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ORIGINAL ARTICLE

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Post exercise hot water immersion and hot water immersion in isolation enhance vascular, blood marker, and perceptual responses when compared to exercise alone

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Abstract

Exercise and passive heating induce some similar vascular hemodynamic, circulating blood marker, and perceptual responses. However, it remains unknown whether post exercise hot water immersion can synergise exercise derived responses and if they differ from hot water immersion alone. This study investigated the acute responses to post moderate-intensity exercise hot water immersion (EX+HWI) when compared to exercise (EX+REST) and hot water immersion (HWI+HWI) alone. Sixteen physically inactive middle-aged adults (nine males and seven females) completed a randomized cross-over counterbalanced design. Each condition consisted of two 30-min bouts separated by 10 min of rest. Cycling was set at a power output equivalent to 50% $\dot{V}O_{2\ peak}$. Water temperature was controlled at 40°C up to the mid sternum with arms not submerged. Venous blood samples and artery ultrasound scans were assessed at 0 (baseline), 30 (immediately post stressor one), 70 (immediately post stressor two), and 100 min (recovery). Additional physiological and perceptual measures were assessed at 10-min intervals. Brachial and superficial femoral artery shear rates were higher after EX+HWI and HWI+HWI when compared with EX+REST ($p < 0.001$). Plasma nitrite was higher immediately following EX+HWI and HWI+HWI than EX+REST ($p < 0.01$). Serum interleukin-6 was higher immediately after EX+HWI compared to EX+REST ($p = 0.046$). Serum cortisol was lower at 30 min in the HWI+HWI condition in contrast to EX+REST ($p = 0.026$). EX+HWI and HWI+HWI were more enjoyable than EX+REST ($p < 0.05$). Irrespective of whether hot water immersion proceeded exercise or heating, hot water immersion enhanced vascular and blood marker responses, while also being more enjoyable than exercise alone.

KEYWORDS

blood markers, enjoyment, exercise, passive heating, physical inactivity, vascular responses

1 | INTRODUCTION

Cardiovascular disease is the number one cause of mortality worldwide.[1] The pathogenesis of cardiovascular disease occurs over a period of decades, which is evidenced by impairments in endothelial function and arterial stiffness in middle-age.[2,3] Considering that deaths due to cardiovascular disease are predominantly caused by artery related diseases,[4] the initiation of preventative lifestyle strategies in middle-age to delay impairments in cardiovascular and cardiometabolic health are of great importance.

Remaining physically active across the lifespan is well-established to reduce cardiovascular disease risk.[5] Yet it is estimated that 25% of adults are either unable or unwilling to meet the minimum recommended weekly physical activity guidelines (150 min moderate intensity activity or 75 min of vigorous intensity activity, or a combination of both).[6] However, some middle-aged adults take part in infrequent, shorter and less intense bouts of physical activity and/or exercise in their weekly routine (e.g., brisk walking and cycling).[7] As the transition from an inactive state to a moderately active one elicits the largest reduction in all-cause mortality and cardiovascular disease risk,[8] it is crucial to identify complementary therapies that augment the physiological responses from smaller amounts of exercise for physically inactive populations.

Evidence suggests that heat therapy can support cardiovascular, cardiometabolic, and skeletal muscle health.[9–11] These diverse health benefits are thought to be underpinned by passive heating replicating some of the physiological responses of light to moderate-intensity exercise, such as increases in core and skin temperature, heart rate, and blood flow.[12,13] In light of these findings, we recently proposed that such overlapping responses may underpin mutual improvements in cardiorespiratory fitness, vascular health, and cardiometabolic health.[14] Despite the apparent overlap in physiological responses, two important questions remain: (1) Can post exercise hot water immersion synergize exercise derived physiological responses? (2) Do the physiological responses of post exercise hot water immersion differ from time matched hot water immersion? These questions warrant investigation as hot water immersion as an adjunct to exercise could offer a more attractive option than the continuation of exercise for many physically inactive populations that typically have poor exercise adherence.[15]

Preliminary findings have revealed that post exercise passive heating may further improve cardiorespiratory fitness, systolic blood pressure, and total cholesterol when compared to exercise alone.[16] However, the acute mechanisms which underpin these health benefits are unclear. Repeated episodic increases in body temperature and subsequent elevations in shear stress may play a vital role. In this regard, episodic elevations in shear stress underpin improvements in vascular function following both exercise training[17] and heat therapy.[18] Specifically, post exercise antegrade shear rate is positively correlated with improvements in resting vascular function.[19] Furthermore, acute reductions in systolic and diastolic blood pressure, after hot water immersion and moderate-intensity exercise, are also correlated with reductions in resting blood pressure.[20] Given the predictive value of these acute responses, it is important to investigate if longer durations of hot water immersion or using post exercise hot water immersion can synergize vascular responses.

Acute elevations in vascular shear stress[21] and temperature[22] stimulate the release of blood markers into circulation through exercise and passive heating.[14] Notably, nitric oxide is released in a shear dose dependent manner,[23] and achieving high core temperatures through hot water immersion (38.5–39°C for 60 min) is suggested to promote angiogenesis by enhancing nitric oxide bioavailability.[24] Similarly, elevations in core temperature during exercise may modulate the release of signaling molecules, such as angiogenic factors, anti-inflammatory cytokines, and catecholamines into the bloodstream.[25] The magnitude of the acute circulating vasoactive and anti-inflammatory milieu could be important for vascular health, glycemic control and chronic low-grade inflammation when repeated over a period of months. However, many of these signaling molecules are particularly sensitive to the intensity and duration of the stimulus[26]; indeed 30 min of moderate intensity exercise does not appear sufficient to induce changes in nitric oxide[27] or anti-inflammatory cytokines.[28] Such findings emphasize the need to assess whether either extending the hot water immersion stimulus or using hot water immersion after smaller amounts of exercise can augment circulating angiogenic and anti-inflammatory responses.

The purpose of this study was to investigate the acute vascular, circulating blood marker, and perceptual responses to post moderate-intensity exercise hot water immersion in physically inactive middle-aged adults, in comparison to exercise and hot water immersion alone. It was hypothesized that: (1) Post exercise hot water immersion will augment vascular and blood marker responses when compared to exercise alone, (2) hot water immersion alone will replicate the vascular responses of post exercise hot water immersion and (3) hot water immersion

after exercise and independently will be perceived as more enjoyable than exercise alone.

2 | MATERIALS AND METHODS

2.1 | Ethical approval

The study was approved by Coventry University ethics committee (P97102) and preregistered at ClinicalTrials.gov (NCT05035004). All participants completed written informed consent and health questionnaires prior to enrolment on the study. All procedures conformed with the Declaration of Helsinki.

2.2 | Participants

Sixteen physically inactive middle-aged adults (sex: nine males and seven females; age: 54 ± 6 years; body mass: 82 ± 15 kg; height: 1.69 ± 1 m; body mass index: 29 ± 4 kg/m^2, $\dot{V}O_{2\ peak}$: 24 ± 6 mL/kg/min) were recruited for this study. Prior to starting the study, participants completed health, and physical activity screening forms. Physical inactivity was assessed according to the International Physical Activity Questionnaire and self-reported activity over the 6 months prior to study enrolment (<150 min of moderate-intensity activity or < 75 min of vigorous intensity activity, or a combination of both per week). All participants were nonsmokers with no history of cardiovascular, metabolic, or renal disease, nor any contraindications to exercise. Those on medications were excluded from the study (e.g., blood pressure, cholesterol, anti-inflammatory, and hormone replacement therapy). Women were post-menopausal to minimize the potential influence of circulating hormones on vascular responses. A resting 12-lead electrocardiogram was performed to confirm normal sinus rhythm, which was followed by resting blood pressure in duplicate to ensure participants were not above the exclusion criteria (systolic blood pressure >160 and/or diastolic blood pressure >100 mmHg). Sit to stand blood pressure was then also assessed to a measure orthostatic hypotension (drop of systolic blood pressure >20 and/or diastolic blood pressure >10 upon standing). Participants were excluded if they suffered from orthostatic hypotension as this increases the risk of syncope and pre-syncope symptoms in the heat. Trials were carried out under continuous supervision due to the risk of thermal intolerance and dizziness upon standing.[29] One participant suffered from pre-syncope symptoms (dizziness and nausea) when exiting the hot tub immediately after the second bout of immersion in the HWI+HWI condition but continued the trial and study.

