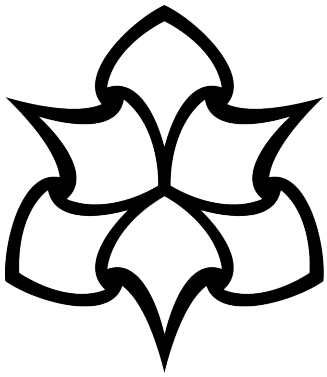

Impact of whole-body passive heat stress and arterial shear rate modification on radial artery function in young men

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Running Title: Heat stress and peripheral vascular function

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ABSTRACT

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

New and Noteworthy:

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

ABBREVIATIONS

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

INTRODUCTION

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

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

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

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

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

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

METHODS

Ethical Approval.

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

Participant characteristics.

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

Experimental measures.

Heart rate (HR) was measured using a standard lead II surface electrocardiogram, and systolic and diastolic blood pressure (BP) obtained non-invasively from left brachial artery by automated sphygmomanometer (Tango+, SunTech Medical Instruments, Raleigh, NC, USA). Core (intestinal) temperature was measured using an ingestible temperature pill telemetry system (T_{pill}; Jonah™ Core Body Temperature, Respironics, Bend, OR, USA). 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

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

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

$$T_{sk}(C°) = 0.3\ x\ (Biceps_{sk}\ C° + Chest_{sk}C°) + 0.2\ x\ (Thigh_{sk}C° + Calf_{sk}C°)$$

201
202 Mean arterial pressure (MAP) was calculated as (39):

$$MAP\ (mmHg) = Diastolic\ BP(mmHg) + [0.33 + (HR\ x\ 0.0012)]\ x\ [Systolic\ BP(mmHg) - Diastolic\ BP\ (mmHg)]$$

203
204 Radial artery blood flow was calculated as:
205 $$Blood\ Flow\ (ml/min) = Mean\ Blood\ Velocity\ (cm/s)\ x\ \pi\ x\ radius\ (cm)^2\ x\ 60\ (s/min)$$
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:

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

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

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

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

Statistical Analysis.

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

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

RESULTS

Core and mean skin temperature.

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

HR and BP.

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

Radial artery characteristics.

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

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

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

Radial artery function responses.

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

DISCUSSION

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

In healthy adults brachial artery FMD has been shown to be enhanced following whole-body passive heat therapy (60 minutes sessions for 8 weeks) (7). Moreover, regular whole-body heating for 3-4 weeks improves endothelial function, maximal O_2 uptake (33), circulating NO metabolite concentrations and reduces oxidative stress markers in chronic 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 $x10^3$ s^{-1} for Pre vs. Post, respectively), and when FMD was
353 corrected for this attenuated SR_{AUC}, 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 completed 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 attention 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.

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

Experimental Considerations

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

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

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

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

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

Conclusions

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

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		P values		
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Trial**	**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

FIGURE LEGENDS

Figure 1. Core and skin temperatures.

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

Figure 2. Cardiovascular responses.

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

Figure 3. Radial artery blood flow pattern.

