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The effect of acute hot water immersion on cutaneous peripheral microvascular responses in males of White-European, Black-African and South-Asian descent

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ABSTRACT

Cardiovascular disease is more prevalent in individuals of Black-African (BA) and South-Asian (SA) descent than White-European (WE) counterparts, with vascular dysfunction identified as contributing to this disparity. Chronic heat therapy can elicit positive vascular adaptations, potentially underpinned by the repeated cardiovascular strain experienced during acute heat exposures. This study examined the cutaneous peripheral microvascular responses following acute hot (HWI) and thermoneutral (CON) water immersion between males of WE, BA, and SA descent. Thirty-one young, healthy WE ($n = 10$), BA ($n = 10$), SA ($n = 11$) males completed 60 minutes of HWI (39°C) and CON (36°C) with thermoregulatory, cardiovascular, and perceptual responses measured throughout. Following 60 minutes of thermoneutral rest, forearm and Great toe cutaneous vascular conductance (CVC) were recorded during cutaneous post-occlusive reactive hyperemia (PORH) and local heating (LH). Baseline CVC was similar between groups ($p \geq 0.08$). During PORH, BA had lower peak forearm and Great toe CVC than WE and SA, and a reduced CVC area under the curve compared to WE ($p \leq 0.01$). Furthermore, BA Great toe CVC was blunted compared to WE and SA during both 42°C ($p \leq 0.033$) and 44°C ($p \leq 0.02$) LH, respectively. Great toe CVC was acutely increased following HWI in responses to 44°C LH compared to CON ($p \leq 0.039$), with no race × condition interaction effects. In conclusion, despite blunted microvascular responses in BA, acute HWI did not elicit distinct effects between males of WE, BA, and SA descent, although microvascular responses to LH were greater following HWI.

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Hot water immersion; microvascular function; racial differences; heat therapy; skin blood flow

Introduction

Cardiovascular disease (CVD) is the leading cause of death globally [1]. In the United States, 48.6% of adults ≥ 20 years of age are afflicted with CVD, although there is a racial disparity in CVD morbidity and mortality [2]. Compared to White-European (WE) descent counterparts, there is a ~ 15% and ~ 32% higher total CVD prevalence in Black-African (BA) descent males and females, respectively [2]. In addition, South-Asian (SA) individuals also have a higher risk of CVD prevalence than WE individuals [3]. Occurrence of primary risk factors of CVD is also greater in BA and SA populations, such as obesity, physical inactivity, and poor nutritional status [2,4], and can onset earlier in life [5].

Impaired vascular function in non-White populations contributes to elevated CVD risk and is a predictor of CVD morbidity and mortality [6,7]. Comparisons of vascular function between racial and/or ethnic groups have primarily involved BA and WE populations. Numerous studies have reported impaired endothelial function in BA compared to WE counterparts through blunted macro- and microvascular responses to infusion of vasodilators [8–11], temporary occlusion [12–14], local cooling [11,15] and local heating [16,17]. Evidence of this discrepancy has been reported in diseased populations as well as in young healthy individuals, suggesting the development of vascular dysfunction occurring before clinical diagnosis and persisting throughout life [18]. Although vascular function in the SA population has received less attention, there is evidence of reduced flow-mediated dilation [19] and endothelial-dependent vasodilation in response to acetylcholine [20] in SA compared to WE. Importantly, microvascular dysfunction has been identified as an early marker of CVD and often precedes macrovascular dysfunction [21].

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Vascular dysfunction is characterized by an attenuated production and bioavailability of nitric oxide (NO), a key vasodilator, via impairments in endothelial nitric oxide synthase (eNOS), as well as an impaired smooth muscle relaxation response [22,23]. Although multifactorial, oxidative stress and inflammation have been proposed as underlying physiological mechanisms driving vascular dysfunction and atherosclerosis [22,24]. Oxidative stress and inflammation reduce NO synthesis through eNOS uncoupling and formation of asymmetric dimethylarginine, as well as impair NO bioavailability through superoxide scavenging of NO to form peroxynitrite [25]. Indeed, elevated circulating markers of systemic inflammation and oxidative stress have been described in BA and SA populations [26–28].

Heat therapy refers to the passive elevation of systemic whole-body core temperature, such as sauna bathing and hot water immersion (HWI), with regular use associated with a reduced risk of all-cause and cardiovascular-related mortality [29,30]. Heat therapy has received recent attention as a prevention strategy for CVD [31,32], although this therapy has been utilized by many cultures for centuries [33]. There is a plethora of experimental evidence indicating beneficial cardiovascular adaptations from chronic heat therapy [34]. For example, improved macrovascular and blood pressure responses [35–38], alongside enhanced microvascular function [39–41] have been observed. It has been proposed that the repeated cardiovascular strain experienced during acute heat exposure underpins the chronic adaptations [42]. Indeed, microvascular function has been improved following acute heat exposure [43,44]; however, it has also been reported to be unchanged in young [45], middle and older age groups [46] as well as clinical populations [47,48]. These conflicting findings may be due to substantial heterogeneity among participant groups, heating protocols, and measurement timing [49].

Despite heat therapy eliciting beneficial cardiovascular adaptations, to our knowledge, only one study has investigated the acute effects of heat exposure on subsequent vascular responses between individuals of distinct racial backgrounds [44]. It was reported that 60 minutes of whole-body heating using a water-perfused suit similarly increased peak forearm reactive hyperemia and flow-mediated dilation in BA and WE females. These results would suggest that acute heat therapy improves vascular function independent of racial background in females. However, microvascular responses following acute HWI have not yet been investigated in males of WE, BA and SA descent, which is of interest given potential sex differences in the underlying mechanisms of vascular dysfunction between races [17]. Therefore, this study aimed to investigate the effects of acute HWI compared to a thermoneutral control (CON) on subsequent microvascular responses between males of WE, BA and SA descent. It was hypothesized that BA and SA individuals would have reduced microvascular function compared to WE, and HWI would elicit improvements in microvascular responses to a greater extent in BA and SA individuals.

Methods

Participants

Thirty-one males (10 WE, 10 BA, 11 SA) volunteered for the study (Table 1). Participants were classified into racial groups by self-reporting all four grandparents were as follows: White and born in Europe (WE), Black and born in Africa (BA), or born in South Asia (SA), defined as Afghanistan, Bangladesh, Bhutan, India, Maldives, Nepal, Pakistan, and Sri Lanka. All participants were recreationally active (≥ 30 minutes of moderate-intensity exercise ≥ three times per week for at least three months, self-reported), nonsmoking, free from any cardiovascular, metabolic, or skin conditions, and provided written informed consent. A favorable ethical

Table 1. Participant characteristics of White-European (WE), Black-African (BA) and South-Asian (SA) groups.

	WE ($n = 10$)	BA ($n = 10$)	SA ($n = 11$)
Age (years)	23 ± 4	22 ± 5	24 ± 4
Height (cm)	176.8 ± 7.0	174.9 ± 7.2	175.3 ± 7.0
Body Mass (kg)	75.7 ± 13.8	82.7 ± 15.1	72.8 ± 8.0
BMI (kg/m^2)	24.2 ± 3.5	27.0 ± 4.4	23.7 ± 2.0
Body fat (%)	15.8 ± 5.8^#	20.8 ± 5.3	21.3 ± 3.6
Body surface area (m^2)	1.92 ± 0.19	1.97 ± 0.18	1.88 ± 0.13
Estimated VO_{2max} (mL/min/kg)	56 ± 9^#	47 ± 6	45 ± 8

Data are mean ± SD. Significant difference between ^WE and BA, #WE and SA ($p < 0.05$).

opinion was obtained from Loughborough University's ethics committee (code: 13094) and experimental procedures conformed to the Declaration of Helsinki in all aspects apart from registration in a database.

Experimental design

Participants attended the laboratory on three occasions separated by at least 48 hours. During visit one, anthropometric measurements were recorded, and a submaximal exercise test was completed. Visits two and three were randomized and counterbalanced, whereby participants completed either HWI or CON using a crossover design. The start time of visits were conducted at the same time of day (±1 hour) and counterbalanced between morning and afternoon (before and after 12 pm, respectively) to account for the circadian effects on blood flow [50]. Participants were administered a food diary to record the volumes of food and drink consumed in the 24 hours before visit two and asked to replicate this before visit three. No food was consumed at least 2 hours before arriving at the laboratory. For 24 hours before testing, participants were asked to refrain from alcohol, caffeine, vigorous activity, foods high in nitrate, and anti-bacterial mouthwash.

