-FULL TITLE: EXERCISE TRAINING REDUCES THE FREQUENCY OF MENOPAUSAL HOT FLUSHES BY IMPROVING THERMOREGULATORY CONTROL

SHORT TITLE: Exercise reduces hot flushes

TOM G. BAILEY, PHD[1,2]
N. TIMOTHY CABLE, PHD[1,3]
NABIL AZIZ, MD[4]
REBECCA DOBSON, MD[5]
VICTORIA S SPRUNG, PHD[5]
DAVID A. LOW, PHD[1]
HELEN JONES, PHD[1*]

[1]*Research Institute for Sport and Exercise Sciences, Liverpool John Moores University*, Tom Reilly Building, Byrom Street, Liverpool L3 3AF, UK
[2] *School of Health and Sport Sciences,* University of the Sunshine Coast, Australia.
[3]*Department of Sports Science,* Aspire Academy, Qatar.
[4] *Department of Gynaecology and Reproductive Medicine*, Liverpool Women's Hospital, UK.
[5]*Department of Obesity and Endocrinology*, University of Liverpool, UK

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FIGURES: 3 **TABLES:** 3

AUTHOR FOR CORRESPONDENCE (REPRINTS UNAVAILABLE):

*Dr Helen Jones, Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Tom Reilly Building, Byrom Street, L3 3AF. Email: h.jones1@ljmu.ac.uk

ABSTRACT

Objectives: Post-menopausal hot flushes occur due to a reduction in oestrogen production causing thermoregulatory and vascular dysfunction. Exercise training enhances thermoregulatory control of sweating, skin and brain blood flow. We aimed to determine if improving thermoregulatory control and vascular function with exercise training alleviated hot flushes.

Methods: Twenty one symptomatic females completed a 7-day hot flush questionnaire and underwent brachial artery flow-mediated dilation and a cardiorespiratory fitness test. Sweat rate and skin blood flow temperature thresholds and sensitivities, and middle cerebral artery velocity (MCA*v*) was measured during passive heating. Females performed 16-weeks of supervised exercise training or control, and measurements were repeated.

Results: There was a greater improvement in cardiorespiratory fitness (4.45 $ml \cdot kg^{-1} \cdot min^{-1}$ (95% CI: 1.87, 8.16; P=0.04) and reduced hot flush frequency [48 hot flushes·week (39, 56) P<0.001] following exercise compared to control. Exercise reduced basal core temperature [0.14°C (0.01, 0.27) P=0.03] and increased basal MCA*v* [2.8 cm/s (1.0 to 5.2) P=0.04] compared to control. Sweat rate and skin blood flow thresholds occurred ~0.19 and 0.17°C earlier, alongside improved sweating sensitivity with exercise. MCA*v* decreased during heating [P<0.005], but was maintained 4.5 cm/s (3.6, 5.5 P<0.005) higher during heating following exercise compared to control [0.6 cm/s (-0.4, 1.4)].

Conclusions: Exercise training that improves cardiorespiratory fitness reduces self-reported hot flushes. Improvements are likely mediated through greater thermoregulatory control in response to increases in core temperature and enhanced vascular function in the cutaneous and cerebral circulations.

KEY WORDS: Hot flushes; exercise training; vascular function; thermoregulation; brain blood flow

INTRODUCTION

Hot flushes are experienced by the vast majority of menopausal women and are associated with increased cardiovascular disease risk [1]. Menopausal hot flushes can seriously disrupt the lives of symptomatic females [2] with ~70% of females experiencing hot flushes 1-5 years after the onset of the menopause [3]. A hot flush is typically defined as the subjective sudden intense sensation of heat causing cutaneous vasodilation and profuse sweating [4]. Hormone therapy (HT) is an effective treatment for hot flushes and can reduce hot flush frequency by 50-72% [5,6] but has poor uptake[7]. Furthermore, not all females can be prescribed HT due to time since the menopause and a history of cardiovascular disease or breast cancer [8]. The current alternatives are limited but one non-pharmacological option is exercise training.

The mechanisms causing hot flushes are not completely understood, yet it is thought that the reduction in oestrogen due to ovarian failure causes thermoregulatory and vascular dysfunction, leading to the occurrence of hot flushes [9]. An elevation in basal core body temperature and a narrowed thermo-neutral zone are thought to be primary explanations [2], with a reduced skin vascular reactivity to increases in core body temperature also proposed as a mediator [9,10]. No research study to date has simultaneously investigated the impact of exercise training on thermoregulatory and vascular dysfunction observed in symptomatic post-menopausal females and the effect of improvements in these systems on hot flush symptomology.

A number of research studies, but not all, have shown exercise training can reduce the frequency of self-reported hot flushes [11-17] and improve other non-vasomotor symptoms including depression, anxiety and insomnia [14,18,19]. Nevertheless, these studies have solely relied on subjective questionnaires as the primary outcome. It is also important to highlight that the most recent randomised control trial investigating the impact of exercise training (home based and general advice) using subjective frequency of hot flushes reported a lack of impact of exercise training despite finding a clinically meaningful decrease of 9 weekly hot flushes compared to control [17].

It is well established that exercise training can improve the thermoregulatory control system by decreasing core body temperature, and by changing both the temperature threshold for the onset, and sensitivity of sweating and cutaneous vasodilation in pre-menopausal females [20]. Whilst HT reduces hot flushes it also affects thermoregulatory control mechanisms via lowering core body temperature and altering the threshold at which cutaneous vasodilation and sweating responses are initiated [21,22]. If the thermoregulatory control system can be altered with exercise training in symptomatic post-menopausal women, this may also reduce the frequency of hot flushes. Moreover, exercise training improves endothelial function in the cutaneous and conduit vessels in post-menopausal females [23-25], and cerebral blood flow (CBF) in older individuals [26,27]. Endothelial dysfunction is associated with hot flush severity [28], suggesting that if endothelial function is improved with exercise training this may contribute to a reduction in the occurrence and severity of hot flushes. Therefore, the aim of this study was to determine whether improving thermoregulatory control and systemic vascular function with exercise training alleviates the frequency and severity of menopausal hot flushes. We hypothesised that exercise training reduces the frequency and severity of hot flushes via improving sweat rate and skin blood flow responses to increases in core body temperature.

