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ORIGINAL ARTICLE

Effect of whole-body mild-cold exposure on arterial stiffness and central haemodynamics: a randomised, cross-over trial in healthy men and women

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Abstract Aortic pulse wave velocity (PWV) and augmentation index (AIx) are independent predictors of cardiovascular risk and mortality, but little is known about the effect of air temperature changes on these variables. Our study investigated the effect of exposure to whole-body mild-cold on measures of arterial stiffness (aortic and brachial PWV), and on central haemodynamics [including augmented pressure (AP), AIx], and aortic reservoir components [including reservoir and excess pressures (P_{ex})]. Sixteen healthy volunteers (10 men, age 43 ± 19 years; mean ± SD) were randomised to be studied under conditions of 12 °C (mild-cold) and 21 °C (control) on separate days. Supine resting measures were taken at baseline (ambient temperature) and after 10, 30, and 60 min exposure to each experimental condition in a climate chamber. There was no significant change in brachial blood pressure between mild-cold and control conditions. However, compared to control, AP [+2 mmHg, 95 % confidence interval (CI) 0.36–4.36; $p = 0.01$] and AIx (+6 %, 95 % CI 1.24–10.1; $p = 0.02$) increased, and time to maximum P_{ex} (a component of reservoir function related to timing of peak aortic in-flow) decreased (−7 ms, 95 % CI −15.4 to 2.03; $p = 0.01$) compared to control. Yet there was no significant change in aortic PWV (+0.04 m/s, 95 % CI −0.47 to 0.55; $p = 0.87$) or brachial PWV (+0.36 m/s; −0.41 to 1.12; $p = 0.35$) between conditions. We conclude that mild-cold exposure increases central haemodynamic stress and alters timing of peak aortic in-flow without differentially affecting arterial stiffness.

Keywords Aortic reservoir function · Augmentation index · Central blood pressure · Climate

Abbreviations

PWV	Pulse wave velocity
AIx	Augmentation index
AP	Augmented pressure
BP	Blood pressure
CV	Cardiovascular
LV	Left ventricle
P_{res}	Reservoir pressure
P_{ex}	Excess pressure
PWA	Pulse wave analysis
PP	Pulse pressure
MBP	Mean blood pressure

Introduction

Acute cold exposure results in peripheral vasoconstriction, accompanied by increased arterial pressures measured using the brachial cuff method (Kingma et al. 2011; Stocks et al. 2004). Brachial systolic and diastolic blood pressures (BPs) are also affected by seasonal changes in air temperature, with studies reporting that brachial BPs increase in the winter and decrease in the summer (Alperovitch

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et al. 2009; Charach et al. 2004; Halonen et al. 2011). Moreover, cold air inhalation (Muller et al. 2011) and whole-body cold exposure using a water-perfused suit (Gao et al. 2012; Wilson et al. 2010) have been shown to increase myocardial oxygen demand and decrease coronary perfusion. However, little is known about the effects of cold exposure on measures of central haemodynamics such as aortic pulse wave velocity (PWV) and augmentation index (AIx).

Aortic PWV and AIx are independent predictors of cardiovascular (CV) risk and CV mortality (Vlachopoulos et al. 2010a, b). The aorta is the body's largest elastic artery and its stiffness is predominantly affected by degenerative changes that occur with ageing or disease (Nichols and O'Rourke 2005). Thus, aortic PWV is a measure of passive or chronic changes in localised stiffness of elastic arteries (e.g. aorta or carotid arteries) (Nichols et al. 2008). PWV can also be measured in the brachial artery, which is acutely reactive to interventions causing changes in muscular arterial tone (Kelly et al. 2001). Therefore, brachial PWV is a measure of transient changes in muscular arterial stiffness (Nichols et al. 2008). AIx, however, is more a marker of central haemodynamic stress and left ventricular (LV) afterload (Saba et al. 1993), and is strongly influenced by changes in aortic reservoir function (Davies et al. 2010) as a result of peripheral vasomotor changes (Sharman et al. 2009).

The elastic aorta acts as a buffer, or *reservoir*, which expands during LV ejection (systole) and recoils during diastole, effectively smoothing pulsatile flow from the left ventricle into smaller downstream arteries before reaching a steady flow through the microcirculation (Westerhof et al. 2009). Thus, *reservoir function* describes the cushioning effect and pressure-flow–time relationships in the proximal aorta caused by LV contraction and relaxation (Davies et al. 2007; Wang et al. 2011). While it is currently unknown what effects low environmental temperatures have on aortic reservoir function, peripheral vasoconstriction and increased arterial pressures during cold exposure (Stocks et al. 2004) have been suggested as one possible cause of increased AIx (Kelly et al. 2001). Evidence of alterations in aortic and brachial PWV in response to interventions causing peripheral vasoconstriction, however, remains controversial (Edwards et al. 2008; Hess et al. 2009; Kelly et al. 2001).

While the few studies that have directly investigated the effects of cold exposure on PWV or AIx have reported increases in these measures (Casey et al. 2008; Edwards et al. 2006, 2008; Geleris et al. 2004; Hess et al. 2009; Moriyama and Ifuki 2010), the majority of these studies used localised cold, i.e. frozen gel packs (Edwards et al. 2008), or a cold pressor test (Casey et al. 2008; Geleris et al. 2004; Moriyama and Ifuki 2010). To our knowledge only two studies have investigated whole-body cooling effects on central haemodynamics (Edwards et al. 2006; Hess et al. 2009). One of these used a water-perfused suit for 20 min (Hess et al. 2009). However, the other study used methods that approximated a more realistic environmental cold exposure model (Edwards et al. 2006). This latter study used a controlled climate chamber to affect whole-body cooling for 30 min and reported increased heart rate, AIx, and brachial and aortic systolic BP during cold exposure (Edwards et al. 2006). However, that study did not measure aortic or brachial PWV, and the cold stimulus used was severe (4 °C plus fans to create wind chill of 6.1 m/s). All participants in that study commenced shivering after ~5 min of cold exposure (Edwards et al. 2006), which may have influenced the haemodynamic changes that were observed (Sessler 2009).

Day-to-day exposure to cold is not typically a challenging experience due to behavioural conditioning to dress appropriately and avoid shivering (Blatteis 2012). By controlling for the systemic effects of shivering and using a whole-body mild-cold exposure in a controlled climate chamber, our study was designed to more realistically reflect haemodynamic changes from exposure to a cool environment. The aim of our study was to test the hypothesis that compared to a control condition (21 °C), 60 min exposure to mild-cold (12 °C) would increase central haemodynamic stress in a healthy adult population. To this end we measured regional arterial stiffness (aortic and brachial PWV) and measures of central haemodynamics including augmented pressure (AP), AIx, BPs, and aortic reservoir components including reservoir pressure (P_{res}), excess pressure (P_{ex}), and timing of P_{ex} in each condition.