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

Figure 4. Radial artery characteristics

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

Figure 5. Radial artery function

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

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FIGURES

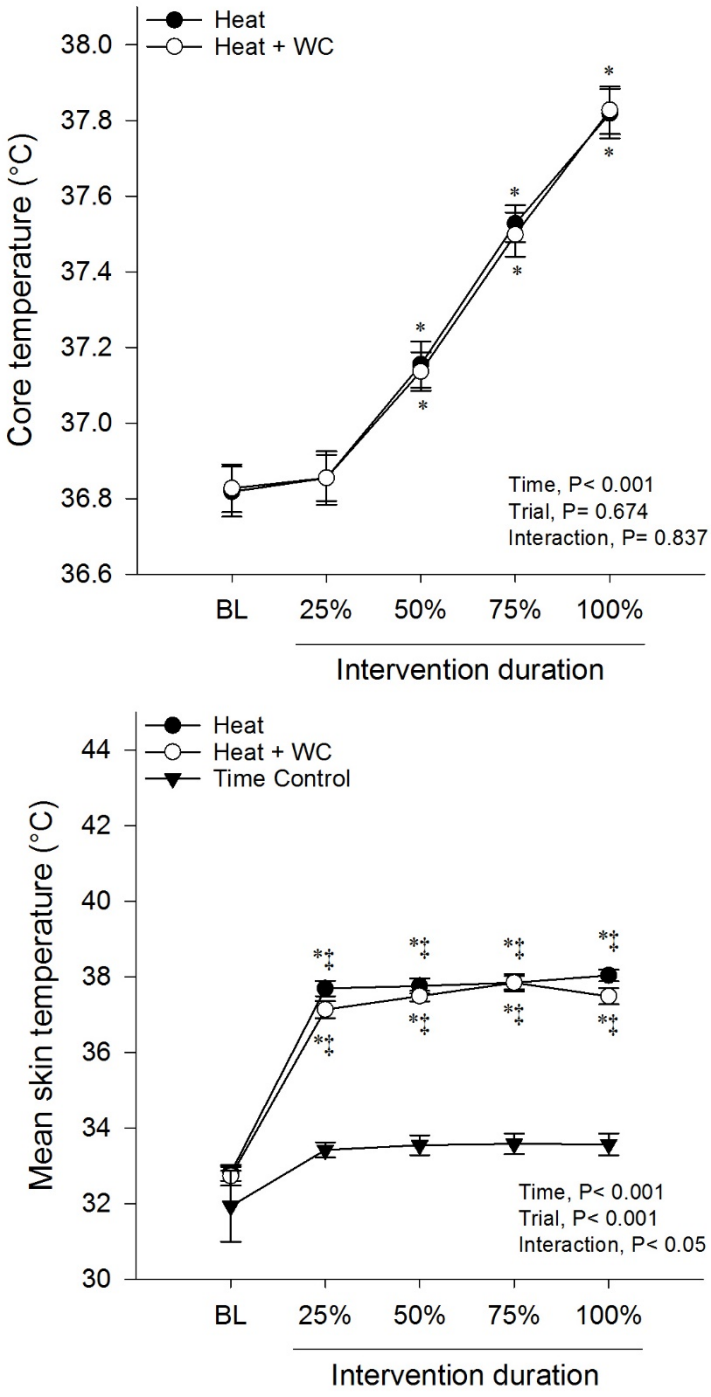
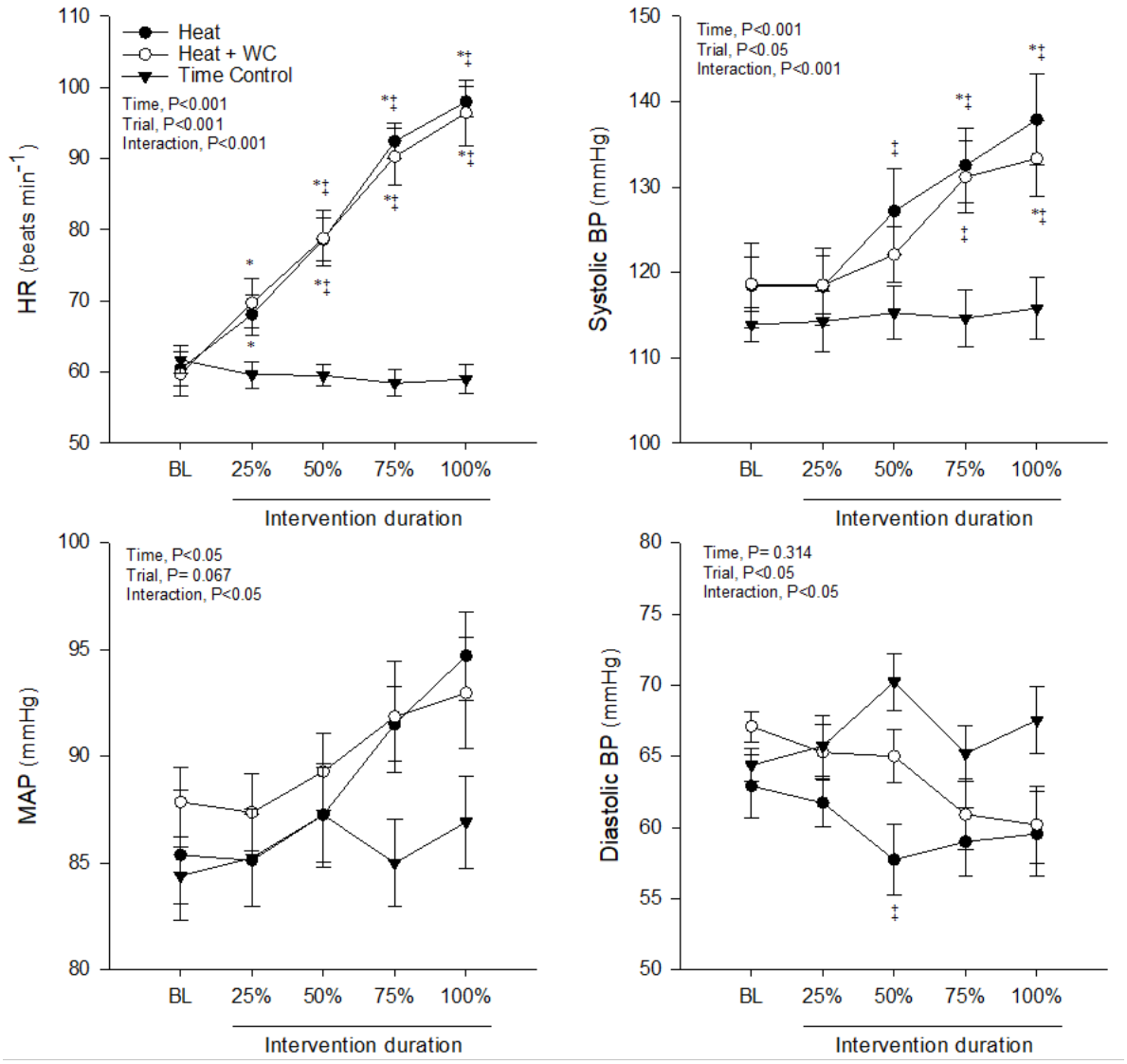