2.3 | Experimental design

The study was a randomized cross-over counterbalanced design (Figure 1). Participants first took part in a preliminary visit for health assessments and $\dot{V}O_{2peak}$ testing, after which participants were randomized to three conditions: (1) Exercise followed by hot water immersion (EX+HWI), (2) exercise followed by rest (EX+REST) and (3) hot water immersion followed by hot water immersion (HWI+HWI). Each condition consisted of two 30-min bouts which were separated by a 10 min rest period. All outcome measures were assessed within a 10 ± 1-min window post stressor. Based on pilot testing, this was the minimum duration required to assess all measures, while also maintaining elevations in core temperature. Measures were assessed in order of importance: (1) brachial artery ultrasound scan (1–4 min), (2) venous blood sample (3–7 min) and (3) superficial femoral artery scan (6–10 min). Exercise consisted of 30 min cycling at a power output equivalent to 50% $\dot{V}O_{2peak}$ (3.4 ± 0.8 METs) on a cycle ergometer (Lode Corival, Groningen, Netherlands). The hot water immersion bout consisted of 30 min immersion up to the mid sternum, with arms unsubmerged, at a water temperature of 40 ± 0.5°C. The same commercially available hot tub (Lay-Z-Spa Vegas, Devon, UK) was used for all sessions, with water temperature maintained by the generator set to 40°C and additional hot/cold water added or removed from the tap as required. Pre, during and post each visit, a range of outcomes were assessed, including rectal temperature, skin temperature, heart rate, blood pressure, brachial and superficial femoral artery shear rates, venous blood markers, thermal sensation, thermal comfort, basic affect, and enjoyment. Each visit was separated by a > 1-week washout period. All laboratory visits took place in the morning (±1 h) to control for the potential influence of circadian rhythm on all measures. Room temperature and relative humidity were 20 ± 1°C and 47 ± 12%, respectively (5400 Kestrel, Boothwyn, USA).

2.4 | Preliminary procedures

2.4.1 | Maximal exercise test

Participants completed a maximal exercise test on a cycle ergometer (Lode Corival, Groningen, Netherlands). The protocol started at 40 W and increased by 20 W every 3-min, with a cadence of 70–80 rpm. Respiratory gases were measured continuously by a breath-by-breath gas analyzer (Ultima™ Series PFX, Medical Graphics, Tewkesbury, UK), with heart rate and rating of perceived exertion[30] measured at 3-min intervals. Peak

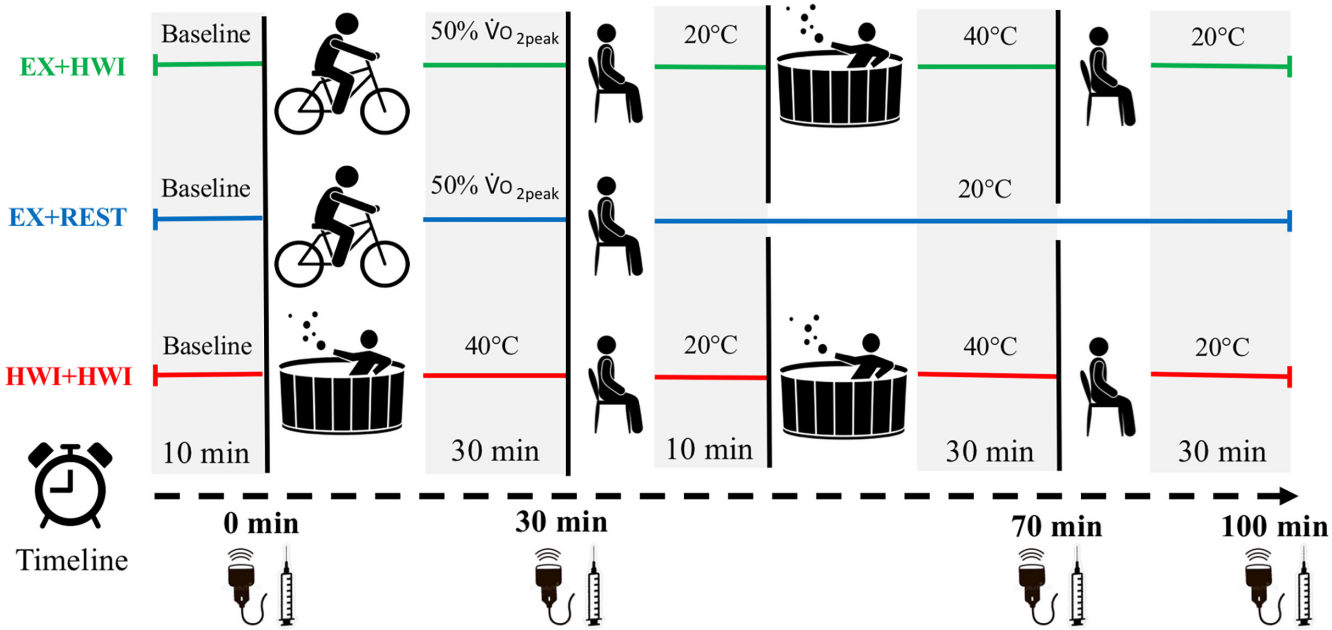

FIGURE 1 A schematic representing the randomized cross-over counterbalanced design for the EX+HWI, EX+REST and HWI+HWI conditions. EX+HWI: Stationary cycling for 30 min at a power output equivalent to 50% $\dot{V}O_{2peak}$ (3.4 ± 0.8 METs), followed by 10 min of rest at 20°C, 30 min of hot water immersion at 40°C and then 30 min of seated rest at 20°C. EX+REST: Stationary cycling for 30 min at a power output of 50% $\dot{V}O_{2peak}$ (3.4 ± 0.8 METs), followed by 70 min of seated rest at 20°C. HWI+HWI: Hot water immersion for 30 min at 40°C, followed by 10 min of rest at 20°C, 30 min of hot water immersion at 40°C and then 30 min of seated rest at 20°C. Brachial and superficial artery ultrasound scans and venous blood samples were taken at 0 min (baseline) 30 min (immediately post stressor one), 70 min (immediately post stressor two) and 100 min (recovery) in all conditions.

oxygen uptake was the average of the final 30 s of cycling before termination. To calculate the power outputs required in the experimental sessions, mean oxygen consumption in the final 30 s of each incremental stage was plotted against power output and a linear regression was performed. The subsequent equation was used to establish a standardized power out for each participant (50% $\dot{V}O_{2peak}$).

2.4.2 | Diet control and anthropometric measurements

In the 24 h prior to the experimental visits, participants were asked to avoid strenuous exercise, antihistamines, caffeine, alcohol, and nitrite/nitrate rich foods (e.g., beetroot, rocket, spinach, lettuce cabbage radishes and highly processed meats). All participants adhered to these instructions. Participants arrived at the laboratory after an overnight fast and drank 5 mL/kg of water upon arrival to help control hydration levels. Anthropometric parameters including height and nude body mass were measured to allow for the calculation of body mass index. Participants then inserted a rectal probe 10 cm beyond the anal sphincter to monitor rectal temperature.