Materials and methods

Study design

The study design was approved by the Human Research Ethics Committee (Tasmania) Network and adhered to the principles of the declaration of Helsinki. All participants provided written informed consent. Trial order was randomised and individually sealed envelopes were produced by a biostatistician not connected with the study, and delivered prior to any data collection. The experiment was carried out as a cross-over design and 16 participants completed the two test sessions in random order, with approximately 7–14 days between tests. The two test conditions were: control at 21 °C with 40 % relative humidity (RH), and mild-cold at 12 °C with 40 % RH (ASHRAE 2010; Australian Bureau of Meteorology 2011). The temperature difference between the two test conditions

was representative of the temperature change when moving from an indoor climate-controlled environment to outdoors on a cool day (Australian Bureau of Meteorology 2011). This temperature difference was expected to elicit haemodynamic responses, but deemed tolerable and safe for participants over the 60-min experimental session.

Participants

Healthy adult volunteers were sought to participate in the study using local media. Inclusion criteria were: male or females aged >18 years with a resting brachial BP ≤150/80 mmHg. Potential participants were excluded if they had a self-reported clinical history of CV or metabolic disease.

Experimental protocol

Study participants fasted overnight (water ad libitum) prior to each test session, and were instructed to avoid caffeine, fried/fatty foods, strenuous exercise, and alcohol in the 24 h prior to a test session. The menstrual cycles of female participants were not taken into account during this study as the effect of ovarian hormones on reflexive BP control during haemodynamic perturbations has been found to be negligible (Hayashi et al. 2006). To ensure consistency between trials, participants were instructed to wear the same (or similar) light clothing (i.e. a short-sleeved top and light long pants) to each test session, irrespective of the outdoor weather. If a participant was wearing socks, these were removed to allow access to the skin for temperature measurements. After height and weight measures, and consumption of 150 mL of water, participants rested supine on a vinyl-covered massage table for 10 min in ambient laboratory conditions then had baseline physiological measures. Following this, participants walked (~8 steps) into the climate-controlled chamber, which was pre-set at one of the two experimental conditions. Participants then rested quietly in a supine position on a vinyl-covered massage table inside the climate chamber while physiological measures were taken at 10, 30, and 60 min post-entry to the climate chamber. To avoid adaptive thermogenesis, stress responses, and movement due to shivering in the mild-cold condition, a light cotton blanket was placed over the feet between 5 and 20 min of exposure and pulled up to the neck if participants thought they were about to shiver. The blanket was removed and placed back over the feet when participants felt comfortable again. No blankets were requested in the control condition. Laboratory temperature and RH were recorded at the participant's arrival time (between 8.00 a.m. and 9.00 a.m.) each test day (Vantage VUE weather station console 6351, Davis Instruments, CA, USA). There was minimal air velocity in the climate chamber and the air inflow vent was directed away from the resting participant. Climate chamber temperature and RH were recorded in close proximity to the participant at 10, 30, and 60 min during each test session (Perception II weather station, Davis Instruments, CA, USA). Venous bloods were collected at the first visit into serum clot activator tubes (for serum cholesterol) and sodium fluoride/potassium oxide tubes (for plasma glucose) in order to describe the metabolic profile of the participants. Samples were processed and stored at −80 °C for later analysis using spectrophotometric enzymatic methods (Konelab 20XT, Thermo Fisher Scientific, VA, USA) using commercially available kits (Thermo Fisher Scientific, VA, USA), according to the manufacturer's instructions.

Physiological measures

At each timepoint (−10 min/baseline in ambient laboratory temperature, then 10, 30, and 60 min in both climate chamber conditions) participants had their core temperature taken with an infrared tympanic thermometer (Genius 3000A, Covidien, MA, USA). Skin temperatures were taken at four sites (central forehead, 10 cm to the side of the umbilicus, central–dorsal aspect of the hand, and central–dorsal aspect of the foot) using taped 2-plug wire thermocouples (QM1284, Digitek Instruments, HK, China) and a digital multimeter (QM1538, Digitek Instruments, HK, China) and averaged. Subjective perception of thermal comfort was taken at each timepoint with a thermal sensation and comfort scale, which asked the user to provide a number corresponding to their overall feelings of thermal comfort (ASHRAE 2010). Scale ratings were in 0.5 increments from −4 "unbearably cold", through 0 "neutral (comfortable)", and to +4 "unbearably hot" (ASHRAE 2010).

Standardised (Laurent et al. 2006; Van Bortel et al. 2002), non-invasive haemodynamic measures were made in duplicate at each timepoint and averaged for analyses. To reduce inter-observer bias, one trained operator performed all haemodynamic measures on the same participant for both experimental sessions. All artery waveforms were collected for a minimum of 12 s each with a Millar tonometer (SPC-301, Millar Instruments, TX, USA) and processed with dedicated software (SphygmoCor Vs 8.2, AtCor Medical, Sydney, Australia). Aortic PWV was calculated by simultaneously recording electrocardiogram-gated carotid and femoral artery waveforms, while brachial PWV was similarly calculated from the carotid and radial artery pulse sites (O'Rourke et al. 2002; Wilkinson et al. 1998). Pulse wave analysis (PWA) was performed on the radial artery waveform using brachial BP as the calibrating value. Measures of central haemodynamics were derived using a generalised transfer function previously validated

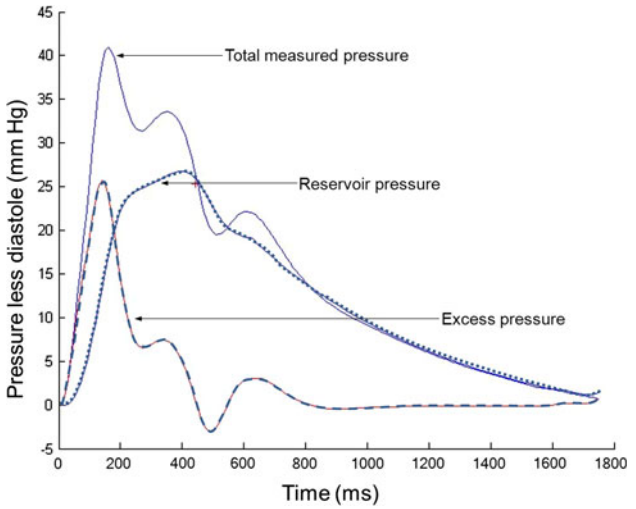

Fig. 1 The components of the human arterial pressure waveform. Representative radial pressure waveform was taken from a single participant in ambient lab temperatures. Total measured pressure (minus diastole; *solid line*) is equal to the reservoir pressure (*dotted line*) plus the excess pressure (*dashed line*). Reservoir pressure matches total measured pressure closely during late diastole while excess pressure is similar to the measured pressure during early systole, but approaches zero during diastole

during rest and haemodynamic perturbations including the Valsalva manoeuvre, nitroglycerin, and exercise (Chen et al. 1997; Gallagher et al. 2004; Sharman et al. 2006). These measures included aortic BP, AP (the difference between the first and second systolic pressure peaks), pulse pressure (PP; systolic pressure minus diastolic pressure), mean BP (MBP: calculated using customised SphygmoCor software as the mean value of the area under the curve for the averaged radial pressure waveforms), and AIx (AP divided by PP expressed as a percentage).

