EXERCISE TRAINING REDUCES THE ACUTE PHYSIOLOGICAL SEVERITY OF POST-MENOPAUSAL HOT FLUSHES

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- A post-menopausal hot flush consists of profuse physiological elevations in cutaneous vasodilation and sweating that are accompanied by reduced brain blood flow. These responses can be used to objectively quantify hot flush severity.

- The impact of an exercise training intervention on the physiological responses occurring during a hot flush is currently unknown.

- In a preference controlled trial involving 21 post-menopausal women, 16 weeks of supervised moderate-intensity exercise training was found to improve cardiorespiratory fitness and attenuate cutaneous vasodilation, sweating and the reductions in cerebral blood flow during a hot flush.

- It is concluded that the improvements in fitness that are mediated by 16 weeks of exercise training reduce the severity of physiological symptoms that occur during a post-menopausal hot flush.

ABSTRACT

A hot-flush is characterised by feelings of intense heat, profuse elevations in cutaneous vasodilation and sweating, and reduced brain blood flow. Exercise training reduces self-reported hot-flush severity, but underpinning physiological data are lacking. We hypothesised that exercise training attenuates the changes in cutaneous vasodilation, sweat rate and cerebral blood flow during a hot flush. In a preference trial, 18 symptomatic post-menopausal women underwent a passive heat stress to induce hot-flushes at baseline and follow-up. Fourteen participants opted for a 16-week moderate intensity supervised exercise intervention, while 7 participants opted for control. Sweat rate, cutaneous vasodilation, blood pressure, heart rate and middle cerebral artery velocity (MCA*v*) were measured during the hot-flushes. Data were binned into eight equal segments, each representing 12.5% of hot flush duration. Weekly self-reported frequency and severity of hot flushes were also recorded at baseline and follow-up. Following training, mean hot-flush sweat rate decreased by 0.04 $mg \cdot cm^{2} \cdot min^{-1}$ at the chest (95% CI: 0.02-0.06, P=0.01) and by 0.03 $mg \cdot cm^{2} \cdot min^{-1}$ (0.02-0.05, P=0.03) at the forearm, compared with negligible changes in control. Training also mediated reductions in cutaneous vasodilation by 9% (6-12) at the chest and by 7% (4-9) at forearm ($P \leq 0.05$). Training attenuated hot flush MCA*v* by 3.4 cm/s (0.7-5.1, P=0.04) compared with negligible changes in control. Exercise training reduced the self-reported severity of hot-flush by 109 arbitrary units (80-121, $P<0.001$). These data indicate that exercise training leads to parallel reductions in hot-flush severity and within-flush changes in cutaneous vasodilation, sweating and cerebral blood flow.

ABBREVIATIONS:

BMI, Body Mass Index; **BP,** Blood Pressure; **CBF,** Cerebral Blood Flow; **CBVC,** Cerebro-Vascular Conductance; **CI,** Confidence Interval; **CO,** Cardiac Output; **CV,** Coefficient of Variation; **CVC,** Cutaneous Vascular Conductance; **CVD,** Cardiovascular Disease; **DBP,** Diastolic Blood Pressure; **DICOM,** Digital Imaging and Communications in Medicine; **eNOS,** Endothelial Nitric Oxide Synthase; **FMD,** Flow-Mediated Dilation; **HR,** Heart Rate; **HRT,** Hormone Replacement Therapy; **HRR,** Heart Rate Reserve; **LDF,** Laser Doppler Flux; **LSD,** Least significant difference; **MAP,** Mean Arterial Pressure; **MCA,** Middle Cerebral Artery; **MCA*v*,** Middle Cerebral Artery Velocity; **MSNA,** Muscle Sympathetic Nerve Activity; **NO,** Nitric Oxide; **NOS,** Nitric Oxide Synthase; $\mathbf{P_{ET}CO_2}$, End-tidal of Carbon Dioxide; **RCT,** Randomised Control Trial; **RPE,** Rating of Perceived Exertion; **ROI,** Region of Interest; **SBP,** Systolic Blood Pressure; **SD,** Standard Deviation; **SKBF,** Skin Blood Flow; **SR,** Sweat Rate; **SRAUC,** Shear Rate Area Under the Curve; **SV,** Stroke Volume; $\mathbf{T_{body}}$, Mean Body Temperature; $\mathbf{T_{core}}$, Core Body Temperature; **TCD,** Transcranial Doppler;$\mathbf{VO_{2peak}}$, Maximal Oxygen Consumption

INTRODUCTION

The primary symptom of the menopause is the experience of multiple hot flushes, which are associated with elevated lipids and, potentially, insulin resistance - all of which are risk factors for cardiovascular disease (Thurston *et al.*, 2012a; Thurston *et al.*, 2012b). Self-reported hot flush severity is linked to vascular inflammation and endothelial dysfunction in post-menopausal women (Bechlioulis *et al.*, 2010). A hot flush is described as a sudden and intense sensation of heat causing skin reddening and profuse sweating (Freedman, 2002). The observed elevations in cutaneous vasodilation (~80% increase from baseline) and sweating (5-fold increases) during a hot flush that lasts 2-3 min are comparable to those mediated by 30 min of moderate-intensity cycling or 60 min of passive heating, which tend to raise core body temperature by ~0.4-0.6°C (or greater) (Wingo *et al.*, 2010). Hot flushes can therefore be considered to be moderate-to-severe thermoregulatory events.

