Haemodynamic Responses to Climate Variations in Healthy Individuals and those with Type 2 Diabetes Mellitus

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BHM (Ex. & Sp. Sci.) Hons

Dissertation submitted in fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

In the School of Human Life Sciences

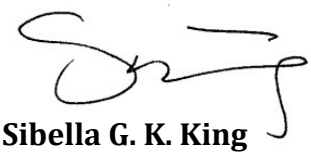
University of Tasmania, Launceston, Tasmania

JULY, 2013

DECLARATIONS

Statement of Originality

I hereby declare that this thesis entitled "haemodynamic responses to climate variations in healthy individuals and those with type 2 diabetes mellitus" contains no material which has been accepted for a degree or diploma by the University of Tasmania or any other institution, expect by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material has previously been published or was written by another person except where due reference is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.



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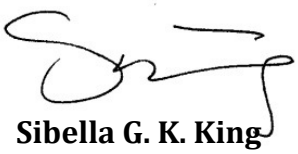

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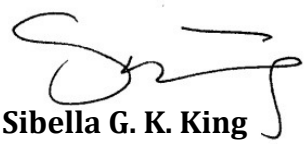
Statement of Ethical Conduct

The research associated with this thesis abides by the guidelines of the Human Research Ethics Committee (Tasmania) Network: Approval numbers: H0010849 and H0011347.



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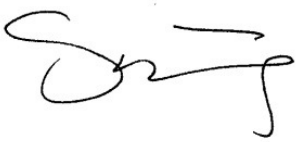
Statement of Co-Authorship of Jointly Published Work

I was first author of the manuscript, an edited version of which comprises Chapter 4, that was published as:

> KING, S., AHUJA, K., WASS, J., SHING, C., ADAMS, M., DAVIES, J., SHARMAN, J. & WILLIAMS, A. 2013. Effect of whole-body mild-cold exposure on arterial stiffness and central haemodynamics: A randomised, cross-over trial in healthy men and women. *Eur J Appl Physiol, 113 (5), 1257-1269*. DOI: 10.1007/s00421-012-2543-1.

Candidate



Sibella G. K. King

Date

7th July 2013

Primary Supervisor

Andrew D. Williams

Date

7th July 2013

Statement of Candidature Contribution to Thesis

The thesis comprises two research investigations which have been completed almost entirely by the candidate, **Sibella King**. However, the following people also contributed to each of the studies as detailed:

- **Sibella King (Human Life Sciences, University of Tasmania):** Study design, lead role in recruitment, data collection, data analysis and interpretation, first author on all manuscripts (Chapters 4 to 7).
- **Andrew Williams (Human Life Sciences, University of Tasmania)**: Study design, data collection (cannulations), statistical analysis (planning), data interpretation, manuscript revisions.
- **Kiran Ahuja (Human Life Sciences, University of Tasmania)**: Study design, data collection (cannulations), statistical analysis (planning), data interpretation, manuscript revisions.
- **Murray Adams (Human Life Sciences, University of Tasmania):** Study design, manuscript revisions
- **Cecilia Shing (Human Life Sciences, University of Tasmania):** study design, manuscript revisions
- **James Sharman (Blood Pressure Group, Menzies Research Institute, Tasmania):** Study design, data interpretation, manuscript revisions
- **Justin Davies (National Heart and Lung Institute, Imperial College London, UK):** Data analysis, data interpretation, manuscript revisions
- **Jezreel Kay (Human Life Sciences, University of Tasmania):** Assisted with data collection, revisions on manuscripts 1 and 2 (Chapters 4 and 5).
- **Laura Nicholson (Human Life Sciences, University of Tasmania)**: Assisted with some data collection
- **Melissa Williams (Human Life Sciences, University of Tasmania)**: Assisted with some data collection

Specific contributions to Chapters 4 and 5

- From October 2009 to October 2010 Jezreel Wass assisted with data collection in the Healthy Group Study for her Honours project which was completed on results from a small sample of participants in this study. Some additional assistance with data collection was provided by Melissa Williams as part of a practicum unit.

- Aortic reservoir component data were calculated in collaboration with Dr. Justin Davies from provided pulse wave analysis data from the Healthy Group Study.

Specific contributions: Chapter 6 and 7

- From March to May 2012, Laura Nicholson, an undergraduate Exercise Science Summer Scholarship student, providing general assistance for data collection on the T2DM Group Study.

- Aortic reservoir component data were again calculated in collaboration with Dr. Justin Davies from provided pulse wave analysis data from the T2DM Group Study.

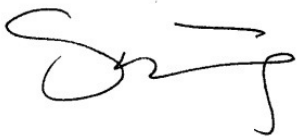
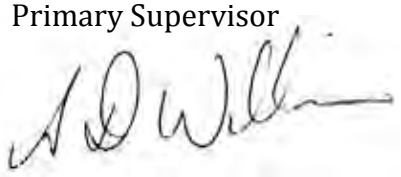
Percent contributions to the Healthy Group Study

Sibella King (70%), Kiran Ahuja (7%), Jezreel Wass (1.8%), Melissa Williams (0.2%), Cecilia Shing (4%), Murray Adams (3%), Justin Davies (3%), James Sharman (4%), and Andrew Williams (7%).

Percent contributions to the T2DM Group Study

Sibella King (71.5%), Kiran Ahuja (7%), Laura Nicholson (0.5%), Cecilia Shing (4%), Murray Adams (3%), Justin Davies (3%), James Sharman (4%), and Andrew Williams 7%).

We, the undersigned agree with the above stated "proportion of work undertaken" for each of the published or submitted peer-reviewed manuscripts which contribute to this thesis:

Candidate	Primary Supervisor
	
Sibella G. K. King	**Andrew D. Williams**
Date	Date
7th July 2013	7th July 2013

CONFERENCE PRESENTATIONS

The following presentations arising from the thesis have been delivered by the candidate:

September 2010: University of Tasmania, Confirmation Presentation to Faculty of Human Life Sciences; Oral. King, Sibella, *PhD Overview: Investigations into Haemodynamic Function in Type 2 Diabetes Mellitus*

October 2010: University of Tasmania, Collaborative Graduate Research Symposium; Oral. King, Sibella, *PhD Overview: Investigations into Haemodynamic Function in Type 2 Diabetes Mellitus*

November 2010: University of Tasmania, Student Excellence in Research Conference; Oral. King, Sibella, *Effect of acute temperature and humidity changes on haemodynamic function*

November 2011: University of Tasmania, Student Excellence in Research Conference; Poster defence. Sibella G. King, Kiran D.K. Ahuja, Jezreel Kay, Cecilia M. Shing, James E. Sharman, Justin E. Davies, Andrew D. Williams. *Effect of acute mild cold exposure on central haemodynamics* (a copy of this poster is presented in Appendix 1).

ACKNOWLEDGEMENTS

Foremost, I would like to thank and acknowledge the support of my supervisors; Dr. Andrew Williams, Dr. Kiran Ahuja, and Dr. Cecilia Shing for their encouragement, feedback and invaluable help with all aspects of this thesis, for keeping me motivated when I needed it (Kiran!), and for always giving their time when asked. Sincere thanks to Assoc. Prof James Sharman for discussions and feedback regarding data interpretation and manuscript direction. My thanks to Jezreel Wass, Melissa Williams and Laura Nicholson for their help with data collection. Thanks to my co-authors on the manuscripts, Dr. Murray Adams, and Dr. Justin Davies for their valuable contributions and excellent feedback on drafts. I am grateful to the Clifford Craig Medical Research Trust, Tasmania for funding (Grant number: W0018838), and to the School of Human Life Sciences, University of Tasmania for providing additional funding to undertake my studies. The assistance of Dr. Iain Roberston is acknowledged for statistical planning, design, and randomisation of participants into the climate trials. Thanks to Assoc. Prof. Dominic Geraghty for his advice throughout on thesis planning and for providing some stress relief in the shape of a Nerf gun. The 'MacGyver' award for technical ingenuity goes to Steven Stone and Samantha Clarke for their assistance on devising data collection methods out of available lab equipment. Deepest gratitude goes to my study participants who kindly gave me so much time and tolerance; this thesis would not have been possible without you. Thanks to my fellow PhD and masters students for a sympathetic ear and a laugh throughout this journey. Thanks to my sisters and brothers at Devil State Derby League for being my rock and my roll. A very special thank you to my family and friends: My mum Helen, who has been incredibly supportive throughout, providing endless food, sympathy and babysitting. To my beautiful kids, Mia and Jamie for being so patient for the past several years; I'll be back soon! To my best friends throughout this rollercoaster ride; Dan, Chris, Denise, and Ange, thanks for your patience in putting up with my absence, and keeping me sane. Finally, to every person mentioned above, for all you have taught me during this journey, thank you.

GENERAL ABSTRACT

Background and aims

Environmental cold and heat exposure are linked to increased cardiovascular (CV) morbidity and mortality. People with impaired vascular function and thermoregulation, such as individuals with type 2 diabetes mellitus (T2DM) are at higher risk of heat or cold-related illness. To date, very few studies on whole-body cold or heat exposure have included individuals with T2DM. Even fewer have used central haemodynamic indicators of CV risk such as aortic pulse wave velocity (PWV), which is a marker of aortic stiffness, or augmentation index (AIx), which signifies left ventricular (LV) load. Moreover, there are no data available of the effects of high humidity, with or without heat, on resting central hemodynamic measures in any population. The studies that comprise this thesis aimed to determine the effects of whole-body exposure to differing air temperature (cold and heat) and relative humidity (RH) on measures of central haemodynamics and arterial stiffness in resting healthy individuals, and those with T2DM.

Methods

Five climate trials were undertaken in two participant groups; a Healthy and a T2DM group. The Healthy group comprised 16 adults (10 men), aged 43±19 years, and the T2DM group included 14 participants with T2DM (8 men), aged 63 ± 7 years. Supine, resting measures included aortic and brachial PWV, aortic AIx, brachial and aortic blood pressures (BPs), and measures of aortic reservoir function including reservoir pressure (P_{res}), excess pressure (P_{ex}), and timing of P_{ex}. The five climate conditions were 21˚C with 40% RH (control), 21˚C with 80% RH (humid), 12˚C with 40% RH (mild-cold), 36˚C

with 40% RH (hot-dry), and 36˚C with 80% RH (hot-humid). Every participant in both groups completed all five climate trials on separate days, with a washout of at least 7 days between each trial. Time points for data collection were ambient baseline, then at 5 (T2DM group only), 10, 30, 60, and 90 minutes while in each climate condition. 300mL (Healthy group) and 250mL (T2DM group) of water was consumed following the 60 minute measures in each climate condition in every participant. For analysis and presentation of results, data were split into *mild-cold vs. control*, and *heat and humidity vs. control* results. Data from baseline to 60 minutes were used for main analyses, and data from 60 to 90 minutes for *heat and humidity* results were analysed separately in order to account for any possible effect of dehydration and rehydration in the hot conditions.

