The effects of exercise training in the cold on cerebral blood flow and cerebrovascular function in young heathy individuals

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Abstract

Exercise elicits acute increases in cerebral blood flow velocity (CBFv) and provokes long-term beneficial effects on CBFv, thereby reducing cerebrovascular risk. Acute exposure to a cold stimulus also increases CBFv. We compared the impact of exercise training in cold and thermoneutral environments on CFBv, cerebrovascular function and peripheral endothelial function.

Twenty-one (16 males, 22±5 years) individuals were randomly allocated to either a cold (5 ºC) or thermoneutral (15 ºC) exercise intervention. Exercise consisted of 50-minutes cycling at 70% heart rate max, three times per week for eight weeks. Transcranial Doppler was used to determine pre and post intervention CBFv, dynamic cerebral autoregulation (dCA) and cerebrovascular reactivity (CVR_{CO2}). Conduit endothelial function, microvascular function and cardiorespiratory fitness were also assessed.

Cardiorespiratory fitness improved (2.91 $ml.min.kg^{-1}$, 95%CI 0.49, 5.3; P=0.02), regardless of exercise setting. Neither intervention had an impact on CBFv, CVR_{CO2}, FMD or microvascular function (P>0.05). There was a significant interaction between time and condition for dCA normalised gain with evidence of a decrease by 0.192 $\%cm.s^{-1}.\%mmHg^{-1}$ (95%CI -0.318, -0.065) following training in the cold and increase (0.129 $\%cm.s^{-1}.\%mmHg^{-1}$, 95%CI 0.011, 0.248) following training in the thermoneutral environment (P=0.001). This was also evident for dCA phase with evidence of an increase by 0.072 radians (95%CI -0.007, 0.152) following training in the cold and decrease by 0.065 (95%CI -0.144, 0.014) radians following training in the thermoneutral environment (P=0.02).

Both training interventions improved fitness but CBFv, CVR_{CO2} and peripheral endothelial function were unaltered. Exercise training in the cold improved dCA whereas thermoneutral negated dCA.

Introduction

Cerebral blood flow (CBF) declines with age (Stoquart-ElSankari et al., 2007) and reductions in CBF are associated with clinical conditions including stroke (Markus et al., 2004), cognitive impairment (Benedictus et al., 2017) and Alzheimer's disease (Kisler et al., 2017). Higher cardiorespiratory fitness is associated with an increased CBF velocity (v) across a broad age range (Ainslie et al., 2008, Bailey et al., 2013, Brown et al., 2010), thus exercise may be a useful non-pharmacological intervention for offsetting age-related CBFv reductions or improving cerebrovascular health. Research studies examining the impact of exercise interventions on CBFv and cerebrovascular health have however, yielded contrasting results. Moderate intensity exercise increased CBFv in post-menopausal women (Akazawa et al., 2012), but did not alter CBFv in healthy young or older individuals (Murrell et al., 2013b, Lewis et al., 2019a). High intensity interval training mediated a small detriment in dynamic cerebral autoregulation (dCA) (Drapeau et al., 2019) whereas moderate intensity endurance exercise training improved cerebrovascular reactivity (CVR_{CO2}) in both healthy older and younger individuals (Murrell et al., 2013b) and also in stroke survivors (Ivey et al., 2011), but not in those with chronic obstructive pulmonary disease (Lewis et al., 2019a). Possible explanations for contrasting findings may relate to the exercise stimulus, age/disease status and the cerebrovascular assessment. One way to enhance the exercise training stimulus is to add an environmental stressor (e.g. cold), the combination of exercise and cold may improve the training related cerebrovascular outcomes in all ages without increasing the exercise workload.

During an acute bout of sub-maximal continuous exercise, CBFv increases linearly with exercise intensity from rest up to ~60-70% of maximal oxygen uptake (Smith et al., 2014, Moraine et al., 1993), although the magnitude of effect appears greater in younger individuals

(Klein et al., 2019). Increases in CBFv mediate elevations in shear stress to the cerebral vessel walls (Smith et al., 2019), likely resulting in shear mediated intracellular signalling cascades similar to that observed in the peripheral vasculature and micro vessels of the skin (Barnes and Corkery, 2018, Carter et al., 2016, Hoiland et al., 2017) (Green et al., 2017). Increasing the exercise stimulus during an acute bout of exercise by adding an environmental cold stimulus may mediate changes in arterial diameter and/or larger blood flow and shear stress thus enhance vascular adaptation to chronic exercise training. Acute cold exposure mediates cardiovascular responses including sympathetically driven cutaneous vasoconstriction (Alba et al., 2019b) and blood pressure increases (Modesti, 2013). The cutaneous vasoconstrictor response represents a primary mechanism to limit heat loss and maintain core temperature at a cost of reduced peripheral tissue temperature (Castellani and Young, 2016). In the cerebrovasculature, unlike in the peripheral and microvasculature, acute exposure to a cold stimulus elicits a decrease in vascular resistance (Wilson and Metzler-Wilson, 2018) and an increase in CBFv (Brown et al., 2003, Doering et al., 1996) and the addition of face cooling during exercise amplifies the exercise induced increase in CBFv (Kjeld et al., 2009). Performing a bout of exercise in a cold environment may cause a greater increase of CBFv relative to a normothermic environment and may translate into enhanced functional improvements in the cerebrovasculature.

Our primary aim was to assess the impact of 8 weeks of exercise training in a cold environment on CBFv and cerebrovascular function compared to exercise training in a thermoneutral environment in young healthy individuals. Our secondary aim was to examine the acute effects of a single exercise bout in the different environmental conditions on CBFv. Given the differences in acute exercise changes in cerebral compared to peripheral vessels with cold we also employed assessment of peripheral conduit and microvascular function to provide insight

into systemic vascular health. It was hypothesised that performing exercise in a cold environment would elicit an enhanced CBFv response and would improve measures of cerebrovascular function (dCA and (CVR_{CO2}) to a greater degree than performing exercise in a thermoneutral environment.

Methods

***Participants*:** Twenty-one (16 males & 5 females) physically active individuals who engaged in low to moderate intensity exercise 2-3 times per week were recruited. Participants were normotensive (BP <140/90 mm Hg), non-smokers with no history of cardiovascular disease. Female participants were initially assessed during the early follicular phase of their menstrual cycle. Two female participants recruited were currently taking hormonal contraception; these participants were also tested during the 7-day gap from hormonal contraception (Shenouda et al 2018). All participants were informed of the methods verbally and in writing before providing written informed consent prior to any assessments being performed. The study conformed to the Declaration of Helsinki and was approved by the local university ethics committee (15/SPS/033).

***Research Design*:** Participants attended a temperature-controlled laboratory (19-21^{o}C) for two experimental visits no more than 5 days apart having fasted overnight (12 hours), abstained from exercise for 24 hours and caffeine for 12 hours. Experimental visit 1 consisted of an assessment of CBF, cerebrovascular function, brachial artery endothelial function and cutaneous microvascular function. Experimental visit 2 consisted of an incremental treadmill test to assess maximal cardiorespiratory fitness ($\dot{V}O_{2peak}$). Participants were then randomly assigned (computer generated sequence) to either 8-weeks of exercise training in a cold (5^{o}C,

n=10) or a thermoneutral (15ºC, n=11) environment (Figure 1). Visits 1 and 2 were then repeated in the same order and at the same time of day following the intervention (Ainslie *et al*, 2007; Jones et al., 2010) (Figure 2a; chronic experiment). In addition, participants attended a single visit at the midpoint of the intervention to examine cerebral, conduit and cutaneous response during an acute bout of exercise in their randomised environment (acute experiment) (Figure 2b). Data collection took place during October to December to minimise the possible impact of seasonal variation in the measurements (Brennan et al., 1982, Widlansky et al., 2007).

