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Alali, Mohammad H, Vianna, Lauro C, Lucas, Rebekah AI, Junejo, Rehan T and Fisher, James P (2020) Impact of whole-body passive heat stress and arterial shear rate modification on radial artery function in young men. *Journal of Applied Physiology*, 129 (6). pp. 1373-1382. ISSN 8750-7587

**DOI:** <https://doi.org/10.1152/jappphysiol.00296.2020>

**Publisher:** American Physiological Society

**Version:** Accepted Version

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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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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 ( $8.6\pm 1.2$  vs.  $2.2\pm 1.4\%$ ,  $P<0.05$ ), whereas no change in FMD  
42 was observed in Heat+WC ( $7.8\pm 1.2$  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, antegrade shear rate and FMD  
85     (44). While studies in animals and *in-vitro* studies of human endothelial cell cultures have  
86     shown an increased antegrade 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).

## **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  $\geq 6$  hours, no alcohol or caffeine for  $\geq 12$  hours, no polyphenol rich food/beverages for  $\geq 18$  hours, no vigorous exercise for  $\geq 48$  hours and no vitamin supplements for  $\geq 72$  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 ( $T_{\text{pill}}$ ; Jonah™ Core Body Temperature, Respironics, Bend, OR, USA). Data were transmitted wirelessly to monitoring device (EQ02+ LifeMonitor, Equivital,



Hidalgo, Cambridge, U.K) and then gathered with embedded application software (eqView mobile, Equivital, Hidalgo, Cambridge, U.K). Skin temperature was measured by using thermistors located at four sites (chest<sub>sk</sub>, biceps<sub>sk</sub>, thigh<sub>sk</sub> and calf<sub>sk</sub>) (Squirrel SQ2010 Data Logger; Grant, Cambridge, UK).

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

### *Experimental Protocol.*

Prior to experimental trials, participants attended a familiarization session during which study procedures were explained and methods demonstrated. Participants then returned for three separate experimental trials to investigate the impact of whole-body passive heat stress on radial artery endothelial function and blood flow pattern. Trials were conducted on three days separated by at least 24 hours and completed within 14 days. The three experimental trials were; 1) whole-body passive heat stress sufficient to raise core temperature by 1 °C (Heat), 2) whole-body passive heat stress sufficient to raise core temperature by 1 °C with concurrent inflation of a cuff placed around the right wrist to 75 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  $\geq 220$  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

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

#### *Data analysis*

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

$$T_{sk}(C^{\circ}) = 0.3 \times (Biceps_{sk} C^{\circ} + Chest_{sk} C^{\circ}) + 0.2 \times (Thigh_{sk} C^{\circ} + Calf_{sk} C^{\circ})$$

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

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

Radial artery blood flow was calculated as:

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

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

Radial artery wall shear rate was defined as:

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

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

Core temperature (Heat and Heat + WC only), skin temperature, HR, BP and radial artery characteristics were obtained prior to the start of intervention, and then every 5 min during the intervention (Heat, Heat + WC, Time Control trials). In order to make between trial comparisons of the temporal response pattern for temperature and cardiovascular 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- $\Delta$  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 ( $78.6 \pm 9.5$  min) and Heat + WC ( $76.3 \pm 12.2$  min) and Time Control ( $77.5 \pm 9.1$ ) 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 ( $32.8 \pm 0.68$  °C) and Heat + WC ( $32.7 \pm 0.83$  °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<sub>2</sub> 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

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

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

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

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

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
<b>Baseline</b>									
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 <sup>-1</sup> )	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
<b>L-FMC</b>									
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 <sup>-1</sup> )	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 <sup>-1</sup> )	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
<b>FMD</b>									
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 <sub>AUC</sub> (x10 <sup>3</sup> s <sup>-1</sup> )	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 <sub>AUC</sub> 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 <sub>AUC</sub> -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

459 Values are means (SD). L-FMC, low-flow mediated constriction; FMD, flow mediated dilatation; SR<sub>AUC</sub>, 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<sub>AUC</sub>-corrected-FMD (%) represent ANCOVA results. \* P < 0.05 vs. Pre; † P < 0.05 vs. Heat + WC; ‡ P < 0.05 vs. Time Control.

