Jones, H, Bailey, TG, Cable, NT, Sprung, VS, Low, DA and Miller, GD

REPEATED WARM WATER IMMERSION INDUCES SIMILAR CEREBROVASCULAR ADAPTATIONS TO 8-WEEKS OF MODERATE-INTENSITY EXERCISE TRAINING IN FEMALES

REPEATED WARM WATER IMMERSION INDUCES SIMILAR CEREBROVASCULAR ADAPTATIONS TO 8-WEEKS OF MODERATE-INTENSITY EXERCISE TRAINING IN FEMALES

SHORT TITLE: Exercise vs. warm water immersion training

FIGURES: 2 **TABLES:** 3

Key Words: cerebral blood flow, thermoregulation, exercise training, warm water immersion, vascular function

ABSTRACT

Exercise training has potential to positively impact cerebrovascular function in healthy and diseased individuals. Passive heat training using warm water immersion has recently been shown to enhance systemic vascular function including the cerebrovascular response to heating. We suggest that a passive heating intervention can be a useful adjunct or alternative to exercise training. Our aim was to directly compare the effects of exercise with warm water immersion training on cerebrovascular and thermoregulatory function. 18 females (25±5y) performed 8-weeks of moderate-intensity cycling (70% HR_{max}) or warm-water immersion (42°C) for 30 min three times per week. Brachial artery flow-mediated dilation (FMD) and cardiorespiratory fitness were measured prior to and following both interventions. A passive heat stress was employed to obtain temperature thresholds (T_b) and sensitivities for chest and forearm sweat rate (SR) and cutaneous vasodilation (CVC). Middle cerebral artery velocity (MCAv) was measured at rest and throughout heat stress. FMD (P=0.003) and VO_{2peak} (P<0.001) improved following both interventions. MCAv and cerebrovascular conductance were higher at rest (P<0.001 and 0.05, respectively) and during passive heating (P<0.001 and <0.001, respectively) following both interventions. Chest and forearm SR occurred at a lower T_b post-intervention with no difference between interventions. Chest and forearm SR sensitivity were increased after both interventions with no differences between interventions at the forearm but a larger increase at the chest (P<0.001) following water immersion compared to exercise training. Chest and forearm CVC occurred at a lower T_b (P<0.001) following both interventions with no differences between interventions or over time. Warm water immersion training elicits favourable and similar cerebrovascular, conduit- and thermoregulatory adaptations compared to a period of moderate-intensity exercise training over 8-weeks.

Abbreviations: BP, blood pressure; CBF, cerebral blood flow; CBVC, cerebral vascular conductance; %CVC_{max}, percentage of maximal cutaneous conductance; FMD, flow-mediated dilation; HR, heart rate; HRmax, maximal heart rate; LSD, least significant difference; MAP, mean arterial pressure; MCA*v*, middle cerebral artery velocity; NO, nitric oxide; PETCO2, partial pressure of end tidal carbon dioxide; RPE, rating of perceived exertion; SkBF, skin blood flow; SV, stroke volume; T_b, mean body temperature; T_c, core body temperature; Tskin, skin temperature; $VO2_{peak}$, cardiorespiratory fitness.

INTRODUCTION

The preservation of cerebral blood flow (CBF) is critical for maintaining appropriate neural and cognitive function [58]. Precipitous acute reductions in cerebral perfusion can have significant consequences, e.g., syncope [55]. Chronic reductions in CBF, such as that observed with aging, are also detrimental as they have been linked with an increased risk of cerebrovascular disease and dementia [48]. Exercise training and physical fitness have been shown to offset the age related decline in CBF, e.g., in lifelong endurance trained individuals [1]. The effect of exercise training interventions on resting CBF and cerebrovascular function are conflicting with improvements observed in young and older healthy individuals [32], as well as those with known cerebrovascular disease, e.g., stroke patients [27], but no changes following ~3 months of endurance training in young tennis players [54] or obese males [42]. The mechanisms by which exercise training could alter CBF and cerebrovascular function have been proposed to include favourable alterations in cerebral volume, CO_2 reactivity, angiogenesis, cerebral vessel compliance, neuronal mass, dendritic density, neuroplasticity, neurovascular coupling, as well as reduced blood pressure, arterial stiffness, inflammation and improved metabolic control [31]. Nevertheless, the benefits of exercise training on CBF may only be evident during physiological stressors, such as during acute exercise, as observed during low-to-moderate intensity exercise [8]. Whether these benefits are also observed during other acute stressors, e.g., during passive heating, is currently unknown.

Recently, it has been demonstrated that repeated sauna bathing is associated with a decrease in fatal cardiovascular and all-cause mortality [29]. A number of studies have examined the impact of repeated heat exposure using immersion in warm water [10,22,33] and the combination of

exercise and heat exposure [17] in an attempt to maximise the cardio- and cerebrovascular responses to exercise training or to establish a suitable alternative in those that are not able to exercise, but are at increased risk of vascular dysfunction, e.g., spinal cord injured or chronic heart failure. Conduit and cutaneous blood flow increases acutely during exercise [15,52] and passive heating [16], with consequent chronic improvements in shear stress mediated endothelial function due to repeated exposure of elevations in conduit and cutaneous blood flow [9,22,52,53]. The potential therapeutic benefits of repeated passive heating on cerebrovascular function are less clear, with the response of CBF during acute passive heating inverse to that of exercise. In contrast to elevations during acute bouts of moderate exercise [36], CBF is significantly reduced during passive heat stress [7,30], but is acutely elevated during whole-body immersion in thermo-neutral water [11]. A 6-day period of daily exercise training in the heat attenuates cerebral hypoperfusion during acute passive heat stress in young healthy tennis players [17] whereas 16-weeks of endurance training in the same group did not alter resting CBF [54]. It is therefore currently unclear if an improved CBF at rest, and potentially in response to passive heat stress in healthy individuals is exercise or temperature-mediated. No research study to date has directly compared passive heating with an exercise training intervention matched for frequency and duration of exposures to ascertain the independent effects of each on CBF at rest, and in response to heat stress. Such information would provide important insight into the impact of passive heating interventions as an adjunct or alternative intervention for treatment of individuals with heat related illnesses (e.g., heat-induced syncope), thermoregulatory dysfunction (e.g. symptomatic postmenopausal women, heart failure patients or spinal cord injured individuals) or cerebrovascular dysfunction (e.g. stroke or vascular cognitive dementia). Therefore, the aim of this study was to examine the impact of a passive heat training intervention

using warm water immersion compared to 8 weeks of moderate-intensity exercise training on CBF using a randomised control trial. We hypothesised that a warm water immersion intervention would elicit similar cerebrovascular adaptations at rest and in response to a passive heat stress to that of exercise training in young healthy individuals.