Procedures

During the first visit, participant height (Invicta, Bishop, UK) and body mass (KCC150, Mettler Toledo, USA) were measured. Additionally, body fat percentage was measured through 4-point bioelectrical impedance (MC-780 MA, Tanita, Japan) which has been used previously in experimental research to measure body composition [51]. Despite mixed evidence of agreement when comparing body composition measurements between bioelectrical impedance and the gold standard dual-energy X-ray absorptiometry method [52], accuracy is greater within non-obese individuals as well as hydration status was confirmed prior to measurement which is a reported source of variability [53]. Body surface area was calculated using the Du Bois and Du Bois equation [54]. Following this, a heart rate (HR) monitor was positioned directly below the sternum (Polar H10, Polar, Finland). A six-minute Åstrand-Rhyming submaximal exercise test on a cycle ergometer (Lode Excalibur, Groningen, the Netherlands) was then completed, where HR was used to provide an estimate of maximal oxygen uptake (VO_{2max}) [55]. The Åstrand-Rhyming test has been validated to estimate VO_{2max} against the traditional method of collecting metabolic gas exchange during maximal exercise testing [56] and as there was no fitness requirement other than recreational activity, a submaximal test was selected to estimate participant fitness levels. For visits two and three, following 10 minutes of seated rest in an environmentally controlled laboratory [24.2 ± 0.3°C, 43.5 ± 5.3% relative humidity (RH)] (Testo 435, Testo SE, Germany), blood pressure (BP) was recorded in duplicate using an automated sphygmomanometer and averaged and HR was measured via a three-lead electrocardiogram (Tango M2, SunTech

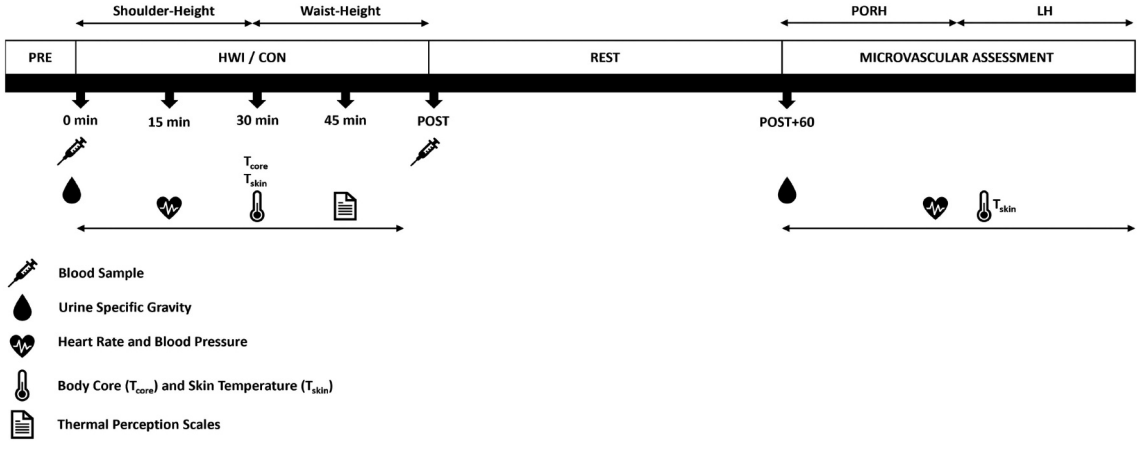

Figure 1. Experimental design and timing of data collection.

Medical, USA; Figure 1). Thereafter, the participants reported their thermal comfort (TC) and thermal sensation (TS) using a 30 cm visual analog scale [57] with 30 corresponding to "very comfortable" and "very hot" and 0 reflecting "very uncomfortable" and "very cold" for TC and TS, respectively.

Euhydration was confirmed from a urine sample as urine-specific gravity (Master Refractometer, ATAGO, Japan) ≤ 1.020 [58]. Body core temperature (T_{core}) was continuously monitored using either a thermistor (YSI 400, DeRoyal, USA) connected to a data logger (Squirrel 2010, Grant Instruments, UK) ($n = 26$), or an ingestible telemetric temperature capsule (e-Celsius, BodyCap Medical, France) exclusively during HWI ($n = 5$, all BA). The thermistor was self-inserted ~ 12 cm beyond the anal sphincter, whereas the capsule was ingested at least 3 hours before immersion. Four wireless thermochrons (DS1922L-F50, Maxim Integrated, USA), which were calibrated using a linear regression equation as described by Hunt et al. [59], were then fitted to the chest, triceps, thigh, and shin with a single piece of adhesive tape (Hypafix, Leukoplast, UK) for calculation of body mean skin temperature (T_{skin}) [60] as:

$$Tskin = 0.3 * chest + 0.3 * arm + 0.2 * thigh + 0.2 * shin$$

Thereafter, participants entered the water bath (Lay-Z-Spa St. Lucia, Bestway, UK) and lay semi-supine immersed to the clavicle at either [mean ± SD]; 36.0 ± 0.3°C (CON) or 39.1 ± 0.3°C (HWI). Water temperature was recorded from the mid-point of the water bath (YSI 400, DeRoyal, USA) and continuously circulated to maintain a homogenous temperature. After 30 minutes of immersion, participants sat upright with their arms at heart level, were towel-dried from the waist up and were immersed to the umbilicus for a further 30 minutes. This specific HWI immersion protocol was employed to elicit at least a ~ 1°C increase in T_{core} which is suggested to maximize the cardiovascular benefits of heat therapy [31,32]. Water was consumed ad-libitum throughout immersion. During immersion, T_{core} and T_{skin} were recorded at 1- and 10-second intervals, respectively, and averaged per minute, with baseline values taken from the final minute of rest in air. Every 15 minutes of immersion, TC and TS were measured and BP was measured from the right brachial artery at 30-, 45- and 60-minutes of immersion, with mean arterial pressure (MAP) calculated as:

$$MAP = diastolic\,pressure + \left(\frac{1}{3} * pulse\,pressure\right)$$

After 60 minutes of immersion, participants exited the water bath, were towel dried, self-removed the rectal probe, changed into shorts and a t-shirt and commenced 60 minutes of seated thermoneutral rest. During this period of rest, euhydration was re-confirmed using the criteria previously described. In the final 10 minutes of rest, participants were fitted with a blood pressure cuff on their right arm, and an occlusion cuff fitted as proximal as possible on the left arm (SC10D, Hokanson, USA) and around the base of the left Great toe (UDC2.5, Hokanson, USA). Exactly 60 minutes following the end of immersion, another BP measurement was recorded (Post+60) and skin blood flow measurements of the left forearm and Great toe pad commenced using a single fiber laser Doppler probe (VP12, Moor Instruments, UK) inserted into an integrated heater (VHP2, Moors Instruments, UK) and connected to a laser Doppler perfusion monitor (moor VMS-LDF2, Moor Instruments, UK). To ensure probe placements were as similar as possible between visits, a combination of site measuring, ink markings, and pictures were used. Laser Doppler flux was recorded at 40 hz with noise spikes, resulting from movement artifacts, removed through linear interpolation and noise filters using computer software (MoorVMS-PC v4.2, Moor Instruments, UK). Subsequently, data were converted to cutaneous vascular conductance (CVC) by dividing flux by MAP.

Post-occlusive reactive hyperemia

Following the period of rest, cutaneous post-occlusive reactive hyperemia (PORH) was assessed using a similar protocol as previously described [61,62] in ambient conditions (24.1 ± 0.2°C, 43.2 ± 4.4%RH). Local skin temperature was clamped at 33°C for the duration of measurement, with baseline measurements lasting 10 minutes. Thereafter,

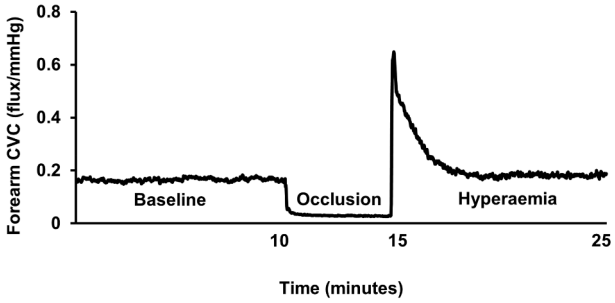

Figure 2. Representative trace of forearm cutaneous vascular conductance (CVC) measured during cutaneous post-occlusive reactive hyperemia.

blood flow was occluded for 5 minutes where occlusion cuffs were rapidly inflated to 220 mmHg through a pressure cuff controller (Moor-VMS-Pres, Moor Instruments, UK). Cuffs were then rapidly deflated and the cutaneous PORH response was measured for a further 10 minutes (Figure 2).

Every 5 minutes, BP was measured from the contralateral brachial artery. Baseline CVC was calculated as the mean CVC over the 5 minutes before occlusion. The amplitude of the cutaneous PORH response was determined as the maximum CVC following occlusion release. The area of hyperemia was quantified as the CVC area under the curve (AUC) between the release of occlusion and CVC returning to baseline, and cutaneous PORH index was calculated by dividing the CVC AUC during the first minute following occlusion release by the AUC during the final minute before inflation.

Cutaneous local heating

Following the completion of the PORH protocol, local heating (LH) measures commenced in comparable environmental conditions (24.1 ± 0.2°C, 43.2 ± 4.1%RH) using a similar protocol to one previously described [61,63]. Initially, probes were clamped at 33°C for 11 minutes at the same location as PORH. Thereafter, the local temperature was increased by 0.1°C/second to 42°C and maintained for 21 minutes 30 seconds, and subsequently increased at the same rate to 44°C for 10 minutes 20 seconds (Figure 3).

Again, BP was measured from the contralateral brachial artery every 5 minutes. To determine CVC plateaus during 42°C and 44°C LH, CVC over the final 5 minutes of heating were averaged.