2.5 | Experimental procedures

2.5.1 | Vascular assessments

In accordance with the European Society of Cardiology,[31] baseline blood pressure (M3 Omron, Kyoto, Japan) was measured in duplicate after 10 min of seated rest, with a 2-min rest period between measurements. An additional recording was taken if the first two readings differed by >10 mmHg. Systolic blood pressure and diastolic blood pressure were reported as an average of the final two measurements. After baseline blood pressure, participants laid supine to allow ultrasound recordings of the brachial and superficial femoral arteries. Ultrasound scans of the brachial and then superficial femoral artery were completed in a supine position at 0 min (baseline) 30 min (immediately post stressor one), 70 min (immediately post stressor two) and 100 min (recovery) by the same trained sonographer. The brachial artery was scanned before the superficial femoral artery at all time points in order to prioritize the systemic rather than local vascular response. Brachial and superficial femoral artery diameter and velocities were recorded continuously for 30 s using a 15-MHz multifrequency linear array probe attached to a high-resolution duplex ultrasound

machine (Terason uSmart 3300, Teratech, Burlington, MA, USA) on the right arm and leg of all participants. Ultrasound parameters were set to optimize the longitudinal B-mode image of the lumen-arterial wall interface with concurrent Doppler velocities collected using the lowest possible insonation angle (always <60°). Transducer location was marked on the skin to ensure the same site was measured during a single trial. External and internal landmarks were used between trials to enhance repeatability of the brachial artery and superficial femoral artery scans, with the distance from the antecubital fossa and common femoral bifurcation standardized (2–3 cm). The depth, focus position and gain settings were standardized for each individual participant and then replicated for each subsequent visit. Analysis of artery diameter and flow was performed using custom-designed edge-detection and wall-tracking software, which is independent of investigator bias and has been previously described elsewhere.[32] From synchronized diameter and velocity data, mean blood flow (the product of lumen cross-sectional area and Doppler velocity) was calculated at 30 Hz and shear rate, an estimate of shear stress without viscosity, was calculated as 4 × mean blood velocity/vessel diameter. Antegrade and retrograde blood flow, and shear rate, were then calculated from the corresponding mean blood velocities per cardiac cycle using the average of only positive or negative data points, respectively. Reproducibility of diameter measurements using this semiautomated software is significantly better than manual methods, reduces observer error, and possesses an intra-observer coefficient of variation of 6.7%.[32]

2.5.2 | Venous blood sampling and analysis

Venous blood samples were taken from the median cubital vein into a 10 mL BD serum and 6 mL BD Lithium Heparin tube (Nu-Care, Bedfordshire, UK) by trained phlebotomists. Venous blood samples were collected at 0 min (baseline) 30 min (immediately post stressor one), 70 min (immediately post stressor two) and 100 min (recovery). In the EX+REST condition, researchers were unable to successfully take one blood sample at 30 and 70 min, and two at 100 min. These missing blood samples were accounted for in the statistical analysis through linear mixed modeling, which is well-established to handle random missing data through maximum likelihood estimation.[33] After collection, Lithium Heparin whole blood samples were centrifuged immediately for 8 min at 1500 × g, and serum samples were left to clot for 45 min and then centrifuged for 12 min at 3000 × g. The resulting plasma and serum were then aliquoted into Eppendorfs and stored at −80°C until later analysis.

Prior to nitrite analysis, plasma was deproteinised using ice-cold methanol. Specifically, 500 μL of plasma was treated with 1000 μL of ice-cold methanol before being centrifuged at 12000 × g with the supernatant used for subsequent analysis. Plasma nitrite concentration was analyzed through injecting 500 μL of deproteinised plasma into a gas tight purge vessel containing glacial acetic acid and aqueous sodium iodide (4% wt/vol). Plasma concentrations of nitrite were established through the reduction of nitrite to gaseous nitric oxide. A thermal electrically cooled red-sensitive photomultiplier tube housed in a Sievers gas-phase chemiluminescence nitric oxide analyzer (Sievers NOA 280i; Analytix, Durham, United Kingdom) was used to determine the spectral emission of electronically excited nitrogen dioxide resulting from the reaction between nitric oxide and ozone. Plotting the signal area (mV) against the sodium nitrite standards allowed the concentration of nitrite to be calculated. Serum samples were analyzed through custom designed Luminex multiplex assays and enzyme-linked immunosorbent assays (R&D Systems Ltd, Abingdon, UK). Samples were analyzed in duplicate, and the manufacturer's instructions were followed at all times. All analytes were corrected for plasma volume changes based upon established criteria.[34] The intra-plate coefficients for the assays were as follows: Nitrite: 4.0 ± 2.3%, vascular endothelial growth factor (VEGF): 6.0 ± 0.6%, endothelin-1 (ET-1): 6.9 ± 0.7%, interleukin-6 (IL-6): 6.7 ± 1.6%, interleukin-10 (IL-10): 7.2 ± 1.7%, interleukin-1 receptor antagonist (IL-1Ra): 5.2 ± 0.9%, matrix metalloproteinase-10 (MMP-10): 4.6 ± 1.6%, matrix metalloproteinase-9 (MMP-9): 1 ± 0.2%, matrix metalloproteinase-2 (MMP-2): 1.2 ± 0.1%, cortisol: 6.1 ± 0.9%, adrenaline: 2.3 ± 0.4% and noradrenaline: 3.1 ± 0.8%.

2.5.3 | Thermo-physiological and perceptual measures

While seated at baseline, and at 10 min intervals during exercise and immersion, heart rate (FT1 Polar, Espoo, Finland), rectal temperature (probe), forehead temperature (thermistor) (Etek 1000 SQ1022) and perceptual measures, including thermal sensation (+5 hot to −5 cold), thermal comfort (+5 very comfortable to −5 very uncomfortable)[35] and basic affect (+5 very good to −5 very bad)[36] were recorded. After each visit, participants completed an 8-item physical activity enjoyment scale, which included the subscales pleasure, fun, pleasant, invigorating, gratifying, exhilarating, stimulating and refreshing (7 positive to

1 negative).[37] Finally, body mass was also assessed pre and post each visit as a surrogate for sweat loss.

2.6 | Statistical analysis

A linear mixed model was used to examine if there was an additive effect of post exercise hot water immersion, with time and condition as fixed effects and participant as the random effect. Parametric and nonparametric data were analyzed through linear mixed modeling due to its robustness to violations of distributional assumptions.[33] If a significant time × condition interaction was observed, post-hoc tests were performed using Tukey's test of multiple comparisons to identify the location of any differences. If there were no interaction effects between time and condition, the main effects of time were examined using the Tukey method to assess the time course of any differences. Physical activity enjoyment results were analyzed through Friedman tests. On the occurrence of a main effect, post hoc pairwise comparisons with Dunn's corrections were conducted. Where appropriate, post hoc results were provided with mean or median differences and confidence intervals (95% CI). Data are presented as means and standard deviations (SD). The alpha value was a priori set at $p < 0.05$ for all tests. All analyses were completed in SPSS (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.), GraphPad Prism 9 and Excel (Microsoft, Version 2019, America).

