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### Article

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RESEARCH ARTICLE

*Physiological Adaptations to Environmental Stressors and Challenging Conditions*

# Sex differences in the thermoregulatory and cardiovascular response to exercise in hot environmental conditions

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## Abstract

Exercise during heat exposure induces skin microvascular and systemic cardiovascular changes. When standardized exercise tasks are completed, such as during military training or in workplace settings, sex differences in responses may be apparent. Nineteen males and 19 females participated in a set-pace laboratory walking test (treadmill walking 5 km/h; 2% incline) in a climate chamber (40°C; 50% RH) for 90 min. Body composition (DXA) and aerobic capacity ( $\dot{V}O_{2\max}$ ) were measured in a preliminary session. Metabolic heat production, skin blood flow (SkBF; laser Doppler flowmetry), limb blood flow (Doppler ultrasound), stroke volume, cardiac output (CO), heart rate (HR), oxygen consumption ( $\dot{V}O_2$ ), and core temperature (Tc) were measured at baseline, 30, 60, and 90 min. No sex difference in Tc at 90 min was evident (male  $38.3 \pm 0.5^\circ\text{C}$  vs. female  $38.5 \pm 0.4^\circ\text{C}$ ;  $P = 0.403$ ) and a similar change from baseline to 90 min ( $\Delta 1.40$  vs.  $1.28^\circ\text{C}$ ;  $P = 0.447$ ) occurred, despite males producing more heat ( $3.4 \pm 1.0$  vs.  $2.1 \pm 0.7$  W/kg;  $P = 0.001$ ), exhibiting higher SkBF ( $192 \pm 50$  vs.  $160 \pm 21$  PU;  $P = 0.026$ ), and higher sweat production rate ( $16.5 \pm 5.1$  vs.  $12.3 \pm 3.3$  mL/min;  $P = 0.009$ ). Males also had higher CO ( $7.25 \pm 1.38$  vs.  $6.11 \pm 1.72$  L/min; group  $P = 0.026$ ) and femoral blood flow ( $1.00 \pm 0.23$  vs.  $0.82 \pm 0.19$  L/min;  $P = 0.026$ ) responses than females. Males compensated for more lean mass and higher metabolic heat production via a larger increase in cardiac output, with more blood flow distributed to active muscle and, as heat and exercise exposure continued, to the skin. Tc in females did not rise more than in males, possibly due to body size and/or anthropometric factors.

**NEW & NOTEWORTHY** In military, workplace, and sporting settings, challenging environmental conditions while performing external workloads are not always avoidable. We assessed sex differences during a 90-min treadmill walk (40°C). Males produced more metabolic heat, had higher skin blood flow, sweat rate, and cardiac output than females. Change in core temperature remained similar between sexes, challenging the proposition that women are more heat-intolerant than men. Our findings underscore the need for tailored heat tolerance strategies for both sexes.

exercise; heat stress; sex difference; skin blood flow; thermoregulation

## INTRODUCTION

In many military, occupational, and some sporting settings, exercise in the heat is difficult to avoid, and risk mitigation requires an understanding of the physiological challenge of the combined stimuli (1). For example, Hunt et al. (2) examined the balance of operational need versus thermal stress mitigation in male soldiers preparing for deployment. During a 10-km timed march carrying 40 kg under demanding field conditions, they noted interindividual variability in the development of heat-related symptoms

and that these outcomes were poorly predicted by change in core temperature (Tc). The authors concluded that an assessment of physiological data beyond Tc was needed to predict exertional heat illness.

As a follow-up to the study of Hunt et al. (2), we recently investigated the integrated physiological responses to an exercise task in the heat in a controlled laboratory environment (3). Confirming the findings of Hunt et al. (2), none of the individuals who became symptomatic for heat illness was hyperthermic (defined as  $T_c > 39^\circ\text{C}$ ), and none of those who was hyperthermic reported symptoms of heat illness.



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These data suggest a dissociation between hyperthermia and heat illness. We also reported (3) that a similar number of males and females became symptomatic and hyperthermic in response to the same absolute workload in the heat, suggesting that sex may not be a major factor in mediating those outcomes. We did not, however, compare the cardiovascular responses between males and females in response to exercise in hot conditions in that article. To provide further insight into the findings of Hunt et al. (2), our aim in the present study was to consider cardiovascular variables that were not possible to assess in the field experiment of Hunt et al. (2), with the additional inclusion of female participants.

Recent data suggest that there may be differences between sexes in the risk factors for heat illness in the same occupations (e.g., in the armed forces) (4). Males present higher rates of heat stroke compared with their female counterparts, and females experience higher rates of heat exhaustion and heat intolerance than males (5). These data suggest that physiological differences may exist in the response to exercise and heat exposure between men and women. However, it was recently reported that only ~15% of studies on human thermoregulation have recruited women (6), despite the fact that women are increasingly integrated into active-duty military roles, that recruitment biases for physically active jobs in industrial settings are diminishing, and that women's sport has become increasingly professionalized. Hence, there is a need to understand the differences and similarities in the response of males and females to exercise in the heat (7, 8).

We compared healthy, physically active, working-age male and female adults who are broadly representative of those involved in military and workplace settings, exposed to exertional heat and exercise. We hypothesized that, despite the same environmental exposure and set-pace task, males and females would exhibit distinct cardiovascular and thermoregulatory responses.