METHODS

Participants

Eighteen healthy females (aged, 25±5 y; BMI, 24.2±4.1 $kg.m^2$) were recruited. Participants were recreationally active and typically engaged in low (e.g., walking) and moderate (e.g., running, stationary cycling) intensity aerobic activities (2–3 days/week) assessed using a self-report questionnaire. Participants reported regular menstrual cycles (~28 days), had no history of cardiovascular or metabolic disease, were non-smokers and not taking any form of medication, including hormonal contraceptives. Participants were informed of the methods and study design verbally and in writing before providing written informed consent. The study conformed to the Declaration of Helsinki and was approved by the institutional ethics committee. Females were exclusively studied because this research formed part of a larger project investigating the cardiovascular and thermoregulatory benefits of warm water immersion or exercise across the female lifespan.

Research Design

Participants underwent two initial visits to the laboratory, following an overnight fast and refraining from alcohol and exercise for 24h and caffeine for 12h before each visit. Visit 1 consisted of assessment of brachial artery endothelial function (FMD) followed by a maximal

cardiorespiratory fitness test (VO_{2peak}). Visit 2 consisted of a passive heat stress challenge to assess thermoregulatory, haemodynamic and cerebrovascular responses. Both visits were completed within 7 days and assessments were conducted in a temperature-controlled laboratory (24 ± 1°C). Participants were then randomly assigned to either an exercise [*n*=9; mid-follicular 6; mid-luteal 3)] or warm water immersion (*n*=9; mid follicular 5; mid-luteal 4) intervention. All assessments described in visits 1 and 2 were repeated in same menstrual phase following the 8-week training program.

Exercise training intervention

Participants exercised on a cycle ergometer (Ergo Bike Premium8i, Daum Electronic; Germany) at ~70% of maximal heart rate (HR_{max}) [average exercise heart rate (HR) during training 135±12 beat·min^{-1}], which was achieved by manipulating power output (watts) until the target HR was attained. Each session was supervised and lasted for 30 min, was conducted 3 times per week (94% compliance) for 8-weeks in a temperature-controlled laboratory (21°C and relative humidity (RH) of 45%). Pilot data from our laboratory illustrate these exercise bouts typically increase core temperature by 0.6-0.8ºC. The intensity of the exercise was based upon previous research in females that effectively improved thermoregulatory function following 8 weeks of cycling exercise [25].

Water Immersion training

Participants were supervised and immersed (seated) in a water tank (ECB, Gloucester, UK) to top-sternal level (arms were not immersed) at a constant temperature (42°C) for 30 min, 3 times per week (91% compliance) for 8-weeks. Laboratory temperature was controlled (21°C and 45%

RH). Pilot data from our laboratory illustrate that these water immersion sessions increase core temperature by 0.6-0.8ºC, which is similar to a recent study [10]. The duration for both interventions was chosen to match the duration of warm water immersion which has been used previously [9,10,33].

Measurements

Maximal oxygen consumption test: The fitness test (VO_{2peak}) was performed on a treadmill (H/P Cosmos, Germany) to quantify peak aerobic capacity. After a 2 min warm up, treadmill speed began at 6 kmh^{-1} for continuous 2-min stages, speed then increased by 2 kmh^{-1} per 2 min to a maximal running speed of 16 kmh^{-1} or until volitional exhaustion. Breath-by-breath expired gases were continuously monitored (Oxycon IV; Viasys, Jaeger, Germany) for oxygen consumption ($mL \cdot kg^{-1} \cdot min^{-1}$) and were averaged over 15 sec. Heart rate was measured continuously using short range telemetry (RS800, Polar, Finland) alongside subjective effort (RPE) using the 6-20 Borg scale. All participants reached the criteria for volitional exhaustion based upon heart rate, borg scale and respiratory exchange ratio.

Brachial artery endothelial-dependent vasodilation: Brachial artery endothelium-dependent function was measured using the flow-mediated dilation (FMD) technique (Thijssen, Black et al. 2011). Measurements were performed in the supine position following 20 min of rest, on the right arm with the cuff placed distal to the olecranon process. A 15-MHz multi-frequency linear array probe, attached to a high-resolution ultrasound machine (T3000; Terason, Burlington, MA) was then used to image the brachial artery in the distal third of the upper arm. Images were optimised and settings were identical between FMD assessments. Following a 1 min recording

period of diameter and flow, the cuff was inflated (>200 mmHg) for 5 min (D.E. Hokanson, Bellevue, WA). Diameter and flow recordings resumed 30 sec prior to cuff deflation and continued for 3 min thereafter, in accordance with recent technical specifications [50]. Analysis 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 [5]. 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% [51].

Passive heat stress challenge: Upon arrival to the laboratory, 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 both forearms. Participants then rested quietly in a semi-recumbent position while water (34°C) was perfused through the suit for a 15 min resting period for the measurement of baseline data. 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 heat stress periods.

Heart rate was obtained from a 3 lead electrocardiogram (Powerlab, AD Instruments, Oxford, UK), alongside continuous beat-by-beat arterial blood pressure (BP) from a digit (Finapres, 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, intermittent arterial BP was also measured by brachial auscultation using an autosphygmomanometer

(Dinamap, Germany). 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 [38]. 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 subject's pre and post trials. Mean body temperature (T_b) was calculated as the weighted product of core and mean skin temperatures [47].

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[57], 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. 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/or 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/body temperature during heating. All data during the heat stress challenge were sampled at 50Hz with a data acquisition system (PowerLab, ADInstruments, Oxford UK).

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$_{max}$). 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 ºC every 5 sec to a temperature of 42 ºC. This resulted in an increase in skin temperature to ~42 Cº at the heating probe-skin surface interface. The protocol was finished after flux at both sites had reached a stable plateau (~30 min).

The mean body and core temperature thresholds for the onset of sweating (Tb) and cutaneous vasodilation (T_c) [6,59] were calculated in a blinded fashion by the same analyst [13]. The sensitivity of the sweating responses were estimated from the slope of the relationships between sweat rate per unit change in body temperature beyond the body temperature threshold, whilst any plateau was excluded from the slope calculation [59]. Skin blood flow sensitivity was estimated in the same way, instead using the rate of CVC per unit change in core temperature [28,59].

Statistical analysis

A two (intervention*time) factor linear mixed model was employed to analyse FMD, VO_{2peak}, resting baseline variables and temperature thresholds and sensitivities of sweat rate and skin blood flow during the heat stress challenge. A three-way (intervention*time*temperature) linear mixed model was employed for the analysis of cerebral blood flow in response to each 0.1°C increase in T_c, during the heat stress. Statistically significant interactions were followed up with the least significant difference (LSD) approach to multiple comparisons. Due to variable increments in T_c during the passive heat stresses, data up to an increase of 0.6°C in core temperature was used for cerebral blood flow analysis (n=18). Analysis was conducted using Statistical Package for Social Sciences (Version 17; SPSS Inc., Chicago, IL). Statistical significance was delimited at P<0.05 and exact P values are cited (P values of "0.000" provided by the statistics package are reported as "<0.001"). Data are presented in the text as mean (95% confidence interval) unless otherwise stated.