Hot flushes are physiologically defined as a transient and pronounced increase in sweat rate that is preceded by a rapid surge in cutaneous vasodilation (Low *et al.*, 2010). Nevertheless, the severity of a hot flush is typically only subjectively defined by the individual, based upon symptom-based interpretations of the magnitude of sweating, cutaneous vasodilation, dizziness and increases in heart rate (Sloan *et al.*, 2001). The physical symptoms that describe hot-flush severity using a self-reported rating scale have recently been confirmed with a series of studies describing physiological elevations in cutaneous vasodilation, sweating and reductions in cerebral blood flow during a hot flush (Low *et al.*, 2008; Low *et al.*, 2010; Lucas *et al.*, 2013). Interventions aimed at relieving menopausal symptoms typically use self-reported rating scales as outcome variables (Stearns *et al.*, 2003; Daley *et al.*, 2015). Nevertheless, no researcher to date has investigated if any of the physiological changes that occur during a hot flush, and define severity, are responsive to intervention.

Exercise training can improve the self-reported frequency and severity of hot flushes (Karacan, 2010; Luoto *et al.*, 2012; Moilanen *et al.*, 2012). Our research group recently demonstrated that the reduction in weekly hot flush frequency with exercise training is associated with improvements in thermoregulatory control in symptomatic post-menopausal women. These improvements were characterised by a lower resting core body temperature, improving the temperature threshold for the onset of cutaneous vasodilation and sweating, alongside improved sweating sensitivity to passive heating (Bailey *et al.*, 2015). Similarly,

exercise training also improves cerebrovascular function in middle-aged and elderly individuals (Ainslie *et al.*, 2008; Bailey *et al.*, 2013; Murrell *et al.*, 2013). The mechanisms of hot flushes have often been attributed to a malfunctioning thermoregulatory control system (either centrally or peripherally) (Charkoudian & Stachenfeld, 2014; Freedman, 2014; Jayasena *et al.*, 2015). If exercise training can improve thermoregulatory and cerebrovascular control in symptomatic post-menopausal women, it is possible to suggest that this may be reflected in the physiological responses observed during a hot flush (e.g. attenuated cutaneous vasodilation, sweating or cerebrovascular responses) which would positively impact upon hot flush severity.

Therefore, the aim of this study was to quantify the acute physiological thermoregulatory and (cerebro)vascular changes that occur during hot flushes prior to and following a 16-week exercise intervention in symptomatic post-menopausal women. We hypothesised that exercise training attenuates the changes in cutaneous vasodilation, sweat rate and cerebral blood flow during a hot flush.

METHODS

Participants

As part of a pilot preference trial, initially powered for the primary outcome of reported hot flush severity, 21 healthy symptomatic post-menopausal women were recruited from the gynaecology and reproductive medicine clinic at Liverpool Women's Hospital, local G.P. practices and via local advertisement. One to four years had elapsed since the participants experienced their last menstrual period, and they suffered more than four hot flushes across a 24h period (self-reported using a 7-day diary). All participants had no history of type II diabetes, cardiovascular or respiratory disease, were non-smokers, drank <14 units of alcohol per week, and had no contraindications to exercise. Participants were excluded if they engaged in regular exercise (>2h per week based on a self-reported questionnaire), had used hormone replacement therapy within the last 6-months, or were currently prescribed metformin, vasoactive or BP lowering medications. 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 National Research ethics committee.

Research Design

Participants reported to the laboratory on two occasions before and after a 16-week exercise training or no-exercise control intervention (four visits in total). For all visits, participants were asked to fast overnight, refrain from alcohol and exercise for 24h, and caffeine for 12h. Visit one consisted of a cardiorespiratory fitness assessment to determine $\dot{V}O_{2peak}$ and a 7-day hot flush severity questionnaire. The second visit consisted of anthropometric measurement (height, weight and body-mass index) and a physiological hot-flush assessment. Thermoregulatory, hemodynamic and cerebrovascular responses were assessed during hot-flushes at rest under normothermic conditions and during passive heating. All participants then undertook either 16-weeks of exercise or a no-exercise control intervention – this choice being based on participant preference (Kowalski & Mrdjenovich, 2013). Visits 1 and 2 were repeated at follow-up. Fourteen (n=14, 52±4y) symptomatic women opted for the 16-week programme of supervised moderate-intensity aerobic exercise training while seven (n=7, 52±6y) symptomatic women opted for the no-exercise control group. Participants in the no-exercise control group were asked to continue with their normal daily routine in terms of physical activity, and received no contact with the research team throughout the 16-week intervention period.

Measurements

Cardiorespiratory assessment for peak oxygen consumption

A test of peak oxygen uptake ($\dot{V}O_{2peak}$) was completed on a treadmill using a modified Bruce protocol. Following a 2-min warm-up at 2.2 km/h on a flat gradient, the initial workload was set at 2.7 km/h 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 (Borg, 1970). Peak oxygen uptake was calculated from expired gas fraction (Oxycon Pro, Jaegar, Hochberg Germany) as the highest consecutive 15-s period of data in the final minute before volitional exhaustion.