## MATERIALS AND METHODS

### Participants

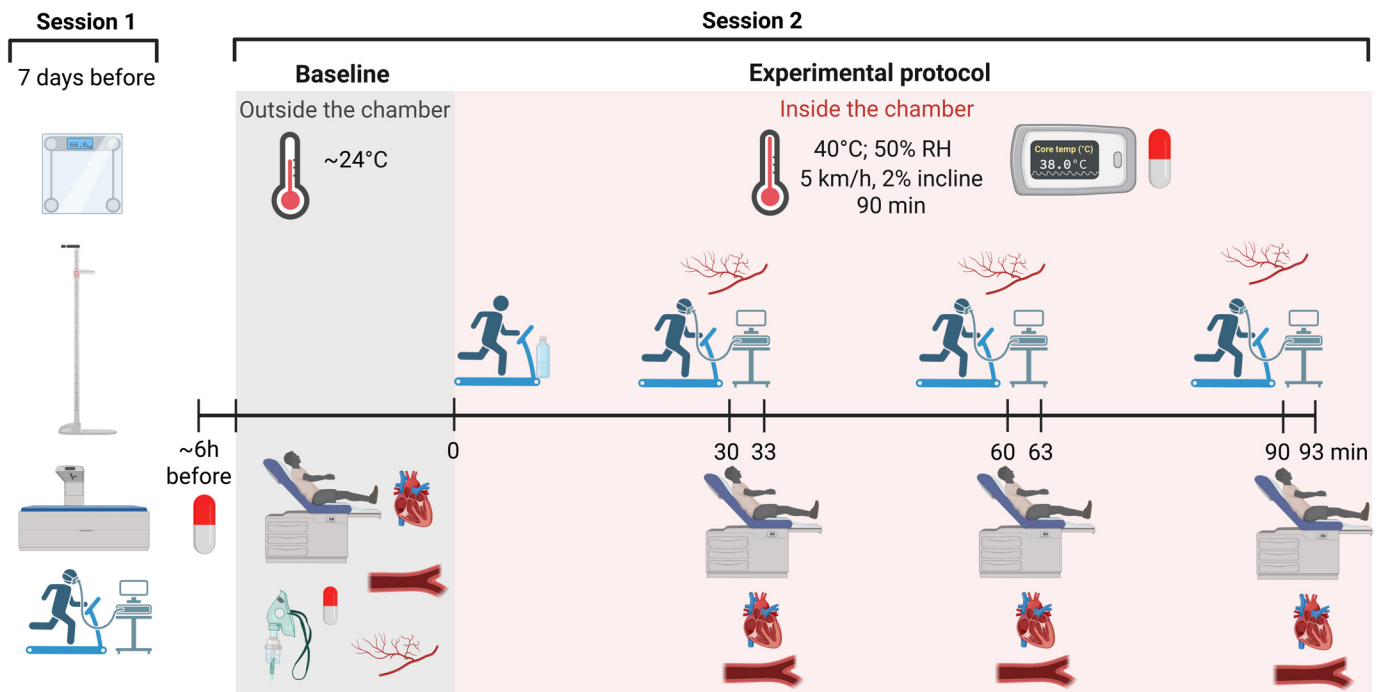
Expected sex differences in end-exercise core temperature (9) were used to calculate ( $\beta = 0.9$ ,  $\alpha = 0.05$ ) a minimum sample size of 38 participants (19 in each group), assuming an effect size of  $f = 0.22$ , for 2 groups and 4 measurements. We report data on 38 healthy adult participants aged 18–40 yr (19 males and 19 females), recruited for this study from the local community through advertising. Inclusion criteria comprised healthy individuals who were nonsmokers, not taking medication, and with no history of cardiovascular, cerebrovascular, metabolic, and/or respiratory disorders or musculoskeletal injuries. In keeping with recent recommendations (10), we report that in all cases the individuals we recruited self-reported their gender as consistent with their sex recorded at birth. The study was approved by the Human Research Ethics Committee of the University of Western Australia (Ref. No. RA/4/20/5716). It conformed to the Declaration of Helsinki, and all participants provided written informed consent before participating in the study. The trial was not registered a priori, as it was not a clinical trial.

### Experimental Protocol

Each participant attended two laboratory sessions (Fig. 1) conducted at the same time of the day for both sessions, starting from 7:00 AM to 10:00 AM.

### Preliminary Assessments

Preliminary assessments were conducted in a thermoneutral laboratory seven days before the subsequent experimental session. This session involved the measurement of height and body mass (used to calculate body mass index), body composition, and aerobic capacity. Body composition was



**Figure 1.** Study design. Figure created with a licensed version of BioRender.com.

measured using dual-energy X-ray absorptiometry (Lunar iDXA, GE Healthcare), which derived indices including fat and lean mass, and visceral adipose tissue. Aerobic capacity ( $\dot{V}O_{2\max}$ ) was assessed during a continuous incremental treadmill exercise test. The intensity of the exercise test (gradient fixed at 10% and speed at 8.0 km/h) was increased by 1 km/h at the end of each 3-min stage, until volitional exhaustion. Heart rate (HR; Polar H10 HR monitor, Polar Electro Oy, Finland) and rating of perceived exertion (RPE) were recorded in the last 15 s of each stage, and at the end of the test. Expiratory flow and gas composition were measured using a metabolic measurement system (Parvomedics TrueOne 2400, Salt Lake City, UT).

## Experimental Session

In the subsequent experimental session, the participants arrived at the laboratory after fasting overnight for a minimum of 8 h and having abstained from caffeine, vigorous physical exercise, and alcohol for a minimum of 24 h. Approximately 6–7 h before the experiment, the participants ingested a temperature sensor telemetry capsule (eCelsius Performance electronic capsule; BodyCap Medical, Hérouville-Saint-Calir, France) to ensure that the sensor was at an appropriate location in the digestive tract during data collection (11). Core temperature ( $T_c$ ) readings were taken as a single value every 5 min with a handheld data monitor.

Water for ad libitum consumption by the participants was available during the 90-min session, and it was placed in the chamber ~60 min before the start of the experiment to ensure no cooling effect. The water bottle was weighed at baseline and at the end of the test to calculate the water ingestion. Dry nude body mass was measured before and after the sessions to calculate mass loss and sweat rate using the formula:  $SR = (\text{pre-exercise weight} - \text{postexercise weight} + \text{fluid intake}) / \text{exercise duration}$  (12). During the baseline assessments, the participants wore a gown and shorts; females were asked to wear a sports bra under the gown. There was no fan placed to generate wind speed.

After initial instrumentation, the participants lay in a semi-reclined position outside the chamber for a 20-min rest period before baseline assessments, during which time the body and arm positioning mimicked the positioning that was required during the subsequent assessments at 30, 60, and 90 min after exercise commenced within the chamber (Fig. 1). Baseline measurements of  $T_c$  and heart rate, systolic and diastolic blood pressure (Dinamap V100, GE Healthcare) were then collected, along with 5 min of expired gas and flow measurement using a metabolic measurement system (Parvomedics TrueOne 2400, Salt Lake City, UT). Metabolic energy expenditure was calculated from minute-average values of oxygen consumption ( $\dot{V}O_2$ ) and the respiratory exchange ratio. Subsequently, metabolic heat production ( $H_{\text{prod}}$ ) was determined by subtracting the amount of mechanical work performed ( $W$ ) from the metabolic energy expenditure ( $M$ ) (13).

After baseline measurements, the participant immediately walked into a climate chamber (at 40°C and 50% relative humidity) and onto a treadmill (5 km/h and 2% incline) for 90 min. During the experimental protocol inside the chamber, skin blood flow (SkBF) and oxygen consumption

were measured for 5 min end of each 30-min stage, while the participants were walking. Vascular and echocardiographic scans were assessed inside the chamber during brief semi-recumbent interludes of 3 min. Participants were removed from the heat chamber if their  $T_c$  exceeded 39°C or if they showed any signs or symptoms of heat-related illness, such as weakness, dizziness, nausea, headache, muscle cramps, or confusion. In such cases, they were closely monitored while they recovered.