3 | RESULTS

3.1 | General physiological responses

A time × condition interaction was observed for rectal ($F = 47.27$, $p < 0.001$) and skin forehead ($F = 15.05$, $p < 0.001$) temperatures, heart rate ($F = 48.78$, $p < 0.001$), systolic blood pressure ($F = 2.64$, $p < 0.001$), diastolic blood pressure ($F = 5.34$, $p < 0.001$) and mean arterial pressure ($F = 4.81$, $p < 0.001$) (Figure 2). There were no differences in the delta changes for sweat loss between the EX+HWI (−0.5 ± 0.3 L), EX+REST (−0.4 ± 0.3 L) and HWI+HWI (−0.6 ± 0.4 L) conditions ($p = 0.144$).

3.2 | Vascular responses

3.2.1 | Mean brachial artery shear rate

A time × condition interaction was present for mean brachial artery shear rate ($F = 40.28$, $p < 0.001$). Mean brachial artery shear rate was higher in the EX+HWI condition compared to the EX+REST condition, peaking at 70 min ($p < 0.001$, mean difference = 351 s^{-1}, 95% CI: 287–415 s^{-1}) and remaining elevated at 100 min ($p = 0.016$, mean difference = 44 s^{-1}, 95% CI: 7–81 s^{-1}). Likewise, mean brachial artery shear rate was higher in the HWI+HWI condition when compared to the EX+REST condition, peaking at 70 min ($p < 0.001$, mean

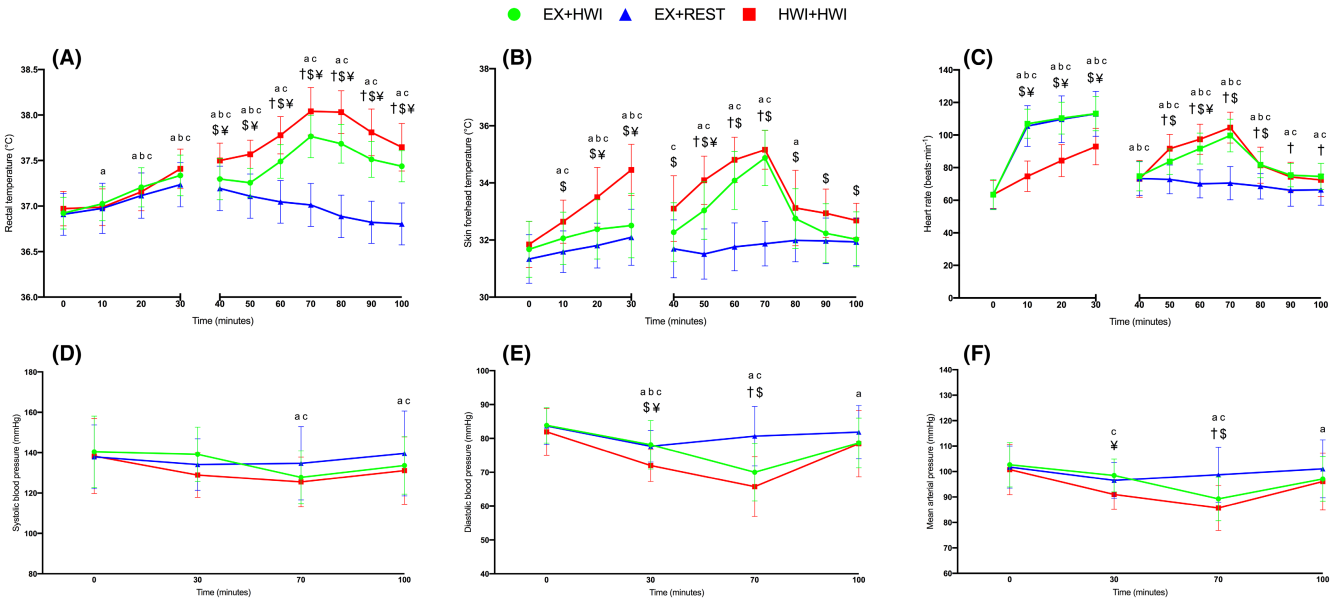

FIGURE 2 Rectal temperature (A), skin forehead temperature (B), heart rate (C), systolic blood pressure (D), diastolic blood pressure (E) and mean arterial pressure (F) in the EX+HWI, EX+REST and HWI+HWI conditions. Post hoc time × condition interactions are symbolized as follows: EX+HWI vs EX+REST († = $p < 0.05$), HWI+HWI vs EX+REST ($ = $p < 0.05$) and EX+HWI vs HWI+HWI (¥ = $p < 0.05$). Post hoc within condition differences from baseline are denoted as follows: EX+HWI (a = $p < 0.05$), EX+REST (b = $p < 0.05$) and HWI+HWI (c = $p < 0.05$). Lines and whiskers represent means and standard deviations ($n = 16$).

difference = 384 s^{-1}, 95% CI: 306–462 s^{-1}) and remaining elevated at 100 min ($p < 0.001$, mean difference = 64 s^{-1}, 95% CI: 26–102 s^{-1}) (Figure 3A). Additional brachial artery parameters assessed in this study are summarized in Table 1.

3.2.2 | Mean superficial femoral artery shear rate

A time × condition interaction was observed for mean superficial femoral artery shear rate ($F = 33.90$, $p < 0.001$). Mean superficial femoral artery shear rate was higher at 30 min in the HWI+HWI condition when compared to the EX+HWI ($p < 0.001$, mean difference = 92 s^{-1}, 95% CI: 41–143 s^{-1}) and EX+REST ($p < 0.001$, mean difference = 94 s^{-1}, 95% CI: 42–147 s^{-1}) conditions. Mean superficial femoral artery shear rate was higher in the EX+HWI condition when compared to the EX+REST condition, peaking at 70 min ($p < 0.001$, mean difference = 151 s^{-1}, 95% CI: 96–206 s^{-1}). Similarly, mean superficial femoral artery shear rate was also higher at 70 min in the HWI+HWI condition in comparison to the EX+REST condition ($p < 0.001$, mean difference = 128 s^{-1}, 95% CI: 77–178 s^{-1}) (Figure 3B). Further superficial femoral artery outcomes are summarized in Table 1.

3.2.3 | Vasoactive markers

A time × condition interaction was observed for nitrite ($F = 8.40$, $p < 0.001$) and a main effect of time for