METHODS

Participants

Twenty-one symptomatic post-menopausal females were recruited from the gynaecology and reproductive medicine clinic at Liverpool Women's Hospital, local G.P. practices and via local advertisement. Participants were 1-4 years since their last menstrual period and suffered >4 hot flushes over a 24h period. All participants had no history of diabetes, cardiovascular or respiratory disease, were non-smokers, drank <14 units of alcohol per week, and had no contraindications to exercise. Participants who had used HT, metformin, vasoactive or BP lowering medications within the last 6 months were excluded from the study. Similarly, females who were currently taking part in regular exercise (>2h a week based on a self-reported questionnaire) were also excluded. Participants were informed of the methods verbally and in writing before providing written informed consent. The study conformed to the *Declaration of Helsinki* and was approved by the local research ethics committee.

Research Design

Participants reported to the laboratory on two separate occasions, and were asked to fast overnight, refrain from alcohol and exercise for 24h and caffeine for 12h before each visit. Visit one included anthropometric measurements, assessment of brachial artery endothelial function using Flow-Mediated Dilation (FMD) and a cardiorespiratory test (VO_{2peak}). Visit 2 consisted of a fasting blood sample and a passive heat stress challenge to assess thermoregulatory, haemodynamic and cerebrovascular responses to increases in core body temperature. Both visits were completed within 7 days of each other with assessments conducted in a temperature-controlled laboratory (24±1°C). Participants then underwent a supervised exercise training intervention or a no-exercise control that was based on participant choice. Fourteen (n=14, 52±4y, BMI; 21.1-41.8 kg/m^2) symptomatic females received a 16-week programme of supervised moderate-intensity aerobic exercise training while seven (n=7, 52±6y, 21.1-41.3 kg/m^2) symptomatic females comprised the no-exercise control group. Following each intervention all measurements were repeated.

Measurements

Hot Flush Frequency and Severity Questionnaire

Participants completed a 7-day hot flush frequency and severity diary [29] prior to, and following the 16-week intervention period. Participants recorded on a daily basis how many hot flushes they experienced as well as information regarding the severity of each hot flush on a scale of 1-4 (1 being mild, 2 moderate, 3 severe and 4 very severe). From this, a 7 day sum of hot flushes provided a weekly hot flush score. A daily severity score was calculated by the sum of hot flushes recorded into each severity rating i.e. [(3*1(mild))+(4*2(moderate))+(1*3(severe))+(0*4(very severe)) = daily severity score of 14]. A hot flush severity index was then calculated by the total sum of daily severity scores over the 7 day period. The use of subjective diaries is established as a valid approach to obtaining data on subjective hot flushes when reporting patient symptoms and perceptions [29] and in a number of hot flush research studies [11,12,16,30].

Cardiorespiratory assessment for peak oxygen consumption

A fitness test (peak oxygen uptake; VO_{2peak}) was performed on a treadmill following a modified Bruce protocol. Following a 2-min warm-up at 2.2 km h^{-1} on a flat gradient, the initial workload was set at 2.7 km h^{-1} at a 5° gradient. Thereafter, stepwise increments in speed and gradient were performed each minute until volitional exhaustion. Heart rate (12-

lead ECG) and rate of perceived exertion were monitored throughout. Peak oxygen uptake was calculated from expired gas fraction (Oxycon Pro, Jaegar, Hochberg Germany) as the highest consecutive 15s period of data in the final minute before volitional exhaustion.

Brachial artery endothelial-dependent vasodilation

Brachial artery endothelium-dependent function was measured using the flow-mediated dilation (FMD) technique [31]. Measurements were performed in the supine position following 20 min of rest and are described in detail elsewhere [31]. Following a 1 min recording period of resting diameter and flow, a rapid inflation pneumatic cuff (D.E Hokanson, Bellevue, UK), positioned on the forearm immediately distal to the olecranon process was inflated (>200 mmHg) for 5 min to provide a stimulus for forearm ischemia. Diameter and flow recordings resumed 30 sec prior to cuff deflation and continued for 3 min thereafter, in accordance with recent technical specifications [32,33].

Analysis of brachial artery diameter was performed using custom-designed edge-detection and wall-tracking software, which is largely independent of investigator bias. Recent papers contain detailed descriptions of the analysis approach [31,32]. From synchronised diameter and velocity data, blood flow (the product of lumen cross- sectional area and Doppler velocity) were calculated at 30 Hz. Shear rate (an estimate of shear stress without viscosity) was calculated as 4 times mean blood velocity/vessel diameter. Reproducibility of diameter measurements using this semi-automated software is significantly better than manual methods, reduces observer error significantly, and possesses an intra-observer CV of 6.7% [34]. We also controlled for the baseline diameter measured before the introduction of hyperemia in each test of FMD. This allometric approach is more accurate for scaling changes in diameter than simple percentage change, which makes implicit assumptions about the relationship between baseline diameter and peak diameter [35].

Passive heat stress challenge

Participants were placed in a tube-lined jacket and trousers (Med-Eng., Ottawa, Canada), which covered the entire body except for the head, feet and the right forearm. Participants rested quietly in a semi-recumbent position while water (34°C) was perfused through the suit for a 15 min baseline period. Participants were then exposed to a moderate heat stress by perfusing water at 48^{o}C through the suit for 60 min or until a rise of ~1°C in core body temperature. The following measurements were taken during the baseline and heating periods.

Heart rate was obtained from a 3-lead electrocardiogram (Powerlab, AD Instruments, Oxford, UK), alongside continuous beat-by-beat finger arterial blood pressure (BP) (Finapress, Amsterdam, Netherlands). Stroke volume (SV) and cardiac output (CO) were calculated using the BP waveform using the Modelflow method, incorporating age, height, sex and weight (Beatscope 1.0 software, TNO, Biomedical Instruments). To verify continuous BP measured at the finger, an automated BP (Dinamap, Germany) reading was collected at regular intervals. Mean skin temperature was obtained from the weighted average of 4 regional temperatures measured from thermocouples (iButtons data logger, Maxim Integrated; San Jose, CA, US) secured to the lateral calf, lateral thigh, upper arm and chest [36]. Core body temperature was measured from an ingestible pill telemetry system taken ~5 h before data collection began (CoreTemp, HQInc; Palmetto, FL, US), with the ingestion time recorded and repeated for each participant's pre and post trials. Mean body temperature was calculated using the weighted product of core and mean skin temperatures [37].

Local sweat rate was recorded continuously from the dorsal forearm and the mid-sternum (not covered by the water-perfused suit) using capacitance hygrometry. Dry 100% nitrogen gas was supplied through acrylic capsules (surface area= 2.32cm^2) attached to the skin's surface at a flow rate of 300mL/min, with the humidity of the gas flowing out of the capsules measured by the capacitance hygrometer (Viasala HMP155, Helsinki, Finland). Local skin blood flow (SkBF) was also measured at the chest and the forearm, using laser-Doppler flowmetry (Periflux System 5001, Perimed; AB, Sweden). Laser-Doppler flow probes were affixed with an adhesive heating ring in close proximity to the ventilated sweat rate capsule. Cutaneous vascular conductance (CVC) was calculated as the ratio of laser-Doppler flux units to mean arterial pressure (MAP) and expressed as both CVC and a percentage of maximum CVC (%CVC_{max}).