Figure 1



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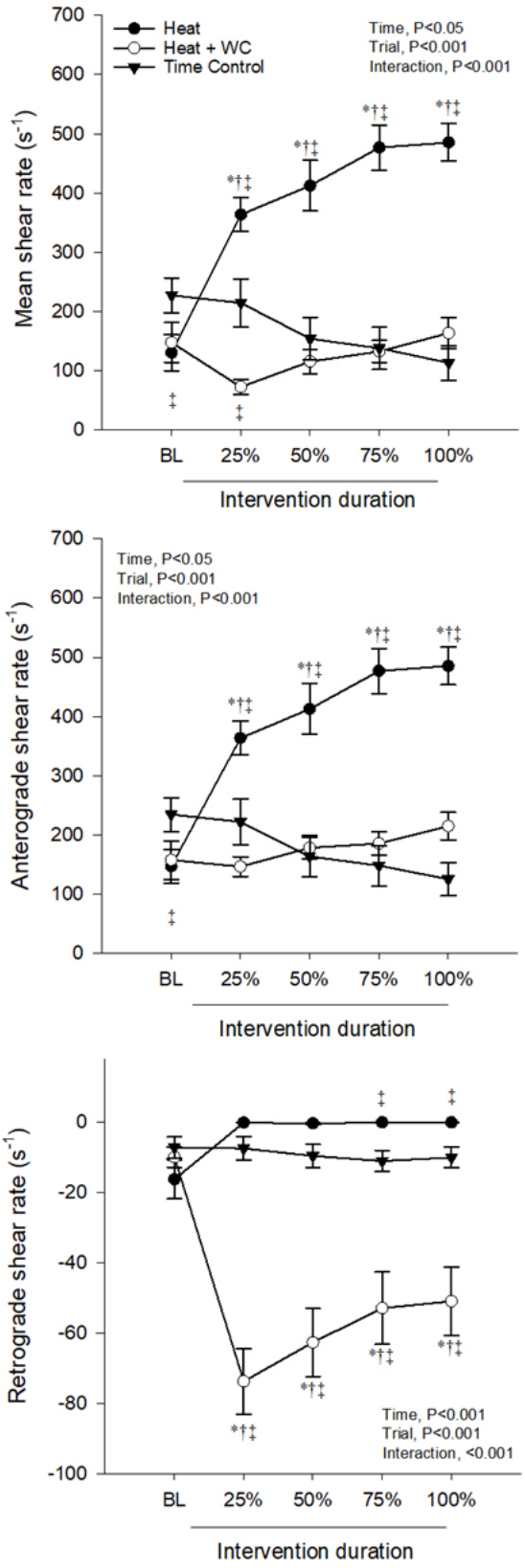