Stroke volume (SV), cardiac output (CO), and heart rate (HR) were assessed via a transthoracic echocardiogram using a commercially available ultrasound system (EPIQ CVx; Philips Ultrasound, Andover, MA) using an X5-1 MHz transducer. Scans were performed by an experienced and accredited sonographer following comprehensive guidelines. Echocardiographic images were obtained with the participants in the semi-recumbent left lateral position at end expiration, with the transducer angled to obtain orthogonal views of the left ventricle (LV). Three complete cardiac cycles were obtained for every image. Images were digitally stored in cine-loop DICOM format and transferred to the Philips Ultrasound Workspace (TOMTEC Imaging Systems GmbH, Freisinger Strasse, Unterschleißheim, Germany) for offline post hoc analysis. SV was calculated as the difference between end-diastolic volume (EDV) and end-systolic volume (ESV).

The diameter of the brachial and femoral arteries and blood velocity were measured on the left side simultaneously for 1 min using a high-resolution Doppler ultrasound (uSmart3300, Terason, Burlington, MA) equipped with a 4–15 MHz linear array transducer probe at an insonation angle of 60°. The site scanned at baseline was maintained during the subsequent scans. Screen recording software (Camtasia Studio; TechSmith, Okemos, MI) captured the screen into a video file for later analysis using a custom-designed edge detection and wall-tracking software package to calculate the blood flow in the brachial and femoral arteries (14). Blood flow was calculated from synchronized diameter and velocity data using the product of lumen cross-sectional area and Doppler velocity.

SkBF was obtained by laser Doppler flowmetry (LDF) (Model 413, Periflux 5000 system; Perimed, Jarfalla, Sweden). LDF is a noninvasive technique that enables estimation of the microcirculatory flux by detecting the Doppler shift in light reflected from moving red blood cells. Two LDF probes were attached to the skin on the volar aspect of the forearm (immediately distal to the cubital fossa) and to the upper back (spinal of scapula), using double-sided adhesive rings. The collected data were exported to a data acquisition system PowerLab (LabChart 7, ADInstruments, Sydney, Australia), in real time and presented in perfusion units (PU).

## Statistical Analysis

Data are expressed as means  $\pm$  standard deviation, unless otherwise indicated. To compare sexes in terms of body composition, cardiorespiratory, hemodynamic,  $T_c$ , and heat production variables at baseline, and sweat rate and water ingestion, we initially performed a test for normality of the data distribution (Shapiro–Wilk). Where data were normally distributed, we compared sex differences using an unpaired



*t* test. Where data were not normally distributed, we report Mann–Whitney test outcomes. Pearson's correlation was used to determine the strength and direction of relationships between SkBF and sweat rate, SkBF and heat production, and lean mass (kg), fat mass (kg), and total body mass (kg) and heat production (W). A two-way mixed model ANOVA was performed to compare the mean differences between groups (males and females) across the four time points for body composition, cardiorespiratory, hemodynamic measures, Tc, and heat production. An a priori decision was made to perform post hoc comparisons between paired data points using Bonferroni correction. For all comparisons, significance was set at  $P < 0.05$ . Statistical analyses were performed using SPSS version 29.0 (SPSS, Inc., Chicago, IL).

## RESULTS

### Participants Characteristics

A total of 38 participants were recruited and completed the baseline assessment (Table 1). The males were slightly younger, taller, heavier, and had more body surface area, lean mass (% and kg), visceral adipose tissue, and less total and limb fat mass (%) than the females. The body mass index (BMI) was not statistically different between groups. The males had larger brachial and femoral artery diameter and flow, but no differences were apparent in brachial or femoral velocity or baseline SkBF. The males were fitter (in terms of absolute, relative, and per lean mass  $\dot{V}O_{2\max}$ ). Males had higher SBP, SV, and CO at rest, with no between-group differences in diastolic BP or HR at rest. The males had a lower baseline Tc than the females. Two females did not complete the 90-min protocol due to heat-related symptoms (nausea and dizziness). The full exercise data analysis, therefore, included 36 participants (19 males and 17 females).

### Sweat Loss and Water Ingestion

The males had a higher rate of sweat loss than the females (male  $16.5 \pm 5.1$  mL/min vs. female  $12.3 \pm 3.3$  mL/min;  $P = 0.009$ ; unpaired *t* test). When the rate of sweat production was adjusted by body surface area (BSA), the sweat loss was not different between sexes ( $8.4 \pm 2.3$  vs.  $7.2 \pm 1.7$  mL/m<sup>2</sup>;  $P = 0.099$ ; unpaired *t* test). The males and females consumed a similar amount of water during the protocol ( $1.0 \pm 0.6$  vs.  $0.8 \pm 0.7$  L;  $P = 0.260$ ; Mann–Whitney *U* test).

### Core Temperature

There was a significant effect of time ( $P < 0.001$ ) and sex ( $P = 0.022$ ) on Tc, with males having lower Tc than females, but there was no interaction ( $P = 0.441$ ) (Fig. 2). Males exhibited significantly ( $P < 0.05$ ) lower Tc at baseline and at 30 min, but no difference between males and females was apparent at 60 and 90 min. Males and females exhibited similar increases in core temperature in response to the 90 min protocol ( $\Delta 90$  min:  $1.40$  vs.  $1.28^\circ\text{C}$ ;  $P = 0.447$ ).

### Oxygen Consumption and Heat Production

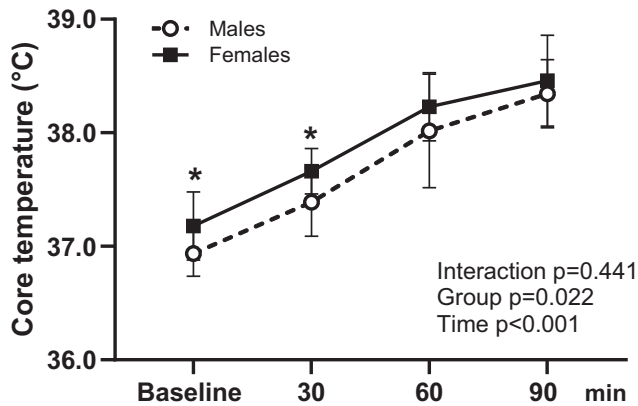
Oxygen consumption (L/min) increased during the protocol (time  $P < 0.001$ ) in the males and the females (group  $P < 0.001$ ), with a larger effect in the males (interaction