Middle cerebral artery blood velocity (MCA_V; 1 cm distal to the MCA-anterior cerebral artery bifurcation) was measured continuously through the temporal window using transcranial Doppler ultrasonography. A 2-MHz Doppler probe (Spencer Technologies, Seattle WA, USA) was adjusted until an optimal signal was identified, as described in detail previously [38-40], and held in place using a headband strap to prevent subtle movement of the Doppler probe and maintain insonation angle accuracy. Once the optimal MCA signal was attained in the temporal window, the probe location and machine settings (depth, gain and power) were recorded to identify the same imaging site during post-intervention assessments. Using these guidelines this technique is a valid and reliable index of cerebral blood flow [38]. Participants were instrumented with a two-way valve-breathing mouthpiece from which peak end tidal CO_2 ($PETCO_2$) was measured every 5 min and at each 0.1°C increase in core body temperature. An index of cerebrovascular conductance (CBVC) was calculated from the ratio of MCA_V to MAP. All data were calculated as 60 sec averages at every 0.1°C increase in core temperature during heating. All data during the heat stress challenge were sampled at 50Hz with a data acquisition system (PowerLab, ADInstruments, Oxford UK).

Following the passive heat stress, local skin heating was performed simultaneously at the chest and forearm laser Doppler flowmetry sites to assess maximal cutaneous blood flow. Temperature of the local heating units was increased at a rate of 0.5 °C every 5 sec to a temperature of 42 °C. This resulted in an increase in skin temperature to ~42 °C at the heating probe-skin surface interface. The protocol was complete once flux at both sites had reached a stable plateau (~30 min).

Data Reduction

The temperature thresholds for the onset of sweating (mean body temperature) and cutaneous vasodilation (core body temperature) were calculated in a blinded fashion by the same analyst [41]. The sensitivity of the sweating responses were estimated from the slope of the relationships between sweat rate per unit change in mean body temperature beyond the mean body temperature threshold, and any sweat rate plateau, or increase during a hot flush episode, were excluded from the slope calculation. Skin blood flow sensitivity was estimated in the same way, instead using the rate of CVC per unit change in core temperature.

Supervised Exercise Training Intervention

Before commencing the exercise intervention, all participants attended a thorough familiarisation session. Participants were required to attend the University gym on a weekly basis during which time they wore a heart rate monitor (Polar Fitness, Polar Electro Oy, Finland) and were provided with full exercise supervision and guidance from a trained

exercise physiologist. During these sessions, participants were issued with a weekly progressive exercise programme that was specific to their own rate of progression [42,43]. On the basis of individual fitness level, participants underwent 30min of moderate-intensity aerobic exercise three-times per week (30% heart rate reserve (HRR)), which progressed weekly based on HR responses and included treadmill walking/running, cycling, cross-training and rowing. At week 12, participants were exercising 4-5 times per week for 45 min at 60%HRR. To facilitate compliance throughout the 16-week intervention, participants were monitored via the Wellness Key® system, a software programme that enables remote and accurate tracking of exercise activity. A moderate-intensity programme was used in line with NHS guidelines and our previous studies that have shown improvements in cardiorespiratory fitness [23,42-44].

Control Intervention

After consent and physiological flush assessment, women who opted for the control group had little contact with the research team throughout the 16-weeks. The research team did not influence lifestyle during the 16-week period. This type of control intervention reflects current convention care for non-pharmacological hot flush treatment in the UK.

Statistical Analyses

For comparison of exercise vs control, delta changes (Δ) from pre-intervention were calculated for each group and entered as the dependent variable in a linear mixed model (ANCOVA), with pre-intervention data entered as a covariate, this allows all differences between changes to be covariate-adjusted for the pre-intervention values[45]. This analysis approach is more statistically precise and adjusts properly for any study group imbalances at pre intervention. Ultimately, this analysis provides one P value for the effect of intervention, which is adjusted for the pre-intervention values. Data are presented in the text for intervention adjusted effects as mean and 95% confidence intervals. Data in the tables are absolute values (point estimates) for v pre and post-intervention and are presented as mean (SD). Correlations between the Δ intervention changes in hot flush frequency and SR and CVC thresholds were quantified using Pearson's correlation coefficient.

For comparison of exercise verses control during the passive heat stress a three-factor ((group*0.1ºC increase*time(pre/post intervention)) linear mixed model was employed for the analysis of the CBF and $PETCO_2$ responses to each 0.1ºC increase in core body temperature. If any hot flushes occurred during heat stress the CBF and $PETCO_2$ data during such episodes were excluded from the CBF and $PETCO_2$ data analyses. Due to variable individual increments in core body temperature during the passive heat stress, data up to an increase of 0.6ºC were used for the CBF and $PETCO_2$ analyses. Statistically significant interactions were followed up with the least significant difference (LSD) approach to multiple comparisons. [46]

Results

Participants undertaking the exercise intervention demonstrated 93% compliance to the exercise sessions. Following adjustment for baseline values, the body mass normalised change in VO_{2peak} was 4.5 (1.9-8.2) $ml \cdot kg^{-1} \cdot min^{-1}$ greater in the exercise group vs control (P=0.04). The absolute change was 21.0 (0.4-41.5) $ml \cdot min^{-1}$ greater in the exercise group vs control (P=0.05). The mean frequency of hot flushes per week was 48 (39-56) events lower

following the exercise intervention vs control (P<0.001). The hot flush severity index was 109 (80-121) AU lower following exercise training vs control (P<0.001).

Conduit brachial artery endothelial function

FMD was 2.3% (0.3 to 4.9) greater following exercise training vs control but this did not reach statistical significance (P=0.08; Table 1). Baseline and peak diameter did not change with either intervention.

Resting measurements

Haemodynamics: Heart rate was 4 (2, 5) beats·min^{-1} lower following exercise training vs control (P=0.003, Table 2). There were negligible differences in mean arterial pressure, cardiac output or stroke volume with the interventions.

Thermoregulatory: Basal core body temperature was 0.14 (0.01, 0.27) °C lower following exercise training vs control in (P=0.03, Table 2). There were negligible differences between interventions for basal skin blood flow (Table 2). Maximal skin blood flow (CVC_{max}) at the arm was 1.2 (-0.1 to 2.4) AU/mmHg greater following exercise training vs control (P=0.05, Table 2). This difference was not evident at the chest CVC_{max}.