Blood analysis

Immediately before and following immersion, blood samples were drawn via venepuncture from the antecubital vein into 6 mL lithium heparin tubes (Vaccutainer, BD, USA). Blood samples were immediately centrifuged at 3500 × g for 10 minutes. Plasma was aliquoted and stored at −80°C until batch analysis of plasma [nitrite]. Blood samples were collected immediately pre and post immersion as the increase of plasma [nitrite] from HWI is transient and following immersion returns to baseline within 30 minutes [64]. Plasma [nitrite] was determined through ozone chemiluminescence as previously described by Higuchi and Motomizu [65]. Samples were deproteinised through the addition of 500 µL of ethanol to 500 µL of lithium heparin plasma and then centrifuged for 10 min at 21,000 × g. The nitrite concentration was determined by its reduction to NO in the presence of glacial acetic acid and aqueous sodium iodide (4% w/v). The spectral emission of electronically excited nitrogen dioxide, derived from the reaction of NO with ozone, was detected by a thermoelectrically cooled, red-sensitive photomultiplier tube housed in a gas-phase chemiluminescence NO analyzer (Sievers NOA 280i, Analytix Ltd, UK). For plasma [nitrite] determination, 200 µL of supernatant was injected into the purge vessel in duplicate. Thereafter, the signal (mV) was smoothed using software (Origin 2019, OriginLab, USA), and peaks were identified and then converted to a concentration

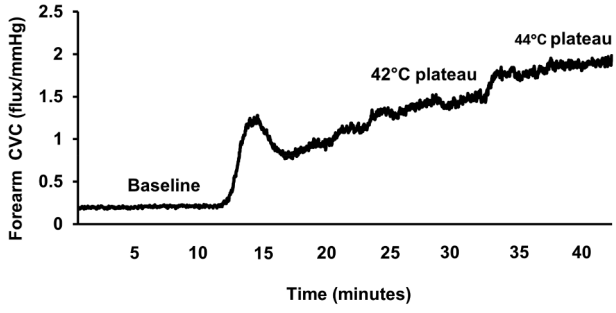

Figure 3. Representative trace of forearm cutaneous vascular conductance (CVC) measured during cutaneous local heating.

using a calibration plot of sodium nitrite standards.

Statistical analysis

The statistical software SPSS (version 28, IBM, USA) was used for all statistical analyses. An a priori power calculation was conducted to determine the number of participants required to observe a significant interaction effect of race (3 levels) and condition (2 levels) in G*Power incorporating a medium effect size [44], power of 0.8 and an α of 0.05 yielded a total sample size of 42. Between-group comparisons of anthropometric and estimated fitness levels were assessed through a one-way ANOVA. Due to missing T_{core} data during CON (BA = 5) resulting in an imbalance of group numbers, a paired samples *t* test was utilized to determine pre/post changes in T_{core} independent of racial group as CON was employed to maintain T_{core}. Group differences in T_{core} throughout HWI were analyzed using a one-way ANOVA, whereas the effect of immersion on T_{skin}, cardiovascular, perceptual, plasma [nitrite], and microvascular responses were analyzed using a 3 (group; WE, BA, SA) x 2 (condition; HWI, CON) mixed-model ANOVA. Where significant effects were observed, differences were identified through LSD post-hoc pairwise comparisons. Effect sizes are reported as partial eta squared (η_p^2) with magnitudes of ≥ 0.01, ≥ 0.059 and ≥ 0.138 interpreted as small, medium, and large, respectively [66]. For all analyses, the statistical significance threshold was set at $p < 0.05$. Data are presented as mean ± SD.

Results

Participants

Participant characteristics of WE, BA, and SA groups are presented in Table 1. Anthropometric measures were similar between groups except for a lower body fat percentage ($p \leq 0.032$; $\eta p^2 = 0.21$) as well as a greater estimated VO_{2max} ($p \leq 0.015$; $\eta p^2 = 0.21$) in WE compared to BA and SA.

Post-occlusive reactive hyperemia

Baseline forearm and Great toe CVC were similar between racial groups (Table 2; $p \geq 0.08$). There was a significant main effect for race ($p < 0.001$; $\eta p^2 = 0.41$), where BA (0.51 ± 0.23 flux/mmHg) had lower peak forearm CVC following occlusion release than SA (0.87 ± 0.26 flux/mmHg) and WE (0.85 ± 0.31 flux/mmHg). A significant main effect of race was also reported where peak Great toe CVC was lower in BA (2.46 ± 0.82 flux/mmHg) than SA (3.32 ± 0.99 flux/mmHg) and WE (3.80 ± 0.67 flux/mmHg; $p \leq 0.013$; $\eta p^2 = 0.37$), in addition to a reduced AUC in BA compared to WE (122.72 ± 104.14 vs 214.83 ± 70.19 CVC·s; $p = 0.014$; $\eta p^2 = 0.21$). Lastly, there was no race × condition interaction effect for cutaneous PORH outcomes ($p \geq 0.24$).

Cutaneous local heating

In response to 42°C and 44°C LH, there were no differences in forearm CVC measures between conditions (Figure 4, Panel a & c; $p \geq 0.60$). There was a main effect for race ($p = 0.033$; $\eta p^2 = 0.22$) where BA had an attenuated Great toe CVC during 42°C LH (1.08 ± 0.51 flux/mmHg) compared to WE (1.90 ± 0.92 flux/mmHg; $p = 0.033$) and SA (2.00 ± 1.2 flux/mmHg; $p = 0.016$). Moreover, BA had a lower Great toe CVC in response to 44°C LH than WE and SA (BA: 1.26 ± 0.63, WE: 2.13 ± 0.93, SA: 2.29 ± 1.10 flux/mmHg, respectively; $p \leq 0.02$; $\eta p^2 = 0.27$). There was a significant main effect of condition where Great toe CVC during 44°C LH was increased following HWI compared to CON (2.07 ± 1.09 vs. 1.72 ± 0.90 flux/mmHg; $p = 0.039$; $\eta p^2 = 0.14$), although there were no race x condition interaction effects for any LH measure ($p \geq 0.12$).

Thermoregulatory responses

There was no change in T_{core} during CON between pre (37.3 ± 0.4°C) and post (37.3 ± 0.4°C; $p = 0.43$). During HWI, T_{core} increased ~ 1°C from pre to post for all racial groups with no differences at any time point (Figure 5, Panel a; $p \geq 0.60$). For T_{skin} (Figure 5, Panel b), there was a main effect of condition throughout immersion where HWI was greater than CON at each timepoint (p all < 0.001; $\eta p^2 \geq 0.90$), with no significant differences between racial groups or race × condition interaction effects ($p \geq 0.06$). Following immersion, there were no

Table 2. Forearm and Great toe Cutaneous Vascular Conductance (CVC) responses during cutaneous post-occlusive reactive hyperemia (PORH) following hot (HWI) and thermoneutral (CON) water immersion in males of White-European (WE), Black-African (BA) and South-Asian (SA) descent.

	Forearm						Great toe					
	WE		BA		SA		WE		BA		SA	
	HWI	CON	HWI	CON	HWI	CON	HWI	CON	HWI	CON	HWI	CON
Baseline CVC (flux/mmHg)	0.18 ± 0.08	0.19 ± 0.06	0.13 ± 0.07	0.11 ± 0.09	0.19 ± 0.16	0.16 ± 0.09	1.00 ± 0.68	0.93 ± 0.51	0.83 ± 0.51	0.66 ± 0.55	0.93 ± 0.74	0.90 ± 0.49
Peak CVC (flux/mmHg)	0.81 ± 0.24	0.88 ± 0.38	0.52 ± 0.27^ †	0.50 ± 0.19^ †	0.91 ± 0.32	0.82 ± 0.21	3.72 ± 0.71	3.88 ± 0.65	2.70 ± 0.81^ †	2.23 ± 0.79^ †	3.44 ±1.13	3.20 ± 0.88
AUC (CVC·s)	31.06 ±12.89	41.62 ± 25.78	33.40 ± 47.75	23.95 ±16.30	40.26 ± 54.60	36.29 ± 22.70	206.18 ± 59.20	223.49 ± 82.44	141.29 ± 108.49^	104.15 ±101.78^	198.78 ± 115.41	152.43 ± 68.62
PORH index (CVC)	3.02 ±1.39	3.19 ±1.19	2.58 ±1.00	3.00 ±1.01	2.88 ±1.12	3.19 ±1.31	4.45 ± 3.04	3.87 ± 2.28	3.40 ±1.79	3.08 ±1.56	3.92 ± 2.55	3.08 ± 1.53

Data are mean ± SD. AUC, area under the curve. Significant difference between ^WE and BA, †BA and SA ($p < 0.05$).