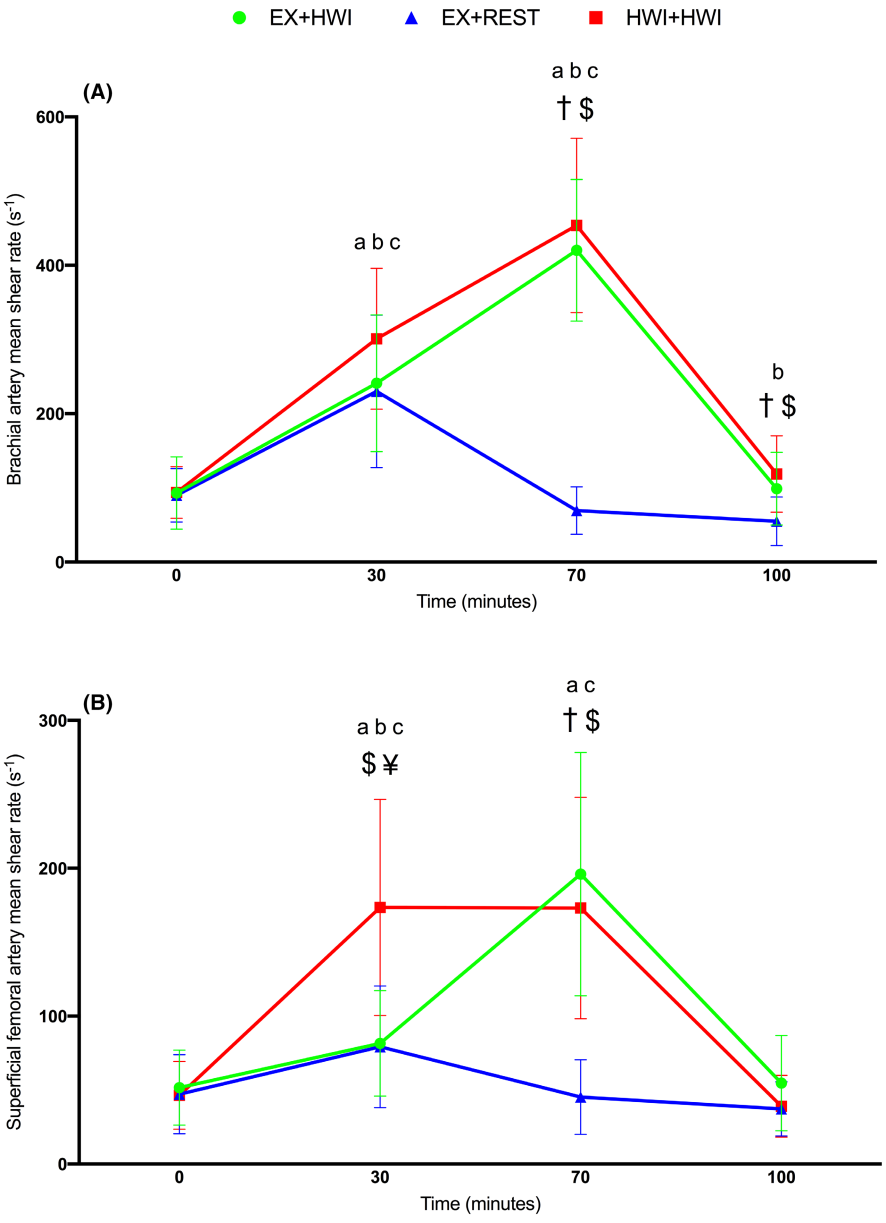

FIGURE 3 Mean brachial artery (A) and superficial femoral artery shear rate (B) responses in the EX+HWI, EX+REST and HWI+HWI conditions. Post hoc time × condition interactions are symbolized as follows: EX+HWI vs EX+REST († = $p < 0.05$), HWI+HWI vs EX+REST ($ = $p < 0.05$) and EX+HWI vs HWI+HWI (¥ = $p < 0.05$). Post hoc within condition differences from baseline are denoted as follows: EX+HWI (a = $p < 0.05$), EX+REST (b = $p < 0.05$) and HWI+HWI (c = $p < 0.05$). Lines and whiskers represent means and standard deviations ($n = 16$).

TABLE 1 Vascular hemodynamic measures at baseline, 30, 70, and 100 min ($n = 16$).

Variable	EX+HWI				EX+REST				HWI+HWI				Statistical significance
	Baseline	30 min	70 min	100 min	Baseline	30 min	70 min	100 min	Baseline	30 min	70 min	100 min	Interaction
Brachial artery													
Antegrade shear rate (s^{-1})	104 ± 47	246 ± 85 a	420 ± 93 †a	109 ± 45	102 ± 37	234 ± 100 b	87 ± 34	79 ± 43 b	105 ± 33	302 ± 91 c	454 ± 114 $c	127 ± 50 $	$p < 0.001$
Retrograde shear rate (s^{-1})	−11 ± 7	−5 ± 7 a	0 †a	−10 ± 8 †	−12 ± 13	−4 ± 7 b	−18 ± 14	−24 ± 16	−11 ± 10	−1 ± 3 c	0 $c	−8 ± 7 $	$p < 0.001$
Blood flow (ml/min)	80 ± 38	218 ± 134 a	476 ± 218 †a	91 ± 61 †	90 ± 56	209 ± 123 b	64 ± 40	41 ± 17	92 ± 61	339 ± 230 c	560 ± 245 $c	115 ± 67 $	$p < 0.001$
Diameter (mm)	4.2 ± 0.8	4.1 ± 0.8	4.5 ± 0.8 a	4.2 ± 0.8	4.3 ± 0.9	4.2 ± 0.7	4.3 ± 0.8	4.1 ± 0.8	4.2 ± 0.9	4.4 ± 0.9 c	4.7 ± 0.7 c	4.3 ± 0.8	$p < 0.001$
Superficial femoral artery													
Antegrade shear rate (s^{-1})	74 ± 34	97 ± 36 ¥a	200 ± 79 †a	79 ± 35	70 ± 33	100 ± 44 b	68 ± 33	62 ± 23	67 ± 27	181 ± 65 $c	182 ± 68 $c	65 ± 22	$p < 0.001$
Retrograde shear rate (s^{-1})	−22 ± 12	−15 ± 8 a	−4 ± 5 †a	−24 ± 13	−23 ± 15	−21 ± 15	−23 ± 15	−25 ± 11	−21 ± 9	−7 ± 13 $c	−9 ± 10 $c	−26 ± 10	$p < 0.001$
Blood flow (mL/min)	159 ± 65	244 ± 85 ¥	647 ± 216 †	155 ± 56	154 ± 82	230 ± 89	134 ± 76	121 ± 58	139 ± 42	626 ± 277 $	573 ± 215 $	118 ± 27	$p < 0.001$
Diameter (mm)	6.5 ± 1.1	6.4 ± 0.8	6.6 ± 1	6.5 ± 1.3	6.5 ± 1	6.4 ± 1.2	6.3 ± 0.9	6.5 ± 1	6.4 ± 0.7	6.7 ± 0.9	6.6 ± 1	6.5 ± 0.8	$p = 0.056$

Note: The vascular hemodynamic responses of the brachial and superficial femoral arteries in the EX+HWI, EX+REST and HWI+HWI conditions. Post hoc time × condition interactions are symbolized as follows: EX+HWI vs EX+REST († = $p < 0.05$), HWI+HWI vs EX+REST ($ = $p < 0.05$) and EX+HWI vs HWI+HWI (¥ = $p < 0.05$). Post hoc within condition differences from baseline are denoted as follows: EX+HWI (a = $p < 0.05$), EX+REST (b = $p < 0.05$) and HWI+HWI (c = $p < 0.05$). Data are expressed as means ± SD.

VEGF ($F = 5.67$, $p = 0.002$) and ET-1 ($F = 3.34$, $p = 0.032$). Nitrite was elevated at 30 min in the HWI+HWI condition when compared to the EX+HWI ($p < 0.001$, mean difference = 68 nM, 95% CI: 36–100 nM) and EX+REST ($p = 0.003$, mean difference = 58 nM, 95% CI: 18–98 nM) conditions. At 70 min nitrite was elevated in the EX+HWI ($p = 0.004$, mean difference = 72 nM, 95% CI: 22–122 nM) and HWI+HWI ($p = 0.002$, mean difference = 79 nM, 95% CI: 28–131 nM) conditions, in contrast to the EX+REST condition (Figure 4A–C).

3.2.4 | Interleukins

A time × condition interaction was detected for IL-6 ($F = 4.57$, $p < 0.001$) and IL-1Ra ($F = 6.89$, $p = 0.002$). A main effect of time was also apparent for IL-10 ($F = 6.06$, $p = 0.002$). IL-6 was higher at 70 min only in the EX+HWI condition when compared to the EX+REST condition ($p = 0.046$, mean difference = 0.37 pg/mL, 95% CI: 0–0.72 pg/mL). This was despite an increase in IL-6 within the HWI+HWI condition at 70 min ($p = 0.001$, mean difference = 0.27 pg/mL, 95% CI: 0.09–0.45 pg/mL). Within the EX+HWI and HWI+HWI conditions, IL-1Ra was higher from 70 min onwards when compared to baseline, peaking at 70 min in the EX+HWI ($p = 0.036$, mean difference = 226 pg/mL, 95% CI: 13–439 pg/mL) and HWI+HWI ($p = 0.015$, mean difference = 106 pg/mL, 95% CI: 19–192 pg/mL) conditions (Figure 4D–F).