Hot Flush Frequency and Severity Questionnaire

At baseline and follow-up, participants completed a 7-day hot flush frequency and severity diary (Sloan *et al.*, 2001). Participants recorded how many hot flushes they experienced on a daily basis 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 in each severity rating e.g. (3*mild)+(2*moderate)+(1*severe)+(0*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 self-report diaries is a valid approach to obtaining data on patient symptoms and perceptions of hot flush (Sloan *et al.*, 2001). The self-reported hot flush and cardiorespiratory fitness data are included in the current manuscript for comparison purposes, and have also been reported by Bailey *et al.* (2015) who focussed on the impact of improving thermoregulatory efficiency during heat stress on self-reported hot flush frequency.

Physiological Hot Flush Assessment

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 30 minute baseline period. Participants were then exposed to a mild heat stress by perfusing ~46^{o}C water through the suit to induce a hot-flush (Kronenberg, 1990; Freedman, 2001; Lucas *et al.*, 2013). HR was obtained from a 3-lead electrocardiogram (Powerlab, AD Instruments, Oxford, UK), alongside continuous beat-by-beat finger arterial 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. 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 post-intervention assessment.

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 150mL/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 (Willie *et al.*, 2011), 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. An index of cerebrovascular conductance (CBVC) was calculated from the ratio of MCA_V to MAP. All data were sampled at 50Hz with a data acquisition system (PowerLab, AD Instruments, 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 Doppler flowmetry units on the chest and forearm was increased at a rate of 0.5 °C every 5 sec to a temperature of 42 °C (Tew *et al.*, 2011). 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).

Physiological hot flush analysis

A hot flush was objectively recorded in 18 participants (11 exercise and 7 control participants) in both normothermia (spontaneous) and during heating. Hot flushes recorded in participants both at baseline and following the exercise training (n=9) and control (n=7) interventions were used for analysis. The onset of a hot flush was objectively identified as a transient and pronounced increase in sternal sweat rate (>0.002 $mg \cdot cm^{-2} \cdot min^{-1}$ per second) as has been used previously (Freedman, 2001; Low *et al.*, 2008; Low *et al.*, 2010). Participants also informed the research team of a self-reported feeling of a hot flush and once the feeling had dissipated. The end of each hot flush was objectively recorded as the return of sweat rate to pre hot flush baseline values. Because of the variance in the length of hot flushes, each hot flush episode was divided into eight equal segments, with each segment representing 12.5%

of hot flush duration (Low *et al.*, 2010; Lucas *et al.*, 2013). Five-second periods of data at the end of each segment, and over a period of 2 minutes before i.e. baseline, and 2 minutes following the hot flush i.e. post hot flush recovery, were used for data analysis.

Supervised exercise training intervention

Before commencing the exercise intervention, all the participants in this study arm 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 (Pugh *et al.*, 2013; Sprung *et al.*, 2013). On the basis of individual fitness level, participants underwent 30 min 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. This type of moderate-intensity programme was informed by NHS guidelines and our previous studies in which improvements in cardiorespiratory fitness were documented (NHS, 2011; Pugh *et al.*, 2013; Sprung *et al.*, 2013).

Control participants

After consent and physiological hot flush assessment, women who preferred to participate in the control group had no contact with the research team throughout the 16-weeks. This type of control reflects the current absence of non-pharmacological treatment for hot flushes in the UK. As such, the research team did not influence any lifestyle factors during the 16-week period.

Statistical Analysis

For self-reported hot flush frequency, severity index, duration and cardiorespiratory fitness (the variables measured once at baseline and once at follow-up), the raw changes from baseline were calculated and intervention vs control differences were quantified with general linear models, with baseline data entered as a covariate. This approach adjusts properly for

any study arm imbalance at pre-intervention (Vickers & Altman, 2001) and is superior to quantifying intervention effects using percentage changes (Vickers, 2001)

For physiological measures during the hot flushes, data were acquired continuously at 50 Hz throughout baseline and passive heating protocol (PowerLab, ADinstruments, UK) and hot flushes were analysed in a blinded fashion by the same observer. A three-factor (intervention, time and segment) linear mixed model was employed for the analysis of hot flush outcomes (BP, HR, sweating skin and cerebral blood flow) during each 12.5% segment, following exercise training or control. Statistically significant interaction terms were followed up with a simple main effects analysis and the least significant difference (LSD) approach to multiple comparisons (Perneger, 1998). Point estimates of each outcome at each time point during the protocol are reported using the mean and standard deviation. Intervention effects are quantified by reporting the difference between exercise and control with associated 95% confidence limits in parentheses, i.e. (lower limit - upper limit) (Gardner & Altman, 1986).

Results

Compliance to the exercise sessions was 93% over the 16-week period. 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). Exercise training reduced self-reported hot flush frequency by 48 (39-56) flushes·week (P<0.001), and hot flush severity index by 109 (80-121) AU (P<0.001) vs the changes in the control group.

Physiological Hot Flush Assessment

Hot flush duration: Exercise training reduced hot flush duration compared to control [61 seconds (-9, 112)], however this did not reach statistical significance (P=0.34).