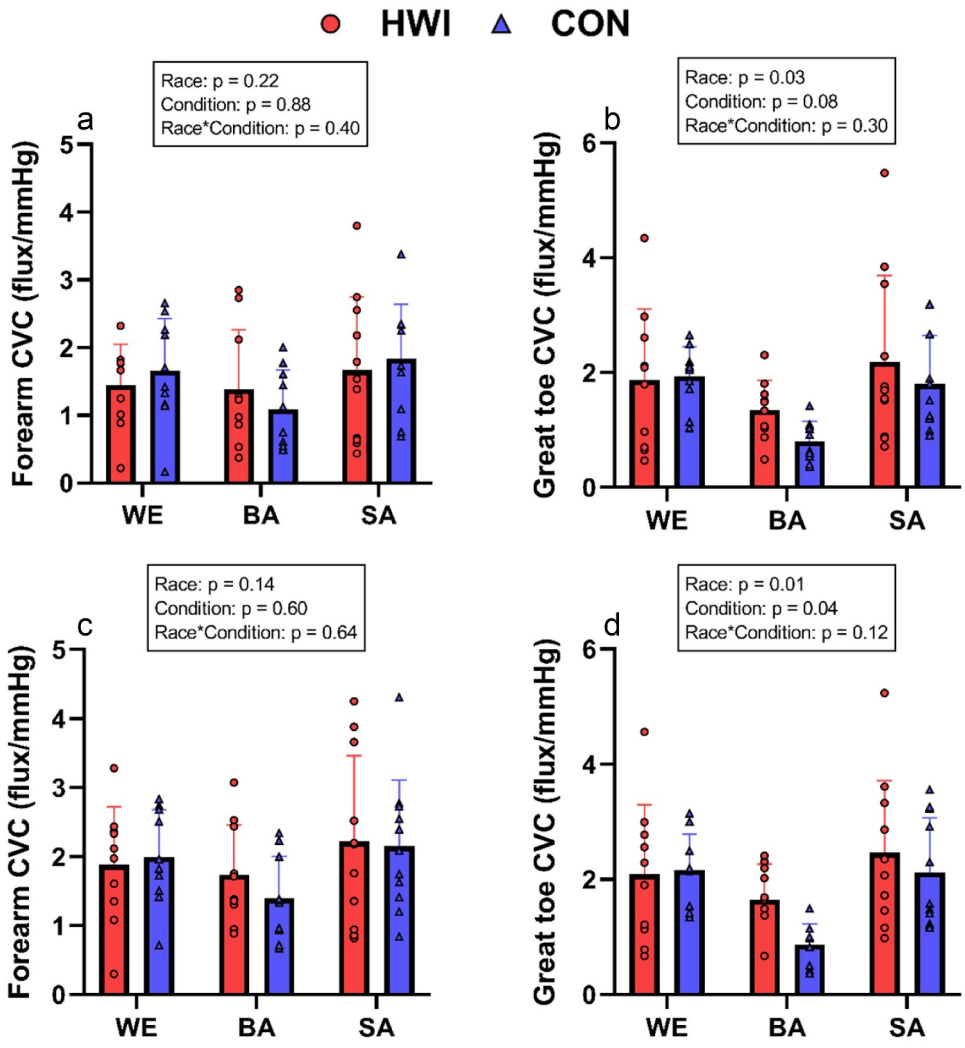

Figure 4. Forearm and great toe cutaneous vascular conductance (CVC) during 42°C (a,b) and 44°C (c,d) local heating following hot (HWI; red bars) and thermoneutral (CON; blue bars) water immersion in individuals of White-European (WE), Black-African (BA) and South-Asian (SA) descent.

significant differences in T_{skin} during PORH and LH.

Cardiovascular responses

There were no baseline differences between racial groups for any cardiovascular measure (Figure 6, Panel a – d; $p \geq 0.19$). There was a main effect of condition where systolic (SBP), diastolic (DBP), and MAP were lower throughout HWI than CON ($p \leq 0.019$; $\eta p^2 \geq 0.18$), whereas HR was elevated ($p \leq 0.014$; $\eta p^2 \geq 0.20$). This reduction in SBP, DBP, and MAP was present 60 minutes following immersion, as was an elevation in HR ($p \leq 0.01$). Throughout immersion, there were no differences between racial groups for any cardiovascular parameter, as well as no race x condition interaction effects ($p \geq 0.20$).

Perceptual responses

Participants experienced a lower TC during HWI compared to CON ($p \leq 0.041$; $\eta p^2 \geq 0.14$) which started in the range of "just comfortable," reduced to "just uncomfortable" 30 minutes into immersion and returned to "comfortable" by the end of immersion (Figure 7, Panel a). At 15 and 30 minutes of immersion, there was a main effect of race where BA had a higher TC compared to WE ($p \leq 0.047$; $\eta p^2 \geq 0.20$), indicating lower perceived discomfort from HWI. Furthermore, TS

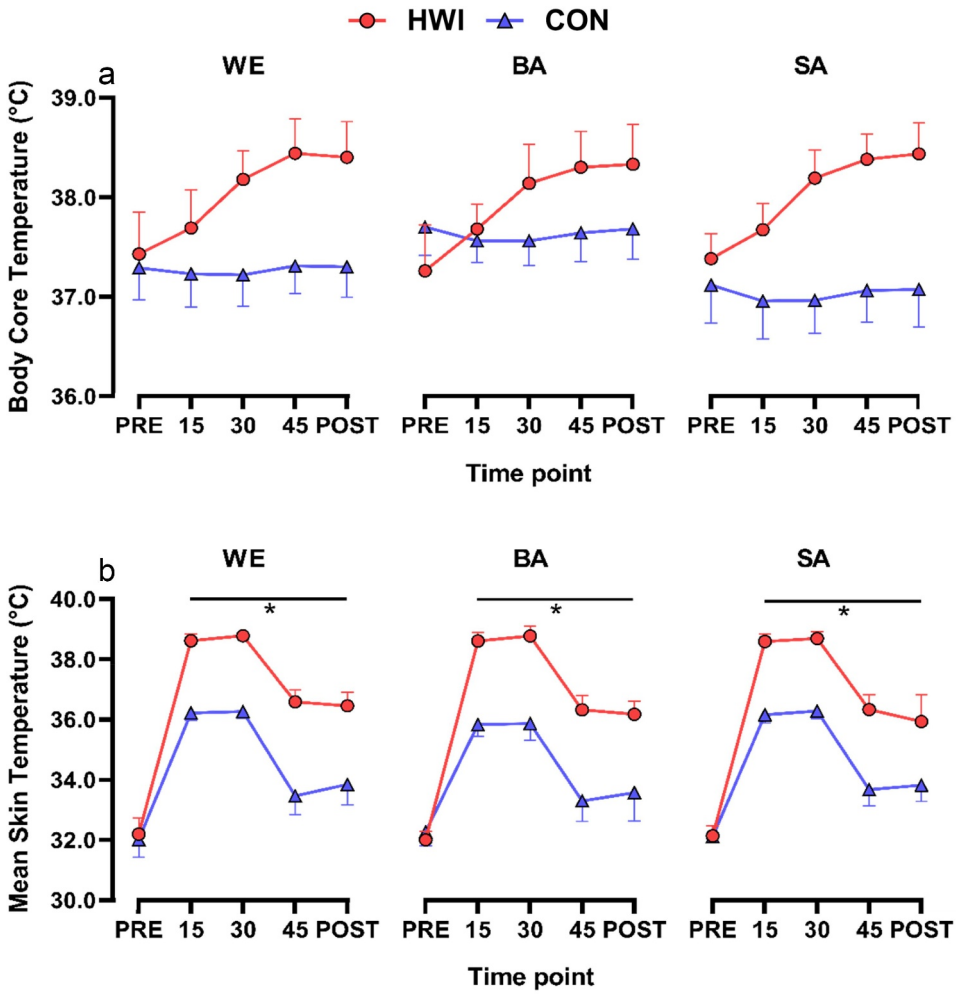

Figure 5. Body core (a) and mean skin (b) temperature throughout hot (HWI; red lines) and thermoneutral (CON; blue lines) water immersion for groups of White-European (WE), Black-African (BA; body core temperature $n = 10$ HWI, $n = 5$ CON), and South-Asian (SA) descent. *significant difference between HWI and CON ($p < 0.05$).

was greater during HWI compared to CON ($p < 0.001$; $\eta p^2 \geq 0.76$) with no differences between racial groups (Figure 7, Panel b). The highest TS was within the range of "hot" 30 minutes into immersion and reduced to "warm" by the end of immersion. For both TC and TS, there were no race x condition interaction effects ($p \geq 0.07$).

Plasma [nitrite] responses

Plasma [nitrite] was unchanged in both conditions for all racial groups (Figure 8; $p \geq 0.14$).

Discussion

To our knowledge, this is the first crossover randomized control trial comparing the effect of acute HWI on microvascular responses between males of WE, BA, and SA descent. The main finding of this study was that despite attenuated microvascular responses during cutaneous PORH and LH in BA, acute HWI elicited no race-specific effects on microvascular function between racial groups. This is in partial agreement with the hypothesis that BA and SA would have reduced microvascular responses compared to WE. However, it was also postulated that HWI would elicit the greatest improvements to microvascular function for these groups which contrasts with our findings.

A plethora of literature report impaired microvascular responses to occlusion [12,14] and local heating [16,17] in BA compared to WE, however this is not a universal finding [44]. In this study, during cutaneous PORH, BA had lower peak