**Table 1.** Participant characteristics at rest

|                                            | Males (n = 19),<br>Means $\pm$ SD | Females (n = 19),<br>Means $\pm$ SD | P Value |
|--------------------------------------------|-----------------------------------|-------------------------------------|---------|
| Age, yr                                    | 26 $\pm$ 4                        | 30 $\pm$ 6                          | 0.0017  |
| Height, m                                  | 1.81 $\pm$ 0.08                   | 1.70 $\pm$ 0.07                     | <0.001  |
| Weight, kg                                 | 74.6 $\pm$ 10.2                   | 62.1 $\pm$ 5.0                      | <0.001  |
| BMI, kg/m <sup>2</sup>                     | 22.83 $\pm$ 2.59                  | 21.61 $\pm$ 1.68                    | 0.092   |
| Body surface area, m <sup>2</sup>          | 1.93 $\pm$ 0.16                   | 1.71 $\pm$ 0.09                     | <0.001  |
| Body composition                           |                                   |                                     |         |
| Fat mass, %                                | 19.5 $\pm$ 6.4                    | 29.0 $\pm$ 6.7                      | <0.001  |
| Lean mass, %                               | 76.3 $\pm$ 6.1                    | 66.9 $\pm$ 6.4                      | <0.001  |
| Fat mass, kg                               | 14.8 $\pm$ 6.0                    | 17.5 $\pm$ 4.8                      | 0.178   |
| Lean mass, kg                              | 57.0 $\pm$ 7.7                    | 41.5 $\pm$ 5.7                      | <0.001  |
| Visceral adipose tissue, g                 | 0.360 $\pm$ 0.307                 | 0.166 $\pm$ 0.136                   | 0.020   |
| Hemodynamic measures                       |                                   |                                     |         |
| Brachial diameter, mm                      | 3.83 $\pm$ 0.46                   | 3.12 $\pm$ 0.38                     | <0.001  |
| Brachial velocity, cm/s                    | 12.7 $\pm$ 6.7                    | 12.9 $\pm$ 5.9                      | 0.888   |
| Brachial flow, mL/min                      | 83.6 $\pm$ 40.8                   | 57.5 $\pm$ 25.0                     | 0.023   |
| Femoral diameter, mm                       | 6.04 $\pm$ 0.67                   | 5.43 $\pm$ 0.62                     | 0.006   |
| Femoral velocity, cm/s                     | 14.2 $\pm$ 5.0                    | 12.2 $\pm$ 5.0                      | 0.225   |
| Femoral flow, mL/min                       | 243.3 $\pm$ 91.4                  | 174.3 $\pm$ 88.3                    | 0.025   |
| Skin blood flux, PU*                       | 22.7 $\pm$ 8.0                    | 23.8 $\pm$ 11.5                     | 0.734   |
| Cardiorespiratory measures                 |                                   |                                     |         |
| Resting O <sub>2</sub> , mL/kg/min         | 3.6 $\pm$ 0.4                     | 3.4 $\pm$ 0.4                       | 0.382   |
| $\dot{V}O_{2\max}$ , L/min                 | 4.5 $\pm$ 1.7                     | 2.6 $\pm$ 0.6                       | <0.001  |
| $\dot{V}O_{2\max}$ , mL/kg/min             | 59.7 $\pm$ 12.1                   | 41.8 $\pm$ 9.6                      | <0.001  |
| $\dot{V}O_{2\max}/\text{lean}$ , mL/kg/min | 74.8 $\pm$ 14.4                   | 62.2 $\pm$ 11.4                     | 0.006   |
| Stroke volume, mL/bp                       | 83 $\pm$ 19                       | 69 $\pm$ 12                         | 0.011   |
| Cardiac output, L/min                      | 5.20 $\pm$ 0.12                   | 3.94 $\pm$ 0.16                     | 0.005   |
| SBP, mmHg                                  | 118 $\pm$ 9                       | 109 $\pm$ 6                         | 0.001   |
| DBP, mmHg                                  | 70 $\pm$ 8                        | 66 $\pm$ 6                          | 0.061   |
| HR, beats/min                              | 63 $\pm$ 12                       | 66 $\pm$ 15                         | 0.540   |
| Core temperature and heat production       |                                   |                                     |         |
| Core temperature, $^\circ\text{C}$         | 36.9 $\pm$ 0.2                    | 37.2 $\pm$ 0.3                      | 0.005   |
| Heat production, W                         | 92 $\pm$ 14                       | 76 $\pm$ 12                         | 0.002   |
| Heat production per kg, W/kg               | 1 $\pm$ 0.2                       | 1 $\pm$ 0.2                         | 0.924   |
| Heat production, W/BSA                     | 47 $\pm$ 6                        | 44 $\pm$ 7                          | 0.187   |

Values are means  $\pm$  standard deviation. Data were compared between groups using an unpaired *t* test. Statistical significance was set at  $P < 0.05$ . BMI, body mass index; BSA, body surface area; DBP, diastolic blood pressure; HR, heart rate; PU, perfusion unit; SBP, systolic blood pressure;  $\dot{V}O_2$ , oxygen consumption;  $\dot{V}O_{2\max}$ , aerobic capacity; W, watts. \*Skin blood flux is the sum of data derived from measures taken from the forearm and back.

$P < 0.001$ ; Fig. 3). When the data were normalized by lean mass, oxygen consumption increased over time (time  $P < 0.001$ ) in both sexes (group  $P < 0.960$ ), and the difference between males and females was no longer statistically significant (interaction  $P = 0.055$ ). The relative exercise intensity increased over time (time  $P < 0.001$ ) in both groups (group  $P = 0.067$ ), but with no interaction ( $P = 0.084$ ). Females performed at a higher % $\dot{V}O_{2\max}$  at 30 min, but no systematic difference existed across the timepoints. Largely as a consequence of the higher absolute  $\dot{V}O_2$ , there was a time ( $P < 0.001$ ), group ( $P < 0.001$ ), and interaction effect ( $P < 0.001$ ) on heat production (W, adjusted per BSA and kg), with the males producing more heat at all of the timepoints. The correlation between heat production (W) at the end of the protocol and lean mass (kg) was moderate and significant ( $r^2 = 0.414$ ;  $P = 0.035$ ), as was the correlation between heat production and body mass ( $r^2 = 0.494$ ;  $P = 0.009$ ). Fat mass (kg) was not significantly correlated with heat production ( $r^2 = 0.202$ ;  $P = 0.322$ ).



**Figure 2.** Responses of  $T_c$  to exercise and in the heat. Data are presented as means  $\pm$  standard deviation. \*Significant difference ( $P < 0.05$ ) between groups.  $T_c$  data acquired from 19 males and 17 females.