Haemodynamics: HR significantly increased by 8 $b \cdot min^{-1}$ (6, 11; P<0.001) during hot flushes. There was no main effect of intervention, time, or intervention*time interaction in HR during hot flushes (P>0.05). MAP significantly decreased by 5 mmHg (1, 10; P<0.001) during the hot flushes with no main effect of intervention, time or intervention*time interaction (P>0.05). The changes in cardiac output and stroke volume were negligible and

not statistically significant during the hot flushes nor between study groups (P>0.05, Table 1).

Core temperature: Core body temperature tended to decrease following hot flushes [by 0.06°C (0.02, 0.11; P=0.15)]. There was no intervention*time interaction for core body temperature (Table 2).

Skin blood flow: Skin blood flow increased during hot flushes (P<0.001; Figure 1). At the chest, there was an intervention*time interaction with a reduction of 9% CVC_{max} (6, 11; P=0.01) during hot flushes following exercise training compared 4% CVC_{max} (-6, 8; P=0.34) in control. Similarly, at the forearm there was an intervention*time interaction with a reduction of 7 %CVC_{max} (4, 9; P=0.05) during hot flushes following exercise training compared to a 3% CVCmax (-3, 6; P=0.44) change following control.

Sweating: Sweat rate increased at the chest and forearm during hot flushes (P<0.001; Figure 2). There was an intervention*time interaction with a reduction of 0.04 $mg \cdot cm^{2} \cdot min^{-1}$ (0.02, 0.06; P=0.01) in chest sweat rate following exercise training compared to 0.01 $mg \cdot cm^{2} \cdot min^{-1}$ (-0.02, 0.03; P=0.19) change in control. There was an intervention*time interaction with a reduction of 0.03 $mg \cdot cm^{2} \cdot min^{-1}$ (0.02, 0.05; P=0.01) in forearm sweat rate following exercise training compared a 0.01 $mg \cdot cm^{2} \cdot min^{-1}$ (-0.01, 0.02; P=0.78) change in control.

Cerebral blood flow: MCAv significantly decreased during hot flushes (P<0.001; Figure 3). There was an intervention*time interaction in MCAv, with the size of the decrease reduced by 3.4 cm/s (0.7, 5.1; P<0.001) following exercise training compared to 0.6 cm/s (-0.7, 1.8; P=0.41) with control. When expressed as CBVC similar findings were evident (P=0.04; Table 2).

Discussion

This is the first study to quantify the effects of an exercise training intervention on the physiological responses observed during hot flushes in symptomatic post-menopausal women. The novel findings of this preference controlled trial were that a 16-week exercise training intervention reduced the sweating and cutaneous vasodilation response typically observed during a hot flush episode. In addition, the acute reduction in cerebral blood flow observed during a hot flush was attenuated with exercise training. Taken together, these

objective physiological responses which define self-reported hot-flush severity in post-menopausal women provide direct evidence that exercise training reduces the physiological hot flush severity by improving thermoregulatory and cerebrovascular responses during a hot flush.

Previous exercise training studies investigating changes in hot flush severity involve self-report questionnaires that rely on self-reported selection of descriptions based on physical symptoms (e.g. (Sloan *et al.*, 2001). The impact of exercise training on hot flush severity has been inconsistent, with some studies reporting reductions in self-reported severity (Lindh-Astrand *et al.*, 2004; Karacan, 2010; Luoto *et al.*, 2012; Moilanen *et al.*, 2012; Reed *et al.*, 2014) and others either reporting no change or have relied solely on hot-flush frequency scores (Sternfeld *et al.*, 2014; Daley *et al.*, 2015). Nevertheless, the physical symptoms on which the self-reported hot flush severity scale is rated, are defined by the magnitude of sweating, cutaneous vasodilation, dizziness and heart rate changes during a hot flush (Sloan *et al.*, 2001). For the first time, our data provide direct evidence that exercise training reduces the objective physiological severity of menopausal hot flushes. The results of the current study suggest exercise training mediates acute amelioration of physiological perturbations (sweating, cutaneous vasodilation and changes in cerebral blood flow) observed during hot flushes, which potentially explains the improvements in self-reported hot flush severity that corresponds with reduced skin reddening, sweating and dizziness as described on the self-reported severity scale following exercise training in this study, and others.

A previous study by our research group recently examined the potential physiological mechanisms for exercise training-mediated improvements in the self-reported frequency and severity of menopausal hot flushes by assessing the thermoregulatory and systemic vascular responses to passive heating (i.e. a protocol to examine thermoregulatory control rather than examine responses during hot flush) before and after an exercise training intervention (Bailey *et al.*, 2015). We found that exercise training improves thermoregulatory control and enhances the function of the cerebral and cutaneous circulations to passive heating alongside improvements in the frequency of menopausal hot flushes (Bailey *et al.*, 2015). More specifically, exercise training mediated a reduction in resting core temperature that enabled an earlier onset of sweating and cutaneous vasodilation, improved sensitivity of sweating and attenuated reductions in cerebral blood flow during passive heating. Given that the mechanisms causing hot flushes have often been attributed to a dysfunctional

thermoregulatory control system (Freedman, 2014) and/or vascular dysfunction (Bechlioulis *et al.*, 2010; Bechlioulis *et al.*, 2012; Sassarini *et al.*, 2012; Sassarini *et al.*, 2014); it is possible that improvements in thermoregulatory control and cerebrovascular function also explain the attenuations in sweating, cutaneous vasodilation and alterations in cerebral blood flow that occur during a hot flush *per se* in the current study. Whilst an increase in core body temperature has previously been proposed as the trigger for hot flushes, the reported elevations are minor (~0.04ºC) (Freedman & Wooodward, 1996; Freedman, 2014) and in the current study we did not observe a core body temperature increase during (or immediately prior to) a hot flush. Therefore, the trigger for a hot flush and importantly the exercise training mediated reductions in thermoregulatory intensity during a hot flush are unlikely mediated by alterations in core temperature unlike the typical cutaneous and sudomotor responses that occur during passive heating.