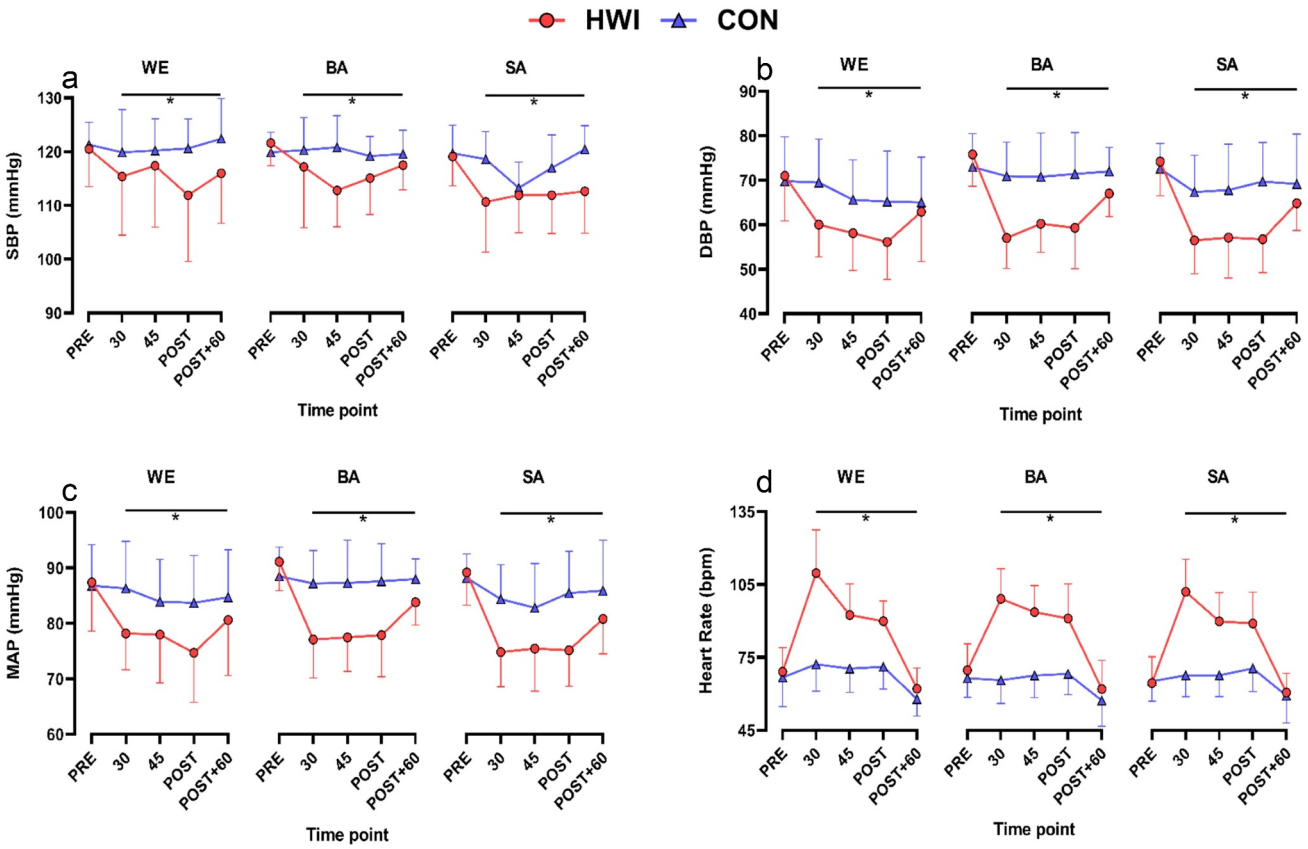

Figure 6. Measurements of systolic (SBP; a) and diastolic (DBP; b) blood pressure, mean arterial pressure (MAP; c) and heart rate (d) throughout hot (HWI; red lines) and thermoneutral (CON; blue lines) water immersion in groups of White-European (WE), Black-African (BA) and South-Asian (SA) descent. *significant difference between HWI and CON ($p < 0.05$).

forearm and Great toe CVC compared to WE and SA, alongside reduced Great toe AUC compared to WE. Additionally, BA had an attenuated Great toe CVC at 42°C and 44°C LH, respectively. This reduced microvascular function identified in BA individuals has often been attributed to reduced NO bioavailability, a key vasodilatory signaling molecule [67]. This is in part due to eNOS uncoupling, where inflammation and oxidative stress have been proposed as underlying physiological mechanisms and are elevated in BA populations compared to WE [27,28] across all ages. However, baseline plasma [nitrite], a circulating marker of eNOS-derived NO [68], was similar between groups, a finding which has been previously reported in young BA and WE males [69]. Another factor which may contribute to the impaired microvascular responses in BA was the lower estimated VO_{2max} and higher body fat than WE. However, SA also had a lower estimated VO_{2max} and higher body fat than WE but reported similar microvascular responses. Additionally, peak forearm and Great toe cutaneous PORH were blunted in BA relative to SA despite similar estimated fitness levels and body fat. Although substantial overlap across groups in estimated VO_{2max} (range: WE = 44–76 mL/min/kg, BA = 39–59 mL/min/kg, SA = 35–63 mL/min/kg), the magnitude of skin blood flow responses can be influenced by fitness levels [70]. Indeed, lower levels of aerobic fitness and greater levels of adiposity in BA and SA populations [71] have been previously identified as contributing to the racial disparity in CVD prevalence.

Despite the positive association between heat therapy and reductions in all-cause and cardiovascular-related mortality [29,30], investigations into vascular responses to acute heat exposures have produced equivocal findings. For example, forearm PORH has previously been reported as unchanged following acute heat stress [45–48] whereas others have described improvements

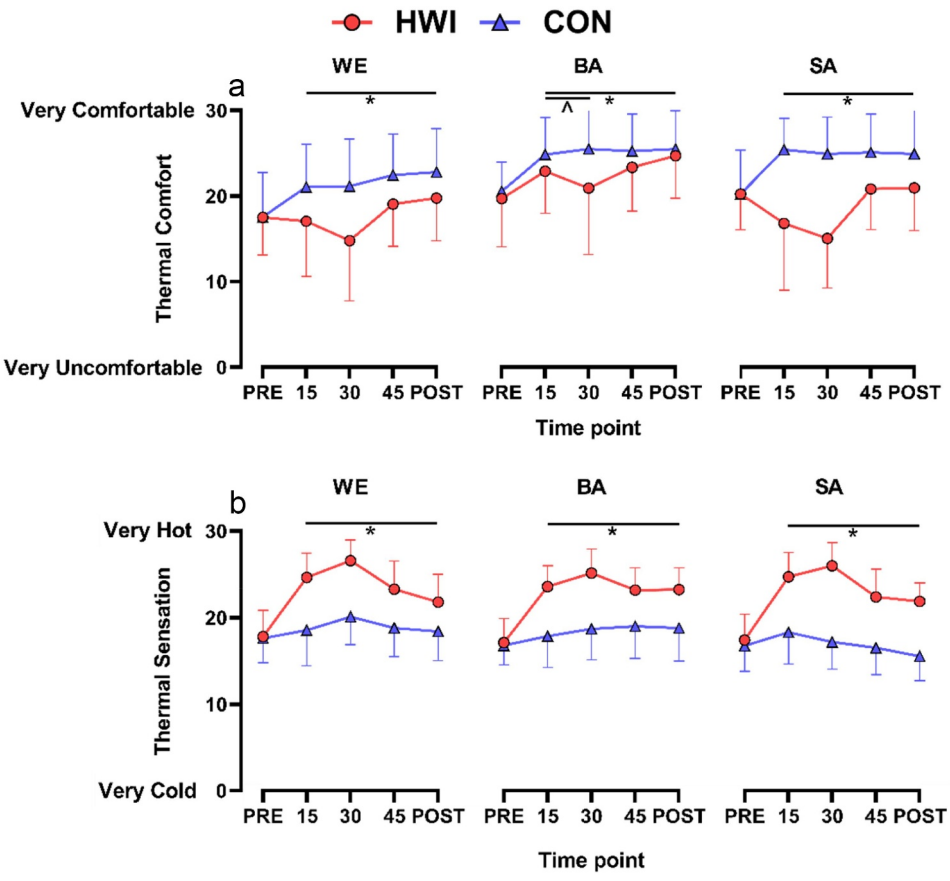

Figure 7. Measurements of thermal comfort (a) and thermal sensation (b) throughout hot (HWI; red lines) and thermoneutral (CON; blue lines) water immersion in groups of White-European (WE), Black-African (BA) and South-Asian (SA) descent. *significant difference between HWI and CON ($p < 0.05$). ^significant difference between WE and BA ($p < 0.05$).

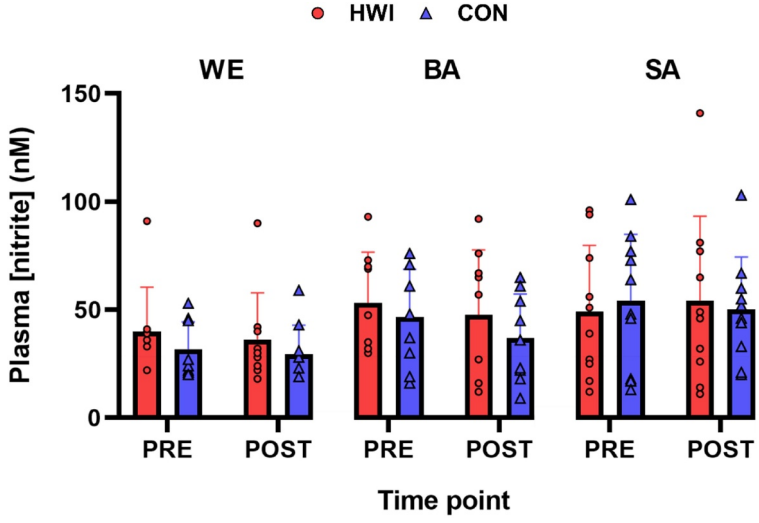

Figure 8. Plasma [nitrite] concentrations pre and post hot (HWI; red bars) and thermoneutral (CON; blue bars) water immersion for White-European (WE; $n = 9$), Black-African (BA; $n = 9$), and South-Asian (SA; $n = 10$) descent individuals.

[43,44]. The inconsistent effect of acute heat exposure on vascular function may be attributed to substantial differences in employed heating modalities and protocols, as well as variation in the time between heating and commencing vascular measurements. For example, Chaseling et al. [49] investigated the impact of measurement timing on forearm microvascular function following 60 minutes of whole-body heating via water-perfused suit and despite a ~ 1°C increase in T_{core}, peak forearm vascular conductance during PORH was only transiently increased (≤ 30 minutes). Nonetheless, Cheng et al. [43] described 45 minutes of 45°C knee-level heating, which increased T_{core} ~ 0.4°C, increased peak forearm vascular conductance within 30 minutes post-heating, whereas the ~ 1.5°C increase in T_{core} elicited during 60 minutes of 40.5°C HWI was not sufficient to increase PORH responses 60 minutes following heating [45]. Further research is warranted to better understand the minimal and optimal time and temperature of HWI required to acutely influence microvascular function, which could then inform longer-term HT interventions.