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## **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  $\pm$  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  $\pm$  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  $\pm$  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  $\pm$  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  $\pm$  SE. \*  $P < 0.05$  vs. baseline (BL); †  $P < 0.05$  vs. Heat + WC; ‡  $P < 0.05$  vs. Time Control.

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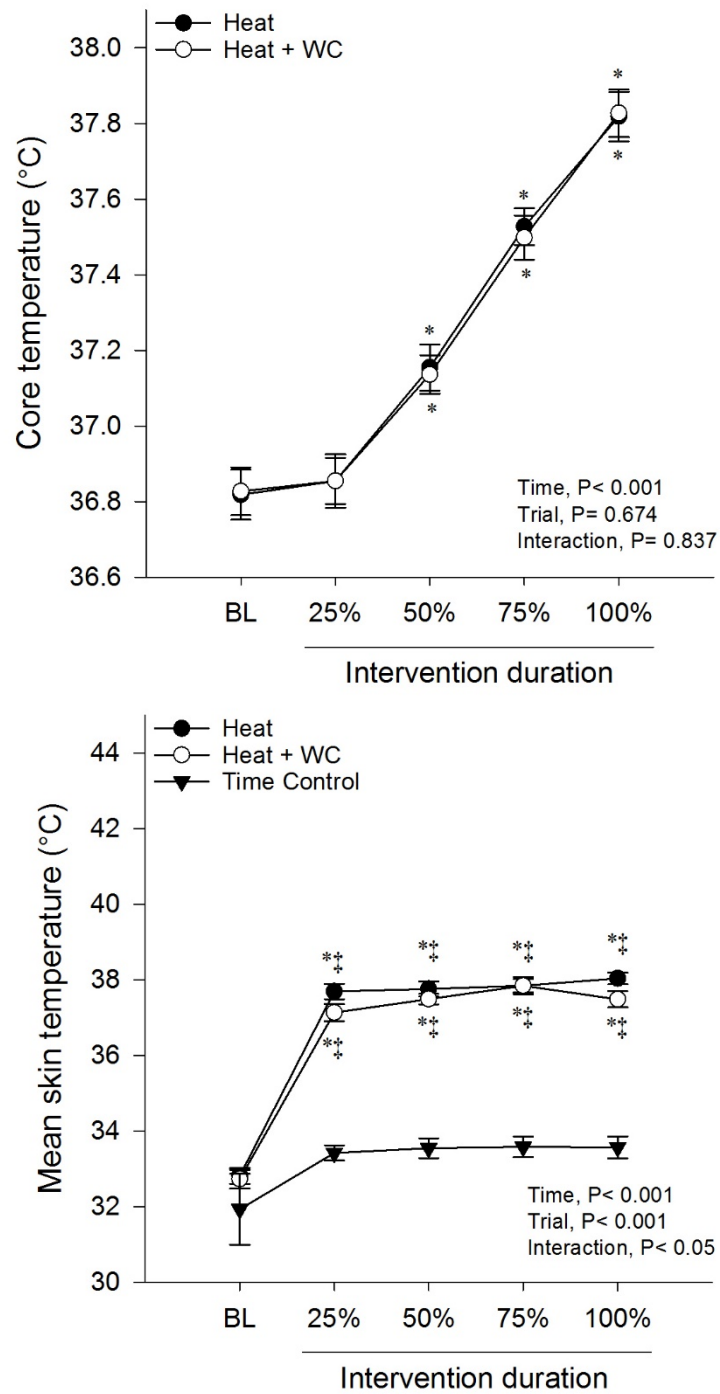
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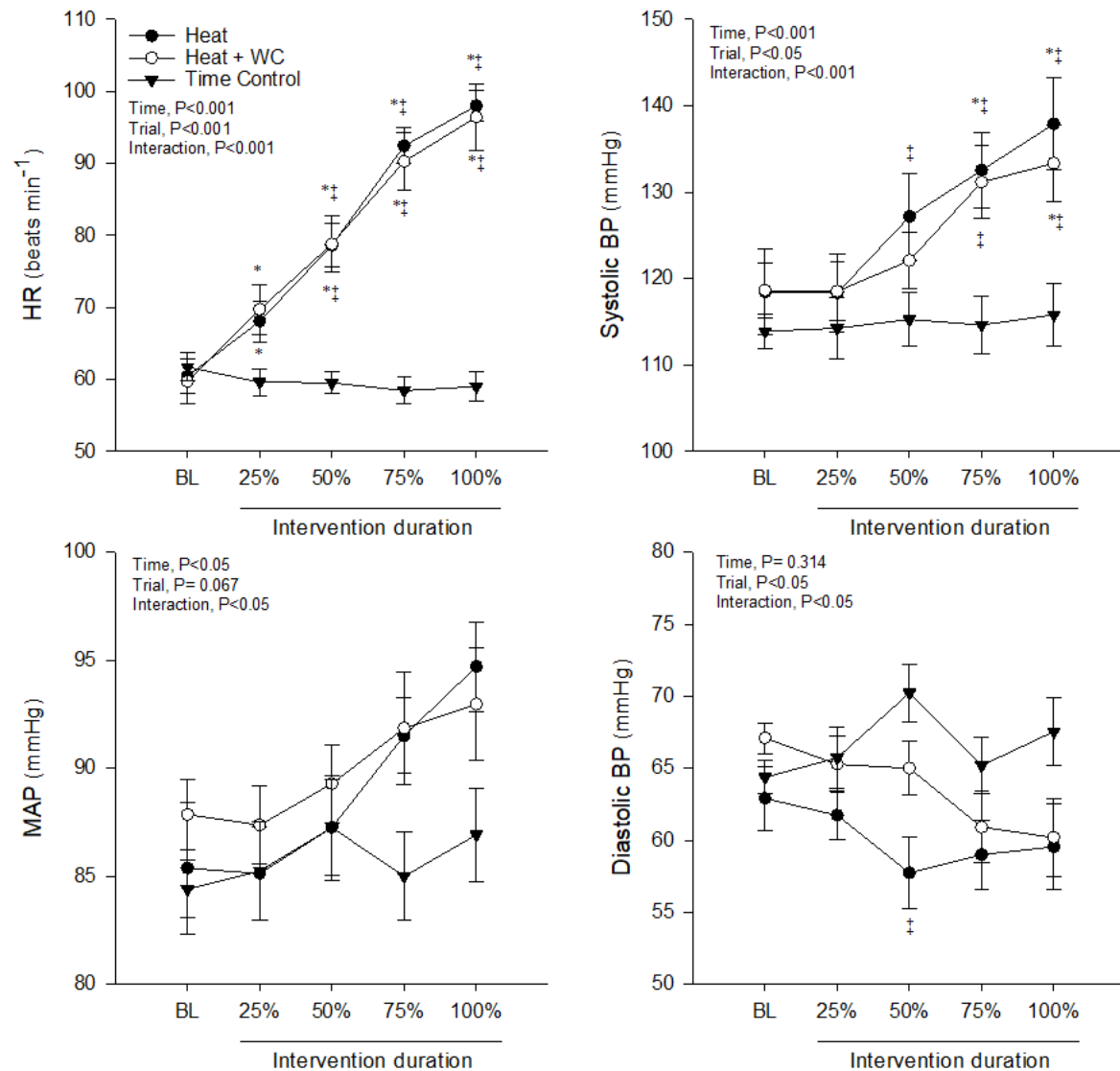
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## FIGURES