An alternative mechanistic explanation that must be considered is an exercise mediated reduction in sympathetic nerve activity. Although we did not measure sympathetic nerve activity in the current study, there is some evidence to suggest that (muscle) sympathetic nerve activity is lower following exercise training in post-menopausal women (Oneda *et al.*, 2014). Cutaneous vasodilation during a hot flush is neurally mediated by a large transient increase in skin sympathetic nerve activity (Low *et al.*, 2010), it is therefore probable that the reduced cutaneous vasodilation (and potentially sweating) may be a consequence of a smaller engagement of the sympathetic cholinergic system, potentially reducing the direct or more likely the ACh-induced release of NO from the endothelium during hot flushes (Hubing *et al.*, 2010; Sassarini *et al.*, 2012). Exercise training has a well-established impact on NO-dependent vasodilation in both large and small vessels, with repeated episodic increases in shear stress during acute exercise causing enhanced NO mediated endothelial function (Green *et al.*, 2004). It is important to highlight that enhanced NO-mediated endothelial function in response to shear stress would cause an increase in cutaneous vasodilation to the same stimulus, whereas cutaneous vasodilation was attenuated during a hot flush in the present study suggesting that the cutaneous NO response is mediated directly by the sympathetic cholinergic system, and not shear stress.

The attenuation of a reduction in cerebral blood flow during a hot flush was not explained by alterations in BP (e.g., a smaller reduction in BP during a hot flush was not evident post training), which is in agreement with the observations of Lucas *et al.*, (2013). Other

modulators of cerebral control consist of cardiac output, arterial CO_2 content and sympathetic nerve activity (Ainslie & Duffin, 2009). Cardiac output (along with SV) remained unchanged during hot flushes following exercise training in the current study. Whilst a hyperventilatory-induced reduction in arterial CO_2 during hot flushes is possible, this was not measured during hot flushes in the current or previous studies (Lucas et al., 2013) and warrants further investigation. Nevertheless, a reduction in basal or hot flush induced sympathetic nerve activity (as discussed above) may also reduce cerebral vasoconstriction and potentially self-reported feelings of faintness.

The current study employs objective physiological measurements of hot flush severity that cannot be influenced by the participant and were analysed in a blinded fashion. Nonetheless, one limitation was that participants only rated weekly self-reported severity and did not also rate severity during the acute hot flushes assessed in the laboratory. Exercise training did reduce weekly severity which supports the physiological acute hot flush data obtained in the laboratory and supports the notion that exercise training reduces the severity of post-menopausal hot flushes.

In summary, we have shown exercise training improves objective physiological markers (sweating and cutaneous vasodilation) of hot flush severity in symptomatic post-menopausal women. These findings confirm that exercise training has a direct influence on thermoregulatory and cerebrovascular responses during hot flushes *per se*, which may explain reductions in self-reported hot flush severity symptoms following exercise training.

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Additional information.

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Table 1. Haemodynamic responses during hot flushes before (pre) and after (post) exercise and control intervention.

Variable	Pre					Post					
	Hot flush (%)					Hot flush (%)					
Exercise	**Baseline (2min)**	**0**	**50**	**100**	**Post (+2 min)**	**Baseline (2 min)**	**0**	**50**	**100**	**Post (+2 min)**	**Time*segment interaction**
Heart rate ($b \cdot min^{-1}$)*	68 (9)	76 (10)	74 (9)	72 (8)	69 (10)	66 (7)	74 (8)	73 (9)	71 (12)	67 (7)	P>0.05
MAP (mmHg)*	75 (7)	70 (9)	71 (10)	73 (8)	73 (7)	74 (7)	71 (8)	70 (9)	72 (8)	73 (7)	P>0.05
Cardiac Output (L·min)	7.4 (2.8)	7.9 (2.6)	7.6 (2.6)	7.2 (2.4)	7.5 (2.2)	7.8 (2.9)	8.0 (2.3)	7.8 (2.2)	7.6 (2.5)	7.3 (2.2)	P>0.05
Stroke Volume (ml)	109 (12)	108 (14)	106 (15)	110 (14)	107 (14)	112 (12)	110 (12)	107 (12)	109 (17)	108 (18)	P>0.05
CBVC ($cm/s^{-1} \cdot mmHg$)*#	0.66 (0.08)	0.62 (0.10)	0.59 (0.09)	0.60 (0.13)	0.63 (0.09)	0.70 (0.06)	0.69 (0.06)	0.67 (0.12)	0.71 (0.08)	0.72 (0.10)	P<0.05
Control	**Baseline (-2min)**	**0**	**50**	**100**	**Post (+2 min)**	**Baseline (-2 min)**	**0**	**50**	**100**	**Post (+2 min)**	
Heart rate ($b \cdot min^{-1}$)*	69 (10)	77 (8)	76 (12)	72 (11)	70 (13)	68 (7)	77 (11)	74 (13)	72 (12)	70 (14)	P>0.05
MAP (mmHg)*	75 (5)	70 (4)	71 (7)	72 (7)	73 (5)	75 (6)	69 (5)	71 (5)	72 (7)	74 (4)	P>0.05
Cardiac Output (L·min)	7.2 (2.4)	7.3 (2.4)	7.4 (2.5)	7.2 (2.2)	7.3 (2.3)	7.4 (2.4)	7.6 (2.8)	7.4 (2.6)	7.2 (2.3)	7.2 (2.6)	P>0.05
Stroke Volume (ml)	103 (11)	107 (20)	104 (13)	106 (11)	105 (17)	106 (18)	110 (12)	108 (12)	109 (14)	108 (16)	P>0.05
CBVC ($cm/s^{-1} \cdot mmHg$)*#	0.67 (0.06)	0.65 (0.05)	0.62 (0.07)	0.68 (0.09)	0.66 (0.05)	0.68 (0.06)	0.65 (0.07)	0.63 (0.11)	0.67 (0.10)	0.65 (0.05)	P<0.05