Results

Results indicate that in the Healthy group, a change from a comfortable ambient climate to a mild-cold climate, as commonly happens in day-to-day life, significantly increased augmentation pressure (AP; $P = 0.01$) and AIx ($P = 0.01$), and reduced time to P_{ex} ($P = 0.01$) compared to control, without significantly altering aortic PWV ($P = 0.87$). Conversely, in the T2DM group, mild-cold exposure significantly increased aortic PWV ($P = 0.03$) but elicited a smaller pressor response compared to that observed in healthy individuals; brachial and aortic systolic BPs, and mean BP increased within condition in mild-cold (all $P < 0.05$) in T2DM participants, but these measures did not change compared to control (all $P > 0.24$).

In the heat and humidity trials, it was observed that humidity at 80% significantly reduced aortic PWV during heating at 36°C in both healthy individuals and those with T2DM (both groups $P < 0.05$); a result that was not apparent when each group was

exposed to hot-dry conditions (each group $P > 0.06$). In healthy individuals, hot-humid conditions did not significantly change measures of LV load (mean BP and AIx both $P > 0.05$). However, in T2DM, mean BP was reduced similarly in all hot comparisons (all $P < 0.005$) and AIx was reduced by hot-humid ($P = 0.03$) but not hot-dry ($P = 0.31$) conditions. In the Healthy group, P_{res} was reduced only in hot-dry ($P = 0.03$) but not hot-humid conditions. However, in the T2DM group P_{res} was reduced in all hot conditions (all $P < 0.006$). The only instance where P_{ex} was significantly affected during any climate trial was during humid-heating in T2DM participants, where P_{ex} was reduced ($P < 0.05$). Finally, the studies into *heat and humidity* demonstrated that compared to control, exposure to high humidity at room temperature (i.e. independently of heat) significantly reduced aortic systolic BP ($P = 0.02$), rate pressure product ($P = 0.02$) and aortic P_{res} ($P = 0.03$) in healthy individuals, and reduced AIx in people with T2DM ($P = 0.04$).

Discussion and Conclusions

The results from the *mild-cold* studies suggest that even a brief exposure to a mild-cold temperature can increase aortic stiffness (aortic PWV) in people with T2DM and increase haemodynamic stress and LV load (AP and AIx) in apparently healthy individuals. In healthy individuals, increased AP and AIx during mild-cold exposure were potentially the result of peripheral vasoconstriction causing reduced peripheral blood run-off and increased impedance to aortic outflow. This may create a transient situation in which aortic in-flow exceeds aortic out-flow volume for the duration of the cold exposure, and this imbalance may have increased AIx and altered timing of P_{ex} in this study. However, in a T2DM population, a greater aortic stiffness and smaller pressor response than observed in healthy individuals during cold exposure is potentially a normal response. This is because of the higher likelihood of autonomic dysfunction in individuals with T2DM which impairs normal vascular reactivity and pressor responses

to cold exposure. Such acute increases in these indicators of CV risk during cold exposure may add to explanations of cold-associated morbidity and mortality in people with T2DM.

The findings of the *heat and humidity* studies show that in healthy individuals, aortic PWV was reduced by humid-heat without affecting brachial or aortic systolic or mean BPs. In T2DM individuals, aortic PWV was similarly reduced by humid-heat, but pressor responses were more variable in the heat and humidity trials than were observed for healthy people. Reductions in aortic PWV in healthy individuals and those with T2DM during humid-heating are potentially due to the increased heat load which accompanies increasing humidity, which in turn may produce a passive relaxation of the elastic aorta. This reduction in aortic stiffness may occur via flow-mediated increases in shear stress which triggers release of nitric oxide and other endogenous vasodilators that decrease large artery stiffness, and can work independently of changes in BP. The more variable pressor responses observed in the T2DM group may be due to impaired vascular reactivity which accompanies T2DM and is due to the toxic effects of chronic hyperglycaemia.

The T2DM *heat and humidity* data in this thesis are the first available that show P_{ex}, a measure of wave-related pressure and longitudinal wave reflections, was reduced only in response to whole-body humid-heat exposure in adults with T2DM. Aortic PWV is thought to be dependent on changes in mean BP, heart rate and AIx, and wave reflections. Given that in the T2DM group, mean BP was reduced and heart rates were increased similarly across all hot comparisons but aortic PWV was only reduced in hot-humid conditions, it is possible that the decreased aortic PWV in hot-humid conditions may be related to reduced wave motion (i.e. P_{ex}), P_{res}, and AIx in patients with T2DM.

Findings from the *heat and humidity* studies in healthy individuals and those with T2DM suggest that high humidity, with and without heat, can reduce measures of aortic stiffness and LV load, which may be beneficial to the CV system. The lowering effect of high humidity on arterial stiffness and haemodynamics may have particular clinical relevance for reduction of CV risk in T2DM individuals.

In conclusion, the results from this thesis show divergent haemodynamic responses between cooling and heating in people with T2DM and healthy individuals. During cooling, some haemodynamic responses to mild-cold were exaggerated in T2DM (i.e. increased aortic stiffness), and some were attenuated (i.e. pressor responses) compared to responses of healthy individuals. Conversely, during humid-heating, people with T2DM had greater pressor reductions yet similar magnitude reductions in aortic stiffness compared to healthy individuals. Results of this thesis highlight the similarities and differences between responses of healthy individuals and people with T2DM during sudden climate changes. The findings demonstrate that cold exposure is potentially detrimental to haemodynamic function, while short-term humid-heating is potentially beneficial to haemodynamic function in healthy individuals, but more particularly in individuals with T2DM.

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COMMON ABBREVIATIONS

°C	Degrees Celsius	AIx	Augmentation index
AP	Augmented pressure	BP	Blood pressure
CAD	Coronary artery disease	CAN	Cardiovascular autonomic neuropathy
CHD	Coronary heart disease	CV	Cardiovascular
CVD	Cardiovascular disease	ECG	Electrocardiogram
LV	Left ventricle / ventricular	MBP	Mean blood pressure
NO	Nitric oxide	P_{ex}	Excess pressure
PP	Pulse pressure	P_{res}	Reservoir pressure
PWA	Pulse wave analysis	PWV	Pulse wave velocity
RH	Relative humidity	RPP	Rate-pressure product
SD	Standard deviation	SEM	Standard error of the mean
T2DM	Type 2 diabetes mellitus	WBGT	Wet-bulb globe temperature
WIA	Wave intensity analysis		

Infrequent abbreviations may be used throughout the thesis and are defined *in situ*.

CHAPTER 1 – THESIS OVERVIEW

1.1 Background

There are numerous factors, both exogenous and endogenous, that affect human haemodynamic function. Sudden whole-body cold or heat exposure causes circulatory adjustments that are intended to preserve optimal core temperature and protect the vital organs from hypo- or hyper-thermia. As a consequence, blood is diverted either to the core (in the case of cold exposure) or away from the core (in the case of heat exposure) which profoundly affects brachial blood pressures (BP), and thus, left ventricular (LV) work.

Climate change is causing global temperatures to reach new record highs every year. Increasing global temperatures leads to increased weather variability and extreme environmental heatwaves are becoming more common in many parts of the world, while extreme cold events are becoming less common (IPCC, 2007). Environmental cold is strongly associated with peaks in cardiovascular (CV) morbidity and mortality which are apparent during winters and coldwaves (out-of-season extreme cold snaps). Furthermore, the association of cold with CV mortality persists after adjustment for peaks in respiratory mortality during winter. Winter excess mortality rates are highest among the frail, the elderly, and those with chronic disease (Semenza et al., 1999). Type 2 diabetes mellitus (T2DM) is Australia's fastest growing chronic disease (Diabetes Australia, 2012) and over time T2DM patients suffer autonomic and vascular dysfunction which impairs their ability to thermoregulate and adjust BP in response to external climate change (Vinik et al., 2003).

To date, very little is known about the physiological effects of exposure to sudden reductions in temperature on measures of central haemodynamic function, including aortic arterial stiffness, aortic BPs and on components of aortic reservoir function. Moreover, current evidence suggests that temperate regions of the world with definite seasonal variability have even larger cold-related mortality rates than regions with consistently cold climates. The effects of mild-cold (ranging from 10 to 15°C) which is typical of a winter day in temperate regions of the world, on central haemodynamic function is at present, a particularly under-researched area.

Short and long duration exposure to cold can increase CV morbidity and mortality risk. However, heat exposure has differing effects on human CV risk which appears to be related to duration of exposure. Prolonged exposure to heat is linked to increased morbidity and mortality, which is largely due to increased CV strain and heart rates, and to haemostatic factors (i.e. increased tendency to blood coagulation), and also to hyperthermia and heat illness. Conversely, there is increasing evidence that short-term exposure to heat has beneficial lowering effects on brachial BPs and on improving endothelial function, and thereby, vascular function. To date, studies on short-term heat therapy have largely focussed on cardiac function in healthy people and people with cardiovascular diseases (CVDs) particularly, heart failure. Thus evidence is lacking on whether beneficial effects of short-term heat are more widely applicable in other chronic disease settings such as T2DM. Scant data are available on the effects of heat, with or without humidity on measures that are indicative of CV risk such as aortic stiffness, and central haemodynamics [i.e. aortic BPs and augmentation index (AIx)] in healthy or T2DM populations, and no heat exposure studies in humans have yet included measures of aortic reservoir function. Moreover, data on the effects of humidity (whether in normal room temperatures, or combined with heat) on resting human haemodynamics

are almost entirely absent in the literature. The research presented in this thesis aimed to address specific gaps in current knowledge regarding the unique influences of external environmental climate on specific measures of haemodynamics in healthy individuals and on people living with an increasingly common chronic condition, T2DM.

Two core studies were undertaken. The first was in a group of apparently healthy participants (*n*=16). The second was in a group of people with T2DM (*n*= 14). Five climate test conditions were completed by all participants from each group in randomised order with 7 to 14 days between each test. Test conditions were: control (21˚C/40% RH; 21/40), humid (21˚C/80% RH; 21/80), mild-cold (12˚C/40%; 12/40), hot-dry (36˚C/40% RH; 36/40), and hot-humid (36˚C/80% RH; 36/80). For each test session, haemodynamic measures were taken at baseline in ambient temperatures before participants moved to a climate chamber which was set at one of the five randomised test conditions. Measures were then repeated at 5 (T2DM group only), and 10, 30, and 60 minutes once inside the climate chamber. Details of study protocols are Chapter 3, General Methods.

Given the complexity of measures and results, data from each study (healthy and T2DM groups) were split into two, and each of these are presented as two manuscripts for each group in the thesis; the aim of which was to present the results in the most logical, easy-to-read manner. For each group, data are presented as *mild-cold vs. control* (12/40 vs. 21/40) and as *heat and humidity vs. control* (21/40, 21/80, 36/40 and 36/80). For the *heat and humidity* manuscripts, each test condition was compared to the others in order to locate the source of the effect on haemodynamic measures, if any (i.e. was the influence coming from heat, or humidity, or a cumulative effect of both). The study results are presented as a series of published, and soon-to-be published

manuscripts, and as such there is a certain amount of unavoidable repetition where it was deemed necessary to explain the studies. All efforts have been made to minimise any unnecessary repetition in thesis topic content.