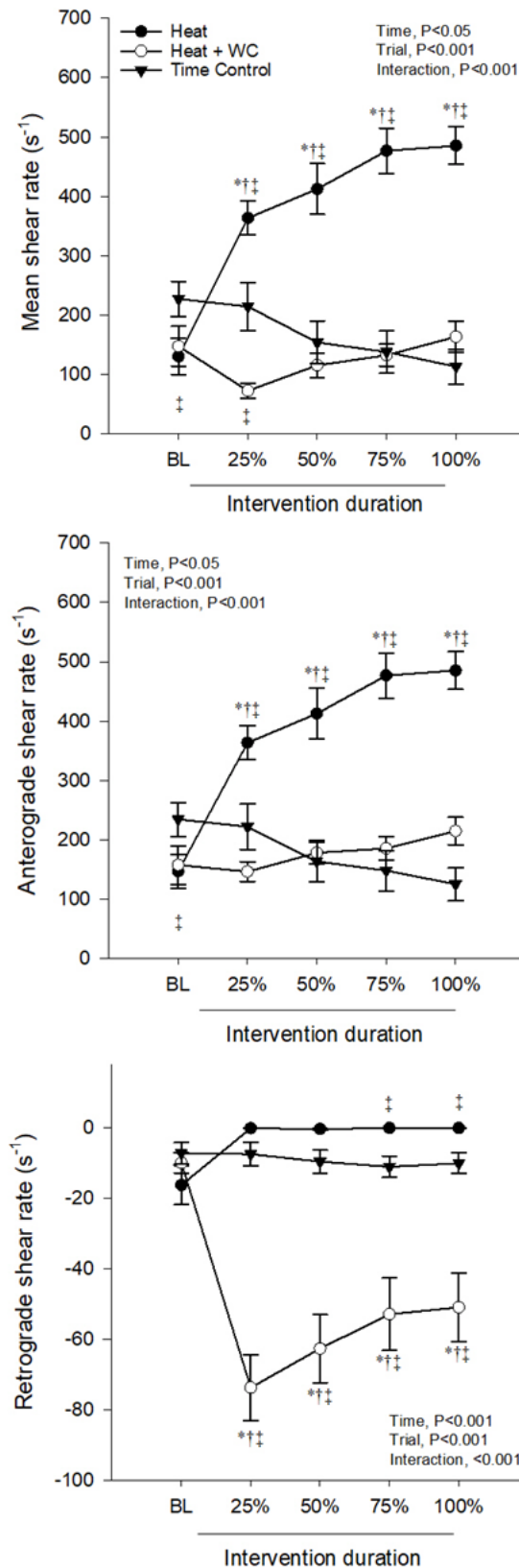


**Figure 1**

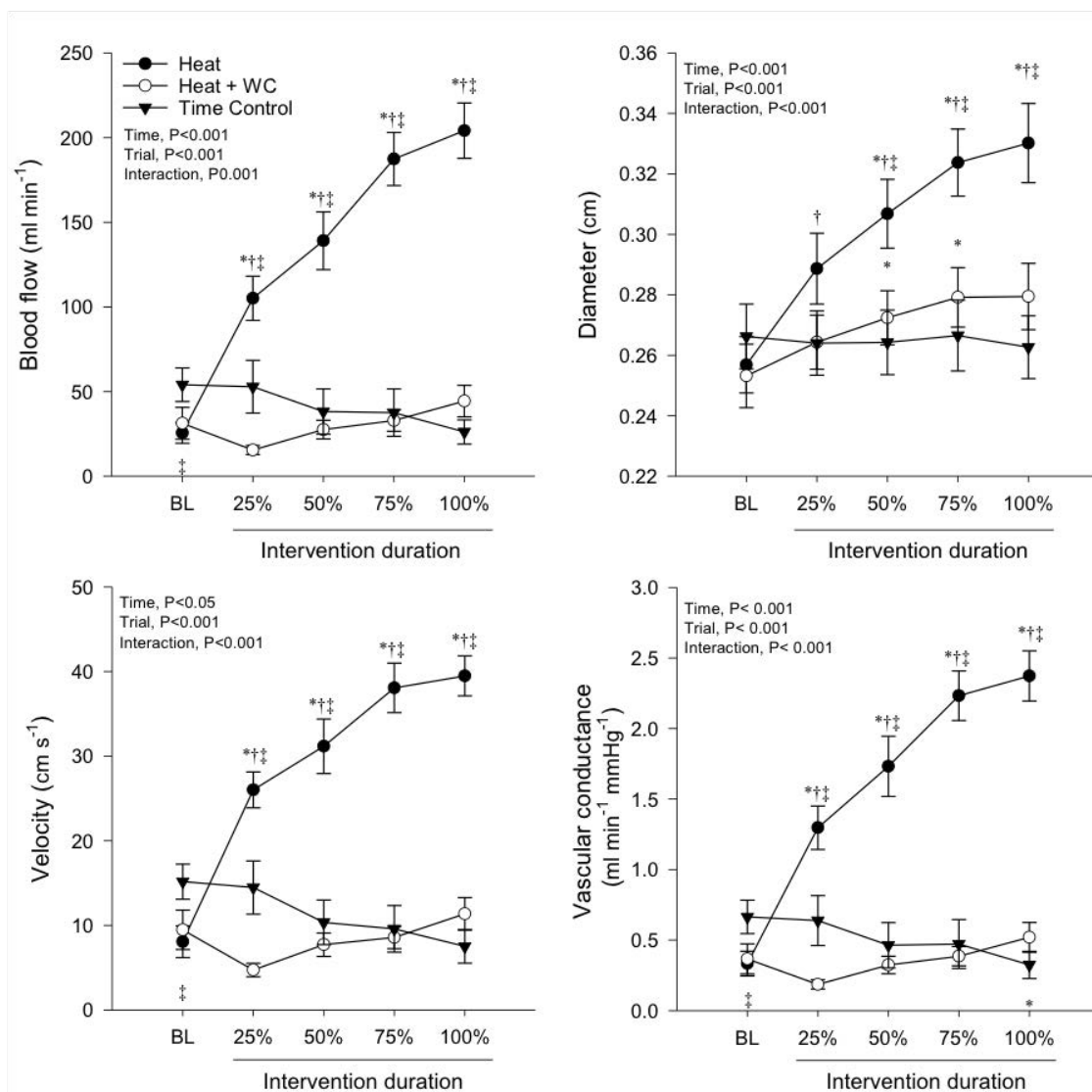