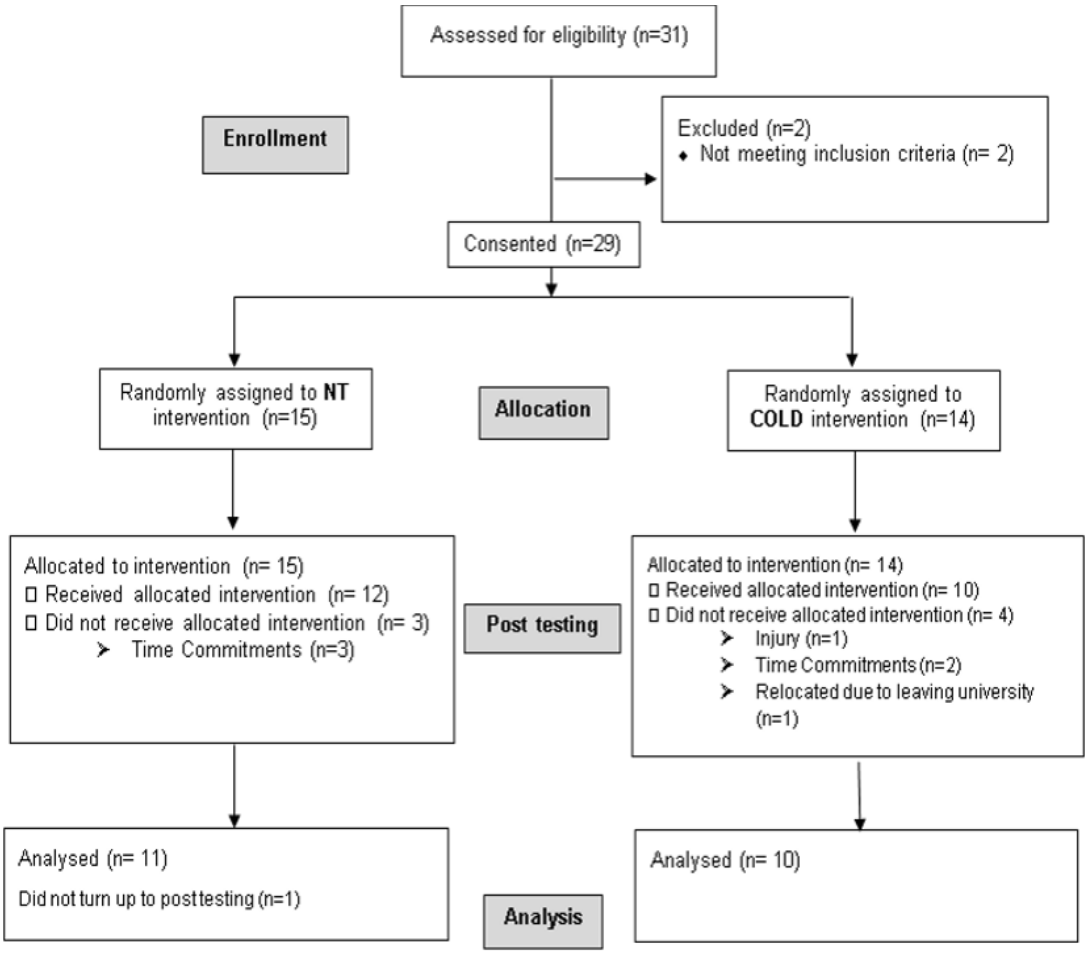

Figure 1. Flowchart of the study design. Abbreviations; NT; thermoneutral

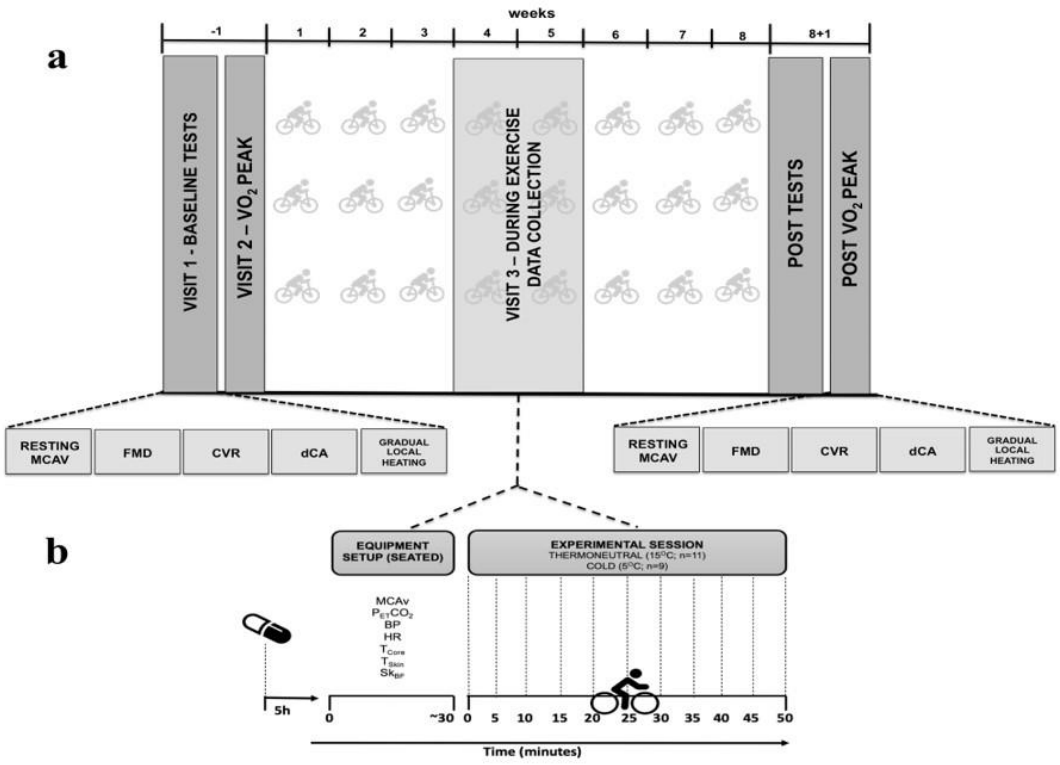

Figure 2. Schematic of experimental design (a) and experimental procedures of during exercise data collection (b). Abbreviations; MCAv; Middle cerebral artery velocity, FMD; flow mediated dilatation, CVR; cerebrovascular CO2 reactivity, dCA; dynamic cerebral autoregulation, $P_{ET}CO_2$; Partial pressure end tidal carbon dioxide, BP; Blood Pressure, HR; Heart rate, T_c; Core temperature, T_{sk}; skin temperature & SkBF; skin blood flow.

Measurements

Chronic experiment (n=21): the effects of moderate intensity cycling exercise on cerebral, conduit and micro-vascular function in a cold vs neutral environment.