Data are presented as mean (SD). *Significant main effect of time, #Significant intervention x time interaction ($P<0.05$). NB. Hot flushes were statistically analysed over 8 segments, but are represented over 3 time segments (0, 50, 100%) above.

Table 2. Thermoregulatory responses during hot flushes before (pre) and after (post) the exercise and control intervention

Variable	Pre					Post					
	Hot flush (%)					Hot Flush (%)					
Exercise	**Baseline**	**0**	**50**	**100**	**Post**	**Baseline**	**0**	**50**	**100**	**Post**	**Time*segment interaction**
Core temperature (°C)	37.1 (0.4)	37.1 (0.3)	37.1 (0.4)	37.0 (0.4)	37.0 (0.3)	37.0 (0.3)	37.0 (0.3)	37.0 (0.3)	36.9 (0.4)	36.9 (0.3)	P>0.05
CVC_{chest} (AU/mmHg)*#	1.3 (0.7)	2.5 (0.9)	1.8 (1.1)	1.7 (0.9)	1.4 (0.8)	1.1 (0.8)	1.9 (0.9)	1.4 (0.9)	1.3 (0.9)	1.2 (0.8)	P<0.05
CVC_{arm} (AU/mmHg)*	0.5 (0.4)	1.4 (0.5)	0.9 (0.7)	0.8 (0.6)	0.8 (0.6)	0.4 (0.3)	1.3 (0.5)	0.7 (0.6)	0.6 (0.5)	0.6 (0.4)	P>0.05
Control											
Core temperature (°C)	37.0 (0.3)	37.0 (0.3)	37.0 (0.2)	36.9 (0.2)	36.9 (0.3)	37.0 (0.2)	37.0 (0.2)	37.0 (0.3)	36.9 (0.3)	36.9 (0.3)	P>0.05
CVC_{chest} (AU/mmHg)*	1.6 (1.0)	2.7 (1.2)	1.9 (1.2)	1.8 (1.1)	1.3 (0.4)	1.3 (0.8)	2.6 (0.7)	1.7 (0.9)	1.6 (1.0)	1.3 (0.9)	P>0.05
CVC_{arm} (AU/mmHg)*	0.7 (0.4)	1.4 (0.3)	0.9 (0.5)	0.8 (0.4)	0.9 (0.5)	0.8 (0.2)	1.5 (0.4)	0.8 (0.3)	0.7 (0.9)	0.7 (0.3)	P>0.05

Data are presented as mean (SD). *Significant main effect of time (P<0.05). #significant interaction between time and HF segment (P<0.05). NB. Hot flushes were statistically analysed over 8 segments, but are represented over 3 time segments (0, 50, 100%) above.