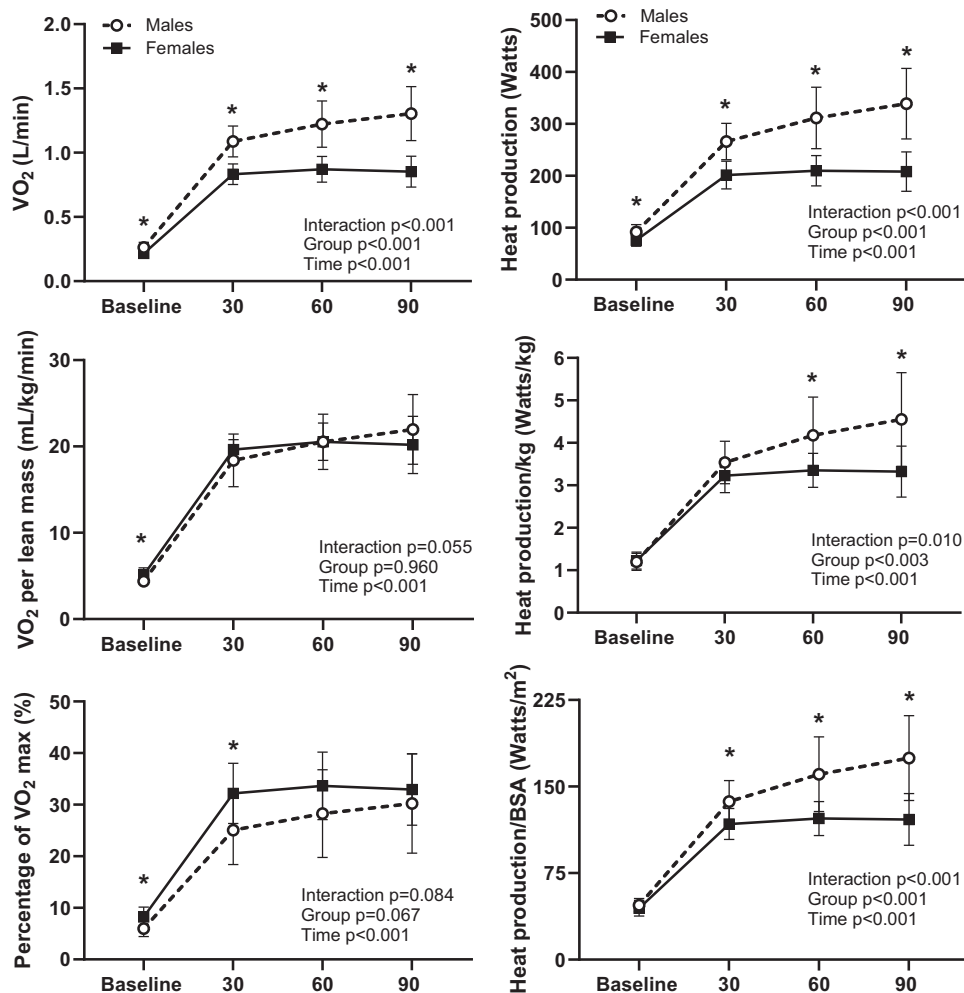
### Cardiac Response to Exercise in the Heat

Heart rate increased similarly in both sexes (group  $P = 0.491$ ) over the 90 min (time  $P < 0.001$ ), but no interaction effect was apparent ( $P = 0.763$ ; Fig. 4). SV decreased in both groups over time ( $P < 0.001$ ), but no interaction ( $P = 0.423$ ),

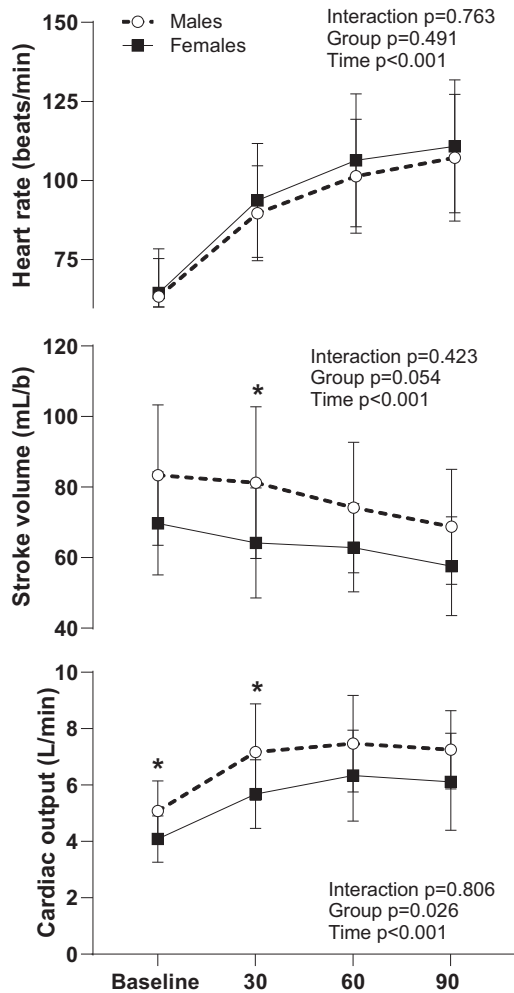
or group effect ( $P = 0.054$ ), was present. There was a time ( $P < 0.001$ ), group ( $P = 0.026$ ), but no interaction effect ( $P = 0.806$ ) on CO, with both sexes exhibiting an increase over time, a plateau at 60 min, with males presenting a larger magnitude of response than females (group  $P = 0.026$ ). Males exhibited higher CO at baseline and at the 30-min timepoint. When BSA adjusted SV and CO, no interaction or group effect was shown, with a similar increase over time ( $P < 0.001$ ).

### Response of the Conduit Arteries to Exercise in the Heat

Brachial artery diameter exhibited a time ( $P < 0.001$ ), group ( $P < 0.001$ ), but no interaction effect ( $P = 0.074$ ) (Fig. 5A). Males had a larger brachial ( $P < 0.001$ ) diameter at all time points than females. Brachial velocity increased over time ( $P < 0.001$ ) in both sexes ( $P < 0.030$ ), with no interaction ( $P = 0.166$ ). Brachial blood flow increased over time ( $P < 0.001$ ), with no difference between sexes (group  $P = 0.334$ ), and no interaction ( $P = 0.695$ ). Femoral artery diameter (Fig. 5B) also increased over time ( $P < 0.001$ ) with a larger impact in the males (group  $P = 0.004$ ), exhibiting a larger diameter at all timepoints, but no interaction effect was present ( $P = 0.601$ ). Femoral velocity increased ( $P < 0.001$ ) similarly in both sexes (group  $P = 0.817$ ), with



**Figure 3.** Oxygen consumption and heat production in response to exercise in the heat. Data are presented as means  $\pm$  standard deviation. \*Significant difference ( $P < 0.05$ ) between groups. Data acquired from 13 males and 14 females at all timepoints. BSA, body surface area;  $\dot{V}O_2$ , oxygen consumption;  $\dot{V}O_{2max}$ , aerobic capacity.