All measures were also taken at 90 minutes of exposure in each of the five conditions in every participant, in each group, with the aim of determining any haemodynamic effects of water intake which occurred after the 60 minute measures (300 mL for healthy group, 250 mL for T2DM group). The effect of dehydration during the cold exposures was deemed to be negligible, thus the 60 to 90 minute data were not used in the analysis of results for the mild-cold studies in both groups (Chapters 4 and 6). However, for the heat and humidity data, a sub-analysis was performed on the 60 to 90 minute data in both groups, and results are reported as effect of dehydration and rehydration in results sections of Chapters 5 and 7.

Another sub-analysis of data of the main variables was undertaken in the T2DM group to account for any potential influence of background, or "baseline" cardiovascular autonomic neuropathy (CAN) in that group. However, as three T2DM participants tested positive to more than one CAN test (out of the three CAN tests undertaken), it was decided to use the unadjusted data for the main analysis as it was deemed invalid to base conclusions on a dataset with only three participants. Details of CAN testing and criteria for likelihood of CAN are in Chapter 3, General Methods, and results of the CAN sub-analyses in T2DM data are in Results sections of Chapters 6 and 7.

1.2 Thesis Organisation

- This chapter **(Chapter 1)** contains a general introduction of the themes comprising the thesis and expresses the rationale, aims, study designs, and general layout of the thesis.

- **Chapter 2** presents a review of prior literature which explores the themes of the thesis. Here I describe how haemodynamic measures have evolved to the current non-invasive methods used in this thesis. I introduce T2DM; the co-morbidities and current treatments for the disease, and describe determinants of vascular function in healthy and T2DM individuals. Additionally, the latest knowledge on the specific topics that underpin the thesis - the effects of heat, cold, and humidity on haemodynamic function in healthy individuals and people with T2DM - are detailed in this chapter.

- **Chapter 3** presents the general methods used in the studies. Included here are detailed recruitment methods, data collection timelines, data collection methods, daily protocols, and statistical analyses used for each study.

- **Chapter 4** presents the first manuscript from the Healthy Group Study, which aimed to determine how a sudden change from a comfortable ambient climate, to a mild-cold climate affects central haemodynamics in resting, healthy individuals. It was hypothesised that, compared to a control condition (21°C), 60 minutes of exposure to mild-cold (12°C) would increase central haemodynamic stress in a healthy adult population. Sixteen healthy men and women participated in this study.

An edited version of this manuscript was published as:

> Sibella G. King, Kiran D. K. Ahuja, Jezreel Wass, Cecilia M. Shing, Murray J. Adams, Justin E. Davies, James E. Sharman, and Andrew D. Williams. (2013). Effect of whole-body mild-cold exposure on arterial stiffness and central haemodynamics: a randomised, cross-over trial in healthy men and women. *Eur J Appl Physiol*. 113 (5), 1257-1269. DOI 10.1007/s00421-012-2543-1

The novel findings from this study were that whole-body mild-cold exposure in healthy individuals increased brachial PWV, and measures of central haemodynamic

stress and LV systolic load (AP and AIx), and altered timing of peak aortic in-flow [time to excess pressure (P_{ex})] without changing peak in-flow volume (P_{ex}) or aortic PWV.

- **Chapter 5** presents the second manuscript from the study in healthy individuals, comparing the results of exposure to a control condition, a humid condition, a hot-dry, and a hot-humid condition. The hypotheses tested were that 60 minutes of whole-body heat exposure at low and high RH would reduce LV afterload including AIx, aortic systolic BP and mean BP, aortic and brachial PWV and aortic P_{res} in healthy individuals.

The novel findings from this study were that heat at either high and low humidity reduced brachial PWV, but aortic PWV was reduced only in hot conditions with high humidity. At room temperature, brachial and aortic systolic BPs, aortic PP and P_{res} were lower in conditions with high humidity compared to low humidity, but this reducing effect of humidity did not occur when conditions were already hot. Contrary to the hypothesis, AIx was not affected by any combination of conditions in this study. An edited version of this manuscript is currently under review for publication.

- **Chapter 6** presents a manuscript which details how sudden exposure to mild-cold affects central haemodynamics in people with T2DM. The hypotheses tested were that 60 minutes whole-body, mild-cold exposure would increase markers of CV risk and LV load (i.e. aortic PWV, aortic AIx and aortic systolic BP and mean blood pressure) in a group of individuals with T2DM . The novel findings from this study were that whole-body exposure to mild-cold for 60 minutes increases aortic PWV (a measure of CV risk), without increasing brachial artery stiffness, and produces a modest pressor response in measures of LV load (mean, brachial and aortic systolic BPs), compared to the control condition. An edited version of this manuscript will shortly be submitted for publication.

- **Chapter 7** presents the manuscript of results from the second part of the T2DM group study, which details and compares the effects of exposure to identical hot-dry (36/40), hot-humid (36/80), humid room temperature (21/80) and control conditions (21/40) as in the Healthy group. The hypotheses tested were that 60 minutes of whole-body heat exposure (at low and high RH) would reduce arterial stiffness (aortic and brachial PWV) and measures of LV afterload (AIx, aortic systolic BP and mean BP) in adults with T2DM. The novel findings from this study were that aortic PWV was reduced by heat only when humidity was high, and brachial PWV was not affected by any combination of condition. Pressor responses were varied between conditions, with brachial and aortic pulse pressures, and P_{ex} reduced only by heat with high humidity, while aortic and brachial systolic BPs were reduced equally by dry (36/40) and humid-heat (36/80). However, diastolic and mean BP and P_{res} had slightly larger reduction in dry-heat than in humid-heat. Finally, AIx was not affected by dry-heat, but was reduced by humid-heat compared to control (i.e. 36/80 vs. 21/40), and was the only variable affected by high humidity *per se*, in normal room temperatures (i.e. 21/80 vs. 21/40). An edited version of this manuscript will soon be submitted for publication.

- **Chapter 8** presents a contrast and general discussion of the results of the Healthy and T2DM studies. This chapter outlines the novel contributions and implications of the findings to current knowledge on the impacts of acute changes in environmental climate on haemodynamics in healthy individuals and those with T2DM.

- Finally**, Chapter 9** presents the potential limitations and future studies arising from the thesis, and concluding comments.

CHAPTER 2 – LITERATURE REVIEW

2.1 Haemodynamics

2.1.1. Overview of blood pressure measurement

The human circulatory system is essentially composed of a pump, the heart, which forces blood at regular intervals into a branched system of tubes, the arteries and veins. The term "haemodynamics" refers to the adaptable control of blood pressure (BP; the pulsatile force exerted by the blood onto the artery walls) and blood flow through the heart and vessels (Nichols and O'Rourke, 2005). Despite normal and frequent fluctuations due to endogenous and exogenous stimuli, BP is kept relatively stable in healthy individuals by means of several homeostatic mechanisms, involving cardiovascular (CV) and hormonal adjustments. However, when BP is very low, also known as hypotension, or higher than optimal, i.e. hypertension, health complications can ensue (U.S. Department of Health and Human Services, 2004). Hypotension is not considered pathological unless neurologic symptoms, for instance dizziness, syncope or seizure, are present (American Heart Association, 2012). On the other end of the scale, a hypertensive crisis is a rare, yet life-threatening acute increase in BP with evidence of organ damage, which may accompany cerebral haemorrhage or acute myocardial infarction (Feldstein, 2007). Current resting brachial BP classifications and ranges (U.S. Department of Health and Human Services, 2004) are presented in Table 2.1.

Table 2.1. Brachial blood pressure classifications

Category	Systolic BP (mm Hg)	Diastolic BP (mm Hg)
Hypotensive	≤ 90	≤ 60
Optimal/normal	< 120	< 80
Pre-hypertensive	120 – 139	80 – 89
Hypertension	≥ 140	≥ 90
Hypertension stage 1	140 – 159	90 – 99
Hypertension stage 2	160 – 180	100 – 110
Hypertensive crisis	> 180	> 110

Table modified from (U.S. Department of Health and Human Services, 2004)

Hypertension can be categorised as primary, or essential hypertension which has no other known cause, and secondary, which is hypertension caused by another condition (U.S. Department of Health and Human Services, 2004). Uncontrolled chronic hypertension is a major modifiable risk factor for atherosclerosis and arterial disease, stroke, aortic aneurism, acute myocardial infarction, heart failure, and chronic renal disease (Carretero and Oparil, 2000). Hypertension is associated with older age and type 2 diabetes mellitus (T2DM) (Redon et al., 2008), and is considered a risk factor for cardiovascular disease (CVD) and death as it increases the work that the heart must perform with each beat in order to maintain adequate cardiac output for tissue perfusion (Lewington et al., 2002). As hypertension and vascular dysfunction are often concomitant with T2DM, the regular monitoring of brachial BP is regarded as an essential health management tool for people with chronic conditions, such as diabetes (Potenza et al., 2009).

2.2 Haemodynamic measurement

2.2.1. History of blood pressure measurement

Analysis of the pulse was central to diagnosis and therapy in the ancient cultures of Egypt, Greece, India and China (Nichols and O'Rourke, 2005). However, the documented chronicle of haemodynamic study began with the work of Galileo Galilee (1564-1642) who influenced a student of his, William Harvey (1579-1657) who went on to write on the motion of the heart and blood flow in animals. Isaac Newton (1642-1727) then advanced understanding of fluid viscosity and influenced modern views of arterial pressure and flow relationships (O'Rourke, 1990).

2.2.2. Brachial and aortic blood pressures

The measure of brachial BP as we know it today can be traced back to the work of Scipione Riva-Rocci (1863 – 1937) and then Harvey Cushing (1869-1939) who each successively improved upon the basic brachial cuff sphygmomanometer originally invented by Samuel von Basch (1837-1905) over 100 years ago (Roguin, 2006). Current medical practice utilises the exact same methods today; an inflated cuff placed around the upper arm, occludes blood flow in the brachial artery, and a stethoscope or an automated device is used to detect the sounds characteristic of distorted blood flow in the brachial artery as the occluded arteries are released. The pressure at which the first sounds of the heart beat are heard, and when the sound muffles, or disappears, determines systolic and diastolic pressures respectively (O'Rourke, 1990). The resulting measures give an estimate of the BPs as the heart is at the peak of contraction, i.e. systole, and at its' lowest pressure during relaxation and filling, i.e. diastole (O'Rourke, 1990).