Results

Brachial artery endothelial function: FMD increased by 1.71 % (0.56, 2.19) following both interventions (P=0.003; Figure. 1). There was no main effect of intervention (P=0.11) or interaction between intervention and time (P=0.18).

Cardiorespiratory Fitness (VO_{2peak}): Fitness improved by 2.26 $ml \cdot kg^{-1} \cdot min^{-1}$ (1.27, 3.25) following both interventions (P<0.001; Figure 1). There was no main effect of intervention (P=0.78) or interaction between intervention and time (P=0.43). When expressed in absolute

units, fitness still improved by 0.12 $L \cdot min^{-1}$ (0.04, 0.20) following both interventions (P=0.007) with no main effect of intervention (P=0.79) or interaction between intervention and time (P=0.92). There was no main effect of time for body mass (P=0.13) or for intervention (P=0.87). There was a significant interaction between intervention and time (P=0.014) with a reduction evident in the exercise group (1.67 kg, 0.52, 2.83; P=0.007) but no change in the warm water group (0.46 kg (-0.70, 1.61; P=0.42).

Resting baseline measurements

Cerebral Blood Flow: Resting MCAv was 2.30 cm/s (1.20, 3.34) higher following the interventions (P<0.001; Table 1), with no main effect of intervention (P=0.55) or intervention*time interaction (P=0.83). Cerebral vascular conductance was also 0.03 $cm \cdot s^{-1} \cdot mmHg^{-1}$ (-0.01 to 0.05) higher following the interventions (P=0.05; Figure 2). There was no main effect of intervention (P=0.73) or intervention*time interaction (P=0.89). There was no main effect of intervention (P=0.12), time (P=0.17) or intervention*time interaction (P=0.43) for $PETCO_2$ at rest.

Haemodymanics: Heart rate [2 $beats \cdot min^{-1}$ (-1, 4)] and mean arterial pressure [3 mm Hg (0.2, 5)] were lower following the interventions, although this did not reach statistical significance (P=0.07; Table 2). There was no main effect of intervention or intervention*time interaction for heart rate and mean arterial pressure. Stroke volume was 3.8 ml (0.6, 4.9) higher following the interventions (P=0.02), with no main effect of intervention (P=0.24) or interaction between intervention and time (P=0.17). Cardiac output was 0.6 $L \cdot min^{-1}$ (-0.1, 1.1) higher following the

interventions (*P*=0.07), with no main effect of intervention (*P*=0.26) or interaction between intervention and time (*P*=0.51; Table 2).

Thermoregulatory: T_c was 0.14 ºC (0.04, 0.23) lower following the interventions (*P*=0.004), with no main effect of intervention (*P*=0.74) or intervention*time interaction (*P*=0.70). $\bar{T}_{sk}$ was not different between interventions or over time (*P*>0.05; Table 2). T_b was not different over time (P=0.15) or between interventions (P=0.40) with no intervention*time interaction (*P*=0.93). There was no main effect of intervention or time for resting skin blood flow at the chest, skin blood flow at the arm was 2.2% CVC_{max} (0.83, 3.4) higher following the interventions (*P*=0.04) with no main effect of intervention (*P*=0.22), or intervention*time interaction (*P*=0.17).

Responses during the heat stress challenge.

Cerebral blood flow: Cerebral blood flow decreased during heat stress (*P*<0.001, Table 2). There was a significant time*temperature interaction in MCAv (*P*=0.01), where the reduction in MCAv during heat stress was decreased following the interventions. MCAv was on average 6.05 cm/s (1.39, 10.71, *P*<0.001) higher post- compared with pre-intervention. There was no main effect of intervention (P=0.37). CBVC was significantly higher post-intervention during heat stress compared to pre-intervention (*P*=0.001, Figure 2) by on average 0.07 $cm \cdot s^{-1} \cdot mmHg^{-1}$ (0.03, 0.11). There was no main effect of intervention (P=0.63).

Haemodynamics: Changes in heart rate, mean arterial pressure, stroke volume and cardiac output were not different between interventions or over time, whilst there was no interaction between intervention and time (*P*>0.05).

Sweat rate: The onset of chest sweating occurred at a 0.10ºC (-0.14, 0.33, *P*<0.001; Table 3) lower T_b following the exercise and water interventions. Similarly, the onset of forearm sweating occurred at a 0.19ºC (0.12, 0.23, *P*<0.001; Table 3) lower T_b following the interventions. There was no main effect of intervention at either site (chest, *P*=0.49; forearm, *P*=0.43). There was no intervention*time interaction at the chest (*P*=0.18) or forearm (*P*=0.73). When expressed as the increase in T_b from pre-heat stress baseline the onset of chest [-0.12 ºC, (-0.04, -0.21), P=0.006] and forearm chest [-0.13 ºC (-0.01, -0.24) P=0.037] sweating occurred at smaller elevations in T_b after both interventions with no differences between interventions (both P>0.05 for chest and forearm).

The rate of sweating at the chest and the forearm, per 1ºC increase in T_b increased following both interventions (P<0.001). At the chest, a significant main effect for intervention was observed (*P*=0.03), alongside a significant intervention*time interaction (P=0.015), where the intervention mediated change in the rate of sweating per 1ºC increase in T_b was 1.18 $mg \cdot cm^2 \cdot min^{-1}$ (0.68, 1.67; *P*<0.001) compared to 0.38 $mg \cdot cm^2 \cdot min^{-1}$ (0.13, 0.68) higher following the water immersion and exercise training interventions, respectively (Table. 3). Furthermore, the rate of sweating per 1ºC increase in T_b was 0.53 $mg \cdot cm^2 \cdot min^{-1}$ (0.34, 0.74) higher at the forearm following both interventions (*P*<0.001; Table 3). There was no main effect of intervention (*P*=0.48) or intervention*time interaction (*P*=0.39) at the forearm.

Cutaneous blood flow: The onset of chest cutaneous vasodilation occurred at 0.20ºC (0.14, 0.26, *P*<0.001; Table 3) lower T_c following the interventions. Similarly, the onset of forearm

cutaneous vasodilation occurred at 0.19ºC (0.12, 0.25, $P<0.001$; Table 3) lower T_c following the interventions. There was no main effect of intervention at the chest ($P=0.58$) or forearm ($P=0.42$), or intervention*time interaction (chest, $P=0.64$; forearm, $P=0.25$). When expressed as the increase in T_c from pre-heat stress baseline the onset of chest [-0.12 ºC (-0.04, -0.21) P=0.006] and forearm [-0.08 ºC (-0.03, -0.13) P=0.003] cutaneous vasodilation occurred at smaller elevations in T_c after both interventions with no differences between interventions (both P>0.05 for chest and forearm). The change in CVC per 1^{o}C change in T_c was not significantly different between interventions (chest, $P=0.92$; forearm, $P=0.71$), over time (chest, $P=0.74$: forearm, $P=0.84$) with no interaction (chest, $P=0.93$; forearm, $P=0.87$).