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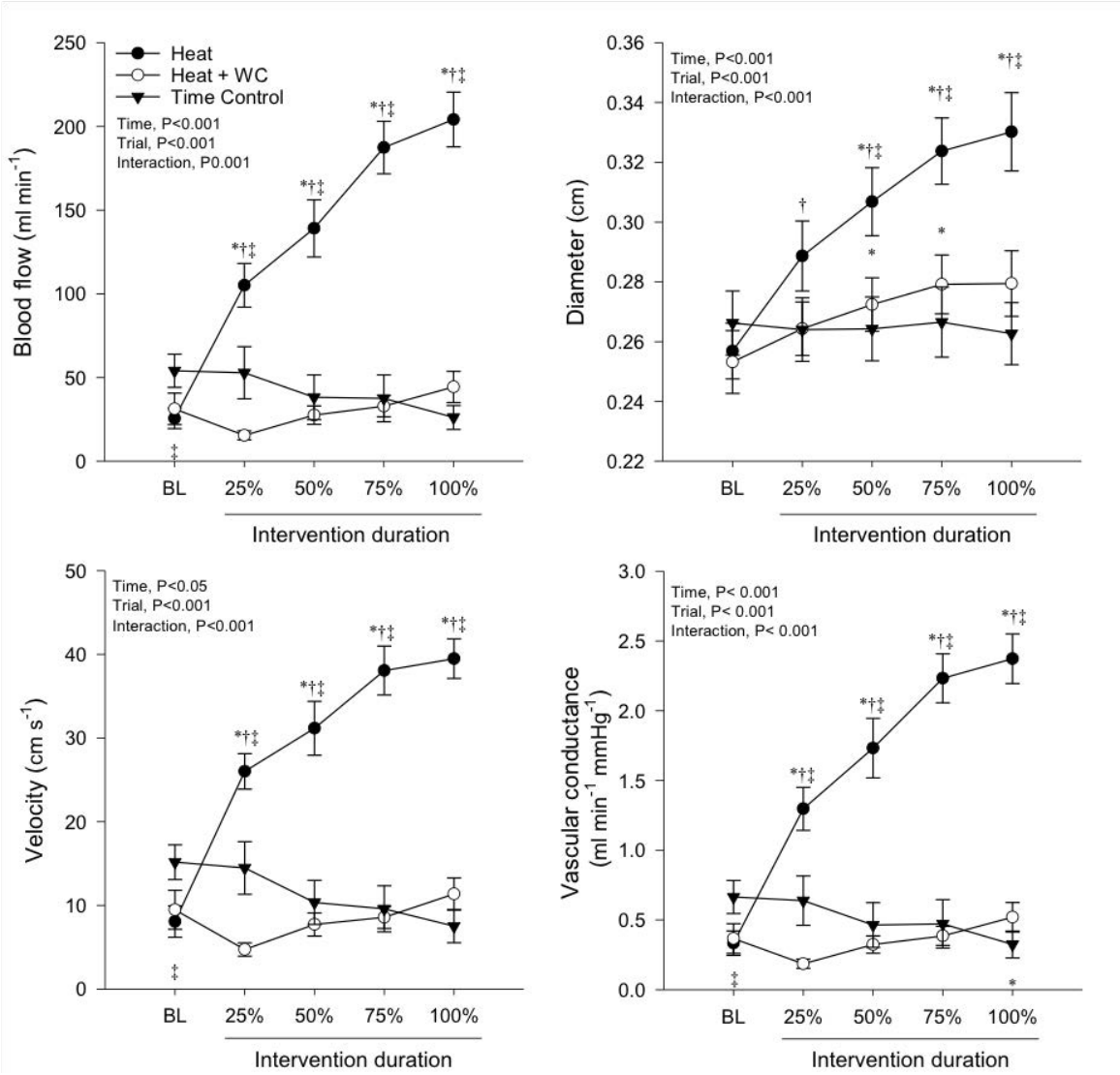