Components of aortic reservoir function (see Fig. 1) were calculated from PWA data by separating the averaged radial pressure waveforms (acquired by the SphygmoCor equipment) using methods described previously (Aguado-Sierra et al. 2008) on customised Matlab software (Mathworks, Inc., Natick, MA, USA). Reservoir components analysed included maximum P_{res} (defined as the mean peak pressure in the aortic reservoir, and the minimal work the LV must perform to overcome net arterial resistance), cumulative P_{res} (a marker for P_{res} over the time of the cardiac cycle and equal to the area under the P_{res} waveform), maximum P_{ex} (defined as the amount of pressure relating to aortic in-flow and wave motion, and is the extra work the LV must perform over and above the P_{res} for a given condition), and time to P_{ex} (defined as the time to peak aortic in-flow and equal to the time taken during the cardiac cycle for P_{ex} to reach maximum). P_{res} is calculated using the following formula:

$$P_{res} - P_{\infty} = e^{-(a+b)t} \int_0^t [aP(t') + bP_{\infty}]e^{(a+b)t'} dt' + (P_d - P_{\infty})e^{-(a+b)t}$$

where P_{∞} is the asymptotic pressure at which flow through the microcirculation is zero, P_d the measured diastolic pressure at $t = 0$, $b = 1/RC$, where $R =$ resistance and $C =$ compliance of the system, and a is a rate constant that is chosen so that the pressure is continuous at the beginning of the exponential fall in pressure during diastole (Aguado-Sierra et al. 2008; Davies et al. 2007; Sharman et al. 2009). Once P_{res} is calculated, P_{ex} can be calculated as:

$$P_{ex} = \text{total (measured) aortic pressure} - P_{res}.$$

Statistical analysis

Prior to commencement of the study a sample size of 16 was determined on the basis of an expected change in AIx. This expected change was based on the mean change in AIx after cold exposure reported by Edwards et al. (2006) (3.4 ± 1.9 to 19.4 ± 1.8 %; a 16 % increase). However, since the cold conditions in our study were considerably milder, we conservatively estimated that AIx would change by half of that reported by Edwards et al. (2006), i.e. by approximately 8 %.

Haemodynamic data were analysed for repeated measures and as panel data via general estimating equations using STATA (version 12, StataCorp LP, College Station, TX, USA). Data were adjusted for order and period effects. Mean for baseline values (at −10 min in ambient laboratory temperatures) and individual timepoints in the climate chamber (at 10, 30, and 60 min) were calculated for each variable in both experimental conditions, and compared. For within-condition change, difference from baseline to the average of all timepoints in the climate chamber (average of values recorded at 10, 30, and 60 min) was determined for all variables in both experimental conditions, and results for each condition were then compared for between-condition changes. Results were corrected for multiple comparisons by the Holm (1979) method. Regression residuals were calculated and used to test the assumptions of linear regression using decomposition tests of heteroskedascity, skewness, and kurtosis (Cameron and Trivedi 1998), and the regression equation specification error test (Ramsey 1969). Where significant violations were found, the affected analyses were replicated using repeated-measures ordinal logistic regression. Results are presented as mean ± SD, and comparative data are presented as mean difference and 95 % confidence intervals (95 % CI). Regression coefficients and 95 % CIs were taken from linear regression analyses, and where data were

found to violate the assumptions of linear regression, p values were taken from ordinal logistic regression post hoc testing.

Results

Demographics

Nineteen adults volunteered for the study and met the inclusion criteria. Three volunteers withdrew prior to commencement of data collection for personal reasons. Therefore, 16 participants (10 men) completed both experimental sessions in random order (baseline means: age 42.8 ± 19.2 years; brachial systolic BP, 122 ± 16 mmHg; brachial diastolic BP, 72 ± 7 mmHg; fasting plasma glucose, 4.7 ± 0.3 mmol/L; and fasting serum total cholesterol, 4.3 ± 0.8 mmol/L). Of the 16 participants, 10 began the study with the control condition (5 men, 5 women), and 6 (5 men, 1 woman) began with the mild-cold condition. After a washout period of approximately 7–14 days between trials, participants returned to complete the remaining test session. All participants were non-smokers and complied with pre-test instructions. There were no differences in baseline measures between trials (all $p > 0.07$) except for diastolic BPs ($p = 0.007$; Fig. 5; Table 1). Mean ambient laboratory environmental temperature was 22.0 ± 1.9 °C. Climate chamber mean temperature for control condition was 20.3 ± 1.0 °C, and 12.5 ± 0.6 °C for the mild-cold condition. To avoid shivering in the mild-cold condition, a blanket was requested by 7 of the 16 participants between ~10 and 30 min of exposure. For those 7 participants, the blanket was used 2 ± 0.4 times per session for 4 ± 1 min per time during the 60-min exposure. No blankets were requested during the control condition.

Thermoregulatory responses

Core temperature

Within condition, there was a decrease in core temperature from baseline in mild-cold (36.2 ± 0.5 to 35.4 ± 0.6 °C; $p < 0.001$), but there was no significant change from baseline in control condition (36.3 ± 0.5 to 36.2 ± 0.6; $p = 0.14$). Between conditions, core temperature was decreased in mild-cold compared to change in control (−0.7 °C; 95 % CI −1.86 to 0.54; $p < 0.001$).

Skin temperature

Skin temperature did not change significantly from baseline in either mild-cold (28.4 ± 1.2 to 27.7 ± 2.4 °C; $p = 0.26$) or control (28.4 ± 1.1 to 27.8 ± 2.2 °C; $p = 0.29$) and between conditions there was no difference in change in skin temperature in mild-cold compared to change in control (+1.14 °C; 95 % CI 0.39–3.27; $p = 0.41$).

Perceived thermal comfort

There was a decline in self-reported thermal comfort from baseline in mild-cold, from feeling "comfortable" to "cold" (−0.12 ± 0.4 to −1.71 ± 0.8 arbitrary units; $p < 0.001$) and a smaller decrease in the control condition, from "comfortable" to approaching "cool" (−0.09 ± 0.4 to −0.62 ± 0.6 arbitrary units; $p = 0.001$). Between conditions, participants felt colder in mild-cold condition, compared to control (−1.1 arbitrary units, 95 % CI −1.5 to −0.5; $p < 0.001$).

Regional arterial stiffness responses

Aortic pulse wave velocity

There was no significant change in aortic PWV from baseline in mild-cold (7.1 ± 2.1 to 7.2 ± 2.1 m/s; $p = 0.56$) or control (6.9 ± 2.0 to 7.0 ± 2.2 m/s; $p = 0.72$; Fig. 2), and between conditions, there was no difference in change in aortic PWV in mild-cold compared to change in control (+0.04 m/s; 95 % CI −0.47 to 0.55; $p = 0.87$; Fig. 2).