Discussion

The primary finding of this study is that passive heat training using warm water immersion elicits similar cerebrovascular improvements to that of a moderate-intensity exercise training intervention. Warm water training also improved conduit and thermoregulatory function to a similar extent to moderate-intensity exercise training of the same duration and frequency. To our knowledge, this is the first study to directly compare an exercise-independent passive heating 'training stimulus' against a moderate-intensity aerobic exercise training intervention. These findings provide direct evidence for warm-water immersion training as a useful alternative for improving cerebral, conduit and thermoregulatory function in young healthy individuals.

This is the first study to demonstrate that repeated passive heating training alone can enhance resting CBF and attenuate heat-induced cerebral hypoperfusion in young healthy individuals. Previous studies have demonstrated that 12-weeks of exercise training can have a small, but

significant impact on resting CBF in young and older previously sedentary individuals [32]. More recently, 16-weeks of exercise training alone and 6-days of exercise training in the heat in moderately trainied individuals did not significalty alter resting CBF [17,54]. Nevertheless, in the current study we found improved CBF at rest when the frequency and duration of the exercise and passive heat interventions were matched. The differences in findings in the present and these previous studies could be a result of various factors, including, different populations, methodologies, sampling procedures and/or different physiological adaptations (see below). The benefits of exercise training/passive heating on CBF may only be evident during physiological stressors, such as observed during low-to-moderate intensity exercise [8]. Similarly, Fujii *et al.*, (2015) previously demonstrated that exercise training in the heat causes an attenutation in cerebral hypoperfusion during passive heat stress. Our findings suggest that both exercise training and passive heat training can independently, and to a similar extent, attenuate the cerebral hypoperfusion typically observed during heat stress. Taken together, these findings suggest resting CBF and cerebrovascular responses to passive heating can be improved with exercise training or with passive heat training using warm water immersion.

The mechanisms by which exercise training could alter CBF and cerebrovascular function have been proposed to include favourable alterations in cerebral volume, CO_2 reactivity, angiogenesis, cerebral vessel compliance, neuronal mass, dendritic density, neuroplasticity, neurovascular coupling, as well as reduced blood pressure, arterial stiffness, inflammation and improved metabolic control [31]. The cerebrovascular respsones in the current study were not explained by differences in $PETCO_2$ (hyperventilatory hypocapnia) and persisted when normalised for changes in blood pressure. One mechanistic explanation for the improved CBF could be related

to improvements in cerebrovascular endothelial function following both interventions via shear stress related mechanisms. In support of this notion we (i) observed an increase in endothelial function in the cutaneous (increased resting cutaneous blood flow), and conduit vessels measured using FMD, both of which are partly mediated by nitric oxide (NO) dilator function [23]; (ii) previous warm water immersion and exercise interventions suggest endothelial function of conduit and cutaneous vessels is improved via repeated increases in episodic shear stress [9,22] on the artery wall which up-regulates endothelial nitric oxide synthase (eNOS) phosphorylation [23] and (iii) cerebrovascular function is partly, NO dependent [37] and is linked to FMD [3]. Nevertheless, during a bout of exercise (up until very high exercise intensities) CBF increases [32], and thus exercise causes increases in cerebrovascular shear stress. Whereas during acute exposure to passive heating a gradual reduction in CBF occurs with increases in core temperature [34] and CBF is elevated during acute thermo-neural water immersion [11]. With these competing responses to water immersion (increased) and passive heating (decreased), it is currently unknown how CBF responds to acute warm water immersion. Despite the potential differences in the responses of CBF during acute exercise and warm water immersion, we observed a greater maintenance of CBF (attenuated hypoperfusion) during the passive heat stress challenge following both interventions, suggesting improvements in CBF control that may not be solely explained by changes in vascular function and associated shear stress mechanisms.

An alternative explanation may relate to chronic increases in stroke volume that may have been driven by plasma volume expansion following the interventions [40]. In respect of chronic adaptations, stroke volume is increased following endurance exercise training [49] and along with venous return has been strongly linked to central blood volume [15]. It is well established

that endurance training [39] and potentially, repeated heating [35] results in blood volume expansion over a number of weeks. Therefore, whilst we did not measure blood volume in the current study, it is possible that both the interventions increased central blood volume, which may have contributed to an attenuated decrease in CBF during heat stress post-intervention. That said, although acute blood volume expansion during heat stress enables a better maintenance of blood pressure and attenuates reductions in cerebral perfusion during simulated haemorrhage it does not increase CBF during heat stress alone [41]. Similarly, greater increases in plasma volume improvements following exercise relative to passive heating interventions have previously been reported and attributed to exercise-induced non-thermal factors such as increased vasopressin, plasma osmotic and resting plasma protein content [14].

Exercise training is a well-established intervention to improve cardio-respiratory fitness ($\dot{V}O_{2peak}$). Whilst this was not the primary aim of the study, it is interesting, and somewhat surprising, that we also observed that cardio-respiratory fitness (absolute and relative $VO_{2peak)}$ was also improved following the warm water immersion intervention. There are a number of potential reasons for this observation. Central physiological improvements to stroke volume, cardiac output and potentially, blood volume represent several physiological mechanisms that influence maximal exercise performance [39]. Analogous to exercise training, repeated elevations in cardiac output and stroke volume during warm water immersion [12] may induce similar favourable cardiac and haemodynamic adaptations and directly influence blood flow during maximal exercise. Additionally, peripheral improvements in conduit vascular function found in this study may also contribute to improvements in maximal aerobic capacity via enhancements in muscle blood flow and oxygen delivery. Interestingly, body mass did not

decrease after warm water immersion, unlike after exercise training, suggesting different mechanisms for increasing cardio-respiratory fitness relative to the moderate-intensity exercise training used in the present study.

Another novel aspect of the current study was that we also assessed central and peripheral thermoregulatory control responses to passive heat stress before and after both interventions, to gain insight into the integrative changes at a systemic level. We found (i) improved central thermoregulatory function during the heat stress challenge evidenced via reduced temperature thresholds for sweating and cutaneous vasodilation, attributed to absolute decreases in resting core and mean body temperatures and elevations in core and mean body temperature prior to the onset of cutaneous vasodilation and sweating; (ii) enhancements in sweat rate sensitivity at the chest and the forearm, with no changes in the sensitivity of cutaneous vasodilation, suggesting peripheral sweat gland adaptations [45]. These thermoregulatory responses have previously been shown with repeated passive heating [46] and exercise training [4,26]. We show for the first time that these thermoregulatory adjustments are of a similar magnitude following 8 weeks of moderate intensity exercise training or passive heating using warm water immersion. One difference between the interventions that was apparent was a greater enhancement in sweat rate sensitivity at the chest following the warm water immersion intervention. Although speculative, this could be explained by chest submersion thus causing a greater local heat stress (e.g., increased chest skin temperature) and reducing the surface area for evaporative heat loss, but it is also possible that warm water immersion is more favourable in improving thermoregulatory function *per se*.