3.2.5 | Matrix metalloproteinases

A time × condition interaction was detected for MMP-10 ($F = 6.98$, $p < 0.001$) and a main effect of time was observed for MMP-9 ($F = 7.67$, $p < 0.001$) and MMP-2 ($F = 5.71$, $p = 0.002$). Within the EX+HWI condition, MMP-10 increased at 70 min in comparison to baseline ($p = 0.003$, mean difference = 117 pg/mL, 95% CI: 40–194 pg/mL). Furthermore, there were opposing responses at 100 min, with MMP-10 decreasing within the EX+REST condition ($p < 0.001$, mean difference = 107 pg/mL, 95% CI: −184 to −30 pg/mL) and increasing within the HWI+HWI condition ($p = 0.028$, mean difference = 53 pg/mL, 95% CI: 5–100 pg/mL) when compared to baseline (Figure 4G–I).

3.2.6 | Catecholamines and cortisol

A time × condition interaction was apparent for cortisol ($F = 4.20$, $p < 0.001$). In addition, a main effect of time was observed for adrenaline ($F = 5.53$, $p = 0.003$) and noradrenaline ($F = 5.34$, $p = 0.006$). Cortisol was lower at

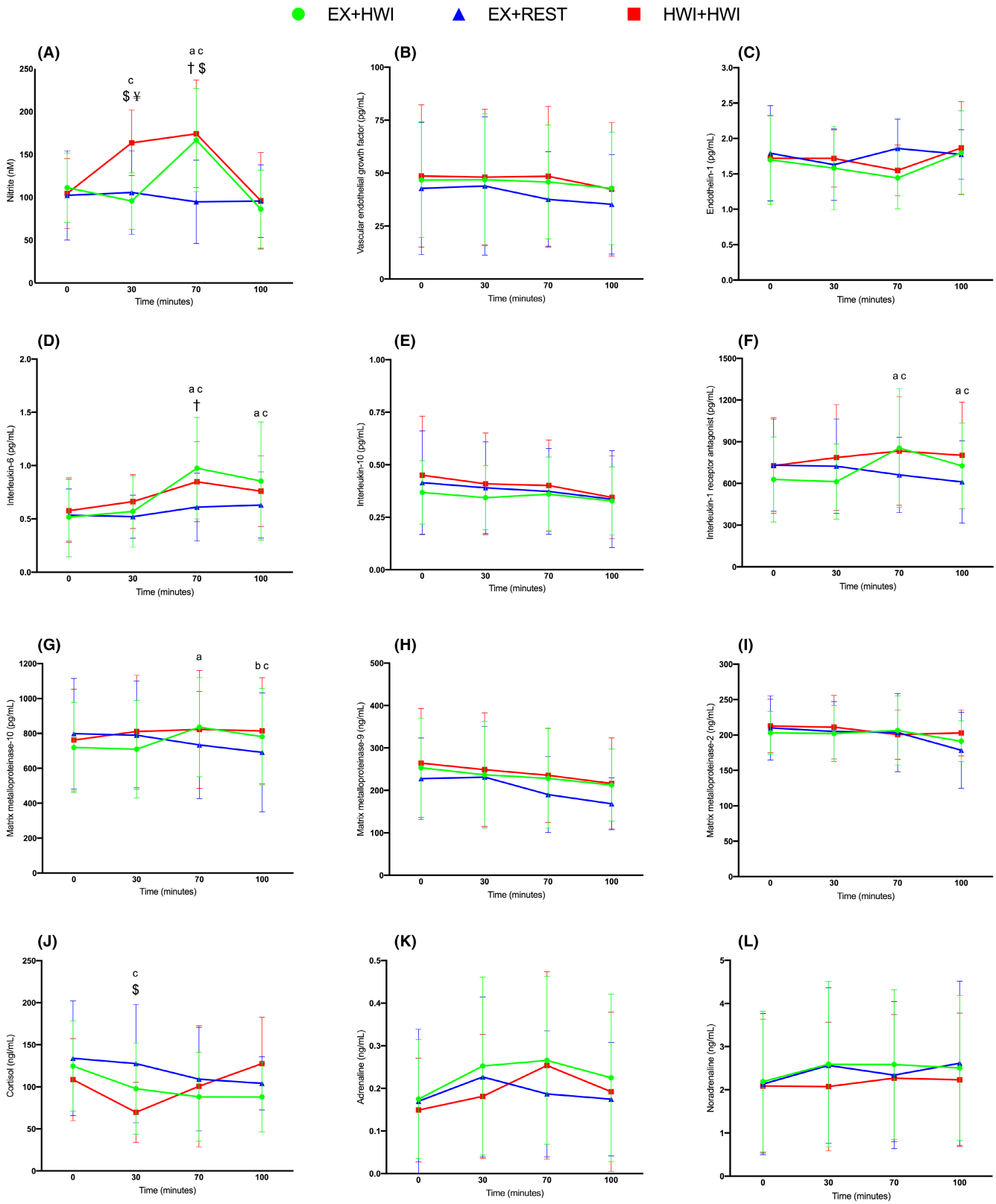

FIGURE 4 Circulating analytes, including Nitrite (A), VEGF (B), ET-1 (C), IL-6 (D), IL-10 (E), IL-1Ra (F), MMP-10 (G), MMP-9 (H), MMP-2 (I), Cortisol (J), Adrenaline (K) and Noradrenaline (L) over time in the EX+HWI, EX+REST and HWI+HWI conditions. Post hoc time × condition interactions are symbolized as follows: EX+HWI vs EX+REST (†=$p<0.05$), HWI+HWI vs EX+REST ($=$p<0.05$) and EX+HWI vs HWI+HWI (¥=$p<0.05$). Post hoc within condition differences from baseline are denoted as follows: EX+HWI (a=$p<0.05$), EX+REST (b=$p<0.05$) and HWI+HWI (c=$p<0.05$). Lines and whiskers represent means and standard deviations ($n=16$). In the EX+REST condition, one sample was not taken at 30 and 70 min, as well as two at 100 min.

30 min in the HWI+HWI condition when compared to the EX+REST condition ($p = 0.026$, mean difference = 58 ng/mL, 95% CI: −109 to −7 ng/mL) (Figure 4J–L).

3.2.7 | Perceptual responses

A time × condition interaction was observed for thermal sensation ($F = 13.96$, $p < 0.001$), thermal comfort ($F = 2.99$, $p < 0.001$) and basic affect ($F = 3.14$, $p < 0.001$). Thermal sensation was higher at 10 min and from 30 to 90 min in the HWI+HWI condition when compared to the EX+REST condition ($p < 0.05$). From 50 to 90 min, the EX+HWI condition also had a higher thermal sensation when compared to the EX+REST condition ($p < 0.05$). EX+HWI and HWI+HWI only differed at 10 min, with thermal sensation higher in the HWI+HWI condition ($p = 0.008$, mean difference = 1 a.u., 95% CI: 0–2 a.u.) (Figure 5A). Thermal comfort was higher at 10 min in the HWI+HWI condition in comparison to the EX+HWI ($p < 0.001$, mean difference = 2 a.u., 95% CI: 1–3 a.u.) and EX+REST ($p < 0.001$, mean difference = 2 a.u., 95% CI: 1–2 a.u.) conditions (Figure 5B). Basic affect was higher at 10 min in the HWI+HWI condition when compared to the EX+HWI condition ($p = 0.040$, mean difference = 1 a.u., 95% CI: 0–2 a.u.) (Figure 5C).