Brachial pulse wave velocity

Brachial PWV increased from baseline in mild-cold (8.7 ± 1.4 to 9.4 ± 1.7 m/s; $p = 0.02$; Fig. 2) with no significant change in control (8.7 ± 1.4 to 8.8 ± 1.4 m/s; $p = 0.32$), yet between conditions, there was no difference in change in brachial PWV in mild-cold compared to change in control (+0.36 m/s; 95 % CI −0.41 to 1.12; $p = 0.35$; Fig. 2).

Haemodynamic responses

Augmented pressure

AP increased from baseline in mild-cold (6 ± 7 to 9 ± 8 mmHg, $p < 0.001$) with no change in control (6 ± 5 to 6 ± 7 mmHg, $p = 0.47$; Fig. 3) and between conditions, AP increased in mild-cold compared to change in control (+2 mmHg, 95 % CI −0.36 to 4.36, $p = 0.01$; Fig. 3).

Augmentation index

There was an increase in AIx from baseline in mild-cold (15 ± 15 to 21 ± 15 %; $p < 0.001$) with no change in

Table 1 Comparison of selected hemodynamic measures in mild-cold (12 °C) and control (21 °C) conditions

Condition	Baseline (−10 min)	10 min	30 min	60 min	Average (10–60 min)[a]	*P* (within condition)[†]	Δ mild-cold vs. Δ control[b]	*P* (between conditions)[‡]
Brachial pulse pressure (mmHg)								
Control	50 ± 12	50 ± 11	49 ± 11	50 ± 10	50 ± 10	0.66	1.29 (−2.26 to 4.85)	0.38
Mild-cold	48 ± 10	47 ± 10	50 ± 10	51 ± 12	49 ± 10	0.57		
Aortic diastolic blood pressure (mmHg)								
Control	71 ± 6	71 ± 7	71 ± 7	71 ± 9	72 ± 7	0.74	1.39 (−2.16 to 4.96)	0.52
Mild-cold	75 ± 9[§]	79 ± 10	75 ± 10	77 ± 10	77 ± 10	0.15		
Aortic pulse pressure (mmHg)								
Control	35 ± 10	35 ± 11	35 ± 11	35 ± 10	35 ± 10	0.81	2.73 (−0.45 to 5.92)	0.24
Mild-cold	34 ± 11	35 ± 11	36 ± 12	38 ± 13	36 ± 12	**0.03**		
Augmentation index standardised to heart rate of 75 beats/min (%)								
Control	7 ± 13	6 ± 17	6 ± 16	6 ± 14	6 ± 15	0.72	5.06 (0.78 to 9.33)	**0.05**
Mild-cold	7 ± 16	12 ± 15	11 ± 18	12 ± 19*	12 ± 17	**0.003**		
Mean blood pressure (mmHg)								
Control	86 ± 10	87 ± 10	87 ± 10	87 ± 11	87 ± 10	0.71	2.18 (−1.39 to 5.77)	0.45
Mild-cold	90 ± 11	95 ± 13	90 ± 13	92 ± 13	92 ± 13	**0.03**		
Mean heart rate (beats/min)								
Control	59 ± 11	57 ± 10	57 ± 10	56 ± 10	57 ± 10	**0.01**	0.20 (−2.26 to 2.66)	0.90
Mild-cold	57 ± 9	55 ± 8	55 ± 9	54 ± 8	54 ± 8	**0.01**		
Rate pressure product (mmHg beats/min)								
Control	7,121 ± 1,751	6,887 ± 1,697	6,840 ± 1,583	6,820 ± 1,624	6,839 ± 1,584	**0.05**	144 (−250 to 538)	0.47
Mild-cold	6,985 ± 1,701	6,963 ± 1,755	6,825 ± 1,700	6,836 ± 1,779	6,844 ± 1,757	0.32		
Cumulative reservoir pressure (mmHg/min)								
Control	11,236 ± 2,200	11,542 ± 1,734	11,734 ± 2,154	11,881 ± 2,481	11,719 ± 2,104	0.12	376 (−486 to 1,239)	0.62
Mild-cold	11,910 ± 2,441	13,072 ± 2,382	12,188 ± 1,933	13,048 ± 2,467	12,770 ± 2,262	**0.005**		
Cumulative reservoir pressure minus diastole (mmHg/min)								
Control	1,652 ± 493	1,664 ± 358	1,709 ± 423	1,756 ± 500	1,709 ± 423	0.51	132 (−115 to 379)	0.65
Mild-cold	1,547 ± 301	1,711 ± 611	1,677 ± 435	1,821 ± 573	1,737 ± 537	**0.03**		
Maximum excess pressure (mmHg)								
Control	33 ± 11	32 ± 11	33 ± 11	33 ± 10	33 ± 11	0.90	−0.29 (−15.4 to 2.03)	0.41
Mild-cold	32 ± 9	31 ± 10	32 ± 9	33 ± 12	32 ± 10	0.90		

$n = 16$; data presented as mean ± SD; Δ, mean change

[§] Significantly different from control at baseline

[†] *P* value of Δ within condition (i.e. from baseline to the average of 10, 30, and 60 min data)

[‡] *P* value of difference between conditions (i.e. comparison of Δ mild-cold and Δ control condition)

* Significantly different from control at specific time point

[a] Data collected at 10, 30, and 60 min in climate chamber were averaged for comparison against baseline data (baseline was at −10 min in ambient laboratory conditions)

[b] Δ mild-cold compared to Δ control condition (95 % confidence interval)