We have shown that warm water immersion could have at least similar benefits on cerebrovascular, conduit and thermoregulatory function, and cardiorespiratory fitness. Whilst we acknowledge the findings of the current study are applicable to healthy young individuals who were free of disease, the findings that both interventions are equally beneficial may be useful and applicable to patients with chronic conditions (e.g. spinal cord injured), those that have a limited exercise capacity (e.g. heart failure) or have cerebrovascular disease (e.g. stroke). Despite a relatively small enhancement in MCAv at rest (2.30 cm/s higher following both interventions), these improvements may have clinical significance, and be magnified in populations whereby CBF is already compromised, such as following acute stroke [27] or with ageing [48].

A number of considerations related to measurements techniques and intervention design are noteworthy. First, the transcranial Doppler assesses MCAv only and does not take into account arterial diameter [43], however previous reports indicate that MCAv is a reliable index of CBF [2,37] particularly when small changes in PETCO2 (±5mmHg) does not appear to influence changes in cerebral arterial diameter [56]. Second, the method of stroke volume and cardiac output estimation employed used in the present study underestimates the changes in these variables during heat stress [44]. Despite this, the changes in stroke volume and cardiac output were similar pre- and post-interventions suggesting that differences in the acute alterations in stroke volume and cardiac output do not explain the better maintained CBF during heat stress post-intervention [34]. Third, the exercise training stimulus did not currently meet the recommended guidelines of 150 min per week [21]. By design, the current study aimed to match the frequency and duration of the interventions to assess the independent impact of both exercise and warm water immersion training. Any increase in exercise training (e.g. to meet

recommended guidelines) or warm water immersion frequency and durations would likely mediate greater changes in cerebrovascular and thermoregulatory function. Finally, only female participants were recruited to this study, and were assessed at the same time period of the menstrual cycle for pre and post intervention assessments. Whilst there are gender differences in vascular and thermoregulatory function [18-20,24], there is no current evidence to suggest that the absolute response to exercise training or passive heat therapy are different between young healthy males and females.

In summary, we show for the first time that warm water immersion training elicits similar cerebrovascular, conduit and thermoregulatory adaptations to a period of exercise training matched for frequency and duration. Our data may serve as an important indication that warm water immersion can be a useful, alternative intervention to enhance systemic vascular function and thermoregulatory control.

CONFLICT OF INTEREST: NONE.

FUNDING: NONE

References

1. *Ainslie PN, Cotter JD, George KP, Lucas S, Murrell C, Shave R, Thomas KN, Williams MJ, Atkinson G*. Elevation in cerebral blood flow velocity with aerobic fitness throughout healthy human ageing. J Physiol 2008; 586: 4005-4010
2. *Ainslie PN, Hoiland RL*. Transcranial Doppler ultrasound: Valid, invalid, or both? J Appl Physiol 2014; 117: 1081-1083
3. *Ainslie PN, Murrell C, Peebles K, Swart M, Skinner MA, Williams MJA, Taylor RD*. Early morning impairment in cerebral autoregulation and cerebrovascular CO2 reactivity in healthy humans: relation to endothelial function. Exp Physiol 2007; 92: 769-777
4. *Armstrong LE, Maresh CM, Keith NR, Elliott TA, VanHeest JL, Scheett TP, Stoppani J, Judelson DA, De Souza MJ*. Heat acclimation and physical training adaptations of young women using different contraceptive hormones. Am J Physiol Endoc M 2005; 288: E868-E875
5. *Black MA, Green DJ, Cable NT*. Exercise prevents age-related decline in nitric-oxide-mediated vasodilator function in cutaneous microvessels. J Physiol 2008; 586: 3511-3524
6. *Brengelmann GL, Savage MV, Avery DH*. Reproducibility of core temperature threshold for sweating onset in humans. J Appl Physiol (1985) 1994; 77: 1671-1677
7. *Brothers RM, Wingo JE, Hubing KA, Crandall CG*. The effects of reduced end-tidal carbon dioxide tension on cerebral blood flow during heat stress. J Physiol 2009; 587: 3921-3927
8. *Brugniaux JV, Marley CJ, Hodson DA, New KJ, Bailey DM*. Acute exercise stress reveals cerebrovascular benefits associated with moderate gains in cardiorespiratory fitness. J Cereb Blood Flow Metab 2014; 34: 1873-1876
9. *Carter HH, Spence AL, Atkinson CL, Pugh CJ, Cable NT, Thijssen DH, Naylor LH, Green DJ*. Distinct effects of blood flow and temperature on cutaneous microvascular adaptation. Med Sci Sports Exerc 2014; 46: 2113-2121
10. *Carter HH, Spence AL, Atkinson CL, Pugh CJ, Naylor LH, Green DJ*. Repeated core temperature elevation induces conduit artery adaptation in humans. Eur J Appl Physiol 2014; 114: 859-865
11. *Carter HH, Spence AL, Pugh CJ, Ainslie P, Naylor LH, Green DJ*. Cardiovascular responses to water immersion in humans: impact on cerebral perfusion. Am J Physiol Regul Integr Comp Physiol 2014; 306: R636-640
12. *Carter HH, Spence AL, Pugh CJ, Ainslie PN, Naylor LH, Green DJ*. Cardiovascular responses to water immersion in humans: Impact on cerebral perfusion. Am J Physiol Regul Integr Comp Physiol 2014, DOI:
13. *Cheuvront SN, Bearden SE, Kenefick RW, Ely BR, DeGroot DW, Sawka MN, Montain SJ*. A simple and valid method to determine thermoregulatory sweating threshold and sensitivity. J Appl Physiol 2009; 107: 69-75
14. *Convertino VA, Greenleaf JE, Bernauer EM*. Role of thermal and exercise factors in the mechanism of hypervolemia. J Appl Physiol Respir Environ Exerc Physiol 1980; 48: 657-664
15. *Crandall CG, González-Alonso J*. Cardiovascular function in the heat-stressed human. Acta Physiologica 2010; 199: 407-423
16. *Crandall CG, Wilson TE*. Human cardiovascular responses to passive heat stress. Compr Physiol 2015; 5: 17-43