While commonly used, the brachial-cuff method of BP measurement has its drawbacks. The cuff size, its placement on the upper-arm, the arm position in relation to

the heart, and many other factors can adversely affect the accuracy of brachial cuff BP when compared to intra-arterial pressures in the brachial artery measured via direct catheterisation (U.S. Department of Health and Human Services, 2004). Moreover, BP at the brachial arteries is typically 20 to 50% higher than BP at the aorta (Karamanoglu et al., 1993). This central-peripheral pressure difference is thought to be due to increasing resistance, or stiffness, and increased pressure wave reflection intensity in the small, muscular conduit arteries of the periphery, compared to the large elastic aorta (Kampus et al., 2011). The amplification of the pressure pulse as it travels from the aorta along the arterial tree to the brachial arteries means that brachial BP does not accurately reflect the BP load at the heart (Nichols and O'Rourke, 2005). Aortic BPs are now well accepted as a better indicator of CV risk and mortality than traditionally measured brachial BPs (Roman et al., 2007, Willum-Hansen et al., 2006, Kampus et al., 2011), and a better indication of the pressures the vital organs are exposed to during the typical activities of daily living (Sharman, 2008).

2.2.3. Arterial pulse waves

The shape of the arterial pulse wave reflects the changes in pressure within the arteries over the time of the cardiac cycle. There are typically two pressure peaks in the arterial pressure wave, the first (P1; Figure 2.1) is due to the pressure of the incident, or forward wave pressure, and the second (P2; Figure 2.1) is typically ascribed to the augmentation of systolic pressure due to the summation of pressure waves which are reflected back towards the heart from distal sites in the aorta (Nichols and O'Rourke, 2005).

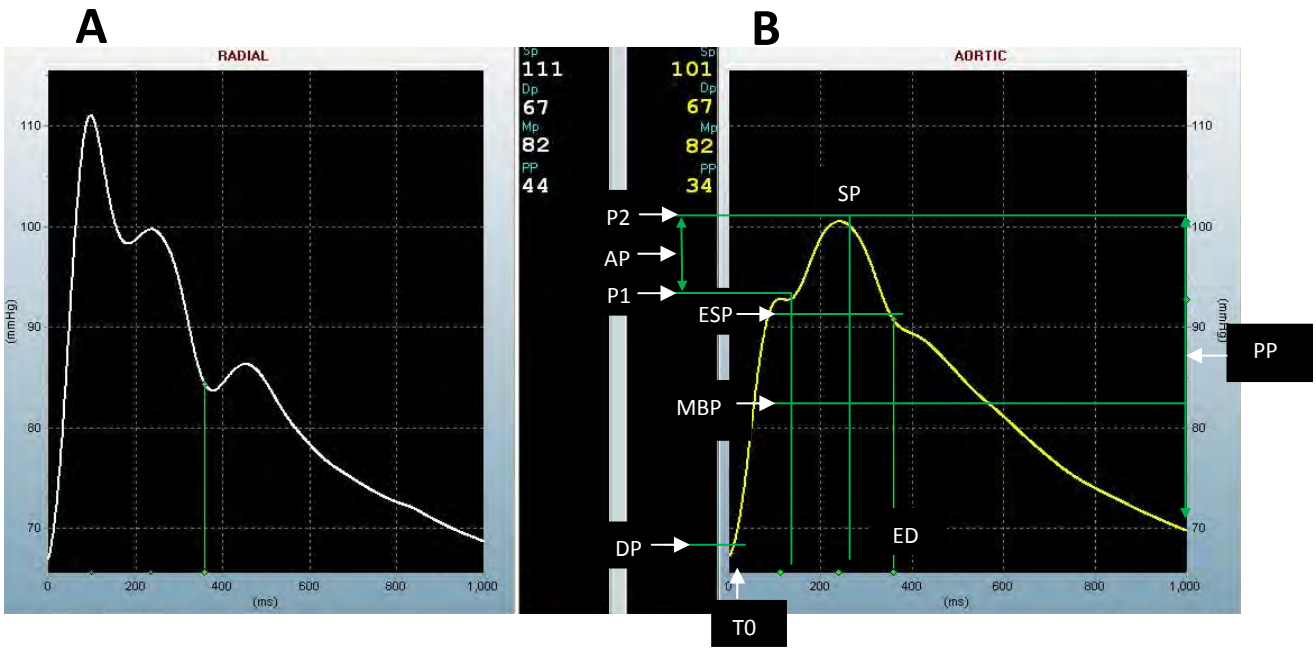
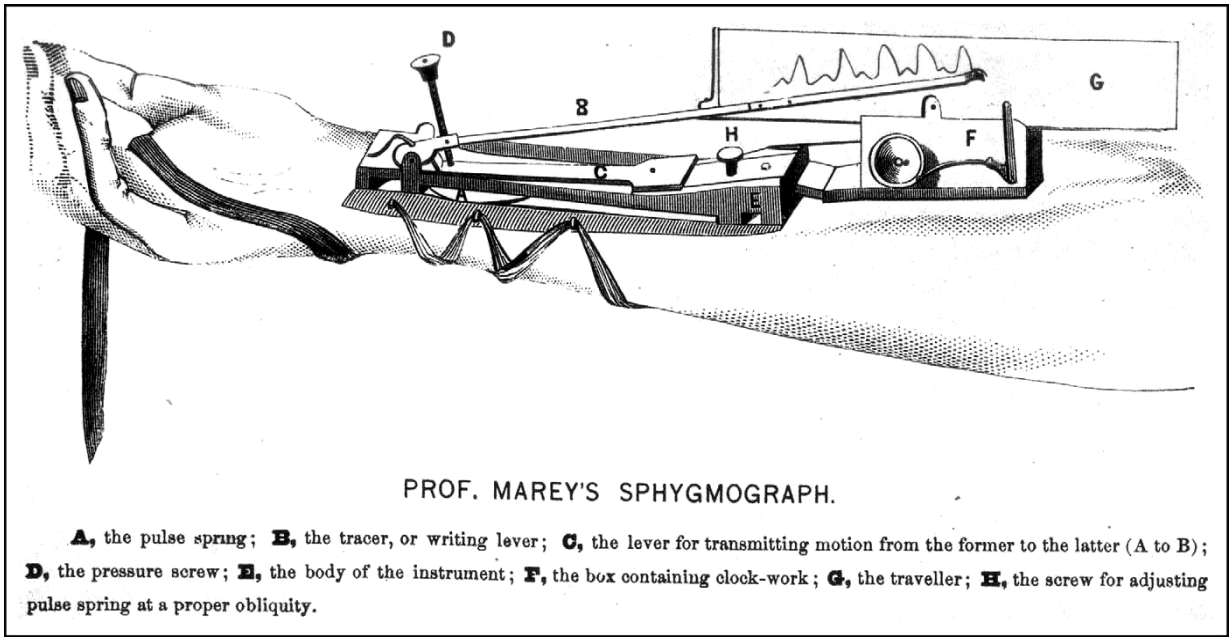


Figure 2.1. Typical contours of the A) peripheral (radial) and B) central (aortic) waveforms over the time of one cardiac cycle.

AP (augmentation pressure) is P2 (pressure at the second systolic peak) minus P1 (pressure at the first systolic peak), SP is systolic pressure, DP is diastolic pressure, PP is pulse pressure (systolic minus diastolic pressure), ESP is end systolic pressure, MBP is the mean blood pressure calculated from the integral of the radial waveforms, T0 is the foot of the pulse at the start of systole, and ED is the ejection duration of systole. In younger, healthy adults, aortic systolic pressure is typically lower than peripheral pressure (compare peak pressures in panel A to B), while diastolic pressure is generally constant between the two sites. Reading taken from one resting healthy female participant from the study presented in Chapter 4.

As early as the 1870's, what we now know as renal and essential hypertension were being diagnosed using a 'sphygmogram' employed by pioneering physicians of the time such as Étienne-Jules Marey (1830 – 1904) (Marey, 1863) and Frederick Mahomed (1849 – 1884) (Mahomed, 1872). The sphygmogram (Figure 2.2) was a device that when strapped to the wrist, traced the radial pulse wave and allowed clinicians of the time to determine features of the pulse waves that were characteristic of aging or disease, such as hypertension.



PROF. MAREY'S SPHYGMOGRAPH.

A, the pulse spring; **B,** the tracer, or writing lever; **C,** the lever for transmitting motion from the former to the latter (A to B); **D,** the pressure screw; **E,** the body of the instrument; **F,** the box containing clock-work; **G,** the traveller; **H,** the screw for adjusting pulse spring at a proper obliquity.

Figure 2.2. An etching of a sphygmograph

As designed by Edgar Holden (1838 – 1909) and used by Marey in the 1860's-70's (Holden, 1874, Marey, 1863).

Mahomed was the first to describe how and why the shape of the pulse wave changes from peripheral to central arteries (Mahomed, 1872). Mahomed noted that the pulse wave contour typical of patients with "hardness of the pulse", now known as hypertension, was similar to that observed in aged patients (Figure 2.3). However, Mahomed observed that the difference between them was that patients with hypertension needed greater pressure to occlude the pulse, while aged healthy arteries needed very light pressure to occlude the pulse (Mahomed, 1872). These early works of pulse wave analysis (PWA) did not find their way into the mainstream medicine of the times due to the widespread acceptance and ease-of-use of the cuff sphygmomanometer (O'Rourke, 1990). Indeed much of Mahomed's work was 'forgotten' and rediscovered decades later, with

much of the credit for his discoveries taken by later physicians who ignored, or were unaware of Mahomed's earlier work (O'Rourke, 1992, Moss, 2006).

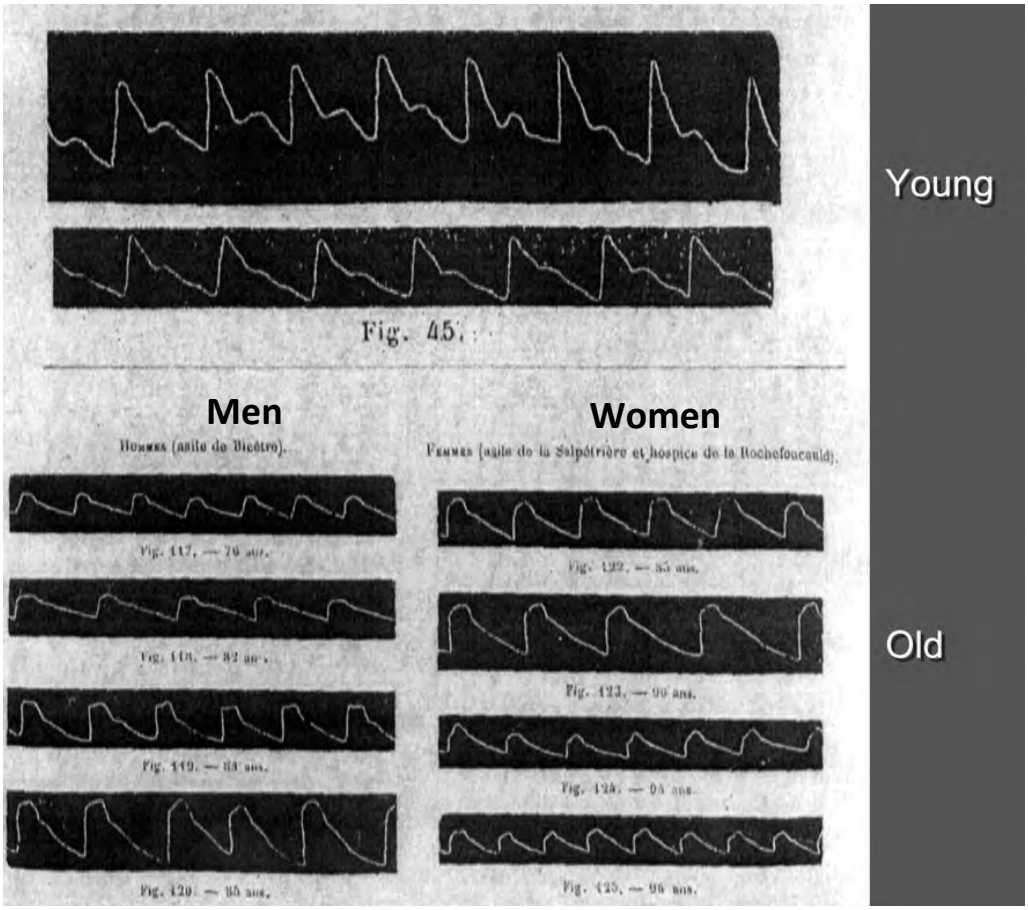


Figure 2.3. Radial tracings from an early sphygmograph (circa 1863) showing pulse waves from young and older individuals.

