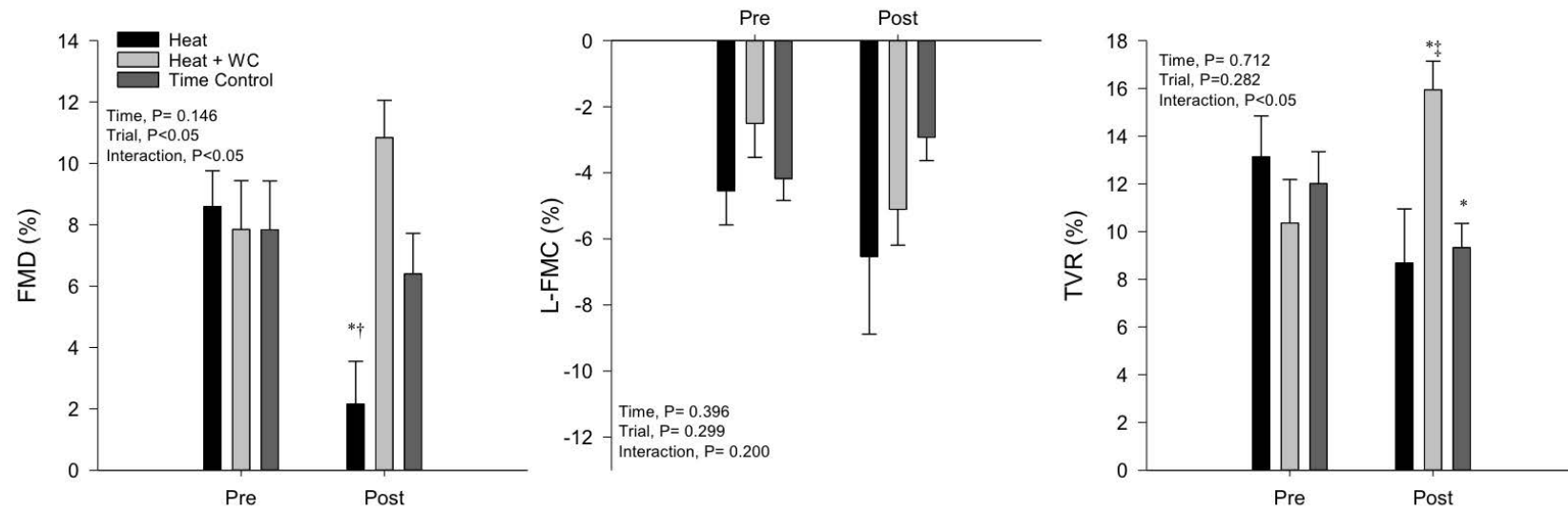


Figure 5.