Following completion of experimental visits 1 and 2, participants cycled (Wattbike Pro/ Trainer, Wattbike, UK) at ~70% of maximum heart rate (HR_{max}), which was calculated from the cardiorespiratory fitness test, for 50 minutes, 3 times per week for 8 weeks. All exercise sessions were supervised by a member of the research team and took place within an environmental chamber (Sporting Edge & TISS, UK) that controlled for the temperature of the

exercise sessions (thermoneutral; 15ºC and cold; 5ºC). Humidity was standardised across interventions at 20% relative humidity. Heart rate (FT1, Polar, Finland), rate of perceived exertion (Borg Scale, UK) and a measure of thermal comfort were recorded every 5 minutes throughout each exercise session.

Cardiorespiratory fitness: An incremental maximal exercise test was performed on a treadmill (Pulsar 4.0, HP Cosmos, Germany). After a 5-minute warm-up (self-paced between 6-8 $km.h^{-1}$), participants started the test at treadmill speed $8km.h^{-1}$ and 1% gradient. Every 3 minutes the treadmill speed increased by $2km.h^{-1}$ until $16km.h^{-1}$. Thereafter the treadmill speed remained the same and the incline gradient increased by 2% until volitional exhaustion. Breath by breath expired gases were measured (Oxycon Pro, Jaeger, Germany) for oxygen consumption ($ml.kg.min^{-1}$) and data were averaged over 15 second blocks. Maximum oxygen consumption was calculated as the highest consecutive 15-second period of gas exchange data occurring in the final minute before volitional exhaustion. Heart rate (HR) was measured continuously using short range telemetry (FT1, Polar, Finland) alongside perceived exertion at each exercise stage (RPE; Borg Scale, 1986).

Cerebral blood flow velocity: Following 15-minutes of supine rest, bilateral middle cerebral artery velocity (MCAv) was continuously measured using transcranial Doppler ultrasound (TCD). A 2-MHz pulsed Doppler ultrasound probe (Spencer Technologies, Seattle, WA, USA) was adjusted through the temporal window until an optimal signal was obtained (Willie et al., 2011) and held in place by a Marc 600 head frame (Spencer Technologies, Seattle, WA, USA). Probe location and parameters (depth, gain and power) were recorded to ensure within-participant consistency of measurement site between visits. Participants were instrumented

with a two-way valve mouthpiece (Hans Rudolph) from which partial pressure of end tidal CO_2 ($P_{ET}CO_2$) was measured using a calibrated gas analyser (ML206 ADinstruments, Colorado Springs, USA). Beat-to-beat arterial blood pressure was measured continuously using finger photoplethysmography (Finapres, Amsterdam, Netherlands) and heart rate was obtained from 3-lead echocardiogram (Powerlab 8.0, AD Instruments, Oxford, UK). To verify continuous blood pressure (BP) readings from finger plethysmography, the average of at least two automated brachial blood pressure measurements were performed using an automated syphygmanometer (Dinamap V100, Germany). All data was sampled at 50Hz using an analog-to-digital converter (Powerlab, ADInstruments, Oxford, UK) interfaced with a computer and analysed using data acquisition software (Labchart version 8, ADInstruments, Oxford, UK). Resting data was averaged over as 5-minutes recording extract of MCAv, BP and $P_{ET}CO_2$ from LabChart.

Cerebrovascular reactivity: Cerebrovascular reactivity to CO_2 was assessed whilst lying in a supine position. Following resting measurements (MCAv, MAP & $P_{ET}CO_2$), participants were coached through a voluntary hyperventilation protocol (approximately 24 breaths per minute for approximately 1 minute) to reach a reduction in $P_{ET}CO_2$ of ~10mmHg from baseline (<20mmHg). Following this, participants were connected to a prefilled bag (Douglas Bag, Hans Rudolph, Oxford) and were instructed to return to their normal breathing rate whilst inhaling a 5% CO_2 (21%O_2, 5% CO_2, N_2 balance) gas mixture for 3 minutes to reach an equivalent increase in $P_{ET}CO_2$. Resting MCAv, $P_{ET}CO_2$ and MAP were calculated as the mean of the minute before the test commenced, and subsequently MCAv, $P_{ET}CO_2$ and MAP values were collected as 10 second averages throughout the 3-minute rebreathing period. Cerebrovascular CO_2 reactivity was calculated both in absolute and relative (%) terms as the

gain of the linear relationship between CBFv and $P_{ET}CO_2$ from baseline to the last 30 seconds of CO_2 inhalation (Battisti-Charbonney et al., 2011) using the equations:

Absolute CVR_{CO2}= ΔMCAv / ΔPETCO2

Relative CVR_{CO2}= % MCAv change from baseline / ΔPETCO2

Where Δ is the change from baseline to the 30 seconds of CO_2 inhalation. To correct for any changes in MAP during CO_2 inhalation, cerebrovascular conductance was calculated (CBFv/MAP) and the absolute and relative (%) gains for CVR_{CO2MAP} vs $P_{ET}CO_2$ were also calculated.

Cerebral Autoregulation: The dynamic relationship between BP and MCAv, referred to as dynamic cerebral autoregulation (dCA), was assessed using a squat-stand procedure to induce transient changes in BP. Participants replicated the experimenter whilst performing these manoeuvres in order to achieve consistent movements. Manoeuvres were performed at 0.10 Hz to create physiologically relevant changes in BP that present challenges to the autoregulatory system that are typically experienced in daily life (Simpson and Claassen, 2018). The BP-MCAv relationship during these manoeuvres were analysed in accordance with most recent guidelines (Classen et al., 2016) using Transfer Function Analysis (TFA). Data from 5-minute recording of squat-stand manoeuvres were extracted from LabChart beat-to-beat using ECG tracing. TFA was applied using MATLAB (2018a; MathWorks-Inc., Natick, MA) to calculate associated power (gain) and timing (phases) and linearity (coherence) at the point estimate of the driven frequency (0.10Hz) (Claassen et al., 2016). Normalised gain is also provided to reflect the relative changes in CBFv to a given change in (%/mmHg) (Claassen et al., 2016). The threshold used for the minimum value of coherence was based on pre-defined TFA settings

and calculated cut off values for coherence that were above the 95% confidence limits of the null hypothesis.

Brachial artery endothelial function: Brachial artery endothelial function was assessed using the flow mediated dilation (FMD) technique strictly following most recent guidelines (Thijssen et al., 2019). Briefly, images of the left brachial artery were acquired using high-resolution ultrasound (T3300; Terason, Burlington, MA). Diameter, flow and shear stress were measured for 1-minute prior to and 3-minutes following 5-minutes of forearm cuff inflation (D.E. Hokanson, Bellevue, WA) (Thijssen et al., 2019). All images were obtained by the same sonographer with a day-to-day coefficient of variation in FMD% of 11% and a coefficient of variation of 3% for baseline artery diameter which is deemed good-excellent based on previous analysis (van Mil et al., 2016). Analysis was performed by a researcher blinded to the group allocation (using a single blinded coding-randomised procedure) and using custom designed edge-detection and wall-tracking software, which is largely independent of investigator bias. Previous articles contain detailed descriptions of this analytical approach (Black et al., 2008a, Woodman et al., 2001). Reproducibility of diameter measurements using this semi-automated software is significantly better than manual methods, significantly reduces observer error, and possesses within-day coefficient of variation of 6.7% (Woodman et al., 2001). FMD analysis was allometrically scaled to control for baseline diameter (Atkinson and Batterham, 2013).