Cerebral Blood Flow: Basal MCAv was 2.8 (1.0 to 5.2) cm/s greater following exercise training vs control (P=0.04; Table 2). This improvement was reduced when accounting for blood pressure, with cerebral vascular conductance (CBVC) 0.05 (-0.02 to 0.13) cm/s higher following exercise training vs control but this did not reach statistical significance (P=0.09; Table 2).

Measurements during the heat stress challenge

Haemodynamics: Heart rate during heat stress was 5 (1, 10) beats.min^{-1} lower following exercise training vs control but this did not reach statistical significance (P=0.08; Table 2).

Thermoregulatory: There were no differences in the changes in core body temperature (0.07 (-0.09, 0.24) °C; P=0.40) or weighted mean skin temperature at the end of heating following the interventions (0.06 (-0.57, 0.66) °C; P=0.88; Table 2).

Sweat rate: Mean body temperature for the onset of chest sweating was 0.19 (0.04 to 0.34) °C lower following exercise training vs control (P=0.01; Figure 2a). Similarly, mean body temperature for the onset of arm sweating was 0.19 (0.05 to 0.36) °C lower following exercise training vs control (P=0.01; Figure 2b]. Mean body temperature onset of sweating at the chest (r=0.688; P=0.006) and the forearm (r=0.688; P=0.006) following the exercise intervention were correlated with the frequency of self-reported hot flushes.

The rate of chest sweating was 0.13 (0.05 to 0.20) mg·cm^{2}·min^{-1} greater following exercise training vs control (P=0.002; Figure 2c). The rate of forearm sweating was 0.19 (0.05 to 0.34) mg·cm^{2}·min^{-1} greater following exercise training vs control (P=0.01; Figure 2d).

Cutaneous blood flow: Mean body temperature onset of chest cutaneous vasodilation was a 0.17 (0.04 to 0.29) °C lower following exercise training vs control (P=0.01; Figure 3a). Similarly, the mean body temperature onset of forearm cutaneous vasodilation was 0.15 (0.02 to 0.28) °C lower following exercise training vs control (P=0.02; Figure 3b).

The rate of cutaneous vasodilation was similar between interventions at the chest (*P*=0.62; Figure 3c) and forearm (P=0.31; Figure 3d).

Cerebral blood flow

CBF decreased during the heat stress (*P*<0.001). There was an intervention*pre/post interaction (*P*<0.001), where the reduction in MCA*v* during heat stress was attenuated in the exercise group vs control (Table 3). MCA*v* was 4.5 cm/s (3.6, 5.5, *P*<0.001) higher during heating following exercise training vs 0.7 (-0.3, 1.7) cm/s in control *(P*=0.27). Similarly, CBVC decreased during the heat stress (*P*<0.001). There was a significant intervention*pre/post interaction (*P*=0.01). CBVC was 0.07 (0.04, 0.09) $cm \cdot s^{-1} \cdot mmHg^{-1}$ higher during heat stress in the following exercise (*P*<0.001) compared to 0.01 (-0.01, 0.03) $cm \cdot s^{-1} \cdot mmHg^{-1}$ in control *P*=0.91). $P_{ET}CO_2$ decreased during heat stress (*P*<0.001), but there was no interaction (Table 3; *P*<0.05).

Discussion

The novel findings of the present study were that reductions in self-reported hot flush frequency and severity with exercise training coincided with improved thermoregulatory and vascular function in symptomatic post-menopausal females. These findings provide evidence that improving thermoregulatory and vascular function with moderate-intensity aerobic exercise training can be effective in the treatment of hot flushes in post-menopausal females.

Exercise training has been shown in a number of studies to improve the subjective ratings of self-reported hot flushes in post-menopausal females [11-15], but the underlying physiological mechanisms responsible have not yet been investigated. The results of the current study suggest that the improvements in the occurrence of post-menopausal hot flushes following 16-weeks of moderate-intensity aerobic exercise training are linked to improvements in thermoregulatory control. We found that exercise training reduces thermoregulatory dysfunction via stabilisation of central thermoregulatory control, i.e., lowering core body temperature and improving heat dissipation thresholds, alongside improvements in peripheral mechanisms that allow for greater heat dissipation (sweating sensitivity). These adaptations likely include increases in the number of sweat expulsions per minute, sweat gland hypertrophy, increased NO availability, and/or enhanced sweat gland recruitment at a given internal temperature, or a combination of all of the above [47]. Importantly, these findings of improved thermoregulatory efficiency with exercise training support previous studies that suggest an improvement in VO_{2peak} in the range of ~15-20% mediates positive adaptations to thermoregulatory function in pre-menopausal females [20,48]. This is the first study to demonstrate that post-menopausal females can improve thermoregulatory function with exercise training, and, importantly, that this contributes to alleviating the frequency and severity of hot flushes with exercise training.

The precise mechanisms underlying the pathophysiology of hot flushes is unclear; however it is acknowledged that thermoregulatory dysfunction is a key contributing factor [4,9,49]. Elevated basal core body temperature and a narrowing of the thermo-neutral zone (where shivering and sweating do not occur) are thought to be responsible for the large, rapid and transient increases in skin blood flow, sweating and flushing that characterise hot flushes [2]. This study

suggests that improving the control, and stability, of the thermoregulatory system through lowering core body temperature and improving heat dissipation mechanisms *per se* reduces the occurrence of hot flushes.

The ability of the blood vessels (including the cutaneous, conduit and cerebral vasculature) to vasodilate and thus deliver blood flow systemically is implicated in the pathophysiology of hot flushes and also contributes to thermoregulatory control. The reduction in oestrogen associated with the menopause causes endothelial dysfunction via decreased NO bioavailability [50] and/or increased reactive oxygen species scavenging NO [51]. Exercise increases eNOS expression via similar mechanisms of transcriptional regulation to that of oestrogen [52] and augments NO-mediated vasodilation [53]. We provide evidence (approaching statistical significance) for an improvement in NO-mediated conduit artery endothelial function, measured using FMD, following exercise training. Previous studies in post-menopausal females have not always observed exercise training-mediated increases in endothelial function using FMD [24,25] but exercise training has been shown to enhance cutaneous endothelial function and microvascular reactivity in post-menopausal females [23,54]. One reason for the lack of statistical significant in FMD with exercise training maybe due to the vascular remodelling that occurs over the intervention period. Previous studies in young healthy males and type 2 diabetic individuals have been shown to improve function (increase in FMD) and then normalise due to changes in artery structure/remodelling [55,56]. Whilst the time course of changes in vascular function have not been investigated in post-menopausal women, this is the first investigation of exercise-mediated changes in endothelial function in symptomatic post-menopausal females. Recent research studies have suggested that menopausal hot flushes are associated with greater vascular impairments, including endothelial dysfunction [1], with FMD a determinant of hot flush severity in early post-menopausal females [28]. Therefore, it is likely that symptomatic females have greater impairments in endothelial function and increased cardiovascular disease risk, a condition that exercise training in this study appears to ameliorate.