The pulse waves of both young and older-aged humans shown in this figure are very similar in shape to modern pulse wave captures, with the older-aged individual's waveforms showing characteristic augmentation (P2 occurs earlier in systole and increases total measured pressure) which is thought to be due to increased resistance and arteriosclerosis from ageing. Adapted from (O'Rourke and Nichols, 2005).

2.3 Novel haemodynamic measures

2.3.1. Arterial tonometry

In the 1960's researchers such as Michael O'Rourke and Edward Freis (1912 – 2005) began to experiment with the old techniques of sphygmography to further explore

the clinical implications of the pulse wave (Nichols and O'Rourke, 2005, O'Rourke, 1990). Then, by the late 1980's, the inaccuracies of traditional brachial cuff BP sphygmomanometry to represent cardiac load and determine or predict CV risk began to be regarded as unacceptable for use in large epidemiological projects that longitudinally study CV risk, such as the Framingham studies, largely due to the pulse pressure amplification which is inherent in brachial BPs (Nichols and O'Rourke, 2005). Thus new, reliable and non-invasive methods to calculate central haemodynamic measures began to be developed and accepted more widely (Nichols and O'Rourke, 2005). Aortic BP and arterial stiffness can now be non-invasively estimated by PWA and pulse wave velocity (PWV). It is accepted that non-invasive measures which estimate central haemodynamic from brachial equivalents have their drawbacks due to the discrepancies between radial and aortic waveforms which are caused by pulse pressure amplification (Gallagher et al., 2004, Chen et al., 1997). Nonetheless, PWA and PWV are sensitive, validated (Pauca et al., 2001) and reproducible (Papaioannou et al., 2007) techniques for use where invasive measures are not ethically viable, and both PWA and PWV are each independent indicators of CV risk and mortality (Vlachopoulos et al., 2010a, Vlachopoulos et al., 2010b). There are several methods of non-invasively acquiring PWA and PWV measures, including Doppler flow ultrasound and finger photoplethysmography (O'Rourke et al., 2001). However, with the adaptation of ophthalmic applanation tonometry to the arteries by Gerald Pressman and Peter Newgard in the 1960's, the current non-invasive standard for obtaining PWA and PWV was born (Pressman and Newgard, 1965).

A tonometer is a device which measures pressure using a high-fidelity micro-manometer at its tip, which applanates, or flattens, a superficial artery against an underlying structure, such as bone or muscle, effectively removing tangential forces and

allowing the internal pressure inside to be measured (Matthys and Verdonck, 2002). Some tonometers are automated, servo-controlled devices which strap onto the radial pulse site, but the most widely-used are hand-held devices, similar in shape to a pen, which allow measurement of pulses at any pulse site. More detail on the tonometer device can be found in Section 3.3.4.2, Chapter 3, General Methods.

2.3.2. Measures acquired by pulse wave analysis

Besides aortic BP, PWA provides several other estimates of central haemodynamic function including augmented pressure [augmented pressure (AP); the difference between the first and second systolic pressure peaks], aortic pulse pressure (PP; systolic pressure minus diastolic pressure), mean BP (MBP; the true mean pressure of the integrated averaged radial arterial waveforms), augmentation index (AIx; AP divided by PP expressed as a percentage). More details on methodological aspects of these measures are in Section 3.3.4.2, in Chapter 3, General Methods.

2.4 Theories of arterial haemodynamics

2.4.1. Windkessel theory

The application of the Windkessel concept to the aorta was first developed by Ernst-Heinrich Weber (1795 – 1878) in 1827 (Belz, 1995). However, it was Otto Frank (1865 – 1944) who mathematically quantified the two-element Windkessel model of haemodynamics (Sagawa et al., 1990). Windkessel theory is known as a "lumped model" which looks at the arterial system as a whole, and is based on resistance and compliance elements (Westerhof et al., 2009, Sagawa et al., 1990). The resistance element refers to total peripheral resistance, which is the sum of all resistance to forward flow from the

heart, into the microcirculation. The compliance element refers to the compliance aspect of the elastic aorta (Westerhof et al., 2009). In Windkessel theory, the heart and aorta are likened to a Windkessel; a hydraulic device employed by fire engines at the time which employed a simple pump system attached to a "Windkessel', an air filled chamber that cushioned the oscillations of the pumping action into a smooth flow at the fire hose nozzle. As water is pumped into the chamber, the water compresses the air and pushes the water through the hose. Frank suggested that the air in the Windkessel performs the same function as the aorta; it expands upon left ventricular (LV) ejection (i.e. systole) and recoils during LV filling (i.e. diastole; Figure 2.4).

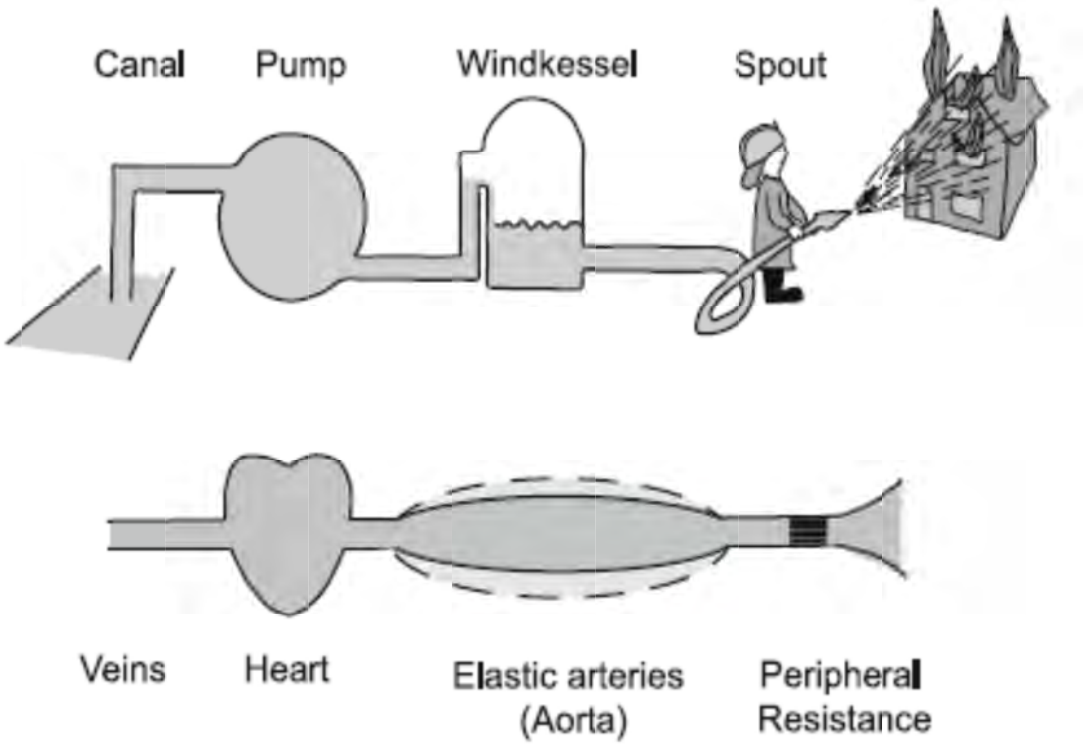


Figure 2.4. The concept of the original two-element Windkessel model

In this model, the air (or aorta) is the buffer that allows the intermittent, pulsatile flow to be relatively smoothed in to a steady flow downstream [from (Westerhof et al., 2009)].

During LV ejection, ~50% of the stroke volume moves directly through the aorta into the peripheral circulation (Belz, 1995). Simultaneously, the aorta expands to store the remaining ~50% of the stroke volume during systole (Belz, 1995). Then, as aortic pressure falls away during diastole, the aorta recoils to propel the remaining stroke volume downstream into the circulation. Thus, during the cyclic highs and lows of pressure in systole and diastole, there is a relatively smooth, continuous flow of blood into in the microcirculation (Westerhof et al., 2009). The Windkessel model accurately predicts that when the aortic valve is closed during diastole, there will be exponential pressure decay. The Windkessel theory is, therefore, excellent in explaining the shape of the pressure waveform in diastole. However, as it is a lumped model, it tends to view the arterial system as a single compartment and this had led many researchers to see the theory as more an explanation of functional principles, rather than a description of actual physiologic events (Belz, 1995). Despite the model's accurate descriptions of exponential pressure decay in diastole, the two-element theory fails to account for the steep pressure rise in early systole, the quantitative differences between predicted and measured systolic pressure and flow, or the second shoulder (P2, Figure 2.1) in late systole, which is commonly said to be due to wave reflections (Nichols and O'Rourke, 2005). The original model has since been expanded to include the three-element, and the four-element Windkessel models (Figure 2.5), which have attempted to rectify the errors inherent in the original.