To our knowledge, the only study to date comparing the vascular effects of HT between racial groups described improved peak forearm PORH in both BA and WE females following 60 minutes of whole-body heating via a water-perfused suit [44]. Contrastingly, neither forearm nor Great toe cutaneous PORH were improved following HWI compared to CON independent of racial group in this study. One important factor which may explain these conflicting results is that Martin et al. [44] measured PORH 45 minutes following heating; in their study internal temperature, forearm blood flow and forearm vascular conductance remained significantly elevated prior to post-heating PORH measures compared to pre-heating baselines. Both studies' heating modalities elicited a similar increase in T_{core} (~ 1°C), although we employed a longer 60-minute period between heating and commencing PORH measurements as this was the time of thermoneutral rest required to allow T_{core} and CVC to return to baseline during pilot testing.

Few studies have investigated the LH response following acute heat exposure; however, chronic interventions have reported positive effects [39,40,41]. Although in this study no race-specific effects were observed from HWI on any microvascular outcome, contrary to forearm CVC responses, Great toe CVC was increased following HWI during 44°C LH. Despite mechanistic differences in the control of skin blood flow whereby active vasodilation is present in non-glabrous (i.e. volar forearm) but not glabrous (i.e. Great toe pad) skin [72], this toe-specific increase in CVC may also be explained by the differences in heating time experienced by measurement sites, where participants' lower body was immersed throughout HWI whereas the upper body was only immersed for the first 30 minutes. Indeed, one of the primary mechanisms proposed to underly the reported beneficial effects of heat therapy on the vasculature pertains to an increase in NO bioavailability [31], and therefore a greater localized heat exposure may explain our findings. The increase in Great toe CVC during cutaneous LH but not PORH following HWI may also be explained by the mechanistic differences between these physiological responses. Following the initial peak, skin blood flow plateaus during LH and is primarily NO-dependent [73,74], whereas PORH responses are primarily mediated by sensory nerves and endothelial-derived hyperpolarising factors [75,76]. However, despite the increase in Great toe CVC following HWI during LH, plasma [nitrite] was not concomitantly increased which contrasts with previous findings [64,77]. This may be in part due to the location of venous sampling (arm) receiving half the heating exposure of the toe, which may point to differences between local and systemic plasma [nitrite] levels. Indeed, further work aiming to understand the mechanisms underlying the effects of heat exposure on the vasculature is needed. Finally, it is important to note that findings from studies investigating the vascular effects of acute heat exposure may not be reflective of chronic heat therapy studies. Therefore, future research should aim to compare the effects of chronic heat therapy between racial groups.

Importantly, one of the reported beneficial hemodynamic effects of heat therapy is a reduction in blood pressure during immersion [34], which can last as long as 60 minutes thereafter [78]. Indeed, MAP was reduced throughout

HWI similarly between racial groups (~ −13 mmHg) and remained lower than pre-immersion values 60 minutes following immersion (~ −7 mmHg). A reduction in BP throughout and acutely following HWI was present in our young, healthy groups; this is of interest as the repeated acute responses to HT have been suggested to underpin chronic adaptations [42]. Therefore, this may be of particular importance for populations more susceptible to hypertension such as BA and SA, respectively [2,4].

During immersion, TC was higher in BA for the first 30 minutes, indicating greater levels of comfort (Figure 7). Although chronic heat therapy is promoted as an intervention to improve cardiovascular health, the thermal discomfort experienced during heat exposure could be a barrier to long-term adherence. Therefore, the greater levels of thermal comfort in BA should be further investigated, particularly as BA, among other non-White racial minorities, are less likely to meet physical activity guidelines [2].

This study is not without limitations. The primary limitation surrounds the underpowered number of participants included within each racial group (n = 10 or 11) relative to our initial aim to detect a significant race x condition interaction effect (n = 14). However, following 31 completed participants a post-hoc power calculation was completed using the smallest p value from the microvascular data (p = 0.12) which reported 129 total participants would be required to reach statistical significance. Therefore, given the non-significant interaction effect as well as the lack of racial differences reported with similar sample sizes (n = 10) in Martin et al. [44], experimental data collection was concluded. Nonetheless, it should be noted that underpowered studies are at risk of bias from type II errors [79]. Another limitation of this study is the between-day comparison of microvascular assessments using laser Doppler flowmetry. Although single-fiber probes have been reported as having moderate-to-low between-day repeatability due to the variability in the skin's microcirculation anatomy [80], greater repeatability has been reported in glabrous (e.g. Great toe pad) than non-glabrous (e.g. volar forearm) skin [62]. We attempted to mitigate this by stringently measuring and recording the placement of laser Doppler flowmetry probes as well as presenting skin blood flow data as CVC which improves repeatability compared to the raw "flux" values [81]. Future studies may aim to also include assessments of the macrovasculature (e.g. flow-mediated dilation) in addition to the microvasculature to better understand how heat therapy may distinctly influence these different areas of the vasculature. However, cutaneous microvascular responses were investigated as changes in reactivity have been identified before clinical signs of microvascular dysfunction [82,83]. Although not a primary outcome of this study, another consideration surrounds the inclusion of rectal and gastrointestinal temperatures as indices of T_{core}. Literature has reported good agreement between gastrointestinal and rectal temperature during exercise [84,85] although a faster response rate has been identified in telemetric pills measuring gastrointestinal temperature [86]. However, using a different telemetric system than was employed in this study, O'Brien et al. [87] reported rectal temperature was lower during HWI than gastrointestinal temperature (mean bias: ~ 0.1°C). Nonetheless, our results are supported by Martin et al. [44] who also described similar gastrointestinal temperature responses during acute heat exposure between racial groups. The last factor which should be considered is that this study focused on males as the investigated population and therefore these findings should not be extrapolated to females. Although racial differences in microvascular function have also been identified in females [12], recent work suggests potential influences of the hormonal environment on microvascular endothelial function [88,89] alongside a potential sex difference in the mechanisms mediating blunted microvascular function between young Black males and females [17]. Nonetheless, Martin et al. [44] also reported no race-specific effects on macro- and microvascular function from acute heat exposure which is in agreement with the present study. Future studies investigating microvascular responses should aim to recruit males and females of distinct racial background to investigate a "sex" x "race" x "condition" interaction.

In conclusion, this study showed that despite reduced cutaneous peripheral microvascular

function in males of BA descent, there were no differences in the acute effects of HWI on microvascular responses between WE, BA and SA populations. Although similar between racial groups, the combined reduction in BP throughout immersion and increase in Great toe CVC following acute heat therapy may support its use as a health-promoting intervention.

Abbreviations

ANOVA	Analysis of variance
AUC	Area under the curve
BA	Black-African
BP	Blood pressure
CON	Thermoneutral control
CVC	Cutaneous vascular conductance
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
eNOS	Endothelial nitric oxide synthase
HR	Heart rate
HWI	Hot water immersion
LH	Local heating
MAP	Mean arterial pressure
NO	Nitric oxide
PORH	Post-occlusive reactive hyperemia
RH	Relative humidity
SA	South-Asian
SBP	Systolic blood pressure
TC	Thermal comfort
T_{core}	Body core temperature
TS	Thermal sensation
T_{skin}	Mean skin temperature
VO_{2max}	Maximal oxygen uptake
WE	White-European
η_p^2	Partial eta squared

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No potential conflict of interest was reported by the author(s).

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References

[1] World Health Organisation. (2024). Global action plan for the prevention and control of noncommunicable diseases 2013-2020. https://www.who.int/publications/i/item/9789241506236

[2] Martin SS, Aday AW, Almarzooq ZI, et al. Heart disease and stroke statistics: a report of US and global data from the American Heart Association. Circulation. 2024;149(8):e347–e913. doi: 10.1161/CIR.0000000000001209

[3] Ho FK, Gray SR, Welsh P, et al. Ethnic differences in cardiovascular risk: examining differential exposure and susceptibility to risk factors. BMC Med. 2022;20(1):149. doi: 10.1186/s12916-022-02337-w

[4] Muilwijk M, Ho F, Waddell H, et al. Contribution of type 2 diabetes to all-cause mortality, cardiovascular disease incidence and cancer incidence in White Europeans and South Asians: findings from the UK biobank population-based cohort study. BMJ Open Diabetes Res Care. 2019;7(1):e000765. doi: 10.1136/bmjdrc-2019-000765

[5] Ferdinand KC. Cardiovascular risk reduction in African Americans: current concepts and controversies. Glob Cardiol Sci Pract. 2016;2016(1):e201602. doi: 10.21542/gcsp.2016.2

[6] Lind L, Berglund L, Larsson A, et al. Endothelial function in resistance and conduit arteries and 5-year risk of cardiovascular disease. Circulation. 2011;123(14):1545–1551. doi: 10.1161/CIRCULATIONAHA.110.984047

[7] Yeboah J, Folsom AR, Burke GL, et al. Predictive value of brachial flow-mediated dilation for incident cardiovascular events in a population-based study: the multi-ethnic study of atherosclerosis. Circulation. 2009;120(6):502–509. doi: 10.1161/CIRCULATIONAHA.109.864801

[8] Campia U, Choucair WK, Bryant MB, et al. Endothelial function assessment reduced endothelium-dependent and-independent dilation of conductance arteries in African Americans. J Am Coll Cardiol. 2002;40(4):754–760. doi: 10.1016/S0735-1097(02)02015-6

[9] Cardillo C, Kilcoyne CM, Cannon Iii RO, et al. Attenuation of cyclic nucleotide-mediated smooth muscle relaxation in blacks as a cause of racial differences in vasodilator function. Circulation. 1999;99(1):90–95. doi: 10.1161/01.CIR.99.1.90

[10] Maley MJ, House JR, Tipton MJ, et al. Vascular responses of the extremities to transdermal application of vasoactive agents in Caucasian and African descent individuals. Eur J Appl Physiol. 2015;115(8):1801–1811. doi: 10.1007/s00421-015-3164-2