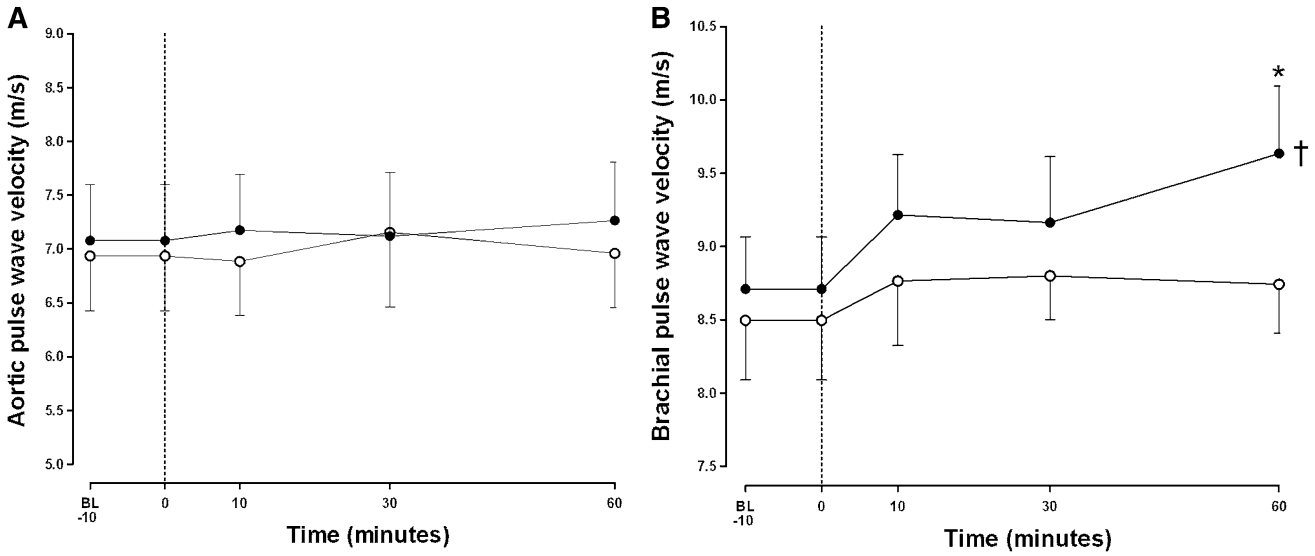

Fig. 2 Aortic pulse wave velocity (**a**) and brachial pulse wave velocity (**b**) in mild-cold (12 °C; *closed circles*) and control (21 °C; *open circles*). Data presented as mean ± SE; $n = 16$; baseline data (BL/−10 min at ambient laboratory temperature) was obtained ~10 min before entry to climate chamber (0 min; *dashed vertical line*); *significantly different from control at specific time point; †significant change within condition (i.e. between baseline and average of data during 60 min in test condition)

control trials (15 ± 11 to 15 ± 14 %; $p = 0.72$; Fig. 3). Between conditions, AIx increased in mild-cold compared to change in control (+6 %; 95 % CI 1.25–10.1; $p = 0.02$; Fig. 3). Moreover, the increase in AIx in mild-cold persisted when standardised to a heart rate of 75 beats/min, with an increase of +5 % in mild-cold compared to change in control ($p = 0.05$; Table 1).

Aortic reservoir function components

P_{res} increased from baseline in mild-cold (103 ± 10 to 107 ± 12 mmHg; $p = 0.01$) with no change in control (100 ± 10 to 100 ± 9 mmHg; $p = 0.93$; Fig. 4), but between conditions, there was no difference in change in P_{res} in mild-cold compared to change in control (+3.73 mmHg; 95 % CI −0.72 to 8.18; $p = 0.33$; Fig. 4). Cumulative P_{res} increased from baseline in mild-cold ($p = 0.005$; Table 1) with no change in control ($p = 0.12$; Table 1) but there was no difference in change in cumulative P_{res} between conditions ($p = 0.35$; Table 1). Additionally, the increase in cumulative P_{res} from baseline in mild-cold was still significant when diastolic pressure was deducted ($p = 0.03$; Table 1), and cumulative P_{res} minus diastole did not change in control ($p = 0.51$; Table 1). However, between conditions, the change in cumulative P_{res} minus diastole was not different compared to change in control ($p = 0.65$; Table 1).

There was no change in P_{ex} from baseline in mild-cold ($p = 0.90$; Table 1), or control ($p = 0.90$; Table 1), and there was no difference in change between conditions ($p = 0.65$; Table 1). However there was a decrease in time to P_{ex} from baseline in mild-cold (116 ± 18 to 106 ± 16 ms; $p = 0.001$) with no significant change in control (116 ± 19 to 113 ± 12 ms; $p = 0.28$; Fig. 4), and between conditions time to P_{ex} decreased in mild-cold compared to change in control (−6.7 ms; 95 % CI −15.4 to 2.03; $p = 0.01$; Fig. 4).

Blood pressure

Brachial systolic BP trended higher from baseline in mild-cold (123 ± 17 to 126 ± 17 mmHg; $p = 0.06$) with no change in control condition (121 ± 15 to 121 ± 15 mmHg; $p = 0.97$; Fig. 5). However, between interventions there was no difference in change in brachial systolic ($p = 0.35$) or diastolic BP ($p = 0.49$) in mild-cold compared to change in control (Fig. 5).

Aortic systolic BP increased from baseline in mild-cold (109 ± 18 to 113 ± 19 mmHg; $p = 0.004$), with no significant change in control (106 ± 15 to 107 ± 16 mmHg; $p = 0.79$; Fig. 5). However, between interventions there was no difference in change in aortic systolic ($p = 0.14$; Fig. 5) or diastolic BP ($p = 0.52$; Table 1) in mild-cold compared to change in control.

Pulse pressure

Aortic PP increased from baseline in mild-cold ($p = 0.03$) with no change in control ($p = 0.81$; Table 1) yet there was no significant change in brachial PP in either mild-cold ($p = 0.57$) or control from baseline ($p = 0.66$; Table 1). However, between interventions, neither aortic ($p = 0.24$)

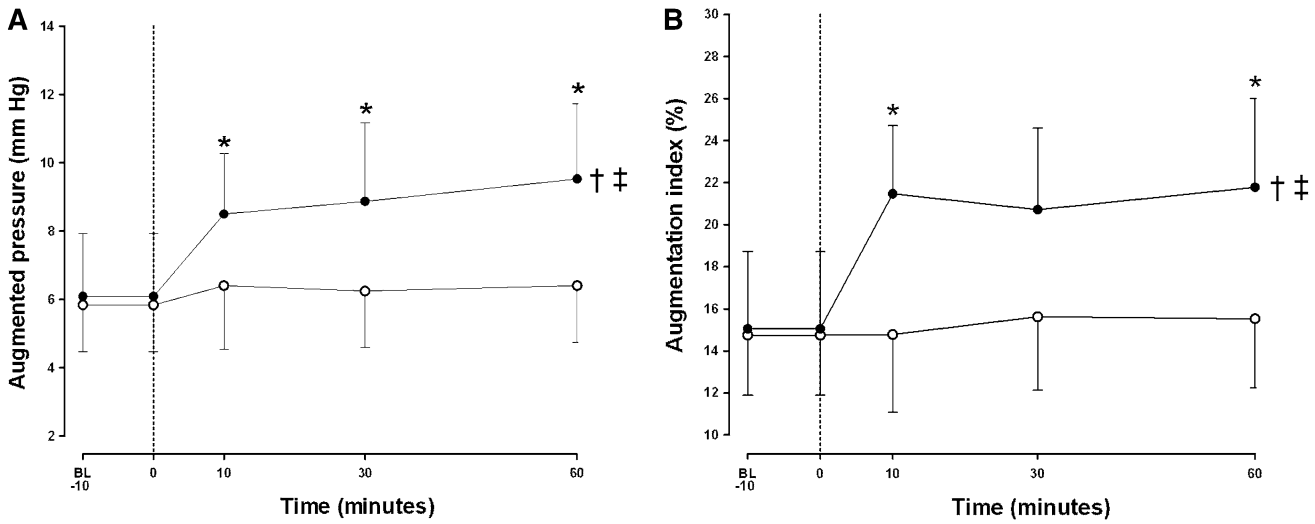

Fig. 3 Augmented pressure (**a**), and augmentation index (**b**) in mild-cold (12 °C; *closed circles*) and control (21 °C; *open circles*). Data presented as mean ± SE; $n = 16$; baseline data (BL/−10 min at ambient laboratory temperature) was obtained ~10 min before entry to climate chamber (0 min; *dashed vertical line*); *significantly different from control at specific time point; †significant change within condition; ‡significant difference between conditions (i.e. difference in change in mild-cold compared with change in control condition)