Cutaneous microvascular function: Cutaneous flux was assessed using laser Doppler flowmetry (LDF) on the non-dominant arm and thigh; one 7-laser array probe was secured into a heating disc and attached to the skin. Skin sites were inspected for any abrasions or skin

damage that may affect cutaneous blood flow responses and measurement sites were chosen, avoiding visible veins and hair follicles (Cracowski et al., 2006). The placement of these probes was recorded to ensure accuracy of placement for the repeat measurement. Following placement and resting measurements (15-minutes at 33^{o}C), the heating discs were gradually heated using an incremental protocol up to 44^{o}C (33^{o}C to 42^{o}C; 1^{o}C every 5-minutes, then 30-minutes at 42^{o}C and 20-minutes at 44^{o}C) (Black et al., 2008c, Roberts et al., 2017). Mean arterial pressure (MAP) and heart rate (HR) data was measured from brachial auscultation using an automated syphygmanometer (Dinamap V100, Germany) on the dominant arm every 5-minutes throughout. Cutaneous vascular conductance (CVC) was assessed by the ratio of laser Doppler flux (LDF)/ MAP. CVC was also calculated as a percentage of max (CVC%$_{max}$ = (CVC/CVC$_{max}$) x100).

Acute experiment (n=21): the effects of acute exercise on cerebral, conduit and cutaneous micro-vascular responses.

Participants underwent measurements of middle cerebral artery velocity (MCAv), end tidal volume of CO_2 ($P_{ET}CO_2$), blood pressure (BP), heart rate (HR), skin temperature (T_{sk}), core temperature (T_c) and skin blood flow (SkBF) and radial artery diameter and velocity during a single exercise session at the midpoint of the training programme. The assessments were performed in the environmental condition to which the individual had been allocated (n=10 cold and n=11 thermoneutral). Measurements were performed pre exercise and every 10-minutes during the 50-minute exercise bout (Figure 2b).

MCAv, $P_{ET}CO_2$ and HR were measured as described previously *(see measurements)*, and BP was monitored from the brachial auscultation using an automated syphygmanometer (Tango,

SunTech, England). Mean skin temperature (T_{sk}) was obtained from the weighted average of 4 regional temperatures measured using thermocouples (iButtons data logger, Maxim Integrated; San Jose, CA, US) secured to the upper thoracic, anterior forearm, anterior mid-thigh and calf (Ramanathan, 1964). The calculation of mean skin temperature (T_{sk}) was calculated using the weighting system for mean surface temperature (Hardy and Dubois, 1938) and the equation: $T_{sk} = (0.3*T_{chest}) + (0.3*T_{arm}) + (0.2*T_{thigh}) + (0.2*T_{calf})$ (Ramanathan, 1964). Core body temperature (T_c) was measured from an ingestible telemetry temperature pill taken ~5 h before data collection began (CoreTemp, HQInc; Palmetto, FL, US) (Goodman et al., 2009). Skin blood flow (SkBF) was assessed as described earlier (*see Cutaneous microvascular function*). CVC was calculated every 5-minutes during the 50-minute exercise and values were also expressed as percentage increases from the baseline [Percentage change (%) = (time point value - baseline value)/(baseline value)*100]. Radial artery diameter and velocity was recorded from the middle third of the forearm using a 15-MHz multi-frequency linear array probe, attached to a high-resolution ultrasound machine (T3300; Terason, Burlington, MA). Images were acquired in accordance with previous methodological guidelines, including ultrasound measurements being acquired by the same sonographer (Thomas et al., 2015) and were analysed as in the same way as brachial artery diameter (*see Measurements*).

Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS version 23.0, Chicago, IL). Between-condition participant characteristics were explored using independent samples t-tests. Fishers exact tests were used to compare the between group proportions of males and females between groups. To examine the changes with exercise training (i.e. not stratified by training environment) two-factor linear mixed models

(intervention*time) were employed to analyse resting haemodynamic variables, fitness, and brachial artery and cerebrovascular function. Three-way linear mixed models (intervention*time*temperature) were employed to analyse cutaneous microvascular function. Covariance structure for each model was determined using a chi-square distribution, with the selection determined by the most parsimonious structure that optimised model fit. To examine the changes during an acute exercise session two-way linear mixed models were employed with condition (cold vs thermoneutral) and time (rest, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 minutes) as fixed effects. Statistically significant interactions between were followed-up with the least significant difference (LSD) approach to multiple comparisons (Perneger, 1998). Distribution data are presented as mean±SD and outcomes of linear mixed models as mean (95% CI). Statistical significance was assumed at $P<0.05$.

Results

Anthropometrics and fitness: At baseline, the groups were similar in age, body mass, BMI, MAP and VO_{2peak} ($P>0.05$, Table 1).

Table 1. Descriptive characteristics of participants in the thermoneutral exercise and cold exercise-training group pre and post exercise intervention

Characteristic	Thermoneutral Group (n=11)		Cold Group (n=10)		P-value			
	Pre	Post	Pre	Post	T-test (on entry)	Time	Condition	Time*Condition
Females	3		2		0.64	-	-	-
Age (years)	22±5		22±5		0.84	-	-	-
Body mass (kg)	66.4±5.1	66.2±5.1	72.8±12.5	72.2±12.1	0.18	0.26	0.16	0.43
BMI (kg/m^2)	22.3±1.5	22.2±1.6	23.6±3.2	23.4±3.0	0.23	0.44	0.20	0.43
SBP (mmHg)	118±11	114±8	122±10	120±12	0.44	0.17	0.28	0.66
DBP (mmHg)	63±5	64±6	63±5	62±6	0.82	0.96	0.57	0.49
MAP (mmHg)	86±7	83±5	84±6	83±7	0.68	0.13	0.93	0.47
Resting HR ($beats.min^{-1}$)	62±8	57±6	57±9	56±6	0.24	0.03*	0.19	0.20
Absolute VO_2 ($l.min^{-1}$)	3.4±0.5	3.6±0.7	3.6±0.6	3.8±0.7	0.54	0.03*	0.36	0.96
Relative VO_2 ($ml^{-1}.min.kg^1$)	50.9±6.9	53.8±9.3	50.4±8.7	53.3±9.2	0.57	0.02*	0.90	0.99

Abbreviations: BMI; body mass index, SBP; systolic blood pressure, DBP; diastolic blood pressure, MAP; mean arterial pressure. *P<0.05.

Chronic experiment (n=21): the effects of moderate intensity cycling exercise on cerebral, conduit and cutaneous micro-vascular function in a cold vs neutral environment.

Adherence to the 24-session exercise intervention was 98 ± 3% in thermoneutral group and 99 ± 2% in the cold group (P=0.51). The efficacy of the training intervention is evident by a decreased resting HR by 4 beats.min^{-1} (-7, -1; P=0.03) and improved VO_{2peak} by 2.91 ml.min.kg^{-1} (0.49, 5.3; P=0.02) after 8 weeks of exercise training, changes that were similar in both groups (P>0.05, Table 1). The exercise interventions did not alter body mass, BMI, SBP, DBP or MAP (P>0.05, Table 1).

Cerebral blood flow and cerebrovascular reactivity: Resting MCAv, $P_{ET}CO_2$, MAP and absolute or relative gain of MCAv to CO_2 inhalation were not altered by exercise training in either environment with no main effects or condition*time interactions (P>0.05, Table 2). CVR_{CO2} was also studied in absolute and relative terms using CVR_{CO2MAP} to account the effects of MAP changes on CVR_{CO2} estimates. There were no effects of condition, time or condition*time interaction for absolute or relative gain of CVR_{CO2MAP} to CO_2 inhalation or MCA CBVC and MCA CBVR (P>0.05, Table 2). Furthermore, a linear mixed model was employed and the change in mean arterial pressure was added to the model as a covariate. This analysis did not affect the previously mentioned results.