Reductions in CBF are evident during a hot flush [57] and during a passive heat stress challenge [58,59], and thus are implicated in thermoregulatory control via a reduction of blood flow to the thermoregulatory centre (hypothalamus) in the brain. Improved basal blood flow to the cerebral circulation was observed with exercise training along with attenuation in the reduction of CBF typically observed with passive heat stress in the current study. Short-term exercise training improves cerebrovascular health across the lifespan [27] and our resting CBF data support this notion in post-menopausal females. No study to date has examined the exercise mediated changes in CBF during passive heat stress, yet given that CBF reductions occur during a hot flush (which could be described as a heat stress response *per se*) it is plausible that an exercise mediated attenuation in CBF decreases during heat stress may positively impact on cerebrovascular function during a hot flush and possibly other perturbations that challenge the maintenance of CBF. The mechanisms responsible for these adaptations could include an exercise-mediated increases in stroke volume [60] , plasma volume expansion [61] and/or improved endothelial function as described above [62].

An alternative although not mutually exclusive explanation for the improvement in hot flush frequency and severity could be related to the central sympathetic nervous system that influences the cutaneous, conduit and cerebral vasculature by activating changes in blood flow via the noradrenergic and cholinergic systems [63,64]. Sympathetic noradrenergic nerve outflow increases following the menopause [65] and elevates peripheral vascular resistance [66], while sympathetic cholinergic nerve activity is also increased during hot flushes [67].

Moreover, muscle sympathetic nerve activity (MSNA), an index of sympathetic nerve activity measured using microneuography, is reduced in post-menopausal females following 6-months of moderate-intensity cycling exercise alongside improvements in basal forearm blood flow [68]. Such a reduction in basal sympathetic nerve activity could have directly reduced the occurrence and/or severity of hot flushes in this study, or indirectly, by reducing vascular resistance.

The impact of a reduction in body mass with exercise training on hot flushes also deserves consideration. Reductions in BMI were evident with exercise training in the current study in accordance with one previous study that reported lower BMI and hot flush symptoms following increases in self-reported physical activity [69]. Whilst the role of obesity on hot flush prevalence is unclear, observational studies have reported that females with low [70] and high [71] body fat are at increased risk of hot flushes. Although speculative, increased adiposity may increase hot flushes due to elevated insulation and/or affect vascular function via the release of adipokines and inflammatory markers from visceral adipose tissue, which could decrease with exercise training. It is also important to highlight that an intervention causing body mass reduction that did not involve exercise would not mediate the thermoregulatory and vascular function improvements observed in the current study [72].

Despite the benefits of the exercise training intervention on reducing hot flushes, they were not completely abolished following exercise training (~62% reduction in weekly frequency). Using various indices, previous studies have reported reductions in hot flush frequency in the range of 8-33% [12,16] and severity in the range of 10-30% [12-14,16]. The reasons for the higher reductions in frequency and severity in the present study are likely due to differences in exercise training programme design (e.g., supervised vs. unsupervised and/or programme duration).[12] In a similar study to ours with supervision and high exercise adherence, Lindh and colleagues reported similar reductions in hot flush frequency and severity with exercise training [15]. The frequency and severity responses of the present study mimic those observed (~50-72% reduction in hot flushes) following HT administration (i.e. usual clinical care) in symptomatic females over 12-weeks [5]. HT administration over 12-months further reduces hot flushes [6,73] and thus the effects of exercise training may also further alleviate hot flushes in a similar dose-response manner. Despite a reduction in hot flushes with both HT and exercise training, it is currently unknown if the effects of exercise and HT act by the same mechanisms, via an increase in oestrogen. Whether the combined effects of exercise training and HT further improve hot flushes and offset the increases in cardiovascular risk observed with HT is worth considering. Furthermore, whether the positive effects of exercise on reducing hot flushes remain following cessation of exercise training is currently unknown; however it can be speculated that the positive effects may be transient in the absence of exercise training i.e. ~4 weeks, in line with the reductions observed in thermoregulatory function following the cessation of exercise training in young females [20]. Nevertheless, the findings of the present study suggest that improving thermoregulatory function in symptomatic postmenopausal females is beneficial for hot flushes. Although exercise training clearly confers improvements in thermoregulatory function these findings also suggest that improving thermoregulatory function *per se* (e.g. passive heat acclimation) may also be of benefit for symptomatic postmenopausal females.

One limitation of this study is that it was not a randomised control trial (RCT), with participants free to choose which treatment group they entered. Whilst this convenience sampling and small sample size limit generalizability these findings are labelled preliminary and need to be confirmed in a larger RCT, the reduction in hot flush frequency in the exercise

group is similar to that observed in previous studies, and the hot flush frequency remained unchanged in the control group. Furthermore, thermoregulatory measurements are objective and cannot be influenced by the participant [41], and were analysed in a blinded fashion. Nonetheless, it is acknowledged that the current findings are specific to early post-menopausal females (1-4y since last menstrual period) that were free of cardiovascular disease and not engaged in regular physical activity. The impact of exercise training in alleviating hot flushes in individuals with cardiovascular risk factors, or disease, or other populations who experience hot flushes (e.g. cancer patients) warrants further research.

Conclusion

In summary, improvements in the occurrence of hot flushes with short-term exercise training are mediated via thermoregulatory and cardiovascular adaptation(s). This study provides mechanistic evidence that exercise training is indeed a useful non-pharmacological alternative intervention in the treatment of hot flushes. These findings suggest that targeting the thermoregulatory and cardiovascular systems with interventions may be useful in treating symptomatic post-menopausal females that suffer from hot flushes.

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TABLES

Table 1 Anthropometric, hot flash and vascular function data following exercise training or control

Table 2. Resting and heating cardiovascular and thermoregulatory data before and after exercise training or control

Table 3. Cerebrovascular responses to 0.1 °C increments in core temperature during passive heat stress before and after 16 weeks of exercise training or no-exercise control.

FIGURES

Figure 1. Delta (Δ) change from pre-training in mean body temperature threshold for the onset of chest (a) and forearm (b) sweating. Delta (Δ) change from pre-training in sweat rate sensitivity (slope) at the chest (c) and forearm (d). Error bars are *SD*.*significant difference between exercise and control ($P<0.05$).