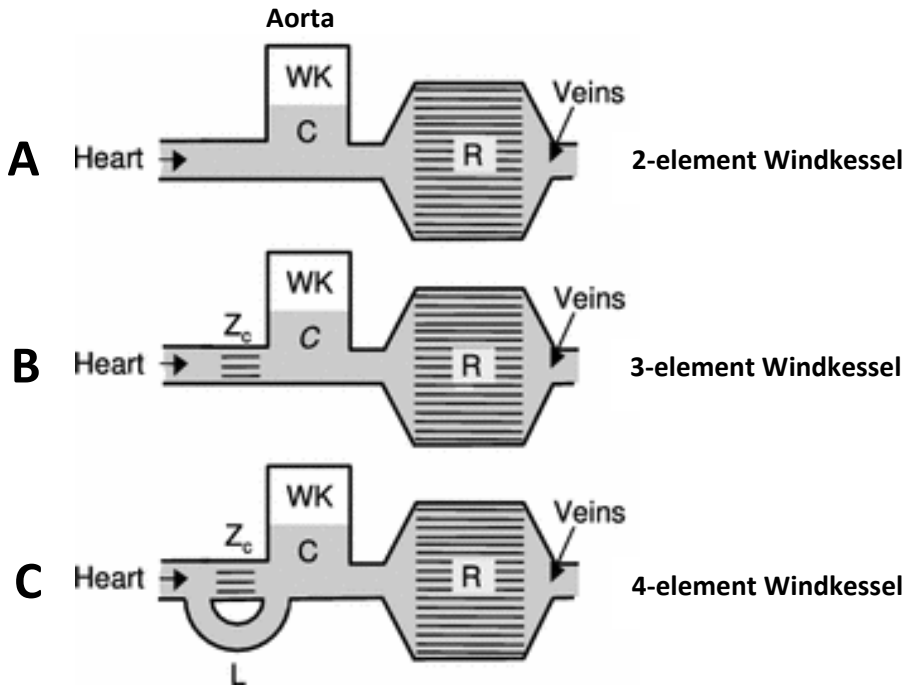


Figure 2.5. A) The two-element, B) the three-element, and C) the four-element Windkessel (WK) models, shown in hydraulic form.

This model depicts that *C* is arterial compliance, *R*, is resistance, Z_c is aortic characteristic impedance and *L* is inertance. Adapted from (Westerhof et al., 2009).

The three-element model includes the characteristic impedance of the proximal part of the arterial tree. Characteristic impedance is equal to wave speed multiplied by blood density, divided by arterial cross sectional area (Westerhof et al., 2009, Kind et al., 2010). When the three-element Windkessel model was applied in the time-domain, i.e. over the time of one cardiac cycle, the difference between predicted and measured systolic pressure that had previously been unexplained, was brought to light (Wang et al., 2003, Westerhof et al., 2009). This pressure difference was termed "excess pressure" (P_{ex}) and the shape of the P_{ex} waveform was found to almost identically match the flow velocity waveform, thus demonstrating that the three-element model was more successful at describing real-time pressure and flow (Wang et al., 2003).

The four-element Windkessel model attempted to further reduce some of the errors in the original model, and introduced "inertance", which is the measure of the arterial pressure gradient that is required to cause a change in flow, or inertia (Kind et al., 2010). An increase in arterial compliance tends to reduce inertance (Westerhof et al., 2009). However the inertance in the four-element model has proved difficult to calculate (Kind et al., 2010, Segers et al., 2005) and has resulted in the three-element model being the more widely-used. Because the Windkessel is a lumped model, it has been seen to have limited physiological applications. As such, the model cannot allow for the study of wave motion and transmission, and localised blood flow changes cannot be represented. For example, an intervention that reduced stiffness of the aorta but did not affect the peripheral arteries could not be effectively studied using the Windkessel models (Westerhof et al., 2009). Consequently, the Windkessel theories have not gained widespread acceptance.

2.4.2. Wave-impedance theory

With its beginnings in the 1950's, the 'Wave-impedance' theory is the most widely accepted model of arterial wave mechanics to date (Nichols and O'Rourke, 2005). Stemming from early work by John Womersely (1907 – 1958) and Donald McDonald (1917 – 1973), Wave-impedance theory is based in the frequency-domain and views the arterial system as being in a steady state of oscillation (Nichols and O'Rourke, 2005). The main focus of Wave-impedance theory is on peripheral resistance, rather than on compliance elements, as is the case with Windkessel theory. Fourier analysis is used to separate aortic pressure and flow waveforms into means and oscillatory, or harmonic components of forward and backwards waves, which are repeated in multiples of the heart rate frequency (Nichols and O'Rourke, 2005). In Wave-impedance theory, total measured pressure is

equal to the pressure of the incident wave, i.e. LV ejection, plus the pressure of the reflected wave (Figure 2.6).

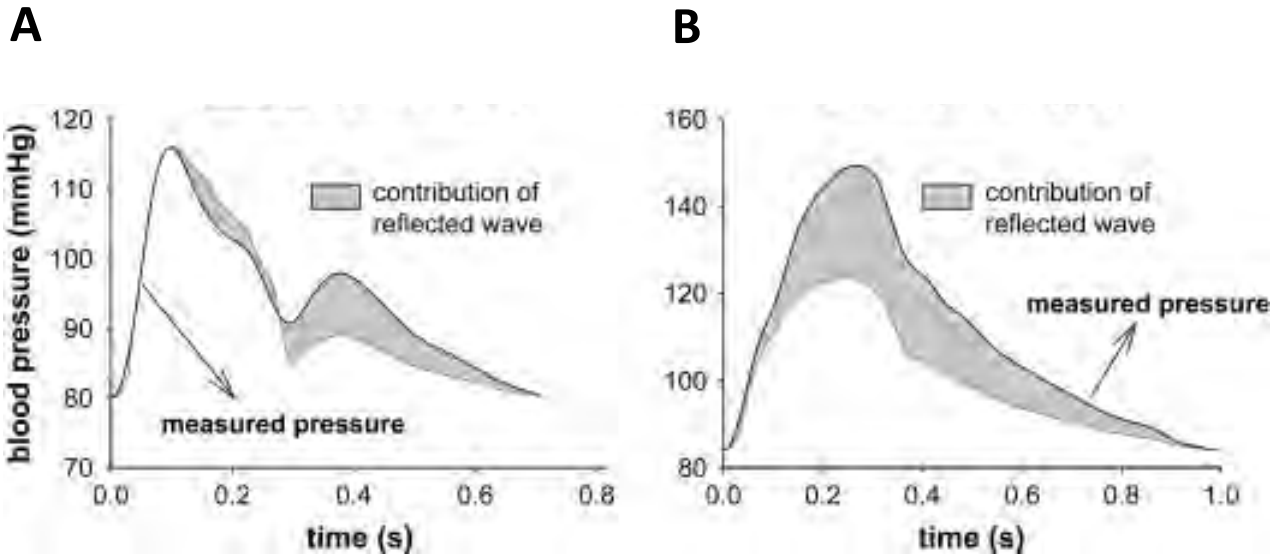


Figure 2.6. The summation of incident pressure wave (in white) and the reflected wave pressure (grey) of total measured radial pressure in a young adult A), and an older adult B) calculated by Fourier analysis.

Note the higher total measured pressure and larger wave reflection components in panel B, which are typically said to be the cause of increased arterial stiffness due to ageing (Swillens and Segers, 2008).

When the LV ejects blood into the aorta, a pressure wave travels with it and is reflected back from distal arterial bifurcations. The precise location of primary reflection sites are still controversial but are often cited as being found near the terminal abdominal aorta and the bifurcations of the femoral and iliac arteries (Westerhof et al., 2008, Murgo et al., 1980). The timing, with respect to LV ejection, and magnitude of these wave reflections are said to change with age and disease states (Nichols and O'Rourke, 2005). Wave-impedance theory states that the differences between the shape of arterial pulse waves in different locations, for instance between aortic and radial sites (Figure 2.1), or in ageing

(Figure 2.6) or disease can be entirely explained by altered timing and magnitude of wave reflections (Murgo et al., 1980, Nichols and O'Rourke, 2005). In young healthy adults, the reflected wave is said to return to the closed aortic valve in diastole which does not augment systolic load. However, according to the theory, increased arterial stiffness, which occurs as a result of natural ageing or chronic disease, is responsible for reflected waves returning to the aortic valve while the heart is still ejecting in systole. The early return of the backwards pressure wave is said to account for augmented aortic systolic pressure, and to cause the late systolic shoulder (P2; Figure 2.1) of the pulse waveform, and is cited as the major determinant of AP and AIx (Nichols et al., 2008).

Wave-impedance theory has been very successful at explaining the shape of arterial pressure and flow waveforms during systole but has not been successful in explaining the pressure and flow waveforms during diastole. When pressure waves are separated into forward and reflected wave components, certain assumptions must be applied which have not yet been adequately explained by proponents of Wave-impedance theory (Tyberg et al., 2009, Tyberg et al., 2008). The first assumption is that the aorta is regarded as a rigid tube (McDonald, 1955). However, if arteries were rigid, pressure changes would occur simultaneously throughout the circulation and blood flow into the microcirculation would equal the blood flow out of the LV, which would mean peak flow during systole, but zero flow during diastole, which is not the case (Aguado-Sierra et al., 2008). Second, in order to satisfy mathematical constraints during diastole, the theory must include large self-cancelling forward and backward waves which travel from both ends of the aorta at the same time (Davies et al., 2007), and which explain the differences in shape of the flow and velocity waveforms in the aorta (Westerhof et al., 1972, McDonald, 1955). In theory, these self-cancelling waves account for a flow velocity of zero despite high pressure in the

proximal aorta during diastole. However, they have never been directly observed (Wang et al., 2011). To attempt to address these inconsistencies of Wave-impedance theory, 'Reservoir-wave' theory has been suggested as a way to unify the undeniable presence of arterial wave motion, with respect to incident and reflected waves, and the buffering, "Windkessel" behaviour of the elastic aorta during the full cycle of systole and diastole (Wang et al., 2003, Tyberg et al., 2008).

2.4.3. Reservoir-wave theory

The Reservoir-wave hypothesis evolved from John Tyberg and Kim Parker's work on wave intensity analysis (WIA) in the late 1980's, and the first experimental data using the theory was published by Juin-Jr Wang and colleagues in 2003 (Wang et al., 2003). Reservoir-wave theory is based in the time-domain and as such can be applied for real-time, beat-to-beat pressure and flow measurements (Tyberg et al., 2008). WIA was initially applied to the study of gasses and now, as applied to arterial haemodynamics, uses equations based on the conservation of mass and momentum in elastic blood vessels (Parker, 2009). WIA uses instantaneous pressure and flow data to separate the measured waves into forward and backwards waves which each have compression and decompression components, that relate to flow acceleration and flow deceleration, respectively (Parker, 2009).

Research using WIA has shown that LV ejection into the aorta begins with a forward compression wave, the 'incident' wave of LV ejection which commences at the start of systole and causes the acceleration of the stroke volume against afterload in the aorta. Following this, the relaxing LV during diastole causes a forward decompression wave, akin

to a backwards suction effect, which decelerates forward flow (Tyberg et al., 2008). There are also backwards compression waves, which are consistent with the 'reflected waves' of classical Wave-impedance theory, and backwards decompression waves, that are associated with diastolic suction and the slowing of forward flow (Tyberg et al., 2008, Davies et al., 2006). As with all measures of arterial pulse waves, the data generated by WIA are dependent on, and specific to the arterial site where the waveforms are collected (Parker, 2009). Reservoir-wave theory states that total measured pressure has two components (Figure 2.7); a reservoir pressure (P_{res}) which is produced by the Windkessel effect of the aorta, plus an excess pressure, P_{ex}, previously called "wave" pressure (Parker, 2009).