17. *Fujii N, Tsuji B, Honda Y, Kondo N, Nishiyasu T*. Effect of short-term exercise-heat acclimation on ventilatory and cerebral blood flow responses to passive heating at rest in humans. J Appl Physiol 2015; 119: 435-444
18. *Gagnon D*. Sex-related differences in local and whole-body heat loss responses: Physical or physiological? Appl Physiol Nutr Metab 2014; 39: 843
19. *Gagnon D, Kenny GP*. Sex modulates whole-body sudomotor thermosensitivity during exercise. J Physiol 2011; 589: 6205-6217
20. *Gagnon D, Kenny GP*. Sex differences in thermoeffector responses during exercise at fixed requirements for heat loss. J Appl Physiol 2012; 113: 746-757
21. *Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee IM, Nieman DC, Swain DP*. American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. Med Sci Sports Exerc 2011; 43: 1334-1359
22. *Green DJ, Carter HH, Fitzsimons MG, Cable NT, Thijssen DH, Naylor LH*. Obligatory role of hyperaemia and shear stress in microvascular adaptation to repeated heating in humans. J Physiol 2010; 588: 1571-1577
23. *Green DJ, Dawson EA, Groenewoud HM, Jones H, Thijssen DH*. Is flow-mediated dilation nitric oxide mediated?: a meta-analysis. Hypertension 2014; 63: 376-382
24. *Ichinose-Kuwahara T, Inoue Y, Iseki Y, Hara S, Ogura Y, Kondo N*. Sex differences in the effects of physical training on sweat gland responses during a graded exercise. Exp Physiol 2010; 95: 1026-1032
25. *Ichinose TK, Inoue Y, Hirata M, Shamsuddin AKM, Kondo N*. Enhanced heat loss responses induced by short-term endurance training in exercising women. Exp Physiol 2009; 94: 90-102
26. *Ichinose TK, Inoue Y, Hirata M, Shamsuddin AKM, Kondo N*. Enhanced heat loss responses induced by short-term endurance training in exercising women. Experimental Physiology 2009; 94: 90-102
27. *Ivey FM, Ryan AS, Hafer-Macko CE, Macko RF*. Improved cerebral vasomotor reactivity after exercise training in hemiparetic stroke survivors. Stroke 2011; 42: 1994-2000
28. *Kuwahara T, Inoue Y, Abe M, Sato Y, Kondo N*. Effects of menstrual cycle and physical training on heat loss responses during dynamic exercise at moderate intensity in a temperate environment. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 2005; 288: R1347-R1353
29. *Laukkanen T, Khan H, Zaccardi F, Laukkanen JA*. Association between sauna bathing and fatal cardiovascular and all-cause mortality events. JAMA Intern Med 2015; 175: 542-548
30. *Low DA, Wingo JE, Keller DM, Davis SL, Zhang R, Crandall CG*. Cerebrovascular responsiveness to steady-state changes in end-tidal CO2 during passive heat stress. J Appl Physiol 2008; 104: 976-981
31. *Lucas SJ, Cotter JD, Brassard P, Bailey DM*. High-intensity interval exercise and cerebrovascular health: curiosity, cause, and consequence. J Cereb Blood Flow Metab 2015; 35: 902-911
32. *Murrell C, Cotter J, Thomas K, Lucas SE, Williams MA, Ainslie P*. Cerebral blood flow and cerebrovascular reactivity at rest and during sub-maximal exercise: Effect of age and 12-week exercise training. AGE 2013; 35: 905-920

33. *Naylor LH, Carter H, FitzSimons MG, Cable NT, Thijssen DH, Green DJ*. Repeated increases in blood flow, independent of exercise, enhance conduit artery vasodilator function in humans. Am J Physiol Heart Circ Physiol 2011; 300: H664-669
34. *Nelson MD, Haykowsky MJ, Stickland MK, Altamirano-Diaz LA, Willie CK, Smith KJ, Petersen SR, Ainslie PN*. Reductions in cerebral blood flow during passive heat stress in humans: partitioning the mechanisms. J Physiol 2011; 589: 4053-4064
35. *Nielsen B, Hales JR, Strange S, Christensen NJ, Warberg J, Saltin B*. Human circulatory and thermoregulatory adaptations with heat acclimation and exercise in a hot, dry environment. J Physiol 1993; 460: 467-485
36. *Ogoh S, Ainslie PN*. Cerebral blood flow during exercise: mechanisms of regulation. J Appl Physiol 2009; 107: 1370-1380
37. *Peebles K, Celi L, McGrattan K, Murrell C, Thomas K, Ainslie PN*. Human cerebrovascular and ventilatory CO2 reactivity to end-tidal, arterial and internal jugular vein PCO2. J Physiol 2007; 584: 347-357
38. *Ramanathan NL*. A new weighting system for mean surface temperature of the human body. J Appl Physiol 1964; 19: 531-533
39. *Sawka MN, Convertino VA, Eichner ER, Schnieder SM, Young AJ*. Blood volume: importance and adaptations to exercise training, environmental stresses, and trauma/sickness. Med Sci Sports Exerc 2000; 32: 332-348
40. *Schlader ZJ, Seifert T, Wilson TE, Bundgaard-Nielsen M, Secher NH, Crandall CG*. Acute volume expansion attenuates hyperthermia-induced reductions in cerebral perfusion during simulated hemorrhage. J Appl Physiol 2013; 114: 1730-1735
41. *Schlader ZJ, Seifert T, Wilson TE, Bundgaard-Nielsen M, Secher NH, Crandall CG*. Acute volume expansion attenuates hyperthermia-induced reductions in cerebral perfusion during simulated hemorrhage. J Appl Physiol (1985) 2013; 114: 1730-1735
42. *Seifert T, Rasmussen P, Brassard P, Homann PH, Wissenberg M, Nordby P, Stallknecht B, Secher NH, Nielsen HB*. Cerebral oxygenation and metabolism during exercise following three months of endurance training in healthy overweight males. Am J Physiol Regul Integr Comp Physiol 2009; 297: R867-876
43. *Serrador JM, Picot PA, Rutt BK, Shoemaker JK, Bondar RL*. MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis. Stroke 2000; 31: 1672-1678
44. *Shibasaki M, Wilson TE, Bundgaard-Nielsen M, Seifert T, Secher NH, Crandall CG*. Modelflow underestimates cardiac output in heat-stressed individuals. Am J Physiol Regul Integr Comp Physiol 2011; 300: R486-491
45. *Shibasaki M, Wilson TE, Crandall CG*. Neural control and mechanisms of eccrine sweating during heat stress and exercise. J Appl Physiol (1985) 2006; 100: 1692-1701
46. *Shido O, Sugimoto N, Tanabe M, Sakurada S*. Core temperature and sweating onset in humans acclimated to heat given at a fixed daily time. Am J Physiol 1999; 276: R1095-1101
47. *Stolwijk JA, Hardy JD*. Partitional calorimetric studies of responses of man to thermal transients. J Appl Physiol 1966; 21: 967-977
48. *Stoquart-ElSankari S, Baledent O, Gondry-Jouet C, Makki M, Godefroy O, Meyer ME*. Aging effects on cerebral blood and cerebrospinal fluid flows. J Cereb Blood Flow Metab 2007; 27: 1563-1572