Figure 2. Delta (Δ) change from pre-training in mean core body temperature threshold values for onset of chest (a) and forearm (b) cutaneous vasodilation. Delta (Δ) change from pre-training in CVC sensitivity at the chest (c) and forearm (d). Error bars are *SD*. *significant difference between exercise and control ($P<0.05$).

Table 1 Anthropometric, hot flash and vascular function data following exercise training or control

Variable	Pre Exercise	Post Exercise	Pre Control	Post Control	*P value*
Weight (kg)	77.9 (18.3)	73.5 (16.5)	75.5 (19.9)	75.2 (20.4)	0.02[a]
BMI (kg/m^2)	29 (5.8)	27 (4.5)	28 (7.2)	28 (7.0)	0.03[a]
Systolic (mmHg)	128 (5)	126 (7)	127 (10)	128 (8)	0.25
Diastolic (mmHg)	78 (8)	75 (7)	77 (11)	77 (9)	0.58
$\boldsymbol{VO_{2peak}}$ (ml/kg/min)	22.5 (3.3)	27.3 (4.1)	23.2 (2.4)	22.6 (3.1)	0.04 [a]
$\boldsymbol{VO_{2peak}}$ (L/min)	1.7 (0.4)	2.0 (0.3)	1.7 (0.3)	1.6 (0.4)	0.05 [a]
Hot Flushes					
Frequency (HF·wk)	64 (20)	23 (13)	45 (21)	49 (36)	<0.001[a]
Severity Index (AU)	137 (49)	37 (22)	91 (49)	102 (70)	<0.001[a]
Vascular Measurements					
FMD (%)	5.0 (1.2)	7.4 (1.5)	5.6 (1.9)	5.5 (1.8)	0.08
Baseline Diameter (mm)	0.37 (0.03)	0.37 (0.05)	0.36 (0.04)	0.35 (0.04)	0.97
Peak Diameter (mm)	0.39 (0.04)	0.40 (0.04)	0.38 (0.04)	0.37 (0.04)	0.86
Shear rate$_{AUC}$ (s^{-1} x 10^3)	16.3 (8.6)	17.8 (10.4)	21.5 (13.9)	20.4 (12.8)	0.95
Time to Peak (s)	69.7 (32.5)	54.2 (34.6)	70.5 (33.2)	76.7 (35.6)	0.19

Data are presented as mean (SD.). [a]Significant difference between change (Δ) in Exercise and Δ in Control values ($P<0.05$).

Table 2. Resting and heating cardiovascular and thermoregulatory data before and after exercise training or control

Variable	Pre Exercise	Post Exercise	Pre Control	Post Control	*P value*
Resting					
Heart Rate ($b \cdot min^{-1}$)	64 (7)	60 (7)	66 (11)	65 (12)	0.003[a]
MAP (mmHg)	75 (7)	75 (5)	76 (4)	75 (6)	0.58
Stroke Volume (ml)	109 (16)	114 (27)	105 (18)	103 (16)	0.47
Cardiac Output ($l \cdot min^{-1}$)	7.2 (1.6)	7.6 (1.8)	7.1 (1.4)	7.3 (1.1)	0.69
Core Temperature (°C)	36.93 (0.19)	36.79 (0.21)	36.86 (0.31)	36.84 (0.27)	0.03[a]
Skin Temperature (°C)	32.2 (0.7)	32.9 (0.6)	32.8 (0.5)	32.9 (0.7)	0.10
MCAv ($cm \cdot s^{-1}$)	51 (6)	54 (7)	51 (5)	51 (5)	0.05[a]
CBVC ($cm \cdot s^{-1} \cdot mmHg^{-1}$)	0.69 (0.11)	0.74 (0.13)	0.68 (0.06)	0.69 (0.04)	0.08
$P_{ET}CO_2$ (Torr)	42 (2)	42 (2)	41 (3)	42 (2)	0.36
CVC_{chest} (%CVC_{max})	10.9 (5.0)	11.2 (6.9)	9.5 (4.5)	8.6 (3.2)	0.93
CVC_{arm}, (%CVC_{max})	9.7 (5.2)	10.2 (5.6)	8.8 (4.5)	9.5 (3.3)	0.13
Chest CVC_{max} (LDF/mmHg)	5.1 (1.6)	5.9 (1.4)	5.4 (1.1)	5.2 (1.4)	0.58
Arm CVC_{max} (LDF/mmHg)	2.9 (0.7)	3.9 (0.9)	3.3 (0.9)	3.4 (0.8)	0.05[a]
Heating					
Heart Rate ($b \cdot min^{-1}$)	93 (10)	88 (12)	89 (9)	93 (9)	0.08
Core Temperature (°C)	37.75 (0.17)	37.71 (0.21)	37.63 (0.22)	37.58 (0.24)	0.40
Skin Temperature (°C)	37.3 (0.7)	37.2 (0.8)	36.9 (0.4)	36.9 (0.5)	0.88

Data are presented as mean (SD). [a]significant difference between change Δ in Exercise and Δ in Control

Table 3. Cerebrovascular responses to 0.1 °C increments in core temperature during passive heat stress before and after 16 weeks of exercise training or no-exercise control.

Variable														
	Exercise Training													
	Pre							***Post***						
Core temp °C	**Rest**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**Rest**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**
MCA*v* $cm \cdot s^{-1}$	51 (6)	50 (7)	47 (7)	45 (7)	43 (9)	43 (8)	42 (8)	54 (7)	54 (7)	51 (8)	50 (8)	49 (9)	48 (9)	48 (10)
CBVC $cm \cdot s^{-1} \cdot mmHg^{-1}$	.69 (.08)	.67 (.08)	.65 (.08)	.63 (.08)	.60 (.09)	.60 (.12)	.59 (.09)	.74 (.06)	.74 (.07)	.70 (.06)	.69 (.04)	.68 (.05)	.67 (.08)	.68 (.05)
$P_{ET}CO_2$ (Torr)	42 (4)	42 (5)	41 (5)	40 (6)	40 (6)	39 (5)	39 (5)	42 (3)	42 (4)	40 (6)	40 (5)	40 (4)	39 (3)	39 (5)
	Control													
	Pre							***Post***						
Core temp °C	**Rest**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**Rest**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**
MCA*v* $cm \cdot s^{-1}$	51 (5)	50 (5)	49 (5)	48 (6)	47 (6)	44 (5)	41 (6)	51 (5)	50 (4)	48 (4)	46 (4)	44 (4)	44 (6)	43 (6)
CBVC $cm \cdot s^{-1} \cdot mmHg^{-1}$	.68 (.06)	.68 (.07)	.67 (.08)	.66 (.05)	.64 (.07)	.61 (.10)	.60 (.09)	.69 (.05)	.68 (.03)	.66 (.04)	.65 (.05)	.64 (.06)	.62 (.07)	.61 (.08)
$P_{ET}CO_2$ (Torr)	41 (5)	41 (6)	41 (6)	40 (8)	39 (6)	39 (5)	38 (6)	42 (5)	42 (5)	41 (5)	40 (7)	40 (5)	39 (5)	39 (5)