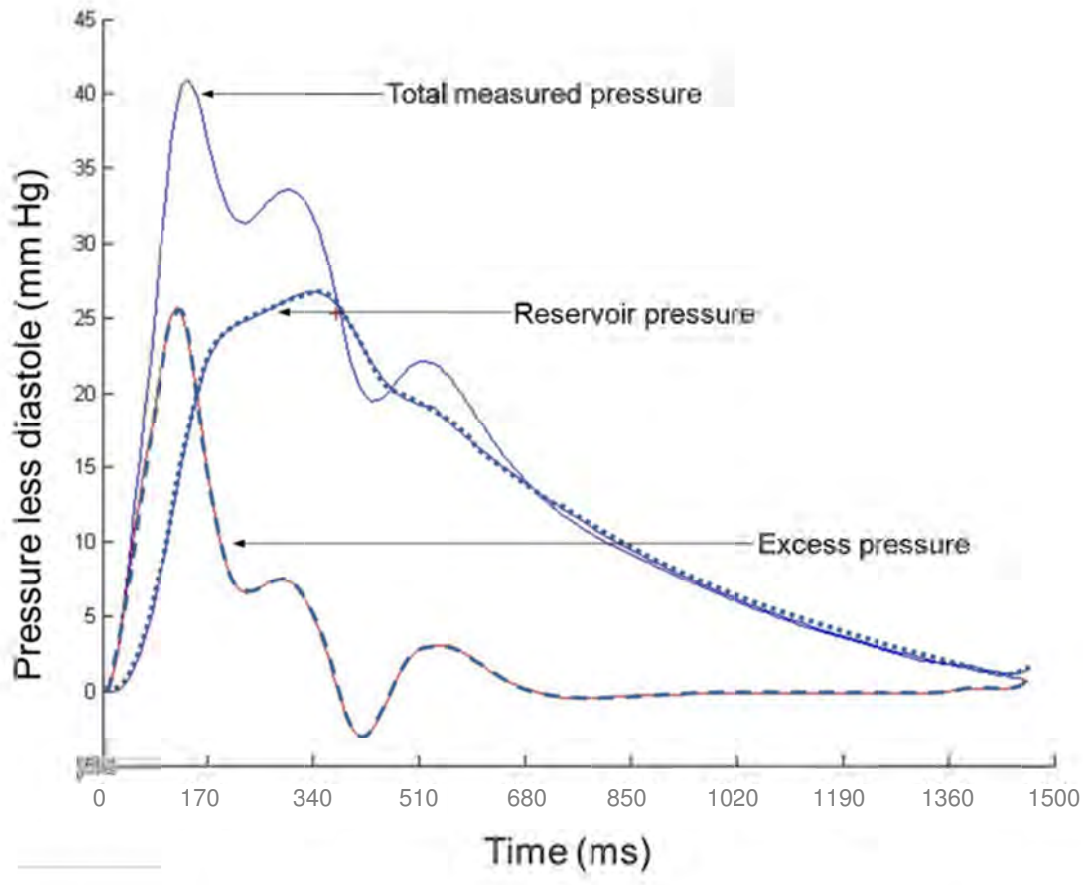


Figure 2.7. Components of the radial arterial waveform according to Reservoir-wave theory.

Measure taken from a single resting healthy female participant from the Healthy Group, aged 40 years.

Reservoir-wave theory demonstrates that the theoretical self-cancelling waves of Wave-impedance theory are not necessary to explain the shapes of aortic pressure and flow waveforms. Indeed, the differences in the shapes of the pressure and flow waveforms are successfully accounted for by deducting the P_{res} from the total measured pressure which reveals that the remaining pressure, the P_{ex}, is identical in shape to the aortic flow wave and is responsible for driving aortic in-flow (Figure 2.8, B) (Wang et al., 2003).

Aortic P_{res} is proportional to aortic volume, and when aortic in-flow is greater than aortic out-flow, P_{res} will increase, and vice-versa (Davies et al., 2007). P_{res} matches total measured pressure closely during late systole and diastole, because it is proportional to flow volume, but the P_{ex} peaks in early systole and is close to zero during diastole, because it closely matches aortic flow velocity (Figure 2.8, A). Thus, P_{res} can be regarded as a summation of pressures from the reflected and re-reflected waves arising from the incident wave as it propagates throughout the arterial system (Hughes et al., 2012). P_{res} is responsible for much of the perfusion of the microcirculation during diastole (Aguado-Sierra et al., 2008), while P_{ex} is more closely associated with longitudinal wave motion, including reflected components, and is the pressure that drives blood into the aorta during systole (Wang et al., 2003).

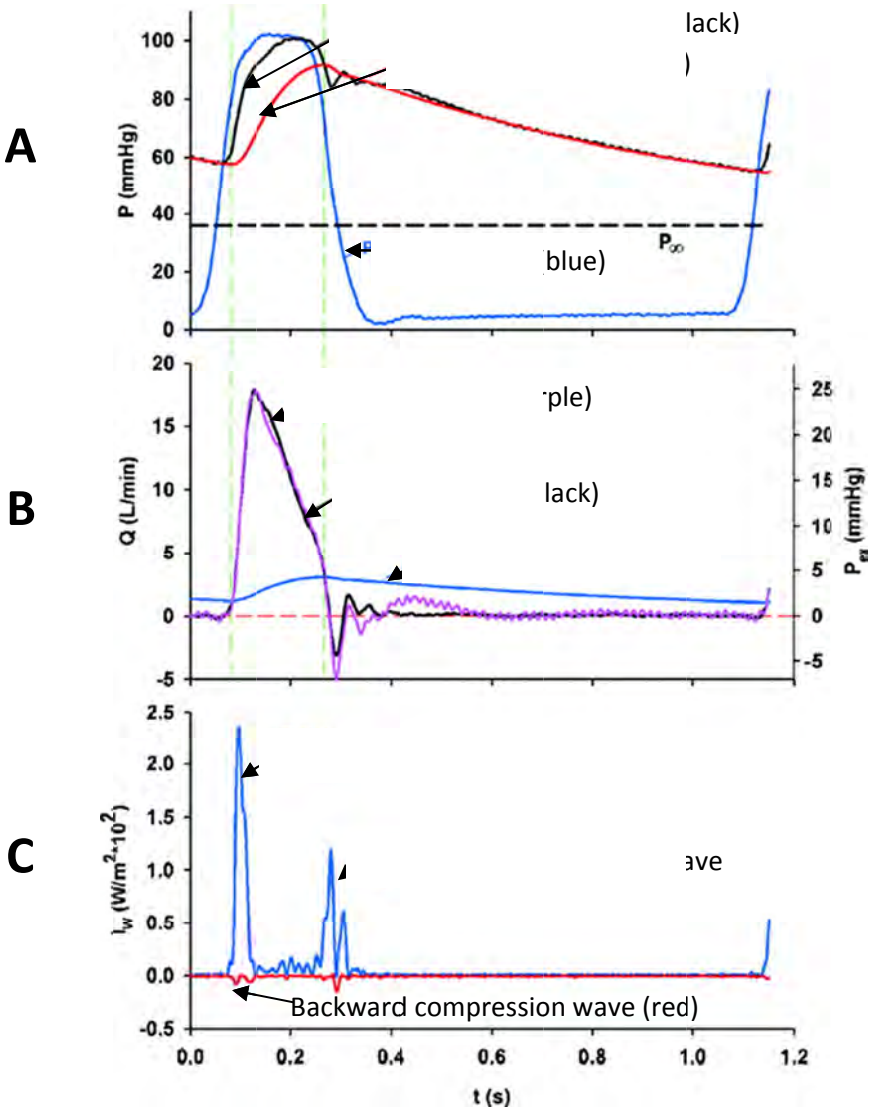


Figure 2.8. Wave intensity analysis and Reservoir-wave theory applied to the canine aorta over the time (t) of a single heartbeat.

The upper panel **A)** shows the measured aortic pressure in black (P), the reservoir pressure in red (calculated from subtracting excess pressure from the measured flow), and the left ventricular (LV) pressure in blue. The middle panel **B)** shows the measured aortic in-flow in black (Q) and the excess pressure (P_{ex}) in purple which are almost identical in shape, and **C)** shows the first peak of wave intensity (I_w) as the forward compression wave (i.e. the incident wave caused by LV ejection), and the second peak is the forward decompression wave, which decelerates flow and is caused by diastolic suction as the LV relaxes and fills. The flatter, red line indicates the backwards compression wave (i.e. the reflected wave) showing some motion but negligible intensity compared to the compared to the forward waves [adapted from (Wang et al., 2003)].

When P_{res} is accounted for, the influence of reflected waves is reduced by ~24%, and the largest component of total measured pressure is found to be P_{res}. Moreover, when P_{res} is taken into account, AP, which Wave-impedance theory asserts is due to reflected waves, is markedly reduced (Davies et al., 2010), or is negligible (Wang et al., 2003) under normal resting conditions (Figure 2.9). This suggests that P_{res} is 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).

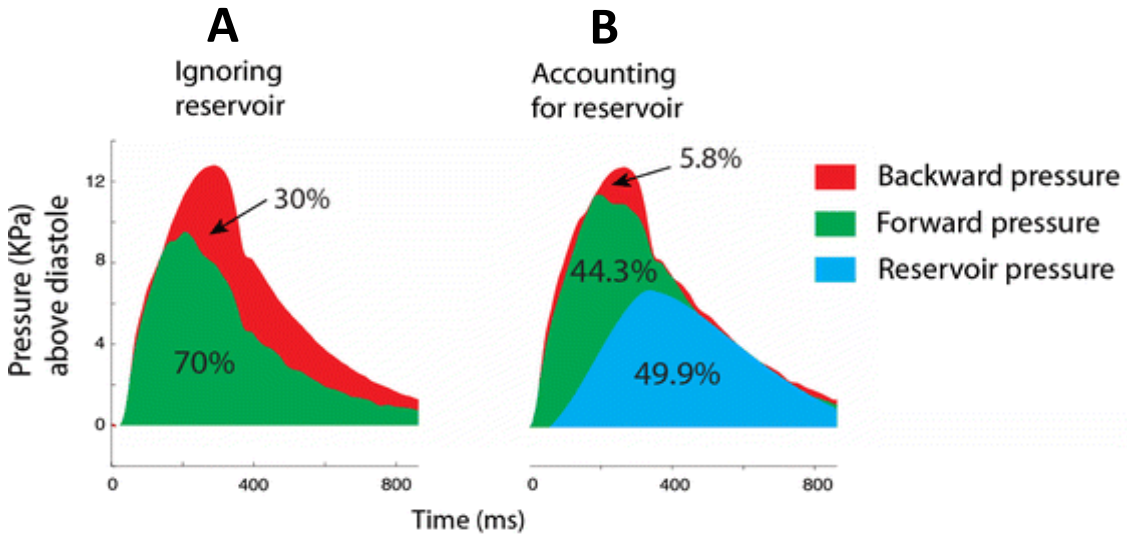


Figure 2.9. Aortic pressure waveforms according to A) Wave-impedance theory which does not account for the contribution of the aortic reservoir, and B) including the aortic reservoir