Cerebral autoregulation: Normalised gain decreased by 0.192 %cm.s^{-1}.%mmHg^{-1} (95% CI =-0.318, -0.065; P=0.01) following exercise training in the cold and increased following exercise

training in the thermoneutral environment [0.129 %cm.s^{-1}.%mmHg^{-1} (0.011, 0.248; P=0.04; condition*time interaction; P=0.001]. Absolute gain decreased by 0.124 cm.s^{-1}.mmHg^{-1} [-0.217, -0.031; P=0.01] following exercise training in the cold and was unaffected (0.016 cm.s^{-1}.mmHg^{-1}; 95% CI = -0.072, 0.103; P=0.71; intervention*time interaction; P=0.03) following exercise training in the thermoneutral environment. Phase increased by 0.072 radians [-0.007, 0.152; P=0.07)] following exercise training in the cold and decreased by 0.065 radians following exercise training in the thermoneutral environment (-0.144, 0.014; P=0.10; intervention*time interaction; P=0.02, Table 2; Figure 3). There were no main effects or intervention*time interactions for coherence (P>0.05, Table 2).

Table 2. Cerebral blood flow, cerebrovascular reactivity and transfer function analysis outputs of dynamic cerebral autoregulation for participants in the thermoneutral exercise and cold exercise-training group pre and post exercise intervention.

Characteristic	**Thermoneutral Group (n=11)**		**Cold Group (n=10)**		**P-value**		
	Pre	**Post**	**Pre**	**Post**	**Time**	**Condition**	**Time*Condition**
Resting							
MCA CBVC ($cm.s^{-1}/mmHg^{-1}$)	0.74±0.11	0.76±0.10	0.80±0.13	0.79±0.10	0.95	0.22	0.68
MCA CBVR ($mmHg^{-1}/cm.s^{-1}$)	1.38±0.21	1.34±0.19	1.28±0.19	1.29±017	0.85	0.23	0.72
CO_2 Reactivity test							
Relative CVR_{CO2} (% $cm.s/mmHg^{-1}$)	4.4±2.1	4.1±2.1	4.0±2.4	3.5±3.0	0.55	0.56	0.82
Absolute CVR_{CO2} ($cm.s/mmHg^{-1}$)	2.8±1.2	2.6±1.6	2.6±1.8	2.5±2.1	0.79	0.82	0.94
Relative CVR_{CO2MAP} (%$cm.s/mmHg^{-1}$. $mmHg^{-1}$)	2.7±4.8	2.4±3.8 [n=10]	3.3±1.9	2.1±3.5 [n=8]	0.18	0.78	0.39
Absolute CVR_{CO2MAP} ($cm.s.mmHg^{-1}$. $mmHg^{-1}$)	1.0±0.6	0.8±0.3 [n=10]	0.9±0.2	0.9±0.2 [n=8]	0.41	0.79	0.50
Cerebral Autoregulation test							
Absolute Gain ($cm.s^{-1}/mmHg^{-1}$)	0.81±0.18	0.80±0.18	0.87±0.16	0.76±0.12	0.09	0.95	0.03*
Coherence	0.7±0.1	0.8±0.1	0.7±0.2	0.7±0.2	0.76	0.09	0.61
CBFv Power	214±104	182±95	201±132	176±131	0.08	0.61	0.28
ABP Power	322±193	250±88	346±149	280±128	0.03*	0.95	0.32

Abbreviations: MCA; Middle Cerebral Artery, CBVC; Cerebrovascular Conductance, CBVR; Cerebrovascular Resistance, CVR_{CO2MAP}; Cerebrovascular reactivity to CO_2, CVR_{CO2MAP}; Cerebrovascular reactivity to CO_2 (MAP accounted for).*$p<0.05$.

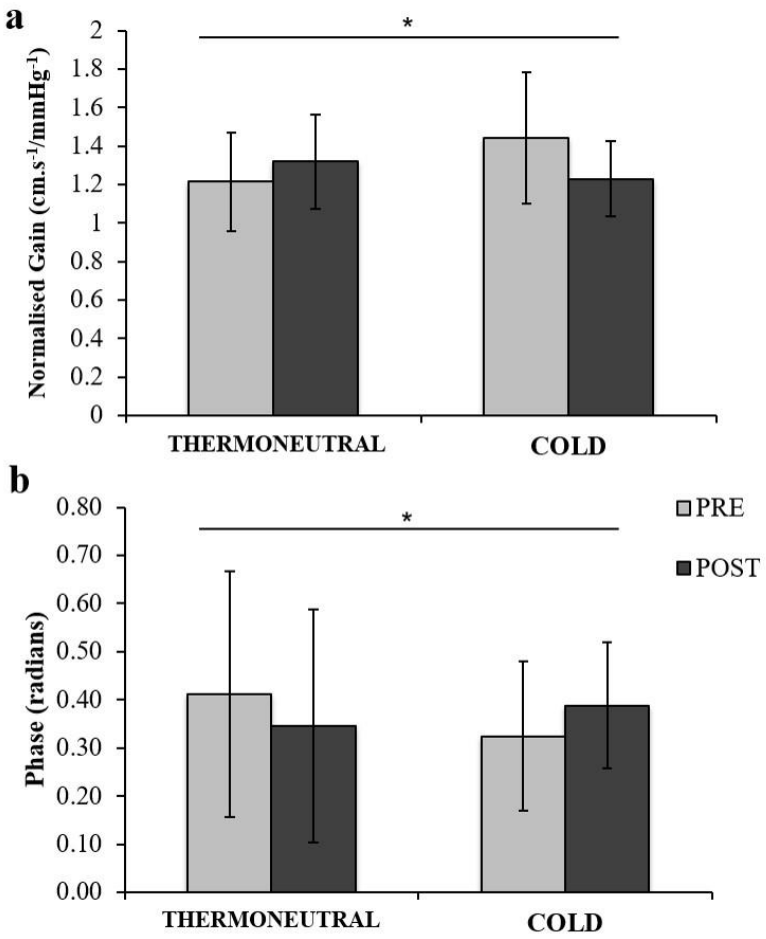

Figure 3. Normalised gain (a) and phase (b) for participants in the thermoneutral exercise and cold exercise-training group pre and post exercise intervention. * denotes significant interaction condition*time (P<0.05). Error bars represent SD.

Brachial Artery Endothelial Function (FMD): Brachial FMD, peak response, time to peak (TTP) or shear rate area under the curve (SRAUC) were not influenced by exercise itself nor when participants were stratified by environment, before or after allometric scaling (P>0.05; Table 3).

Table 3. Vascular function for participants in the thermoneutral exercise and cold exercise-training group pre and post exercise intervention.

Characteristic	**Thermoneutral Group (n=10)**		**Cold Group (n=7)**		**P-value**		
	Pre	**Post**	**Pre**	**Post**	**Time**	**Condition**	**Time*Condition**
Artery diameter (mm)	3.6±0.7	3.6±0.6	3.8±0.7	3.8±0.7	0.91	0.50	0.92
Peak artery diameter (mm)	3.8 ±0.7	3.9±0.5	4.1±0.8	4.2±0.8	0.63	0.33	0.72
Time to Peak (secs)	47.59±16.80	46.24±13.49	50.03±14.41	43.93±15.09	0.50	0.98	0.66
SR_{AUC} ($x10^3$)	20.67±11.47	15.71±8.24	21.14±14.94	20.71±7.08	0.33	0.56	0.61
FMD (%)	6.70±2.84	6.81±4.41	6.58±2.56	7.99 ± 2.14	0.13	0.64	0.44

Abbreviations: SR_{AUC}; Shear rate area under the curve, FMD; Flow mediated dilatation.