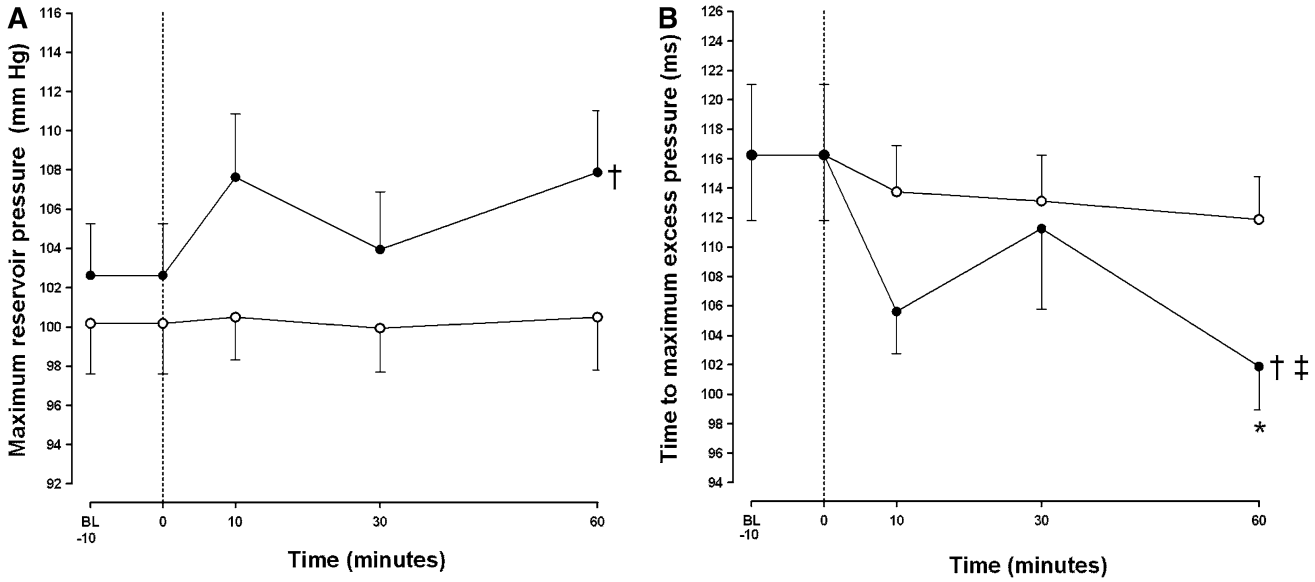

Fig. 4 Maximum reservoir pressure (**a**) and time to maximum excess pressure (**b**) in mild-cold (12 °C; *closed circles*) and control (21 °C; *open circles*). Data presented as mean ± SE; $n = 16$; baseline data (BL/−10 at ambient laboratory temperature) was obtained ~10 min before entry to climate chamber (0 min; *dashed vertical line*); *significantly different from control at specific time point; †significant change within condition; ‡significant difference between conditions

nor brachial PP ($p = 0.38$) changed in mild-cold, compared to change in control (Table 1).

Rate pressure product

Rate pressure product (RPP) was decreased from baseline in control condition ($p = 0.05$), but was maintained (no significant change) in mild-cold, and there was no difference in change in RPP between conditions ($p = 0.47$; Table 1).

Mean blood pressure

MBP increased from baseline in mild-cold ($p = 0.03$) with no significant change in control ($p = 0.71$; Table 1). Yet between interventions there was no difference in change in

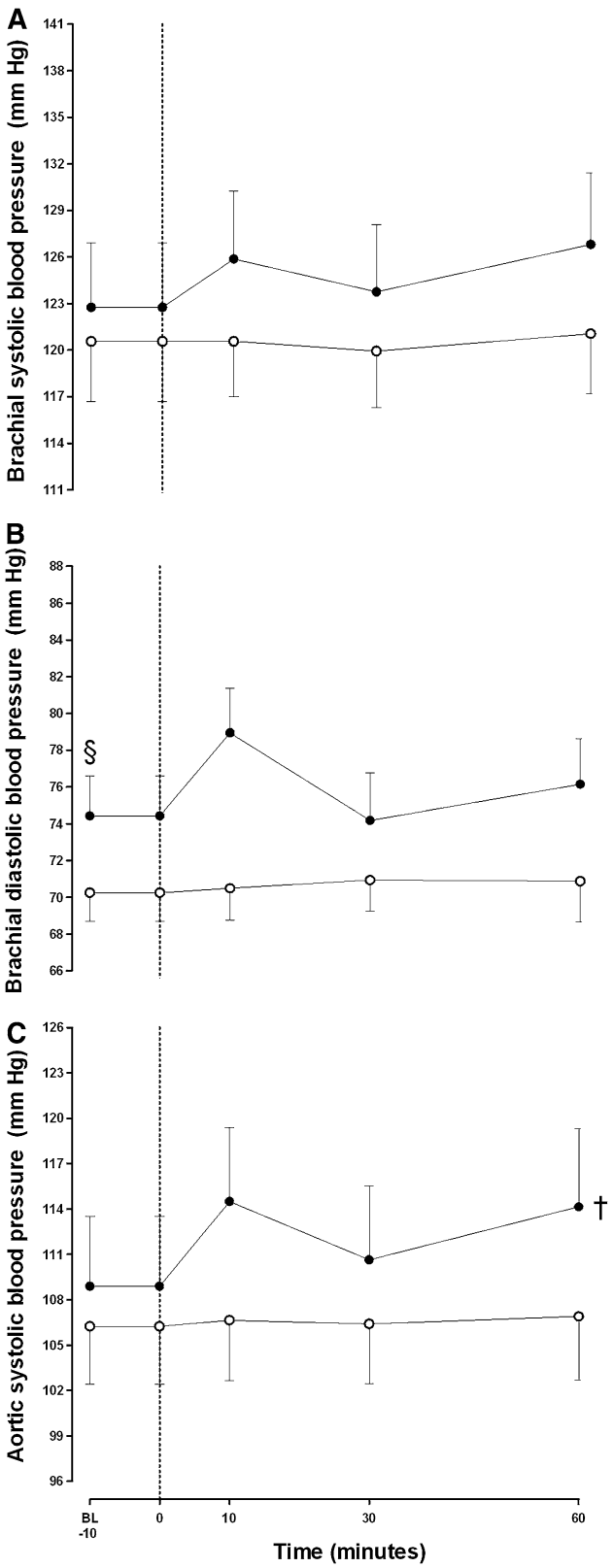

Fig. 5 Brachial systolic (**a**) and diastolic (**b**), and aortic systolic blood pressures (**c**) in mild-cold (12 °C; *closed circles*) and control (21 °C; *open circles*). Data presented as mean ± SE; $n = 16$; baseline data (BL/−10; ambient laboratory temperature) was obtained ~10 min before entry to climate chamber (0 min; *dashed vertical line*); *significantly different from control at specific time point; †significant change within condition; §significantly different from control at baseline

MBP in mild-cold compared to change in control ($p = 0.45$; Table 1).