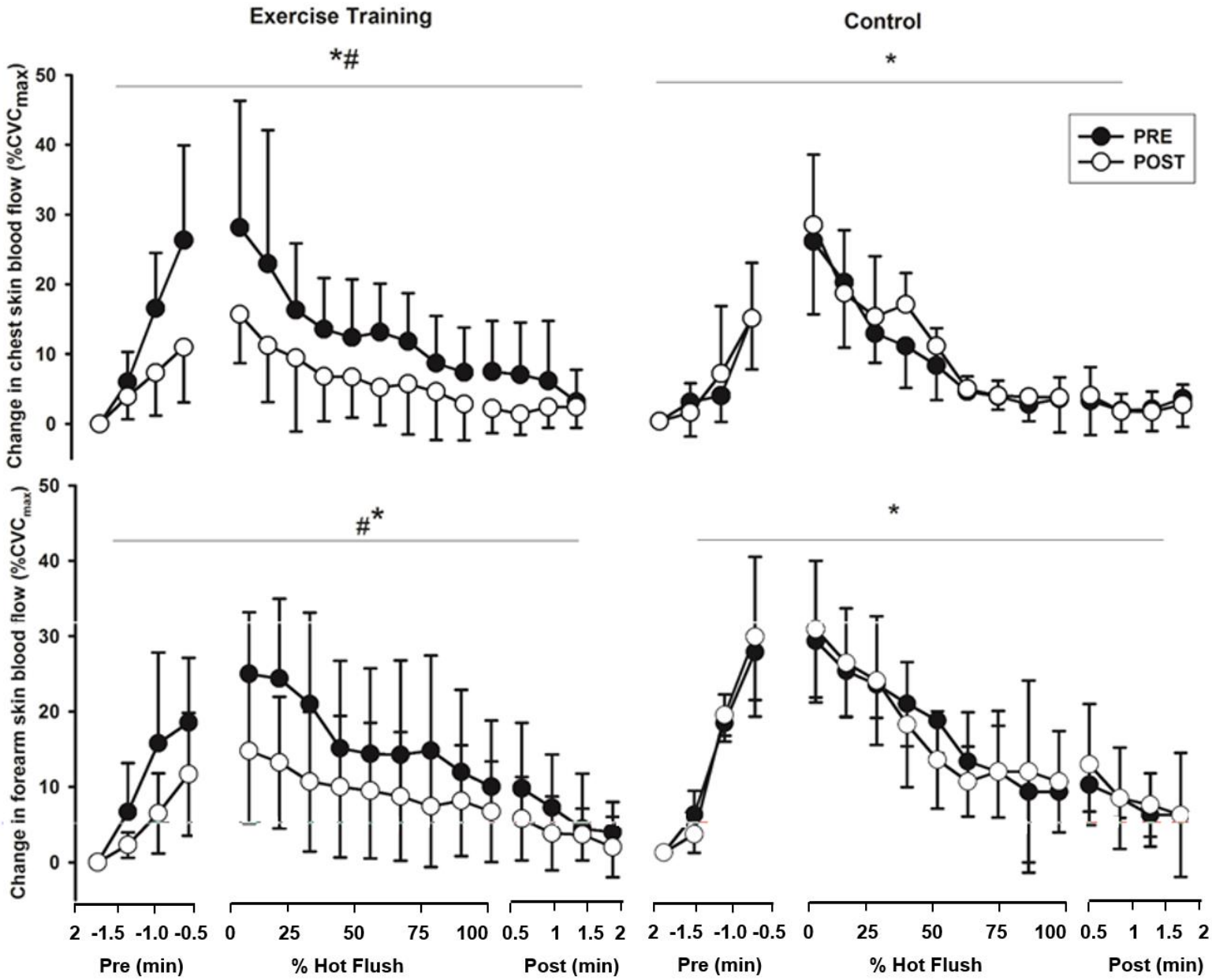

Figure 1 Changes in chest and forearm skin blood flow during hot flushes before and after exercise training and control.
Error bars are SD. *significant change in skin blood flow during a hot flush #significant interaction between intervention and time (P<0.05).