Cutaneous microvascular function: MAP was not different between conditions (P=0.66) and did not change during gradual local heating (P=0.47). A significant main effect of time was evident in the forearm %CVC_{max} decreasing by 5% following exercise training (-9, -1; P=0.03) (Figure 4). There were no main effects of intervention*time or intervention*time*temperature interactions for %CVC_{max} (P>0.05).

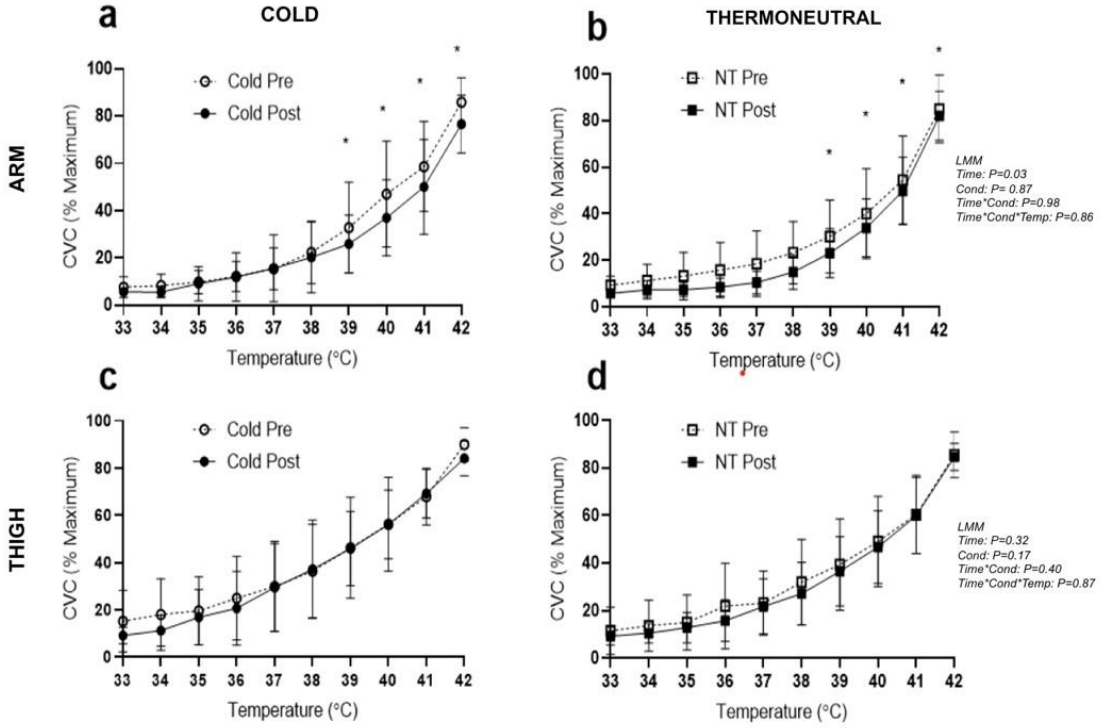

Figure 4. Cutaneous vascular conductance normalised to maximum CVC (%CVC_{max}) across time points (from baseline 33ºC to maximal plateau at 44ºC) following gradual local heating in the arm **(a)** and thigh **(c)** in the cold training group and arm **(b)** and thigh **(d)** in the thermoneutral training group. * denotes significant difference (P<0.05) from pre to post exercise intervention at time point. Error bars represent SD.

Acute experiment (n=21): the effects of acute exercise on cerebral, conduit and cutaneous micro-vascular responses.

MCAv and $P_{ET}CO_2$ increased by 8.4 $cm.s^{-1}$ and 9.1 mmHg during the exercise (P<0.001) but there was no between group differences (Figure 5; P>0.05). MCA CBVC increased by 0.12 $cm.s^{-1}/mmHg^{-1}$ (0.03, 0.21, P<0.01) and MCA CBVR decreased by 0.24 $mmHg^{-1}/cm.s^{-1}$ (-0.39, -0.86; P<0.01) but there was no between group differences (Table 4). SBP increased and DBP decreased significantly (P<0.001) during exercise with no effect of condition or condition*time interaction (P>0.05, Table 4). However, MAP was not altered by exercise (P=0.73) or group allocation (P=0.56.). T_c increased by 0.68 ^oC (0.55, 0.80; P<0.001) during exercise, which was comparable across environmental settings (P>0.05, Figure 5). T_{sk} was lower at resting baseline and during all exercise time points in the cold compared to the thermoneutral temperature (-4.76^oC [-6.89, -2.63], P<0.001) (Figure 5). T_{sk} significantly decreased by 2.82^oC (-4.03, -1.60) during exercise in the cold condition but was unchanged during exercise in the thermoneutral condition (0.69^oC [-0.34, 1.73]) (P<0.001, Figure 5). Thermal comfort increased during the exercise (P<0.001) but was significantly lower in the cold condition at every time point during exercise up until the 40-minute time point (condition*time; P<0.001, Figure 5). Forearm and thigh SkBF significantly increased during the exercise in both environmental conditions (main effect of time; P<0.001), but was significantly lower in the cold condition (main effect of condition; P<0.05). There was a significant condition*time interaction for forearm and thigh SkBF as the rate of increase in flux was higher in the thermoneutral condition compared to the cold condition (condition*time; P<0.001, P=0.01 respectively). Congruent findings were observed when SkBF was expressed as CVC. Radial artery diameter increased by 0.54 mm (0.28, 0.80; P=0.001), blood flow velocity increased by 15.3 $cm.s^{-1}$ {8.4, 22.1; P<0.001) and SR_{AUC} increased by 7.6 (4.1, 11.1; P=0.001) during exercise, comparable across environmental

settings with no effect of group or group*time interaction (P>0.05). Radial artery blood flow was not altered by exercise (P=0.26) or group allocation (P=0.13) (Table 4).

Table 4. Vascular and haemodynamic responses during exercise in a cold or thermoneutral environment.