Discussion

The novel findings of this study were, first, when compared to a control condition (21 °C), exposure to whole-body mild-cold (12 °C) for 60 min leads to increased central haemodynamic stress and LV systolic afterload (AP and AIx). Second, we show for the first time that mild-cold exposure alters peak aortic in-flow timing without changing peak in-flow volume. Third, within condition there was an increase in brachial arterial stiffness in mild-cold, with no influence on aortic arterial stiffness. Finally, we found that within condition, the increase in markers of LV systolic load and central haemodynamic stress (i.e. AP, AIx, aortic BP, aortic PP, and P_{res}) occurred in the absence of a significant change in conventional brachial BP measures.

Our results demonstrating a higher AIx in response to cold exposure are in agreement with previous studies that have investigated the effect of cold stress on measures of central haemodynamics (Casey et al. 2008; Edwards et al. 2006, 2008; Geleris et al. 2004; Hess et al. 2009; Moriyama and Ifuki 2010). However, this is the first study to our knowledge to investigate the effects of whole-body exposure to a mild-cold and a control condition using a climate chamber. Two previous studies (Edwards et al. 2006; Hess et al. 2009) have investigated the effects of whole-body cold exposure on AIx. The present investigation is most similar to Edwards et al. (2006) where a climate chamber was used at 4 °C (with fans to create 6 m/s wind chill) for 30 min, and a ~16 % increase (absolute value) in AIx was reported in the cold condition, with no change in AIx in the control condition (24 °C). The cold temperature used in that study (4 °C) was much lower than the present study (12 °C), which may explain the smaller difference in AIx between our cold and control conditions (+6 % absolute value; Fig. 3) than that observed by Edwards et al. (2006).

Our results for AIx are more comparable to a whole-body cooling study by Hess et al. (2009), where measures from a mild-cold trial using 15–18 °C water (perfused through a whole-body tube-lined suit that covered the body, but not the head, hands, and feet) were compared to those taken during a control trial using 35 °C water. The difference in AIx between experimental conditions in that study was +7 % in young and +8 % (absolute values) in older participants, with no changes observed during control

trials (Hess et al. 2009). The temperature difference between control and mild-cold conditions in the current study was −9 °C, compared to −20 °C in Edwards et al.'s (2006) study, and −18.5 °C in Hess et al.'s (2009) study. Despite the comparable temperature gradients between mild-cold and control conditions in Edwards et al. (2006) and Hess et al.'s (2009) studies, their AIx results are strikingly different. It is possible that the larger effect of whole-body cold exposure on AIx (+16 %) observed by Edwards et al. (2006) was the cumulative effect of the colder conditions than in the present study, plus the systemic effects of shivering (Sessler 2009). Indeed, the more comparable AIx results between our study (+6 %), and with those of Hess et al.'s (+7/8 % (2009), may be because both studies were designed to avoid the physiological stress of shivering.

According to traditional wave-impedance theory, changes in AIx are thought to be highly dependent on altered magnitude and timing of pressure waves reflected from distal bifurcations in the aorta, which may occur chronically or acutely (Nichols et al. 2008; Nichols and O'Rourke 2005). It is believed that these reflected pressure waves affect AIx more so than PWV (McEniery et al. 2005; Nichols and O'Rourke 2005). Kelly et al. (2001) reported that infusion of angiotensin II (a vasoconstrictor) increased AIx relatively independently from aortic and brachial PWV, and suggested this difference was because changes in diameter of small muscular arterioles involved in peripheral vasoconstriction increased the intensity of pressure wave reflections in the aorta and consequently increased AIx. In contrast, acute changes in vasomotor tone in that study did not affect the elastic aorta and thereby, left aortic PWV largely unaffected (Kelly et al. 2001). Additionally, peripheral vasoconstriction in that study was found to have a greater effect on brachial PWV than aortic PWV (Kelly et al. 2001). The findings of Kelly et al. (2001), albeit via different methods, are similar to the current study's findings, and highlight that AIx and PWV may change independently of each other in response to peripheral vasoconstriction.

However, such physiological explanations for increased AIx that are based on the premise that reflected waves from distal arterial sites augment systolic load and thereby, AP and AIx [a concept that is central to traditional wave-impedance theory (Nichols and O'Rourke 2005)], have recently been challenged by an emerging reservoir-wave paradigm (Davies et al. 2010; Tyberg et al. 2008; Wang et al. 2003). Reservoir-wave theory is based on the integration of Otto Frank's Windkessel model (Frank 1899) that accounts for the buffering or *reservoir* effect of the elastic aorta (Davies et al. 2007; Westerhof et al. 2009), plus the effects of reflected waves from classical wave-impedance theory (Nichols and O'Rourke 2005). Recent research suggests that total aortic systolic pressure is composed largely of a P_{res} plus a smaller pressure component (related to aortic in-flow and travelling waves) named P_{ex} (see Fig. 1) (Davies et al. 2007, 2010; Wang et al. 2003).

It has recently been proposed (Wang et al. 2003), and supported by human clinical trials (Davies et al. 2010; Heffernan et al. 2010; Sharman et al. 2009), that the reservoir function of the aorta plays a larger role in determining the shape of the pulse waveform than traditional wave-impedance theory postulates. When P_{res} is taken into account, the AP from reflected waves is markedly reduced (Davies et al. 2010), or negligible (Wang et al. 2003) under normal resting conditions, suggesting P_{res} to be the greatest contributor to AP (and thereby AIx), with only a small contribution from backwards wave motion, and minimal contribution from incident (or forward) pressure waves (Davies et al. 2010). Further, Wang et al. (2011) recently reported that the reflected waves which are supposedly responsible for the late systolic peak of the pressure waveform (AP), were potentially due to proximal negative reflections of the forward decompression wave (a pressure wave resulting from diastolic suction and LV relaxation which decelerates forward flow), and not as a result of pressure wave reflections from any distal arterial site (Wang et al. 2011). However, Wang et al.'s (2011) study used a canine model, therefore human studies are needed to verify these experiments. To date there is minimal human experimental research on reservoir function, and to our knowledge this is the first data available on the effect of temperature changes on aortic reservoir function. Based on the recent findings of Wang et al. (2011) and Davies et al. (2010) it is unlikely that the increase in AP, AIx and aortic systolic BP that we observed during mild-cold exposure was due to reflected waves per se, but more likely from the increase in P_{res}.