**Figure 4.** Cardiac variables in response to exercise in the heat. Data are presented as means  $\pm$  standard deviation. \*Significant difference ( $P < 0.05$ ) between groups. HR data acquired from 19 males and 17 females. SV and CO data were acquired from 13 males and 11 females at all time-points. CO, cardiac output; HR, heart rate; SV, stroke volume.

no interaction effect ( $P = 0.638$ ). Femoral blood flow also increased over time ( $P < 0.001$ ), with a larger impact over time in the males (group  $P = 0.007$ ) but no interaction ( $P = 0.326$ ). Males exhibited higher flows than females at all time points.

#### Skin Blood Flow Response to Exercise in the Heat

SkBF showed significant time ( $P < 0.001$ ) and interaction effects ( $P = 0.002$ ) (Fig. 6A). Post hoc analysis revealed a similar magnitude of change at 30 min and 60 min, and then a significant continuous increase in SkBF in males between 60 and 90 min ( $+13.1 \pm 8.8$  PU), whereas a decrease in SkBF was observed in females ( $-15.4 \pm 8$  PU). A Pearson's correlation was run to assess the relationship between SkBF with heat production and sweat rate (L/min and BSA) at the 90-min timepoint (Fig. 6B). There was a statistically significant, positive, and moderate correlation between SkBF and heat production at 90 min ( $r^2 = 0.402$ ,  $P = 0.046$ ), SkBF and sweat rate ( $r^2 = 0.406$ ,  $P = 0.023$ ), and a significant and positive, but weak, correlation between SkBF and sweat rate when adjusted by BSA ( $r^2 = 0.368$ ,  $P = 0.042$ ).

#### DISCUSSION

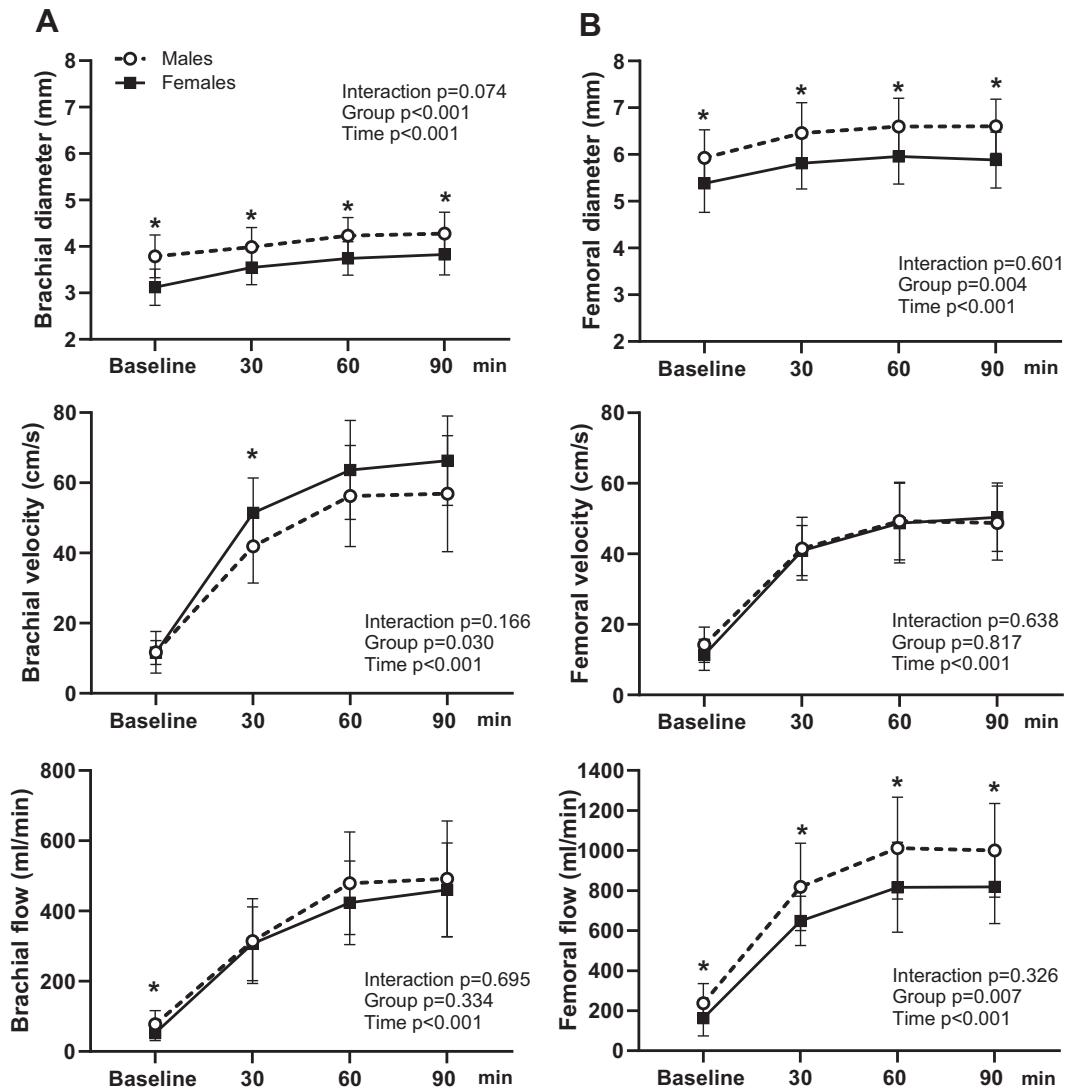
This study assessed whether males and females have similar thermoregulatory and cardiovascular responses to a set-pace laboratory walking test during heat exposure. Our objective was to compare the responses between sexes, recognizing that task demands and environmental conditions cannot always be controlled or modified in military, industrial, or even in elite sport settings. The key outcomes of the study were that males completed the exercise test with higher metabolic heat production and sweat rate than the females, while  $T_{\text{c}}$  was similar during the protocol. In addition, males maintained a higher CO and a higher SKBF at the end of the exercise trial.