Characteristic													P-value		
	CONDITION	**REST**	**5**	**10**	**15**	**20**	**25**	**30**	**35**	**40**	**45**	**50**	**Time**	**Condition**	**Time* Condition**
SBP (mmHg)	*NT*	125±9	159±15	143±44	150±24	158±13	159±16	158±14	151±15	151±14	161±25	148±27	<0.001*	0.94	0.90
	COLD	133±14	157±20	152±22	148±23	148±18	163±33	154±28	144±29	151±36	149±31	141±21			
DBP (mmHg)	*NT*	74±8	61±13	67±12	63±15	68±13	60±13	66±9	67±10	74±18	62±15	65±12	<0.001*	0.32	0.08
	COLD	75±9	61±13	58±15	57±6	63±14	63±19	61±11	65±9	57±14	60±11	63±7			
HR (beat.min^{-1})	*NT*	-	141±6	144±3	144±6	142±5	141±5	144±5	142±9	142±8	142±6	142±7	0.02*	0.004*	0.15
	COLD	-	129±9	137±8	136±6	134±7	138±8	138±7	139±6	139±7	139±7	139±7			
CBVC (cm. s^{-1}.mmHg^{-1})	*NT*	0.67±0.11	-	0.79±0.21	-	0.72±0.13	-	0.72±0.14	-	0.72±0.09	-	0.76±0.12	0.003*	0.84	0.77
	COLD	0.65±0.10	-	0.77±0.18	-	0.79±0.15	-	0.81±0.12	-	0.79±0.13	-	0.77±0.12			
CBVR (mmHg^{-1}.cm.s^{-1})	*NT*	1.53±0.22	-	1.33±0.30	-	1.42±0.22	-	1.44±0.27	-	1.40±0.17	-	1.33±0.19	0.002*	0.85	0.74
	COLD	1.58±0.22	-	1.36±0.31	-	1.31±0.27	-	1.27±0.20	-	1.29±0.22	-	1.33±0.20			
Radial Artery Diameter (mm)	*NT*	4.6±0.4		4.5±0.5		5.0±0.8		5.0±0.7		4.7±0.1		5.1±0.8	0.001*	0.27	0.23
	COLD	4.0±0.1		4.2±0.9		4.2±0.8		4.3±1.3		4.6±1.0		4.4±0.9			
Radial Artery Flow (ml.min^{-1})	*NT*	2.9±5.4		2.7±2.4		3.8±4.1		5.1±3.4		3.8±3.9		4.6±3.3	0.26	0.13	0.86
	COLD	0.3±0.3		1.1±1.2		2.0±1.4		4.0±2.5		2.9±1.7		2.2±1.5			
Radial SRAUC (x10^{3})	*NT*	2.7±2.8		6.9±4.1		6.2±4.0		11.4±2.4		9.0±3.5		9.8±4.8	<0.001*	0.46	0.29
	COLD	1.6±1.3		2.2±1.1		6.3±3.9		12.1±5.0		9.6±6.3		9.6±6.0			

Abbreviations: NT, Thermoneutral; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; HR, Heart Rate; CBVC, cerebrovascular conductance; SRAUC; Shear Rate Area Under Curve.

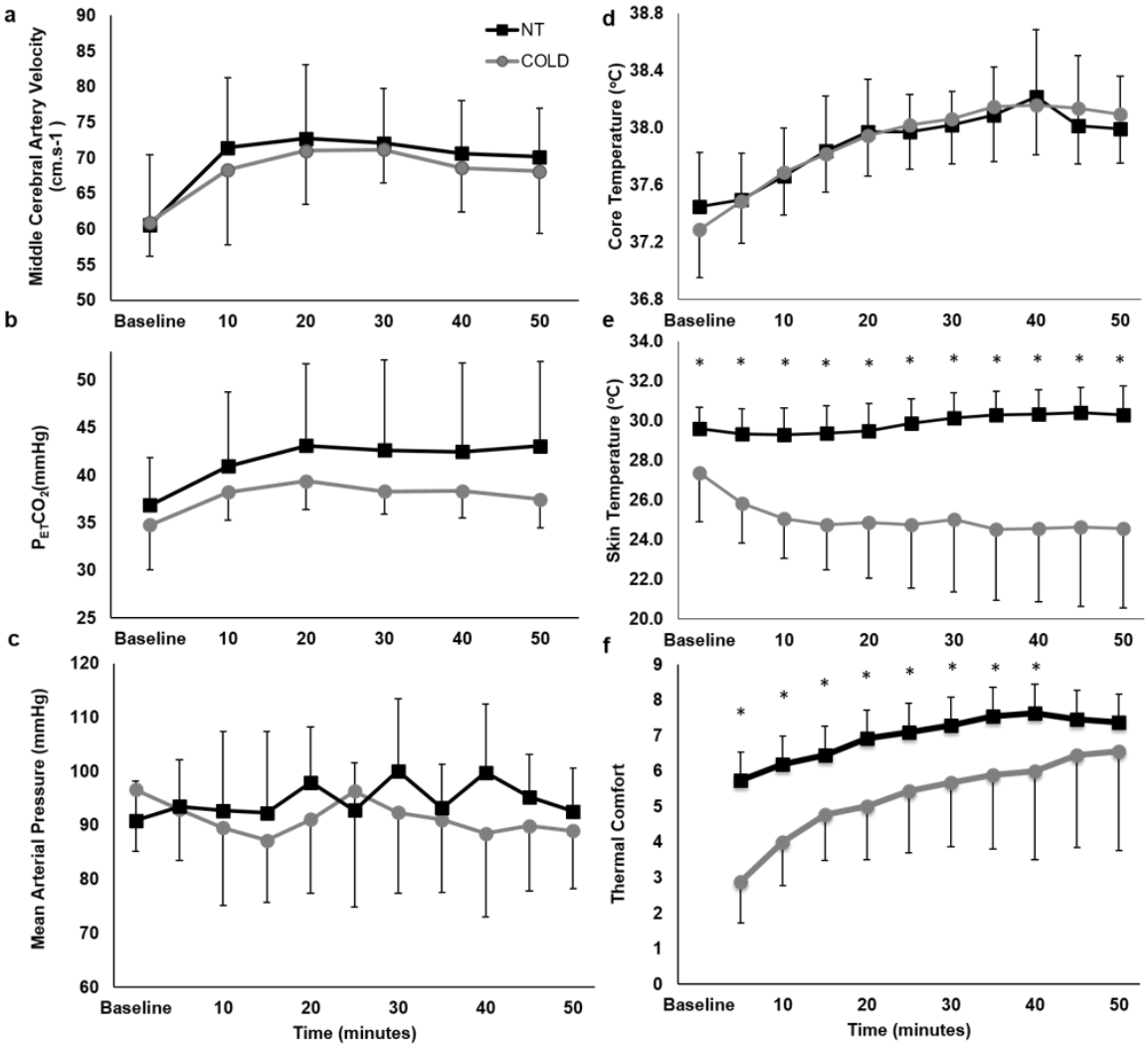

Figure 5. Cerebral artery velocity in the middle cerebral artery (MCA) (a), end tidal volume of CO_2 (b), mean arterial pressure (c), core temperature (d) skin temperature (B) and thermal comfort (C) in response to cycling in a thernoneutral environment vs cold environment (10-minute intervals). *denotes significant difference between groups at time point ($P<0.05$). Error bars represent SD.

Discussion

Our aim was to investigate whether the combination of exercise training in a cold environment could maximise the positive effects of exercise training on CBFv and cerebrovascular function. We show that exercise training in the cold evoked an improvement in the parameters of cerebral autoregulation compared to exercise training in a thermoneutral environment in young healthy

individuals. Nevertheless, the changes in cerebral autoregulation with exercise training in the cold were not directly explained by a greater CBFv or haemodynamics during acute exercise. Taken together, these data suggests that adding cold exposure during exercise could be a potential strategy to enhance or maintain cerebrovascular function in young healthy individuals and investigating the impact on aged individuals or those with cerebrovascular disease is warranted.