The aortic reservoir has recently been found to be responsive to peripheral vasomotor changes in humans (Sharman et al. 2009). Therefore, increased AP, AIx, aortic systolic, and P_{res}s during mild-cold exposure in the current study were possibly the result of peripheral vasoconstriction causing reduced peripheral blood run-off and increased impedance to aortic outflow (Belz 1995). This may create a situation in which aortic in-flow exceeds aortic out-flow capacity and this imbalance may have increased aortic P_{res} and altered timing of P_{ex} in the present study. This theory is consistent with data from previous studies that demonstrated increased preload and afterload (a coronary blood flow mismatch) during cold stress (Muller et al. 2011; Wilson et al. 2010).

Currently very little is known about the timing of P_{ex} in humans. In a canine model, P_{ex} varies with time and location along an artery and peaks during systolic in-flow,

but is at its lowest during diastole (Aguado-Sierra et al. 2008). Other experiments in dogs suggest that the P_{ex} waveform is almost identical to the aortic in-flow waveform (Wang et al. 2003, 2011). Further, it has been shown mathematically that P_{ex} is the additional pressure needed to overcome P_{res} (i.e. afterload) and drive forward flow into the aorta during systole (Alastruey 2010). In the current study, the reduced time to P_{ex} during mild-cold exposure might be associated with increased LV systolic afterload (increased AP, AIx, aortic systolic BP, and maximum and cumulative P_{res}). This, together with the reduced heart rate may have resulted in P_{ex} (i.e. peak aortic in-flow and excess LV work above P_{res}) occurring sooner, but without an increase in peak flow volume (as suggested by the lack of change observed in P_{ex} in the present study). Well-designed prospective human studies using invasive measurement of the aortic pressure waveform during cold exposure are required to confirm these suppositions.

To the best of our knowledge no previous studies have examined changes in aortic or brachial PWV during whole-body mild-cold exposure using a climate chamber. Only one study has investigated whole-body cooling effects on PWV, where a water-perfused suit was used to elicit mild-cooling for 20 min (Hess et al. 2009). Hess et al. (2009) observed an increase of ~11 % in aortic PWV and ~13 % in brachial PWV for older healthy adults (65 ± 2 years) but no change in either aortic or brachial PWV in younger adults (25 ± 1 years) during mild-cold exposure. Although we did not observe any significant differences in aortic or brachial PWV between the mild-cold and control conditions, within condition, brachial PWV increased (+8 % from baseline; Fig. 2) with little change in aortic PWV (+1 % from baseline; Fig. 2) in the mild-cold condition, with no significant change in control trials. A greater increase in brachial PWV than aortic PWV during whole-body mild-cooling is consistent with the results of Hess et al. (2009). Cold exposure leads to peripheral vasoconstriction (Stocks et al. 2004) which can manifest as increased peripheral arterial stiffness as measured in the muscular brachial artery by brachial PWV (Kelly et al. 2001). However, the absence of change in aortic PWV in the current study may indicate that the mild-cold stimulus used was insufficient to cause a passive increase in stiffness of the elastic aorta.

In the current study, certain central haemodynamic measures (i.e. BPs, PPs) increased without a significant increase in the traditional brachial equivalents of these measures. This differential central–peripheral effect has been reported in some (Casey et al. 2008; Edwards et al. 2006, 2008), but not all (Hess et al. 2009) previous cooling studies. Additionally, a peak was observed in certain variables at the 10-min timepoint during the mild-cold trials (i.e. AIx, P_{res}, time to P_{ex}, and BPs; Figs. 3, 4, 5) in the current study. Taken together, these results suggest that even short-term mild-cold stimulus places strain on the CV system which may be masked by the measure of brachial BP alone.

The increase in central haemodynamic stress and LV afterload observed in cooled, resting healthy individuals in the current study may help to explain the higher incidence of cold-related CV mortality (Danet et al. 1999), particularly noted in people with CV risk factors (O'Neill and Ebi 2009). Diseased coronary arteries are known to constrict in vitro, rather than dilate when exposed to cold stimulus (Nabel et al. 1988), and in vivo, cold exposure causes reduced coronary perfusion and increased myocardial oxygen demand in older adults (Gao et al. 2012). Together, cold-induced myocardial ischaemia, combined with increased LV afterload could be potential contributory factors adding to the increased risk of CV events in older individuals with underlying atherosclerosis.

The study has certain limitations which should be considered. First, core temperatures were estimated using infra-red tympanic thermometry which is somewhat dependent on user skill and can over or underestimate actual core temperatures (Farnell et al. 2005). However, core and skin temperature measures were used only to detect any shifts in temperature. Therefore as a surrogate of core temperature, tympanic thermometry was deemed more appropriate than telemetry pill sensors and rectal thermometry, which also have their shortcomings and are less tolerable for participants (Lim et al. 2008). To ensure tympanic temperatures were as accurate as possible, operators were trained in the use of the thermometer according to accepted techniques (Davie and Amoore 2010; McCarthy and Heusch 2006) and the same operator collected data for individual subjects for both test sessions. Second, a light blanket was used at times by some participants to avoid shivering in the mild-cold condition. This extra thermal insulation could have maintained skin temperatures and potentially obscured larger changes in grouped hemodynamic variables that might have been seen without a blanket. However, one of the considerations when designing the protocols of this study was to avoid the physiological stress of shivering, thus the addition of the blanket eliminated the exaggerated haemodynamic stress responses that would have accompanied shivering (Sessler 2009). Lastly, the chosen control temperature of 21 °C may have been cooler than thermoneutral as others have suggested (Laurent et al. 2006). However, 21 °C was similar to our laboratory temperature which was deemed comfortable for people resting in light clothing. The coolness of the control condition may have underestimated the differences in haemodynamic parameters between a thermoneutral and a mild-cold condition.

In summary, whole-body mild-cold exposure for 60 min increases central haemodynamic stress and LV systolic

load (AP and AIx), and alters timing of peak aortic in-flow (time to P_{ex}) in resting, healthy adults. These responses may be associated with peripheral vasoconstriction and increased muscular arterial stiffness (brachial PWV), which alters aortic reservoir function (increased P_{res}) but does not affect large elastic artery stiffness (aortic PWV). While the current study focussed on healthy individuals, patients with stable CV disease or type 2 diabetes mellitus might be suitable future target groups to establish central haemodynamic responses to mild-cold exposure in higher-risk individuals.

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Ethical standards The study design was approved by the Human Research Ethics Committee (Tasmania) Network (approval number H0011347) and adhered to the principles of the declaration of Helsinki. All participants provided written informed consent.

Conflict of interest None of the authors has a conflict of interest to disclose.

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