49. *Stratton JR, Levy WC, Cerqueira MD, Schwartz RS, Abrass IB*. Cardiovascular responses to exercise. Effects of aging and exercise training in healthy men. Circulation 1994; 89: 1648-1655
50. *Thijssen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, Parker B, Widlansky ME, Tschakovsky ME, Green DJ*. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. Am J Physiol Heart Circ Physiol 2011; 300: H2-12
51. *Thijssen DH, Dawson EA, Black MA, Hopman MT, Cable NT, Green DJ*. Brachial artery blood flow responses to different modalities of lower limb exercise. Med Sci Sports Exerc 2009; 41: 1072-1079
52. *Tinken TM, Thijssen DH, Hopkins N, Black MA, Dawson EA, Minson CT, Newcomer SC, Laughlin MH, Cable NT, Green DJ*. Impact of shear rate modulation on vascular function in humans. Hypertension 2009; 54: 278-285
53. *Tinken TM, Thijssen DH, Hopkins N, Dawson EA, Cable NT, Green DJ*. Shear stress mediates endothelial adaptations to exercise training in humans. Hypertension 2010; 55: 312-318
54. *Tomoto T, Sugawara J, Nogami Y, Aonuma K, Maeda S*. The influence of central arterial compliance on cerebrovascular hemodynamics: insights from endurance training intervention. J Appl Physiol 2015; 119: 445-451
55. *Van Lieshout JJ, Wieling W, Karemaker JM, Secher NH*. Syncope, cerebral perfusion, and oxygenation. J Appl Physiol 2003; 94: 833-848
56. *Verbree J, Bronzwaer A-SGT, Ghariq E, Versluis MJ, Daemen MJAP, van Buchem MA, Dahan A, van Lieshout JJ, van Osch MJP*. Assessment of middle cerebral artery diameter during hypocapnia and hypercapnia in humans using ultra-high-field MRI. J Appl Physiol 2014; 117: 1084-1089
57. *Willie CK, Colino FL, Bailey DM, Tzeng YC, Binsted G, Jones LW, Haykowsky MJ, Bellapart J, Ogoh S, Smith KJ, Smirl JD, Day TA, Lucas SJ, Eller LK, Ainslie PN*. Utility of transcranial Doppler ultrasound for the integrative assessment of cerebrovascular function. J Neurosci Methods 2011; 196: 221-237
58. *Willie CK, Tzeng YC, Fisher JA, Ainslie PN*. Integrative regulation of human brain blood flow. J Physiol 2014; 592: 841-859
59. *Wingo JE, Low DA, Keller DM, Brothers RM, Shibasaki M, Crandall CG*. Skin blood flow and local temperature independently modify sweat rate during passive heat stress in humans. J Appl Physiol 2010; 109: 1301-1306

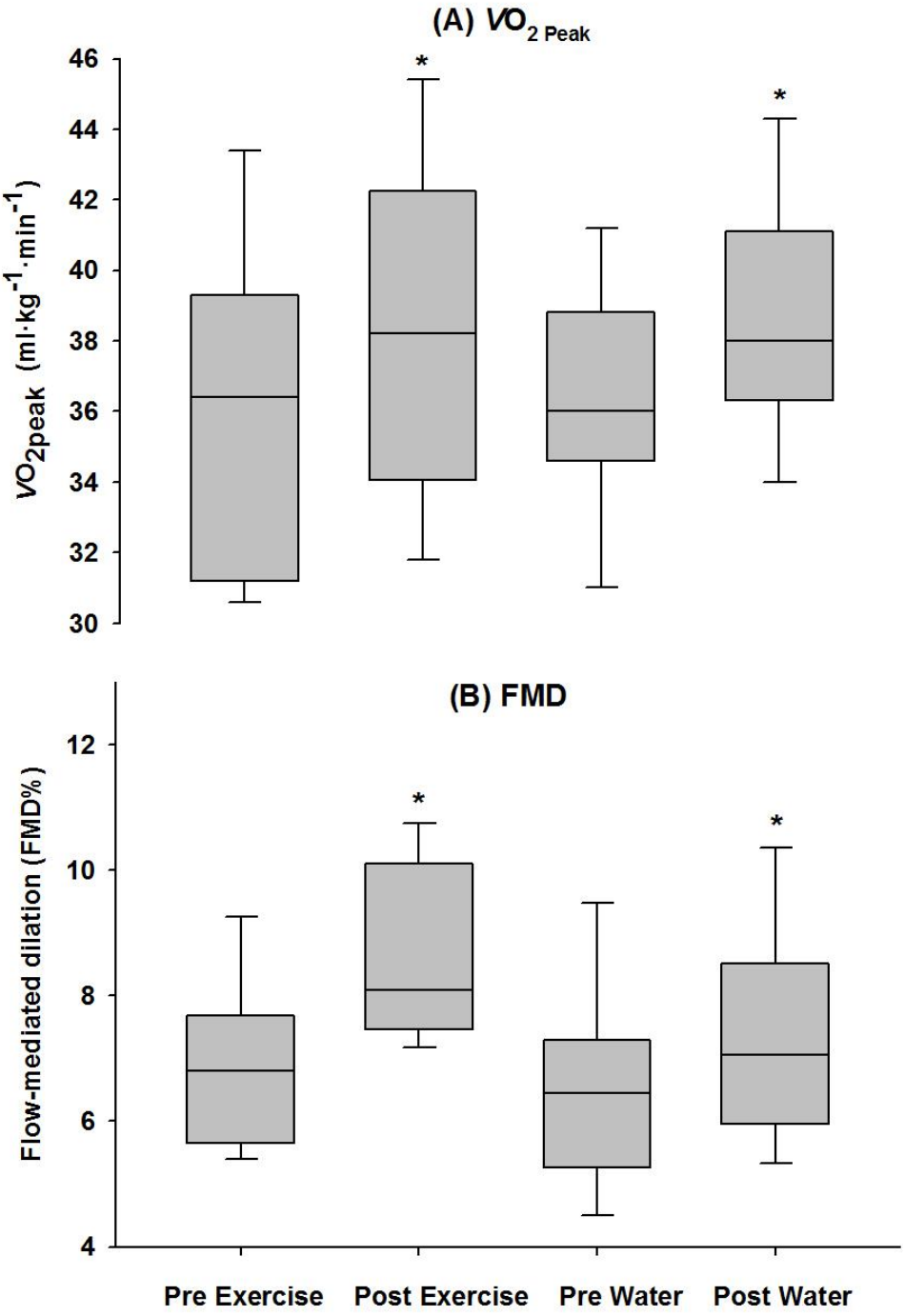

Figure 1. Cardiorespiratory fitness (A; VO_{2peak}) and Flow mediated dilation (B; FMD) before and after exercise and water training interventions. Error bars are 95%CI. *significant difference between pre and post interventions (P<0.05).