[11] Maley MJ, House JR, Tipton MJ, et al. Role of cyclooxygenase in the vascular response to locally delivered acetylcholine in Caucasian and African descent individuals. Microvasc Res. 2017;111:80–87. doi: 10.1016/j.mvr.2017.01.005

[12] D'Agata MN, Hoopes EK, Berube FR, et al. Young black women demonstrate impaired microvascular but preserved macrovascular function compared to White women. Exp Physiol. 2021;106(10):2031–2037. doi: 10.1113/EP089702

[13] Heffernan KS, Jae SY, Wilund KR, et al. Racial differences in central blood pressure and vascular function in young men. Am J Physiol Heart Circ Physiol. 2008;295(6):H2380–H2387. doi: 10.1152/ajpheart.00902.2008

[14] Turner CG, Walker DC, Wong BJ. Contribution of sensory nerves to cutaneous reactive hyperaemia in non-Hispanic Black and White young adults. Exp Physiol. 2023;108(6):802–809. doi: 10.1113/EP091178

[15] Maley MJ, Eglin CM, House JR, et al. The effect of ethnicity on the vascular responses to cold exposure of the extremities. Eur J Appl Physiol. 2014;114(11):2369–2379. doi: 10.1007/s00421-014-2962-2

[16] Hurr C, Patik JC, Kim KY, et al. Tempol augments the blunted cutaneous microvascular thermal reactivity in healthy young African Americans. Exp Physiol. 2018;103(3):343–349. doi: 10.1113/EP086776

[17] Patik JC, Curtis BM, Nasirian A, et al. Sex differences in the mechanisms mediating blunted cutaneous microvascular function in young black men and women. Am J Physiol Heart Circ Physiol. 2018;315(4):H1063–H1071. doi: 10.1152/ajpheart.00142.2018

[18] Brothers RM, Fadel PJ, Keller DM. Racial disparities in cardiovascular disease risk: mechanisms of vascular dysfunction. Am J Physiol Heart Circ Physiol. 2019;317(4):H777–H789. doi: 10.1152/ajpheart.00126.2019

[19] Murphy C, Kanaganayagam GS, Jiang B, et al. Vascular dysfunction and reduced circulating endothelial progenitor cells in young healthy UK south Asian men. Arterioscler Thromb Vasc Biol. 2007;27(4):936–942. doi: 10.1161/01.ATV.0000258788.11372.d0

[20] Shantsila E, Wrigley B, Shantsila A, et al. Ethnic differences in macrovascular and microvascular function in systolic heart failure. Circ Heart Fail. 2011;4(6):754–762. doi: 10.1161/CIRCHEARTFAILURE.111.962365

[21] Hadi HA, Carr CS, Al Suwaidi J. Endothelial dysfunction: cardiovascular risk factors, therapy, and outcome. Vasc Health Risk Manag. 2005;1(3):183–198.

[22] Lakatta EG, Levy D. Arterial and cardiac aging: Major shareholders in cardiovascular disease enterprises: part I: aging arteries: a "set up" for vascular disease. Circulation. 2003;107(1):139–146. doi: 10.1161/01.CIR.0000048892.83521.58

[23] Najjar SS, Scuteri A, Lakatta EG. Arterial aging: Is it an immutable cardiovascular risk factor? Hypertension. 2005;46(3):454–462. doi: 10.1161/01.HYP.0000177474.06749.98

[24] Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation. 2002;105(9):1135–1143. doi: 10.1161/hc0902.104353

[25] Schulz E, Gori T, Münzel T. Oxidative stress and endothelial dysfunction in hypertension. Hypertens Res. 2011;34(6):665–673. doi: 10.1038/hr.2011.39

[26] Chambers JC, Eda S, Bassett P, et al. C-reactive protein, insulin resistance, central obesity, and coronary heart disease risk in Indian Asians from the United Kingdom compared with European Whites. Circulation. 2001;104(2):145–150. doi: 10.1161/01.cir.104.2.145

[27] Deo SH, Holwerda SW, Keller DM, et al. Elevated peripheral blood mononuclear cell-derived superoxide production in healthy young Black Men. Am J Physiol Heart Circ Physiol. 2015;308(5):H548–H552. doi: 10.1152/ajpheart.00784.2014

[28] Farmer HR, Wray LA, Xian Y, et al. Racial differences in elevated c-reactive protein among US older adults. J Am Geriatr Soc. 2020;68(2):362–369. doi: 10.1111/jgs.16187

[29] Laukkanen JA, Kunutsor SK. The multifaceted benefits of passive heat therapies for extending the healthspan: A comprehensive review with a focus on Finnish sauna. Temperature. 2024;11(1):27–51. doi: 10.1080/23328940.2023.2300623

[30] Laukkanen T, Khan H, Zaccardi F, et al. Association between sauna bathing and fatal cardiovascular and all-cause mortality events. JAMA Intern Med. 2015;175(4):542–548. doi: 10.1001/jamainternmed.2014.8187

[31] Brunt VE, Minson CT. Heat therapy: mechanistic underpinnings and applications to cardiovascular health. J Appl Physiol. 2021;130(6):1684–1704. doi: 10.1152/japplphysiol.00141.2020

[32] Cheng JL, Macdonald MJ. Effect of heat stress on vascular outcomes in humans. J Appl Physiol. 2019;126(3):771–781. doi: 10.1152/japplphysiol.00682.2018

[33] Jackson R. Waters and spas in the classical world. Med Hist. 1990;34(S10):1–13. doi: 10.1017/S0025727300070952

[34] Pizzey FK, Smith EC, Ruediger SL, et al. The effect of heat therapy on blood pressure and peripheral vascular function: a systematic review and meta-analysis. Exp Physiol. 2021;106(6):1317–1334. doi: 10.1113/EP089424

[35] Bailey TG, Cable NT, Miller GD, et al. Repeated warm water immersion induces similar cerebrovascular adaptations to 8 weeks of moderate-intensity exercise training in females. Int J Sports Med. 2016;37(10):757–765. doi: 10.1055/s-0042-106899

[36] Brunt VE, Howard MJ, Francisco MA, et al. Passive heat therapy improves endothelial function, arterial stiffness and blood pressure in sedentary humans. J Physiol. 2016;594(18):5329–5342. doi: 10.1113/JP272453

[37] Carter HH, Spence AL, Atkinson CL, et al. Repeated core temperature elevation induces conduit artery adaptation in humans. Eur J Appl Physiol. 2014;114(4):859–865. doi: 10.1007/s00421-013-2817-2

[38] Ely BR, Francisco MA, Halliwill JR, et al. Heat therapy reduces sympathetic activity and improves cardiovascular risk profile in women who are obese with polycystic ovary syndrome. Am J Physiol Regul Integr Comp Physiol. 2019;317(5):R630–R640. doi: 10.1152/ajpregu.00078.2019

[39] Brunt VE, Eymann TM, Francisco MA, et al. Passive heat therapy improves cutaneous microvascular function in sedentary humans via improved nitric oxide-dependent dilation. J Appl Physiol. 2016;121 (3):716–723. doi: 10.1152/japplphysiol.00424.2016

[40] Carter HH, Spence AL, Atkinson CL, et al. Distinct effects of blood flow and temperature on cutaneous microvascular adaptation. Med Sci Sports Exerc. 2014;46(11):2113–2121. doi: 10.1249/MSS.0000000000000349

[41] Green DJ, Carter HH, Fitzsimons MG, et al. Obligatory role of hyperaemia and shear stress in microvascular adaptation to repeated heating in humans. J Physiol. 2010;588 (9):1571–1577. doi: 10.1113/jphysiol.2010.186965

[42] Romero SA, Richey RE, Hemingway HW. Cardiovascular adjustments after acute heat exposure. Exerc Sport Sci Rev. 2022;50(4):194–202. doi: 10.1249/JES.0000000000000304

[43] Cheng JL, Williams JS, Hoekstra SP, et al. Improvements in vascular function in response to acute lower limb heating in young healthy males and females. J Appl Physiol. 2021;131(1):277–289. doi: 10.1152/japplphysiol.00630.2020

[44] Martin ZT, Akins JD, Merlau ER, et al. The acute effect of whole-body heat therapy on peripheral and cerebral vascular reactivity in Black and White females. Microvasc Res. 2023;148:104536. doi: 10.1016/j.mvr.2023.104536

[45] Brunt VE, Jeckell AT, Ely BR, et al. Acute hot water immersion is protective against impaired vascular function following forearm ischemia-reperfusion in young healthy humans. Am J Physiol Regul Integr Comp Physiol. 2016;311(6):R1060–R1067. doi: 10.1152/ajpregu.00301.2016

[46] Gravel H, Coombs GB, Behzadi P, et al. Acute effect of Finnish sauna bathing on brachial artery flow-mediated dilation and reactive hyperemia in healthy middle-aged and older adults. Physiol Rep. 2019;7(13):e14166. doi: 10.14814/phy2.14166

[47] Behzadi P, Ravanelli N, Gravel H, et al. Acute effect of passive heat exposure on markers of cardiometabolic function in adults with type 2 diabetes mellitus. J Appl Physiol. 2022;132(5):1154–1166. doi: 10.1152/japplphysiol.00800.2021

[48] Gravel H, Behzadi P, Cardinal S, et al. Acute vascular benefits of Finnish sauna bathing in patients with stable coronary artery disease. Can J Cardiol. 2021;37 (3):493–499. doi: 10.1016/j.cjca.2020.06.017

[49] Chaseling GK, Debray A, Gravel H, et al. The acute effect of heat exposure on forearm macro- and microvascular function: impact of measurement timing, heating modality and biological sex. Exp Physiol. 2023;108(2):221–239. doi: 10.1113/EP090732

[50] Smolander J, Härmä M, Lindqvist A, et al. Circadian variation in peripheral blood flow in relation to core temperature at rest. Eur J Appl Physiol Occup Physiol. 1993;67(2):192–196. doi: 10.1007/BF00376666

[51] Maley MJ, Hunt AP, Stewart IB, et al. Hot water immersion acutely reduces peripheral glucose uptake in young healthy males: An exploratory crossover randomized controlled trial. Temperature. 2023;10 (4):434–443. doi: 10.1080/23328940.2022.2161242

[52] Achamrah N, Colange G, Delay J, et al. Comparison of body composition assessment by DXA and BIA according to the body mass index: a retrospective study on 3655 measures. PLOS ONE. 2018;13(7): e0200465. doi: 10.1371/journal.pone.0200465

[53] Deurenberg P. Limitations of the bioelectrical impedance method for the assessment of body fat in severe obesity. Am J Clin Nutr. 1996;64(3):449S–452S. doi: 10.1093/ajcn/64.3.449S

[54] Du Bois D, Du Bois EF. A formula to estimate the approximate surface area if height and weight be known. Nutrition. 1989;5(5):303–313.