We did not observe a change in resting CBFv with either exercise training intervention, but we found a small but statistically significant improvement in dCA induced by exercising in a cold environment compared to exercising in a thermoneutral environment in young healthy individuals. Interestingly, the improvements in dCA were accompanied by both normalised gain and phase indicating that cold exercise training improved both the magnitude of the CBFv response and temporal alignment to forced BP oscillations (van Beek et al., 2008, Claassen et al., 2016). Previous exercise training studies, without an additional environmental stimulus, have shown contrasting dCA findings, albeit with different exercise modes (aerobic exercise vs high intensity interval training) and in different participants (endurance trained males and chronic obstructive pulmonary disorder patients and healthy aged-matched controls (Drapeau et al., 2019, Lewis et al., 2019b). Based on these previous findings, our data could suggest that exercise in the cold provides a larger stimulus for cerebrovascular adaption, in young healthy individuals. Whilst these findings are encouraging, the extent of adaptation in cerebral autoregulation across this 8-week exercise intervention is small and may have a limited physiological or clinical relevance healthy individuals. However, this may be a useful strategy for individuals post-stroke as an impaired dCA is identified as a risk factor in post stroke cognitive impairment (Chi et al., 2020)).

The mechanism by which exercise training mediates changes in dCA is unclear, with research evidence indicating contributions from neurogenic, metabolic, myogenic and endothelial adaptation (Tzeng and Ainslie, 2014). There is even less research on the impact of cold on dCA. From our data collected, we can provide some insight into endothelial adaption. We employed assessments to provoke endothelial mediated responses in the cerebral (CVR_{CO2}), conduit (FMD) and microvessels of the skin (response to local heating). We examined difference vessels given that the responses to cold are likely different between cerebral and peripheral vessels and the relationship between endothelial responses of cerebral and peripheral vessels is not strong (Carr et al., 2020). We did not observe differences in the endothelial function in response to exercise training in the cold compared to thermoneutral, nor did we observe a statistically significant change with exercise training (main effect of time) in the cerebral or conduit endothelial assessments. This is unsurprising in the peripheral vessels over an 8 week period, given our participants were young and healthy (Birk et al., 2012, Tinken et al., 2008). There is some evidence of changes in CVR_{CO2} in young individuals following exercise training (Murrell et al., 2013a) which contrasts our findings and could be explained differences in the methodology employed to assess CVR_{CO2} and also differences in the age and fitness of the participants. Nevertheless, our data suggest the addition of the cold stimulus did not enhance the endothelial mediated exercise training response and a change in cerebral endothelial function likely does not contribute to explaining the change in dCA observed in the cold.

A novel aspect of our study is that we also included vascular and haemodynamic measurements during one acute exercise bout to examine if the cold mediated greater changes (e.g. flow or shear stress) during exercise that might contribute to any training related functional adaptations.

Intriguingly, we did not observe greater MCAv, cerebrovascular resistance, conductance or BP during exercise in the cold nor did we observe a greater (peripheral artery) shear stress during exercise in the cold, contrary to our rationale. Taking these acute data together, it is unlikely that these variables contribute to the differences in dCA with exercise training in the cold. However, we did not employ continuous beat-to-beat BP monitoring during acute exercise so cannot quantify the dynamics of the pressure-flow relationship of dCA. More in-depth understanding of the pressure-flow relationship of dCA is required given that (i) the acute dCA response to exercise is complex and poorly understood (Ogoh and Ainslie, 2009); and (ii) the pressure-flow relationship of dCA can be observed in the reverse direction from CBFv to BP (Bari et al., 2017) and is likely under autonomic control (Saleem et al., 2018) . To understand the possible intricacies of the pressure-flow relationship of dCA future research should include direct assessments of autonomic function or autonomic challenges and consideration should be taken of the bi-directional interactions present between CBFv and BP. Given that we did not assess this our finding of an altered dCA response in the cold should be interpreted with caution.

In the current study, an assessment of skin microvascular function was also completed prior to and following the exercise intervention and skin temperature and cutaneous vascular conductance data was collected during the acute exercise. We show increased sympathetic cutaneous vasoconstriction throughout the exercise in the cold, via reduced skin temperature and a blunted increase of cutaneous vascular conductance, likely a response to limit heat loss in the cold environment (Alba et al., 2019a, Castellani and Young, 2016). This data provides indirect information about sympathetic nerve activity. The exact neural contribution to dCA is debated (Ainslie and Brassard, 2014) and one acute increase in sympathetic nerve activity is

unlikely to have an impact on dCA, as shown recently with the cold pressor test (Washio et al., 2020), but it is possible that repeated elevations in sympathetic nerve activity could impact dCA. Direct markers of sympathetic nerve activity and autonomic function would enhance the mechanistic understanding of the dCA response to exercise in the cold but from the current data we cannot rule out any neural impact of on cold on dCA.

As previously outlined, the skin temperature responses during exercise were clearly different between thermoneutral and cold environments, but moderate to large elevations in skin blood flow still occurred in the cold which are key drivers of microvascular adaptation. Intriguingly, the exercise training mediated response was not enhanced in either environmental condition. Previous studies have shown improved exercise training mediated NO endothelial function (Black et al., 2008b, Sprung et al., 2013, Pugh et al., 2013), consistent with regular elevations in skin temperature/or microvascular shear stress required to increase skin blood flow. Our data suggest a similar microvascular response following either exercise intervention despite lower skin blood flow and skin temperatures during exercise in the cold.

Methodological considerations

We acknowledge that some of our methodological limitations may have impacted our results. First, we recruited young, healthy, with little or no evidence of decline in MCAv or cerebrovascular dysfunction, which likely limited exercise training mediated changes. Second, we employed a moderate intensity exercise modality, but a recent research study shows that interval exercise training elicits greater accumulated increases in CBFv compared to moderate continuous training (Klein et al., 2019), it is possible that interval exercise may have greater cerebral changes in both environmental conditions. Fourth, examining the acute during exercise

response at the midpoint of the exercise training program may have resulting in cold habituation or acclimation (Castellani and Young, 2016) and therefore blunting the responses to the exercise performed in the cold (Makinen et al., 2008). It is possible that haemodynamic responses to acute exercise in the cold might have been exaggerated during the early exercise sessions and in fact the improvement in cerebral autoregulation may have occurred early in the exercise training intervention (i.e. between weeks 1-4). Employing a repeated measures design for the acute experiment would have enhanced statistical power and reduced individual participant variability. Finally, we employed environmental temperatures that represented 2 distinct seasons in the UK whereby many individuals are likely to exercise to enhance external validity. We acknowledge that individuals exercise in colder temperatures in other countries, which could alter the responses observed in the current study. Further research is warranted to examine different exercise training modalities in older individuals or those with pre-existing conditions and potentially in a colder environmental temperature.

Limitations

Our primary measurement of CBFv, we acknowledge TCD assesses blood velocity rather than blood flow and relies on assumptions that the diameter of the MCA did not change during the cerebrovascular measurements (CO_2 reactivity & cerebral autoregulation) and throughout the acute exercise. However, whilst structural remodelling is evident following exercise training in the peripheral blood vessels (Green et al., 2017), this has not yet been established in the cerebrovasculature (Green et al., 2021). Research evidence also suggests that MCAv is a reliable index of cerebral blood flow if the insonated vessel maintains a constant diameter

across experimental conditions. MCA diameter has been shown to be consistent during modest changes in CO_2 (±5 mmHg) (Ainslie and Hoiland, 2014) as employed in our study.

Conclusion

In a cohort of young healthy individuals, we show that exercise training in a cold environment can enhance dCA. Whilst these changes were not directly explained by a greater CBFv or haemodynamics during acute exercise, nor were functional changes observed in peripheral vascular beds in the cold, exercise training in cold may be a useful strategy to alter dCA and warrants further investigation especially in those with potentially impaired dCA.

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