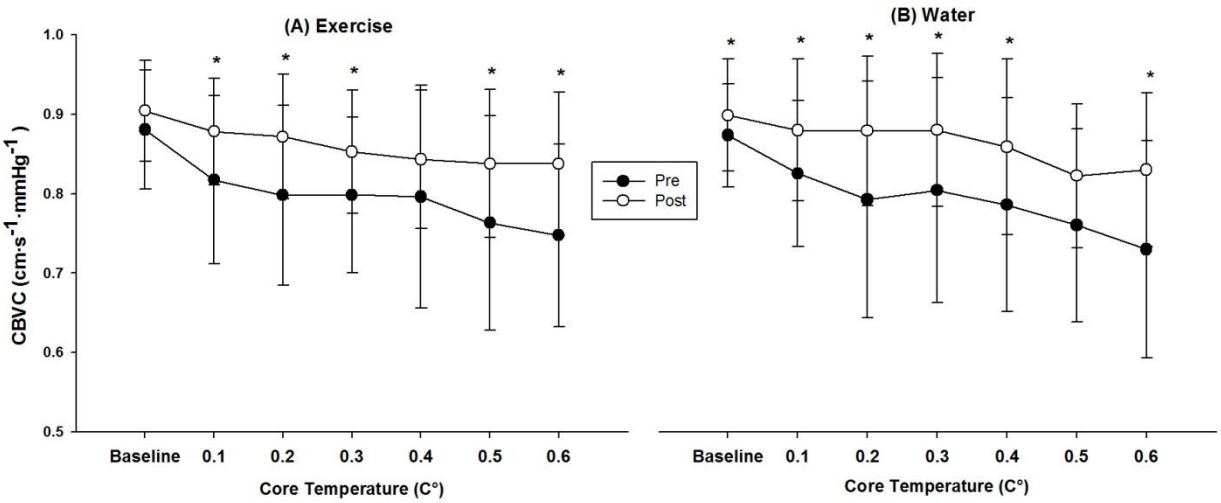

Figure 2. Cerebrovascular conductance (CBVC) during passive heating pre (black circles) and post (clear circles) exercise (A) or water immersion (B) training. Error bars are SD. *significant difference between pre and post interventions (P<0.05)

Table 1. Resting baseline data (mean±SD) before and after exercise (*n*=9) or warm water immersion (*n*=9) training (8-weeks).

Variable	Exercise (n=9)		Water Immersion (n=9)		*P value*		
	Pre	Post	Pre	Post	*Intervention*	*Time*	*Intervention*time*
Heart Rate, beats·min^{-1}	56±8	54±8	58±7	56±4	0.14	0.07	0.55
MAP, mmHg	75±6	73±4	76±5	73±5	0.99	0.07	0.84
Stroke Volume, ml	87±9	93±14	89±7	94±11	0.24	0.02*	0.17
Cardiac Output, l·min^{-1}	6.1±0.9	6.3±1	5.4±0.6	6.1±1.2	0.26	0.07	0.51
Core Temperature, ^{o}C	36.97±0.20	36.88±0.24	37.06±0.25	36.91±0.28	0.74	0.004*	0.70
Skin Temperature, ^{o}C	32.06±1.57	32.13±1.03	32.58±1.52	32.82±1.58	0.14	0.31	0.29
Mean Body Temperature, ^{o}C	36.48±0.9	36.40±0.9	36.57±0.9	36.51±0.9	0.15	0.40	0.93
MCA V_{mean}, cm/s	67±4	69±5	68±5	70±6	0.55	0.000*	0.83
CBVC, cm·s^{-1}·mmHg^{-1}	0.88±0.07	0.90±0.06	0.87±0.06	0.89±0.07	0.73	0.05*	0.89
End Tidal CO_2 (Torr)	42±1	43±3	42±2	43±2	0.12	0.17	0.43
CVC_{chest} %CVC_{max}	9.6±4.6	11.7±9.0	11.4±6.3	9.8±4.2	0.82	0.88	0.50
CVC_{arm} %CVC_{max}	5.6±2.5	7.5±2.7	5.8±2.9	8.9±3.2	0.68	0.003*	0.17

Table 2. Cerebrovascular responses to passive heat stress (increase in Tc of 0.6ºC) before and after 8 weeks of exercise or water immersion training.

Variable	Exercise Training													
				Pre							*Post*			
Tc (ºC)	**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**
MCAv	66±5	61±6	58±7	57±7	56±6	54±9	52±8	69±6	66±7	65±7	63±8	62±9	61±7	60±9
PETCO2	42±3	42±2	41±3	41±3	40±2	40±3	39±4	42±4	43±2	41±2	41±3	40±2	40±1	39±5
	Warm Water Immersion													
				Pre							*Post*			
Tc (ºC)	**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**
MCAv	67±6	63±7	60±9	58±8	56±9	55±8	54±7	69±6	67±6	66±7	66±8	65±8	63±4	62±7
PETCO2	42±2	42±3	41±2	40±4	40±2	40±4	39±3	43±2	42±1	42±3	41±2	40±3	41±1	39±3

Table 3. Thermoregulatory function during passive heat stress pre and post 8-weeks of exercise or warm water immersion training intervention

Variable	Exercise (n=9)		Water Immersion (n=9)		*P value*		
	Pre	Post	Pre	Post	*Intervention*	*Time*	*Intervention*time*
Sweat rate							
Chest T_b threshold (°C)	37.33±0.25	37.16±0.26	37.26±0.22	37.05±0.26	0.49	<0.001	0.18
Arm T_b threshold (°C)	37.36±0.25	37.25±0.29	37.34±0.17	37.12±0.23	0.99	<0.001	0.73
Chest slope, mg·cm^2·min^{-1}/ °C	0.5±0.3	0.8±0.5	0.6±0.4	1.8±1.0	0.03	0.001	0.015
Arm slope, mg·cm^2·min^{-1}/ °C	0.5±0.2	0.9±0.5	0.6±0.4	1.2±0.8	0.48	0.001	0.39
Skin Blood Flow							
Chest T_c threshold (C°)	37.21±0.24	37.02±0.30	37.16±0.21	36.95±0.25	0.58	<0.001	0.64
Arm T_c threshold (C°)	37.23±0.23	37.06±0.32	37.17±0.20	36.95±0.25	0.42	<0.001	0.25
Chest slope, CVC·°C	2.6±1.0	2.8±1.2	2.7±1.2	2.8±1.5	0.92	0.74	0.93
Arm slope, CVC·°C	2.4±0.7	2.5±0.9	2.2±1.2	2.3±1.3	0.71	0.84	0.87