Data are presented as mean (SD). *P* values for MCAv (intervention $P=0.83$, pre/post $P<0.0001$, intervention*pre/post interaction $P<0.001$, intervention*pre/post*temp interaction $P=0.52$), CBVC (intervention $P=0.84$, pre/post $P<0.0001$, intervention*pre/post interaction $P<0.05$, intervention*pre/post*temp interaction $P=0.65$) and $P_{ET}CO_2$ (intervention $P=0.79$, pre/post $P< 0.001$, intervention*pre/post interaction $P=0.47$, intervention*pre/post*temp interaction $P=0.59$).

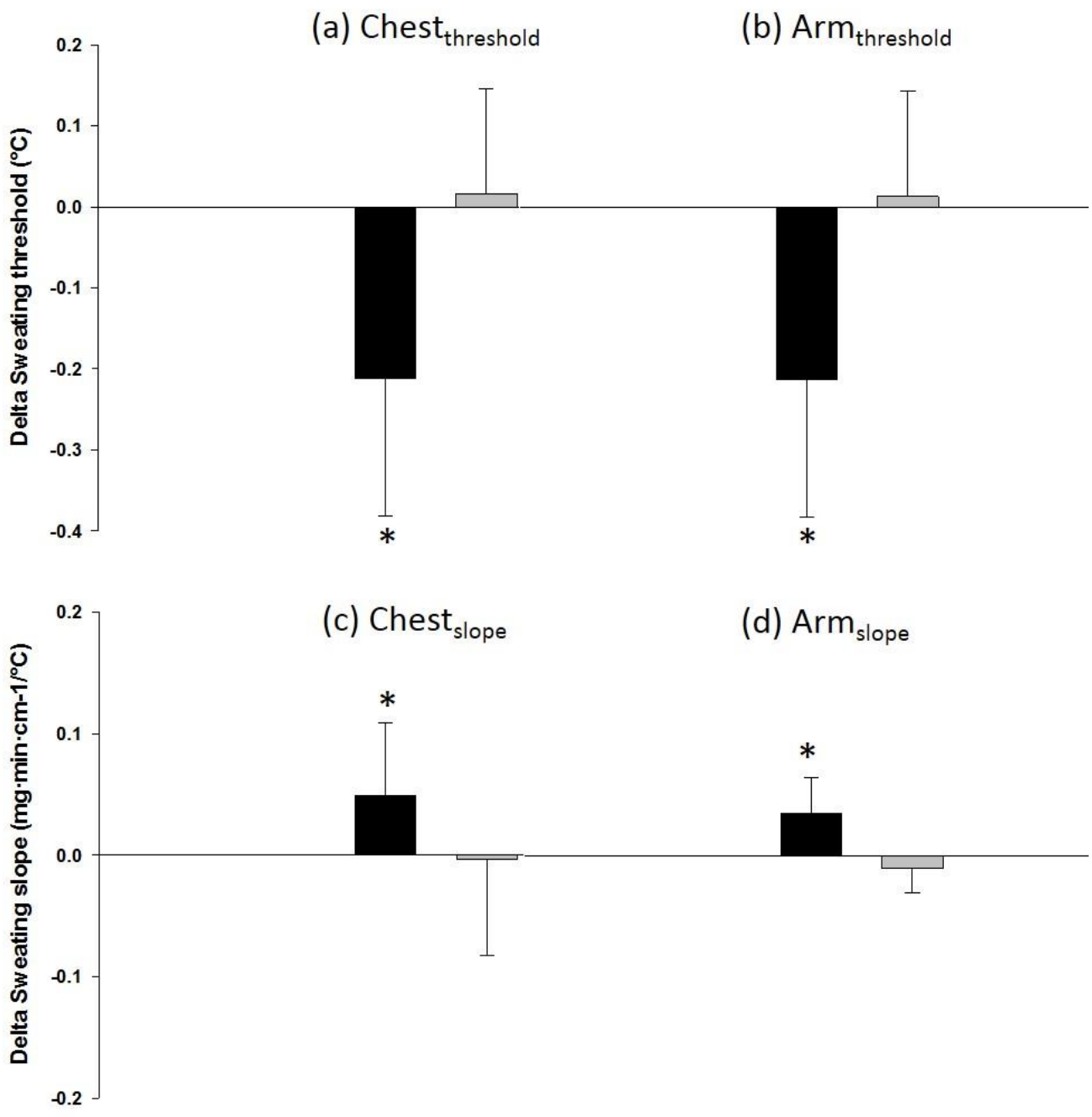

(a) Chest threshold
(b) Arm threshold
Delta Sweating threshold (°C)
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
*
*
(c) Chest slope
(d) Arm slope
Delta Sweating slope (mg·min·cm-1/°C)
0.2
0.1
0.0
-0.1
-0.2
*
*