In our study, males exhibited higher absolute values for cardiac output than females, which, during the advanced stages of exercise during heat exposure, facilitated more distribution of blood flow to the skin, despite a larger blood volume also being directed to the active skeletal muscle in males. This difference is consistent with the study of Ridout et al. (15) in which healthy males ( $n = 29$ ) and females ( $n = 26$ ) performed treadmill and knee extensor exercise while maximal cardiac output and peak femoral blood flow were assessed. They concluded that age and sex-dependent differences exist in systemic cardiovascular regulation during exercise. In men, leg vascular reserve influenced the maximum oxygen delivery and uptake, but that was not the case for women.

Historically, sex differences in performance and function in response to set-paced exercise and heat exposure have been ascribed to physical and/or inherent physiological differences. Morphological differences that can impact exercise capacity include a larger body and lean mass, alongside larger lungs (16), cardiac (17), vascular, and blood volume capacities (18). The males we recruited had larger arterial diameters, SV, and CO than females, which is likely typical of studies that do not prespecify recruitment criteria. These characteristics, alongside the known larger blood volume in males (15, 19), likely contributed to increased cardiac output in males, which may also explain the higher blood flow distribution to the active (femoral) muscle beds during exercise in the heat.

Elevation in  $T_{\text{c}}$  during fixed-duration exercise, where heat stress is uncompensable, reflects an imbalance between heat production and evaporative capacity (20–22). In the current study, heat production was higher in males, but so was their sweat rate, and evaporative potential was also likely higher based on their greater aerobic capacity. This could help to explain why there was a small, but nonsignificant, difference in the change in  $T_{\text{c}}$  between males and females. Our findings suggest that the larger body of males influenced their heat production and, consequently, thermoregulatory responses. This finding is consistent with a study of 16 endurance male runners (55–90 kg), in which those with a lower body mass demonstrated a thermal advantage when running in conditions with compromised heat-dissipation mechanisms, running faster or further before reaching a limiting  $T_{\text{c}}$  (23).

A previous study by Dervis et al. (24) indicated that, in body mass-matched male participants who differed in terms of fat and lean tissue proportions and were exercised at a fixed metabolic heat production, core temperature



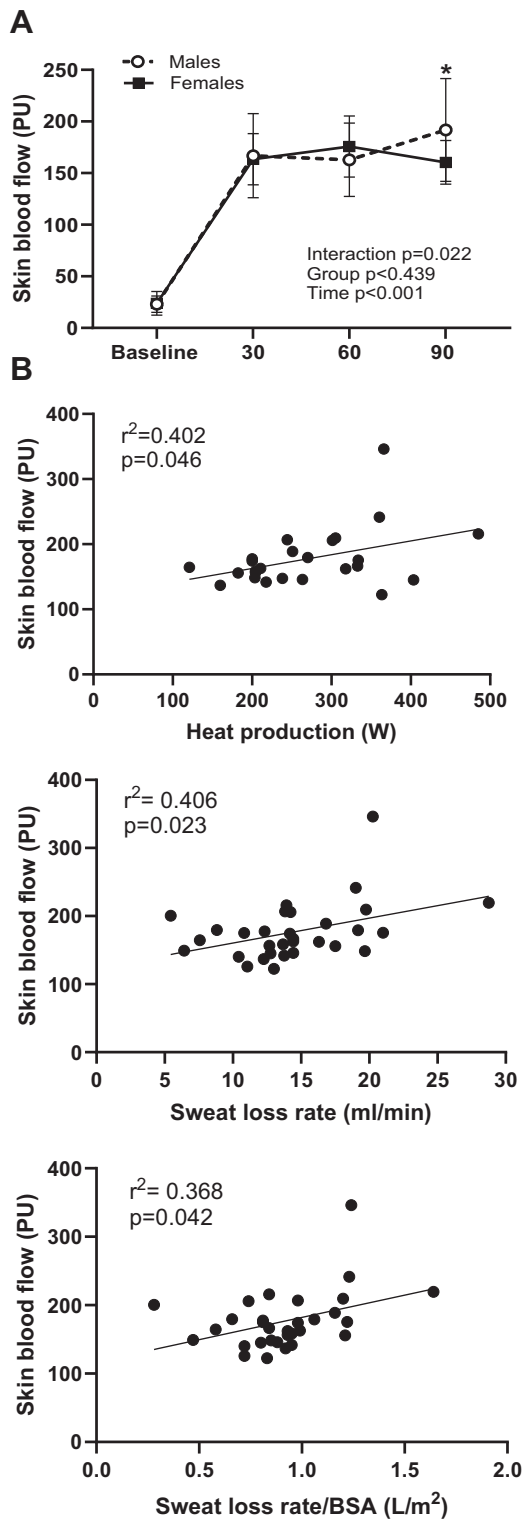
**Figure 5.** Brachial (A) and femoral (B) artery diameter, velocity, and flow in response to exercise in the heat. Data are presented as means  $\pm$  standard deviation. \*Significant difference ( $P < 0.05$ ) between groups. Brachial and femoral data were acquired from 18 males and 17 females at all timepoints.

rose more in higher fat individuals (25). This could not be ascribed to differences between groups in sweat rate or skin surface heat dissipation. These data suggest that, under conditions where rates of heat production and heat loss are similar, individuals with more adipose tissue may heat up to a greater extent because of the lower average heat capacity of the body. Our study was not designed to match male and female participants for body mass or proportions, and males had significantly more total mass (75 vs. 62 kg;  $P < 0.001$ ) and lean mass (57 vs. 41 kg;  $P < 0.001$ ), and generated more heat than the females, both at rest (90 vs. 74 W;  $P = 0.002$ ) and during the exercise bout ( $\Delta$ : 247 vs. 132 W;  $P < 0.001$ ). We observed a significant and positive correlation between total body mass and heat production ( $r^2 = 0.494$ ;  $P = 0.009$ ), as well as lean mass (kg) and heat production ( $r^2 = 0.584$ ;  $P = 0.002$ ), whereas fat mass was not significantly correlated with heat production ( $r^2 = 0.202$ ;  $P = 0.322$ ). These data are consistent with previous evidence that has related heat production to body composition

(26). The augmented physiological responses that we observed in males parallel their higher heat production, requiring exaggerated thermoregulatory responses to dissipate the amount of that was heat produced. Such physiological demands are particularly relevant in contexts where workload and environmental conditions cannot be mitigated, including some military, industrial, and professional sporting settings. A future study, perhaps replicating the approaches adopted by Dervis et al. (24), whereby males and females are matched for body mass and/or composition, would provide further insights into the mechanisms that are responsible for sex differences in response to fixed workload tasks.