A main effect of condition was detected for enjoyment ($p < 0.001$), with EX+HWI ($p = 0.004$) and HWI+HWI ($p = 0.018$) perceived as more enjoyable than the EX+REST condition. The EX+HWI condition was perceived as more pleasurable ($p = 0.024$), pleasant ($p = 0.031$, median difference = 1) and gratifying ($p = 0.040$, median difference = 1) than the EX+REST condition. Furthermore, the HWI+HWI condition was perceived as more pleasant than the EX+REST condition ($p = 0.040$, median difference = 1). No differences were observed for other subscales (fun, invigorating, exhilarating, stimulating and refreshing) (Figure 5D).

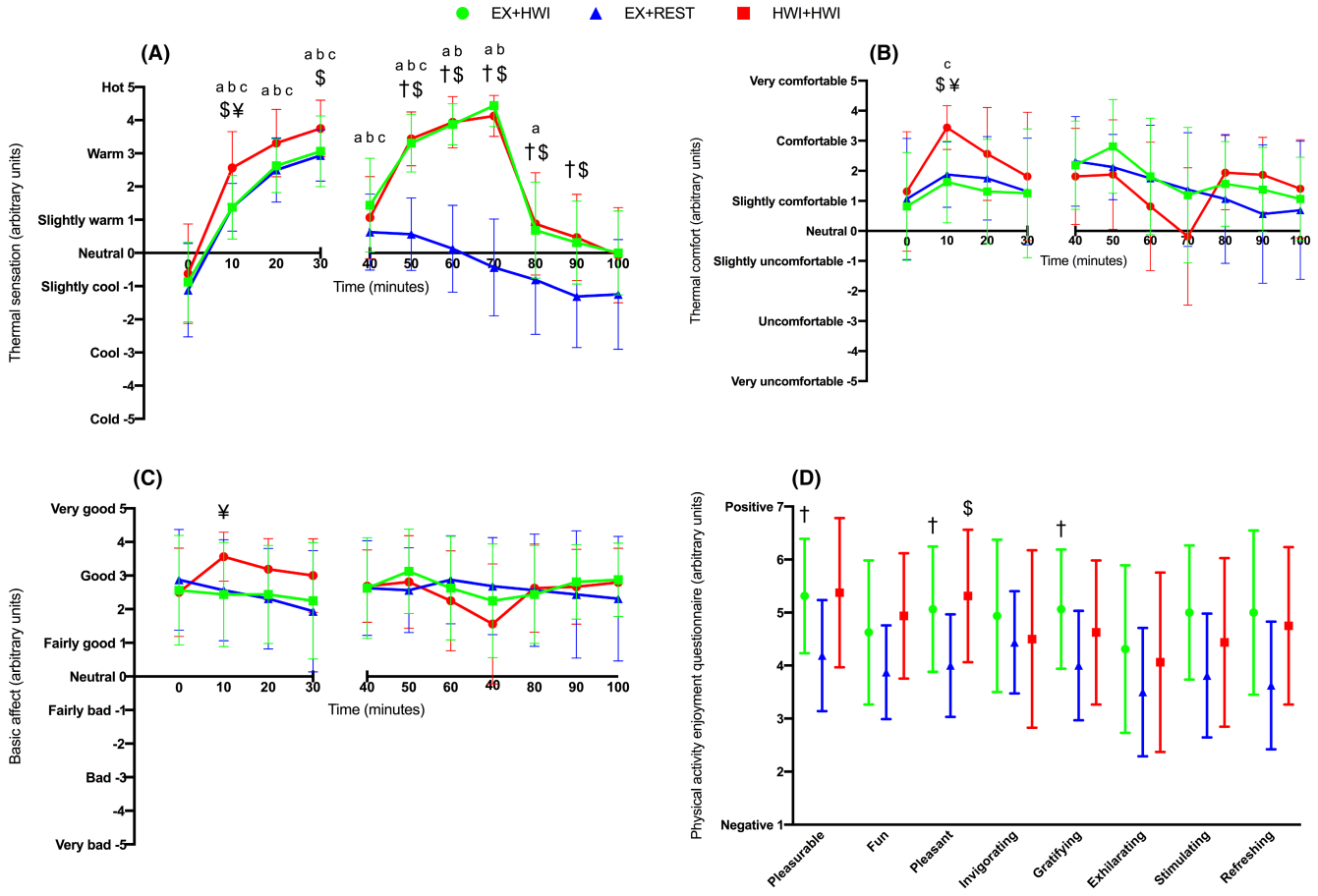

FIGURE 5 Thermal sensation (A), Thermal comfort (B), Basic affect (C) and Physical activity enjoyment questionnaire (D) responses in the EX+HWI, EX+REST and HWI+HWI conditions. Post hoc time × condition interactions are symbolized as follows: EX+HWI vs EX+REST († = $p < 0.05$), HWI+HWI vs EX+REST ($ = $p < 0.05$) and EX+HWI vs HWI+HWI (¥ = $p < 0.05$). Post hoc within condition differences from baseline are denoted as follows: EX+HWI (a = $p < 0.05$), EX+REST (b = $p < 0.05$) and HWI+HWI (c = $p < 0.05$). Lines and whiskers represent means and standard deviations ($n = 16$).

4 | DISCUSSION

The purpose of this study was to investigate the acute vascular, circulating blood marker, and perceptual responses to post moderate-intensity exercise hot water immersion in physically inactive middle-aged adults, in comparison to exercise and hot water immersion alone. In line with our experimental hypotheses, the current study revealed three novel findings: (1) Post exercise hot water immersion extended brachial and superficial femoral artery mean shear rate responses and increased plasma nitrite and serum IL-6 concentrations when compared to exercise followed by rest. (2) Irrespective of whether hot water immersion was used after exercise or heating, hot water immersion extended the mean shear rate responses and stimulated the release of circulating nitrite and IL-6. (3) Hot water immersion after exercise and independently were both perceived as more enjoyable than exercise alone. Based on these findings we accepted our experimental hypotheses. Taken together, hot water immersion was enjoyable and enhanced some of the physiological responses derived from smaller bouts of exercise. Therefore, post exercise hot water immersion may be an effective complementary therapy for populations that are either unable or unwilling to do sufficient amounts of exercise.

The use of EX+HWI and HWI+HWI further increased elevations in rectal and skin forehead temperatures and extended elevations in heart rate. As a collective, these thermo-physiological responses stimulate episodic elevations in shear stress and contribute to beneficial vascular responses.[38] In this regard, we are the first to demonstrate that EX+HWI augmented mean shear rate in the brachial and superficial femoral arteries when compared to EX+REST, which was likely driven by increases in peripheral blood flow in order to assist heat dissipation. This is an important finding given that the duration and peak elevation in episodic shear stress may promote vascular adaptations when repeated. Although it is noteworthy that further increases in shear rate and reductions in diastolic blood pressure occurred in the presence of only modest rises in core temperature. As repeated elevations in shear stress are established to underpin chronic improvements in endothelial function,[17] this highlights the need to move beyond solely using elevations in core temperature to characterize the vascular adaptive potential of hot water immersion protocols.