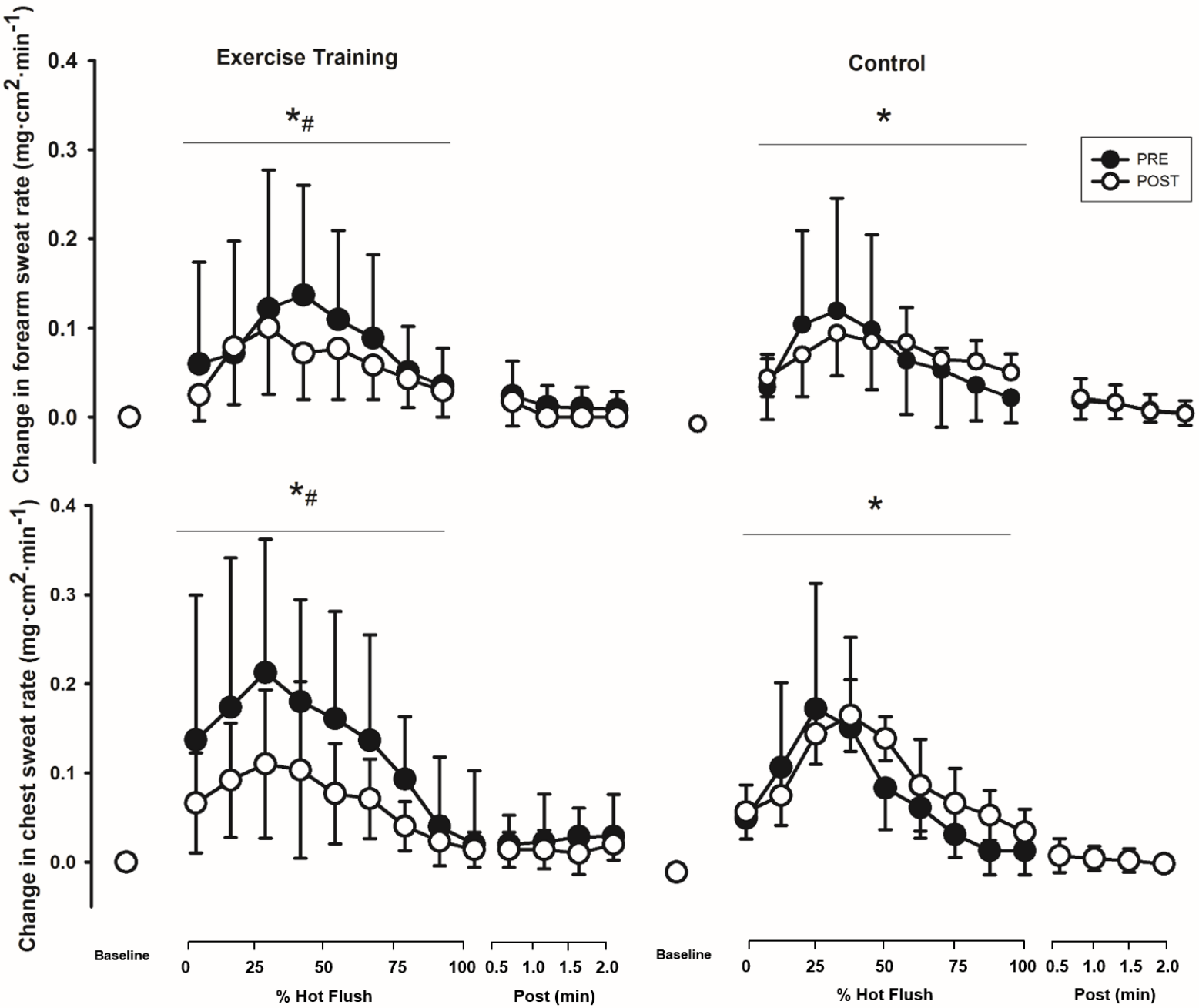

Figure 2 Changes in sweat rate during hot flushes before and after exercise training and control.
Error bars are SD. *significant change in sweat rate during a hot flush #significant interaction between intervention and time (P<0.05).

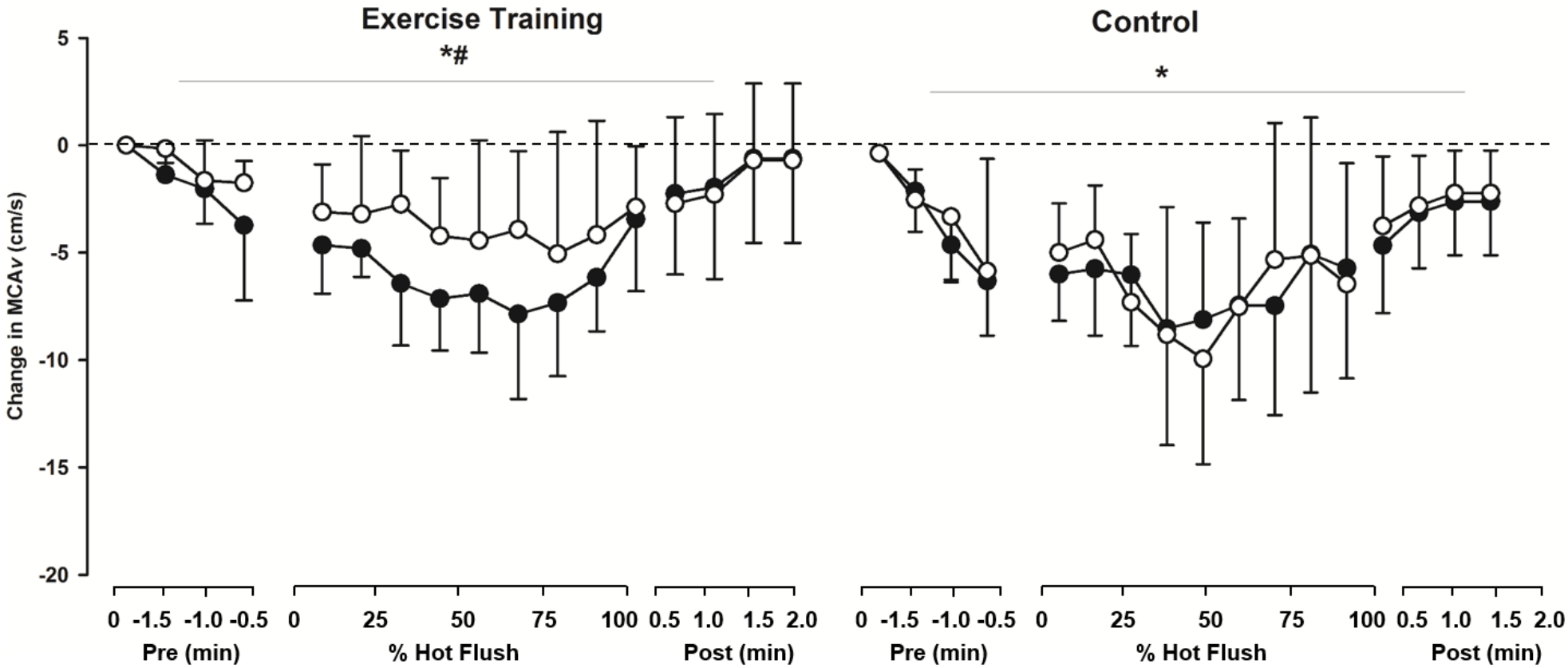

Figure 3 Changes in cerebral blood flow during hot flushes before and after exercise training and control. Error bars are SD.
MCA*v* = middle cerebral artery velocity. *significant change in MCAv during hot flush #significant interaction between intervention and time (*P*<0.05).