Figure 1

Figure 2

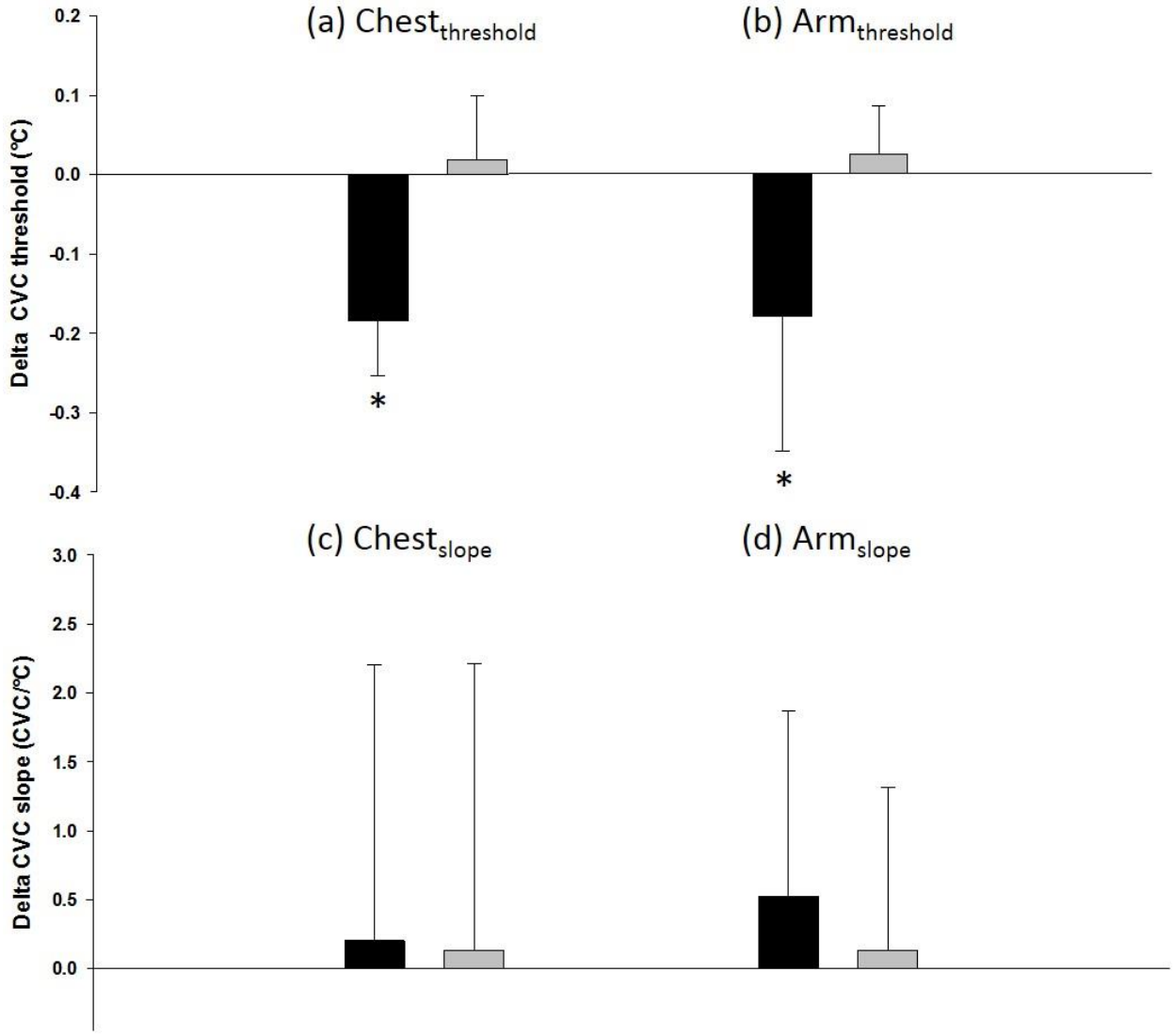
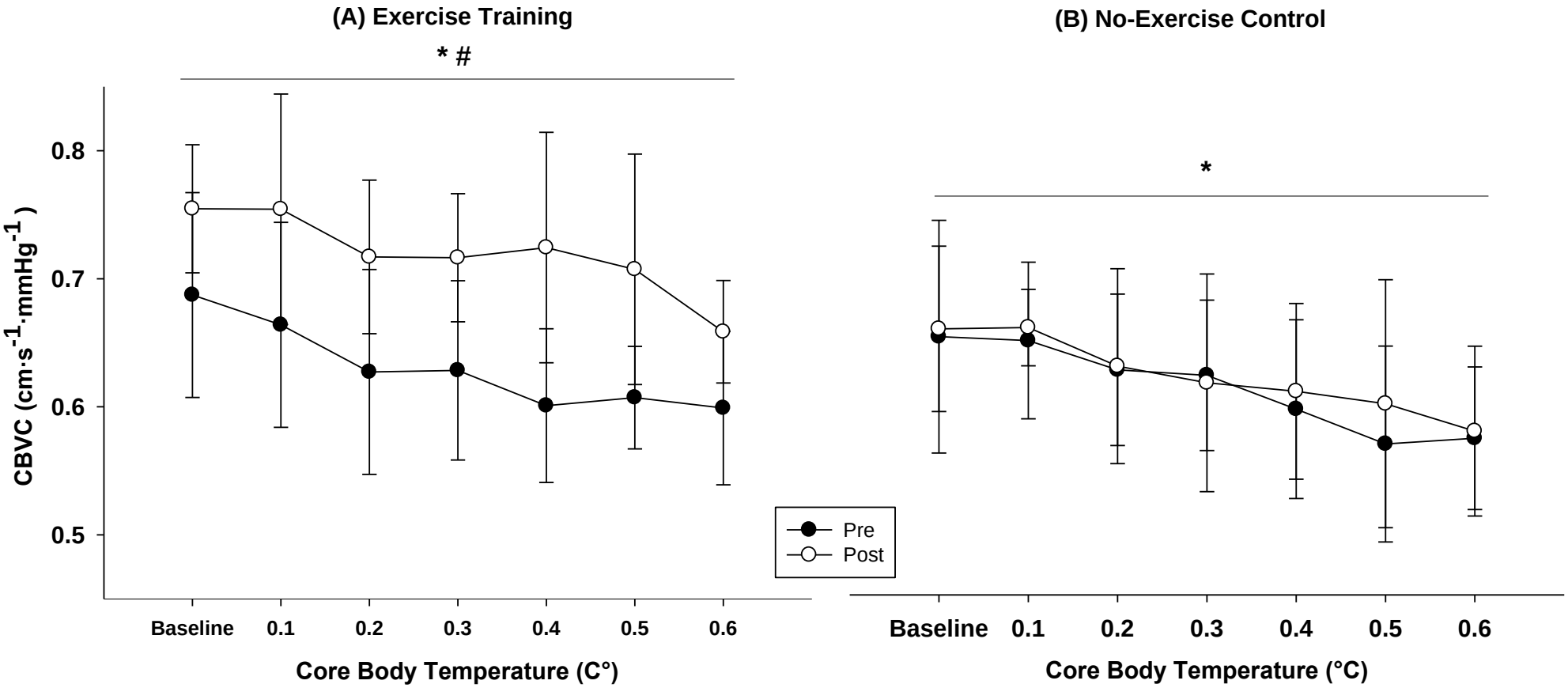

(a) Chest threshold
(b) Arm threshold
(c) Chest slope
(d) Arm slope
Delta CVC threshold (°C)
Delta CVC slope (CVC/°C)
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
*

Figure 3


(A) Exercise Training
* #
(B) No-Exercise Control
*
CBVC (cm·s-1·mmHg-1)
0.8
0.7
0.6
0.5
Pre
Post
Baseline
0.1
0.2
0.3
0.4
0.5
0.6
Core Body Temperature (C°)
Core Body Temperature (°C)