**Figure 2**

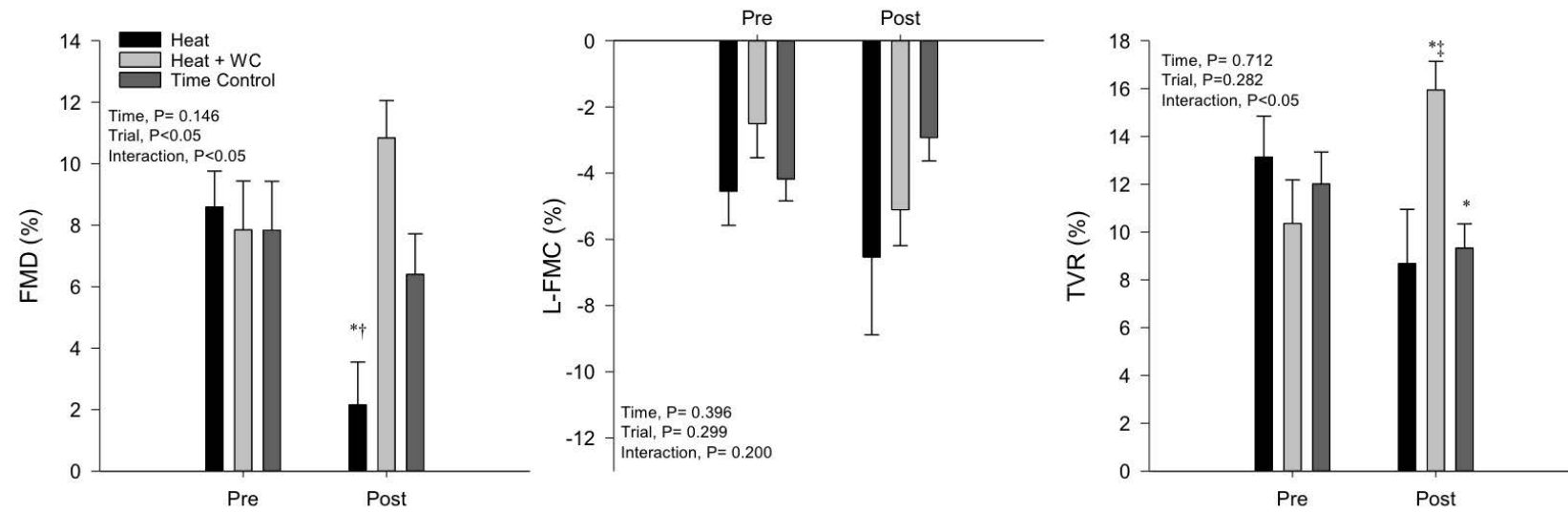




**Figure 3.**



**Figure 4.**



**Figure 5.**