Despite the sex differences outlined above, recent studies (27, 28) have questioned the notion that sex differences can be explained by structural factors alone. Our data showed significant and moderate correlations between SkBF and heat production, SkBF and sweat rate, and SkBF and sweat rate/BSA. In our study, males and females exhibited similar





**Figure 6.** Responses of SkBF to exercise and heat (A) and correlations (B) between SkBF and heat production, sweat rate, and sweat rate adjusted by BSA. Data are presented as means  $\pm$  standard deviation. \*Significant difference ( $P < 0.05$ ) between groups. SkBF data acquired from 18 males and 16 females at all timepoints. BSA, body surface area; PU, perfusion unit; SkBF, skin blood flow.

increases in SkBF with exercise and heat exposure until 60 min, and after that, the males had a higher SkBF at the end of the exercise bout (90 min). These data are consistent with a previous study (9) that reported lower sweat rate in females when matched by BSA with males, after 90 min of continuous cycling exercise (50%  $\dot{V}O_{2max}$ ) at 35°C and 12% RH. In another study (29), sweat gland output in 20 (trained and untrained) females was compared with 17 (trained and untrained) males across five body sites after cycling at 35%, 50%, and 65% of  $\dot{V}O_{2max}$  for 60 min at 30°C and 45% RH. Males exhibited higher sweat rate and higher output per gland than females, regardless of training status, and this sex difference became more pronounced at the higher exercise intensities. Sex steroids likely play a role in those differences because, within females, the sweat rate during exercise is related to the basal testosterone concentration (30).

SkBF is controlled by reflex and localized mechanisms in humans and is closely associated with sweat production (31). In the present study, males lost more sweat than the females (16.5 vs. 12.3 mL/min;  $P = 0.009$ ). One reading of our data is that it concurs with previous studies that have concluded that sex differences exist in sweating in humans (29, 32–34). Other previous studies have suggested that females have more sweat glands per unit area (accessed on the upper back, chest, and forearm) (35), but lower sweat output per gland than males when high levels of heat loss are required (29, 33). The higher sweat rate in males was likely a response to their higher heat production, but the physiological input that drives that response is unclear. Gagnon et al. (36) have suggested that the evaporative requirement for heat balance (of which heat production is the main contributor) largely determines the sweat rate. But no one has been able to find a signal related to metabolic rate that stimulates sweating (37). There is some evidence that muscle temperature acts as an input to sweating (37), and the higher metabolic work rate in the males was likely due to muscle activity, and so it is feasible that a higher muscle temperature provided the stimulus for the higher sweat rate in the males. The fact that males generated more heat but had a similar  $\Delta T_{re}$  in our study, therefore, suggests that the males, on average, had a more sensitive sweat response due to a higher muscle temperature, and/or a higher capacity for evaporative heat loss. It is therefore possible that such a difference would have been observed between small versus large males, a finding that has implications for individuals of distinct body size and shape who perform set-paced tasks. Our findings additionally suggest that, during the advanced stages of exercise during heat exposure, more blood flow was available to distribute to the skin in males, despite a larger blood volume also being directed to the active skeletal muscle. These findings may relate to the larger cardiovascular capacity (SV and CO) in males.

Our study has several limitations. Our results are limited to the exercise and exposure parameters that we imposed. We cannot exclude the possibility that larger differences between men and women may have become apparent under more extreme ambient conditions (e.g., higher temperature or humidity), or as a consequence of a longer or more demanding protocol (e.g., a similar exposure while carrying a 40 kg load, such as that in the study by Hunt

et al. (2). The lack of data regarding the menstrual cycle and use of contraceptives is another limitation. Future studies could target cyclical differences in the impact of exercise in the heat in premenopausal females. As discussed earlier, our male and female participants were not matched a priori for antecedent factors that may have impacted their responses, such as body mass, body composition, or surface area. Although this was intentional, as our aim was to understand differences that may exist in typical military and/or workplace recruits, it may have contributed to some differences in baseline variables, such as those apparent in  $T_{\text{c}}$ . Alternatively, that difference may have been coincidental, given our sample size. Future studies that match males and females for differences in body size and metabolic heat production (and thus the evaporative requirement for heat balance) would be instructive in this regard. It would also be instructive to match groups for acclimatization status to negate evaporative capacity as a confounder. Nonetheless, our observation that males and females had the same  $\Delta T_{\text{c}}$ , despite males generating more heat, suggests that the males in this study had a higher evaporative capacity. Finally, some of our outcome measures may have been impacted by factors such as posture; to optimize image quality, our SV (and corresponding matched HR and CO) data were assessed semi-recumbent during short (~3 min) breaks in the exercise bout. This may explain the failure to observe any increases in SV during the early stages of exercise in the heat and the relatively modest HR responses.

In conclusion, we have demonstrated that males completed a standardized heat and exercise test with higher metabolic heat production, for which they compensated via higher sweat rates, enabling their change in  $T_{\text{c}}$  to remain similar to that of females. In addition, males had higher CO, facilitating elevated skin and muscle blood flows during the exercise trial. An alternative interpretation of our  $T_{\text{c}}$  data is that women had a trend for a slower rate of rise of  $T_{\text{c}}$  compared with men, as they started at higher levels and ended at the same. This is consistent with previous studies (38, 39), suggesting that smaller body size and/or greater surface area-to-mass ratio may be an advantage to heat dissipation and decrease risk of heat illness. The observation in some studies that females may exhibit lower sweating rates may therefore be offset by smaller body size in women, rendering them more efficient in terms of water loss, with a lower risk of dehydration.

## DATA AVAILABILITY

The source data are available to verified researchers upon request by contacting the corresponding author.

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Graphical abstract and Fig. 1 created with a licensed version of BioRender.com.

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## DISCLOSURES

R. A. McLaughlin is a cofounder and Director of Miniprobes Pty Ltd, a company that develops optical imaging systems. Miniprobes Pty Ltd did not contribute to or participate in this study. The remaining authors declare no conflict of interest.

## AUTHOR CONTRIBUTIONS

H.H.C. and D.J.G. conceived and designed research; J.G.C., J.C.L., K.W.W., J.L.C., X.X., J.J.C. and H.H.C. performed experiments; J.G.C., J.C.L., K.W.W., J.L.C., J.J.C., and A.H. analyzed data; J.G.C., J.C.L., J.L.C., J.J.C., S.K.M., R.A.M., K.P.G., and D.J.G. interpreted results of experiments; J.G.C. prepared figures; J.G.C. and D.J.G. drafted manuscript; J.C.L., K.W.W., J.L.C., X.X., J.J.C., L.H.N., A.H., S.K.M., R.A.M., H.J., K.P.G., and D.J.G. edited and revised manuscript; J.G.C., J.C.L., K.W.W., J.L.C., X.X., J.J.C., L.H.N., A.H., S.K.M., H.H.C., R.A.M., H.J., K.P.G., and D.J.G. approved final version of manuscript.

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