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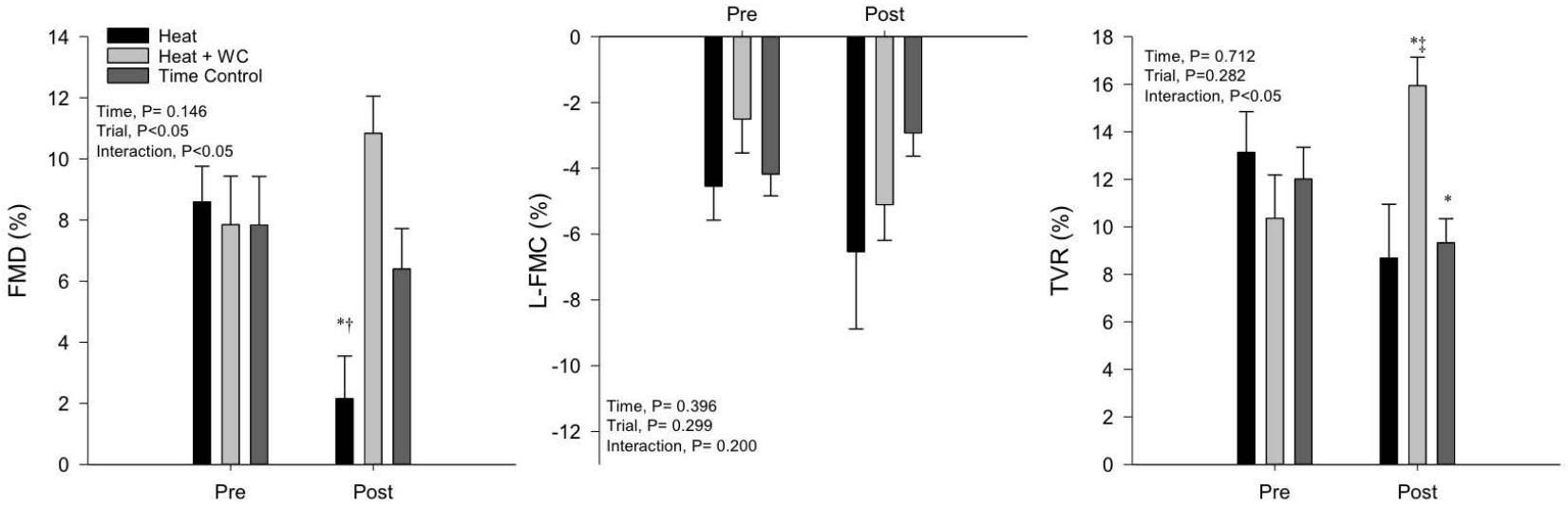

Figure 5.