[55] American College of Sports Medicine. Cardiorespiratory fitness: Estimation from field and submaximal exercise tests. Kaminsky LA, editor. Health-related physical fitness assessment manual. 3rd ed. Philadelphia, USA: Wolters Kluwer Health, Lippincott Williams & Wilkins; 2010. p. 110–137.

[56] Macsween A. The reliability and validity of the astrand nomogram and linear extrapolation for deriving VO2max from submaximal exercise data. J Sports Med Phys Fit. 2001;41(3):312–317.

[57] Leon GR, Koscheyev VS, Stone EA. Visual analog scales for assessment of thermal perception in different environments. Aviat Space Environ Med. 2008;79 (8):784–786. doi: 10.3357/asem.2204.2008

[58] Armstrong LE, Maresh CM, Castellani JW, et al. Urinary indices of hydration status. Int J Sport Nutr. 1994;4(3):265–279. doi: 10.1123/ijsn.4.3.265

[59] Hunt AP, Bach AJE, Borg DN, et al. The systematic bias of ingestible core temperature sensors requires a correction by linear regression. Front Physiol. 2017;8:260. doi: 10.3389/fphys.2017.00260

[60] Ramanathan NL. A new weighting system for mean surface temperature of the human body. J Appl Physiol. 1964;19(3):531–533. doi: 10.1152/jappl.1964.19.3.531

[61] Eglin CM, Wright J, Maley MJ, et al. The peripheral vascular responses in non-freezing cold injury and matched controls. Exp Physiol. 2023;108(3):420–437. doi: 10.1113/EP090721

[62] Roustit M, Blaise S, Millet C, et al. Reproducibility and methodological issues of skin post-occlusive and thermal hyperemia assessed by single-point laser doppler flowmetry. Microvasc Res. 2010;79(2):102–108. doi: 10.1016/j.mvr.2010.01.001

[63] Minson CT, Holowatz LA, Wong BJ, et al. Decreased nitric oxide- and axon reflex-mediated cutaneous vasodilation with age during local heating. J Appl Physiol. 2002;93(5):1644–1649. doi: 10.1152/japplphysiol.00229.2002

[64] Steward CJ, Hill M, Menzies C, et al. Post exercise hot water immersion and hot water immersion in isolation enhance vascular, blood marker, and perceptual responses when compared to exercise alone. Scand J Med Sci Sports. 2024;34(3):e14600. doi: 10.1111/sms.14600

[65] Higuchi K, Motomizu S. Flow-injection spectrophotometric determination of nitrite and nitrate in biological samples. Anal Sci. 1999;15(2):129–134. doi: 10.2116/analsci.15.129

[66] Cohen J. Statistical power analysis for the behavioural sciences2ndRoutledge; 1988, 10.4324/9780203771587

[67] Jin RC, Loscalzo J. Vascular nitric oxide: formation and function. J Blood Med. 2010;2010(1):147–162. DOI:10.2147/JBM.S7000

[68] Lauer T, Preik M, Rassaf T, et al. Plasma nitrite rather than nitrate reflects regional endothelial nitric oxide synthase activity but lacks intrinsic vasodilator action. Proc Natl Acad Sci USA. 2001;98(22):12814–12819. doi: 10.1073/pnas.221381098

[69] Grosicki GJ, Flatt AA, Cross BL, et al. Acute beetroot juice reduces blood pressure in young Black and White males but not females. Redox Biol. 2023;63:102718. doi: 10.1016/j.redox.2023.102718

[70] Simmons GH, Wong BJ, Holowatz LA, et al. Changes in the control of skin blood flow with exercise training: where do cutaneous vascular adaptations fit in? Exp Physiol. 2011;96(9):822–828. doi: 10.1113/expphysiol.2010.056176

[71] Swift DL, Staiano AE, Johannsen NM, et al. Low cardiorespiratory fitness in African americans: a health disparity risk factor? Sports Med. 2013;43(12):1301–1313. doi: 10.1007/s40279-013-0092-3

[72] Johnson JM, Pérgola PE, Liao FK, et al. Skin of the dorsal aspect of human hands and fingers possesses an active vasodilator system. J Appl Physiol. 1995;78(3):948–954. doi: 10.1152/jappl.1995.78.3.948

[73] Kellogg DL Jr, Crandall CG, Liu Y, et al. Nitric oxide and cutaneous active vasodilation during heat stress in humans. J Appl Physiol. 1998;85(3):824–829. doi: 10.1152/jappl.1998.85.3.824

[74] Minson CT, Berry LT, Joyner MJ. Nitric oxide and neurally mediated regulation of skin blood flow during local heating. J Appl Physiol. 2001;91(4):1619–1626. doi: 10.1152/jappl.2001.91.4.1619

[75] Larkin SW, Williams TJ. Evidence for sensory nerve involvement in cutaneous reactive hyperemia in humans. Circ Res. 1993;73(1):147–154. doi: 10.1161/01.res.73.1.147

[76] Lorenzo S, Minson CT. Human cutaneous reactive hyperaemia: role of BK_{Ca} channels and sensory nerves. J Physiol. 2007;585(1):295–303. doi: 10.1113/jphysiol.2007.143867

[77] Hoekstra SP, Bishop NC, Faulkner SH, et al. Acute and chronic effects of hot water immersion on inflammation and metabolism in sedentary, overweight adults. J Appl Physiol. 2018;125(6):2008–2018. doi: 10.1152/japplphysiol.00407.2018

[78] Francisco MA, Colbert C, Larson EA, et al. Hemodynamics of post-exercise vs. post hot water immersion recovery. J Appl Physiol. 2021;130(5):1362–1372. doi: 10.1152/japplphysiol.00260.2020

[79] Mascha EJ, Vetter TR. Significance, errors, power, and sample size: the blocking and tackling of statistics. Anesth Analg. 2018;126(2):691–698. doi: 10.1213/ANE.0000000000002741

[80] McGarr GW, Hodges GJ, Cheung SS. Between-day reliability of local thermal hyperemia in the forearm and index finger using single-point laser doppler flowmetry. Microcirculation. 2017;24(8):e12395. doi: 10.1111/micc.12395

[81] Cracowski JL, Minson CT, Salvat-Melis M, et al. Methodological issues in the assessment of skin microvascular endothelial function in humans. Trends Pharmacol Sci. 2006;27(9):503–508. doi: 10.1016/j.tips.2006.07.008

[82] Ijzerman RG, de Jongh RT, Beijk MA, et al. Individuals at increased coronary heart disease risk are characterized by an impaired microvascular function in skin. Eur J Clin Invest. 2003;33(7):536–542. doi: 10.1046/j.1365-2362.2003.01179.x

[83] Kraemer-Aguiar LG, Laflor CM, Bouskela E. Skin microcirculatory dysfunction is already present in normoglycemic subjects with metabolic syndrome. Metabolism. 2008;57(12):1740–1746. doi: 10.1016/j.metabol.2008.07.034

[84] Casa DJ, Becker SM, Ganio MS, et al. Validity of devices that assess body temperature during outdoor exercise in the heat. J Athl Train. 2007;42(3):333–342.

[85] Hosokawa Y, Adams WM, Stearns RL, et al. Comparison of gastrointestinal and rectal temperatures during recovery after a warm-weather road race. J Athl Train. 2016;51(5):382–388. doi: 10.4085/1062-6050-51.7.02

[86] Bogerd CP, Velt KB, Annaheim S, et al. Comparison of two telemetric intestinal temperature devices with rectal temperature during exercise. Physiol Meas. 2018;39(3):03NT01. doi: 10.1088/1361-6579/aaad52

[87] O'Brien TJ, Goosey-Tolfrey VL, Leicht CA. Rectal and gastrointestinal temperature differ during passive heating and subsequent recovery. J Therm Biol. 2024;119:103755. doi: 10.1016/j.jtherbio.2023.103755

[88] Turner CG, Stanhewicz AE, Nielsen KE, et al. Microvascular endothelial function following cessation of long-term oral contraceptive pill use: a case report. Exp Physiol. 2023;108(1):5–11. doi: 10.1113/EP090861

[89] Turner CG, Stanhewicz AE, Nielsen KE, et al. Effects of biological sex and oral contraceptive pill use on cutaneous microvascular endothelial function and nitric oxide-dependent vasodilation in humans. J Appl Physiol. 2023;134(4):858–867. doi: 10.1152/japplphysiol.00586.2022