A strength of our study design was the assessment of both nonsubmerged/nonexercising (brachial artery) and submerged/exercising limbs (superficial femoral artery) and thus the comparison between local and systemic hemodynamic responses. Consistent with research directly comparing exercise with hot water immersion,[39] we observed similar increases in brachial artery mean shear rate throughout the EX+HWI and HWI+HWI conditions. However, shear rate was higher in the superficial femoral artery following hot water immersion in contrast to exercise. While metabolic increases in shear diminish rapidly after exercise,[12] our observations highlight that the thermal stimulus of immersion maintained the elevation in shear rate for longer than moderate intensity cycling. Analyzing the shear profile in more detail, also revealed that extending the stimulus through hot water immersion induced concurrent increases in antegrade and decreases in retrograde shear rate in the brachial and superficial femoral arteries. This temporary shift in the shear pattern is of relevance as acute increases in antegrade shear transiently improve endothelial function,[17] whereas retrograde shear results in temporary periods of endothelial dysfunction.[40] Taken together, the importance of these beneficial hemodynamic responses for vascular health are well demonstrated by artery occlusion protocols preventing chronic improvements in endothelial function in both exercise training[17] and passive heating models.[18]

Transient elevations in the vasodilator nitrite are often evident in circulation following exercise[27] and passive heating,[24] with similar whole-body hot water immersion protocols (45 min at 39°C) shown to increase circulating nitrite in sedentary adults.[41] Accordingly, we also found that EX+HWI and HWI+HWI increased the concentration of plasma nitrite in comparison to EX+REST. The angiogenic potential of which is evidenced by the serum from a single hot water immersion session stimulating endothelial tubule formation in a nitric oxide dependent manner.[24] From a mechanistic perspective, the supplementary bout of hot water immersion in the present study may have upregulated the expression and activity of endothelial nitric oxide synthase. In this regard, while serum nitric oxide has been shown to increase after hot water immersion, other angiogenic circulating factors, such as VEGF and heat shock proteins remain unchanged.[24] This corresponds with our results as no detectable differences were apparent for VEGF in any condition. Nevertheless, changes in vasoactive circulating factors can occur through a combination of an increase in temperature,[22] mechanical stress, and circulating stimuli,[42] which support an endothelial cell phonotype inducive of releasing vasoactive markers into the bloodstream. Therefore, we endeavored to build on the findings by Brunt and colleagues by analyzing a more extensive range of blood markers to better characterize the circulating milieu. Accordingly, cortisol was lower after 30 min of HWI+HWI compared to EX+REST, which is in agreement with previous whole-body hot water immersion trials for 20 min at 40°C.[43] Cortisol then returned to baseline at 70 min, which may be linked to the gradual rise in thermal burden and thus reflect cortisol's role as

a stress hormone. Furthermore, our results differ from previous findings that observed a decrease in ET-1 after a single water perfused suit session for 90 min at 48°C, in patients with peripheral artery disease.[44] The present findings expand our knowledge by showing that a lower passive heating stimulus, in a comparably healthier population, does not reduce ET-1. Finally, a range of MMPs (MMP-2, MMP-9 and MMP-10) and catecholamines (adrenaline and noradrenaline) were also assessed due to their role in angiogenesis and extracellular matrix remodeling.[45,46] However, in response to the duration and intensity/temperature of exercise and hot water immersion used in the current study, there were no differences between conditions for any of these markers.

Acute elevations in interleukins have a context specific inflammatory and metabolic role after exercise.[47] In particular, IL-6 is a key anti-inflammatory cytokine, which stimulates the down-stream release of other anti-inflammatory mediators, such as IL-4, IL-10 and IL-1Ra.[47] Although IL-6 consistently peaks after hot water immersion,[41] it is 3-fold higher after exercise for the same increase in core temperature.[48] Despite this, the modest bouts of exercise in the current study did not elicit an increase in IL-6, nor its downstream markers IL-10 and IL-1Ra.[49] This is in line with earlier studies demonstrating that moderate-intensity exercise (30 min at 50% $\dot{V}O_{2max}$) failed to increase anti-inflammatory interleukins (IL-6, IL-10 and IL-1Ra).[28] Importantly, we observed that extending the stimulus through hot water immersion, resulted in an increase in serum IL-6 and IL-1Ra in the EX+HWI and HWI+HWI conditions. However, only the EX+HWI condition resulted in a greater increase in IL-6 when compared to EX+REST at the 70-min time point. These findings support the notion that IL-6 is particularly sensitive to the duration of a given stressor, whether that is exercise, heating or a combination.[49] While speculative, it is plausible that the combined metabolic and thermal stimulus of EX+HWI was preferential for increasing circulating IL-6. The current findings are noteworthy given that repeated acute elevations in interleukins may benefit cardiovascular and cardiometabolic health through reducing chronic low-grade inflammation and enhancing glycemic control.[11,47,50,51]

Physically inactive adults face exercise barriers, including tiredness, lack of time, enjoyment, and motivation.[52] Therefore, a major finding was that both EX+HWI and HWI+HWI were perceived as more enjoyable than EX+REST, however only EX+HWI was viewed as more pleasant and gratifying. Such findings could imply that post exercise hot water immersion may promote long-term exercise adherence, which would underpin any potential improvements in cardiovascular and cardiometabolic health. This being said, while 30 min of hot water immersion was well received, two 30-min bouts made some participants feel thermally uncomfortable. One individual suffered from sensations of dizziness and nausea after HWI+HWI, while no adverse responses were recorded following EX+HWI. This is similar to our recent work, wherein we reported thermal discomfort and mild heat mediated adverse responses following 60 min of hot water immersion (39°C).[29] As such, the long-term adherence of post exercise hot water immersion will likely depend on whether an individual is willing to partake in the supplementary heating stimulus. It is therefore recommended that the duration and intensity/temperature of the overall stimulus should be modified based on the tolerance of the individual, with particular care required for clinical populations at higher risks of heat mediated adverse events.

Despite this study comprehensively assessing a range of physiological and perceptual responses, it is not without its limitations. As the order of outcome measures were prioritized based on importance, the short time delay may have resulted in an underestimation of the shear responses in the superficial femoral artery.[12] Furthermore, while blood markers were sampled from the median cubital vein which may better reflect the brachial artery responses, these samples were collected from the venous circulation and thus a different compartment of the vascular tree. Finally, passive heating cannot replicate all of the benefits of exercise, many of which were not measured given our focus on the overlapping responses between exercise and hot water immersion. In the absence of ground reaction forces, and muscle contractions per se, an extended duration of exercise would likely be superior at enhancing bone mineral density, muscle mass and energy expenditure. Therefore, hot water immersion alone should only be considered as a substitute for exercise in populations that have legitimate exercise contraindications. For the majority of populations that do not meet the minimum physical activity guidelines, hot water immersion should be used as a supplementary stimulus to augment the physiological responses of smaller bouts of exercise.

4.1 | Perspectives

Regardless of whether hot water immersion was used after exercise or heating, hot water immersion enhanced a range of vascular (brachial and superficial femoral artery mean shear rate, antegrade and retrograde shear patterns, and diastolic blood pressure) and some blood marker (nitrite and IL-6) responses. Furthermore, both post exercise hot water immersion and hot water immersion in isolation were perceived as more enjoyable than exercise alone. Based on these findings, future studies should

explore whether repeated bouts of post exercise hot water immersion can augment the cardiovascular and cardiometabolic health benefits in populations that are either unable or unwilling to do sufficient amounts of exercise.

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CONFLICT OF INTEREST STATEMENT

No conflict of interest was reported by the authors.

DATA AVAILABILITY STATEMENT

The raw data from this manuscript will be provided by the corresponding author upon reasonable request.

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