From (Tyberg et al., 2008)

Reservoir-wave theory has been successful in unifying and explaining many of the shortcomings of the Windkessel and Wave-impedance theories. However, experimental and theoretical research applying the hypothesis is in its early days. The Reservoir-wave theory does not seek to discount the undeniable existence of reflected waves, but aims to account for the contribution reflected waves make to total measured pressure, and to

brachial BP-derived measures such as the AIx. Acceptance of a new theory does not come without controversy, and as with any emerging theory, there are supporters of traditional Wave-impedance approach who debate its validity (O'Rourke et al., 2010, Mynard et al., 2012, Segers et al., 2012). A recent study conducted computer modelling and experiments on sheep, applying separately, a traditional Wave-impedance and then a Reservoir-wave approach in order to test which method would best predict and describe wave reflections in the arteries (Mynard et al., 2012). Results from modelling and *in-vivo* data suggested that using WIA on Reservoir-wave data reduced or eliminated backwards compression waves, which are the pressure-increasing 'reflected waves', and overestimated or introduced as an artefact, decompression, or pressure-decreasing waves (Mynard et al., 2012). However, in a reply that addressed many of the concerns raised by Mynard and colleagues (2012), the developers of the Reservoir-wave theory accepted that the theory had imperfections, as all theories do, but was still evolving (Hughes et al., 2012). Indeed, a recently published study has used computer modelling and bench-top experiments confirmed that P_{res} and P_{ex} are indeed components of actual measured arterial pressure (Sridharan et al., 2012). Sridharan *et al.,* (2012) reported that their predicted and calculated values for P_{res} and P_{ex} matched the physiological reservoir data published previously by Wang *et al.,* (2003), which adds to the validation of the Reservoir-wave approach (Sridharan et al., 2012). Using a novel theory like the Reservoir-wave model to explore human haemodynamics is important as it may uncover new information about the function of the elastic aorta and its reservoir behaviour, and provide new insight into the characteristics of the arterial pulse. Experimental data, such as those presented in Chapters 4 to 7 of this thesis, assists in further exploring the merits of the emerging Reservoir-wave hypothesis and adds to the growing body of work applying the theory.

2.5 Determinants of Altered Haemodynamics

Haemodynamics is the study of the ever-changing relationships between the pressure and flow of the blood, the arteries, veins and the beating heart. Haemodynamic control, particularly concerning BP, is extremely labile and fluctuates minute-by-minute depending on many endogenous and exogenous factors.

2.5.1 Endogenous determinants of altered haemodynamics

2.5.1.1 Effects of disease states on haemodynamic function

Hypertension is a common co-morbidity of many chronic conditions and is considered a CVD in its own right (World Health Organization, 2012a). In a review of published longitudinal data from several large epidemiological studies undertaken by Cook et. al. (1995), it was found that a sustained reduction in diastolic BP of as little as ~2 mm Hg in the mean population would decrease prevalence of hypertension by ~17%, stroke and trans-ischaemic attacks by ~16% and coronary heart disease (CHD) by ~6%. CVD is currently the number one cause of mortality worldwide and death rates are increasing, which corresponds to the increase in unhealthy, sedentary lifestyles (World Health Organization, 2012a). CVD encompasses many diseases affecting the CV system and includes ischemic heart diseases, peripheral and renal arterial disease, arteriosclerosis (arterial stiffness), heart failure, and stroke (World Health Organization, 2012a). The underlying cause of many CVDs is atherosclerosis, the formation of fatty plaques which decrease the interior lumen of an artery (World Health Organization, 2012a). CVD is often associated with other chronic diseases such Cushing's disease, sleep apnoea, thyroid disorders, dementia, obesity, and T2DM (U.S. Department of Health and Human Services, 2004).

2.5.1.1.1 Type 2 diabetes mellitus - Pathophysiology

T2DM is one of several clinical classes of diabetes and is a metabolic disorder hallmarked by chronic hyperglycaemia, a progressive decline in insulin secretion and sensitivity, and increased glycogenolysis (Kronenberg and Williams, 2008, American Diabetes Association, 2010). T2DM is strongly associated with overweight and obesity, older age, habitual inactivity, ethnicity, and a family history of diabetes (Centers for Disease Control and Prevention, 2011). Impaired glucose tolerance (a deficiency of glucose metabolism) and insulin resistance (a de-sensitisation of insulin receptors resulting from chronic hyperinsulinaemia) typically precede T2DM by 10 to 20 years (Petersen and Shulman, 2002).

Altered Plasma Glucose

The toxic effects of hyperglycaemia on numerous organ systems is known to be one of the main contributors to T2DM-associated morbidities such as retinopathy, neuropathy, and CHD (Vasudevan et al., 2006). The mechanism behind organ 'glucotoxicity' is the formation of advanced glycation end-products (AGE) (Aronson, 2003). AGEs are produced when chronic hyperglycaemia causes excess glucose molecules in the blood to undergo non-enzymatic glycosylation reactions which result in irreversible cross-linked bonds between glucose molecules and tissue proteins, such as the collagen in vascular tissue. This change in structure results in physiological changes to the tissue, i.e., a blood vessel will lose elasticity and flexibility, and will thus become stiffer and less compliant (Aronson, 2003).

Altered Plasma Insulin

Another important element in the pathogenesis of T2DM is insulin (Sesti, 2006). In T2DM, chronic hyperglycaemia causes pancreatic beta cells to continually secrete insulin to reduce blood glucose, but due to insulin resistance of the receptors on the target cells, the

glucose is not taken up and remains in the blood, causing the beta cells to become at first dysfunctional, then eventually exhausted, and unable to produce insulin (Sesti, 2006). Insulin resistance and hyperinsulinaemia have been found to decrease arterial compliance (Stehouwer et al., 2008). At normal physiological concentrations, insulin has acute vasodilatory effects that increase arterial compliance (Stehouwer et al., 2008). However, hyperinsulinaemia in early-stage T2DM has been found to be associated with increased arterial stiffness (Salomaa et al., 1995), increased atherosclerosis and dyslipidaemia (Hadi and Suwaidi, 2007). The mechanisms thought to be responsible for increasing arterial stiffness in T2DM are the changes in endothelial function (Hadi and Suwaidi, 2007) and vascular and ventricular structure due to disordered collagen metabolism (Salomaa et al., 1995), which are in turn, caused by increased AGE formation (Aronson, 2003).

Altered Plasma Lipids

Dyslipidaemia is an independent risk factor for CVD and is among the early cluster of symptoms that comprise the metabolic syndrome, which is a frequent precursor to T2DM (Valabhji and Elkeles, 2003). The most common dyslipidaemias in T2DM are high triglycerides, high low-density lipoproteins (LDLs), and low high-density lipoproteins (HDLs). Additionally, an increase in free fatty acids in the blood plasma can worsen the effects of T2DM by further impairing insulin's ability to stimulate glucose uptake into cells, and increasing the release of very low density lipoproteins (VLDLs) and triglycerides (two precursors of LDLs) by the liver (Kirk and Klein, 2009). Over time, dyslipidaemia contributes to hypertension, atheroma formation and arterial stiffness, and thereby increases CV risk (Kirk and Klein, 2009).

2.5.1.1.2 Common co-morbidities of T2DM

The aetiology of T2DM and its co-morbidities is complex, with numerous and often interlinked determinants. However, it is the chronically uncontrolled hyperglycaemia and hyperinsulinaemia that can predispose individuals with T2DM to a higher risk of developing serious co-morbidities such as CVD. Additionally, nephropathy, retinopathy, and autonomic nervous system disorders such as central and peripheral neuropathies are common complications of T2DM (Schonauer et al., 2008, American Diabetes Association, 2010).

Diabetic neuropathy

Diabetic neuropathy includes several subclasses. Peripheral neuropathy affects peripheral sensory fibres, which include pain, touch/pressure, temperature perception fibres, and the motor nerve fibres. Autonomic neuropathy affects various organs controlled by the autonomic nervous system and has sympathetic and parasympathetic components (Bansal et al., 2006). As with most other diabetes-related complications, neuropathy is the result of damage from the toxic effects of chronic hyperglycaemia and the accumulation of AGEs. Gradually, the tissue of the small blood vessels supplying the nerves is degraded, thereby restricting blood and nutrient supply, and hindering waste removal, which causes axonal demyelination and atrophy of neurons (Bansal et al., 2006). Peripheral neuropathy is the most common and debilitating of the subclasses of neuropathy and affects nearly 70% of patients with T2DM (Dobretsov et al., 2007). Peripheral neuropathy typically progresses to autonomic neuropathy, with the most life-threatening form involving the cardiac nerves (Dobretsov et al., 2007).

Cardiovascular autonomic neuropathy

When cardiac nerves are degraded, the result is cardiovascular autonomic neuropathy (CAN) which may be preceded by peripheral neuropathy, but may also occur in the absence of peripheral neuropathy. To a certain extent, unmyelinated and small myelinated peripheral nerve fibre function can improve with early treatment of peripheral neuropathy (Therapeutics and Technology Assessment Subcommitee of the American Academy of Neurology, 1996). However, CAN is for the most part, irreversible (Dobretsov et al., 2007, Schonauer et al., 2008). CAN is a novel, yet strong risk factor for increased arterial stiffness (Liatis et al., 2011), CVD, and mortality (Vinik et al., 2003), with signs and symptoms including resting tachycardia, exercise intolerance, orthostatic hypotension, and impaired heat / cold tolerance (Vinik et al., 2003). Avoiding progression of CAN is aided by diagnosis and monitoring via non-invasive autonomic tests (Weimer, 2010).

Tests of cardiovascular autonomic function

Diagnosis of CAN is generally via a battery of tests, rather than just one. There are numerous tests of autonomic function which have been detailed elsewhere (Weimer, 2010). Well established tests of cardio-vagal autonomic function include the measurement of heart rate variability, which measures the average range of heart rate change (R-R intervals) during a period of rest via recording of beat-to-beat heart rate during rest, the heart rate response to Valsalva manoeuvre, and the heart rate response to positional change (i.e. supine to standing) (Weimer, 2010).

The heart rate response to the Valsalva manoeuvre, and the heart rate and BP response to postural change are the most reliable and standardised tests of CAN to date (Vinik et al., 2003). These tests, when well controlled, are validated as safe and specific to determine CAN (Weimer, 2010, Vinik et al., 2003, Therapeutics and Technology

Assessment Subcommitee of the American Academy of Neurology, 1996). Further details on CAN tests are in Section 3.3.7, Chapter 3, General Methods.

Heart rate response to Valsalva manoeuvre test

The Valsalva test involves straining, or breathing out, against a closed glottis, or into a small-diameter tube, while heart rate is recorded with an electrocardiogram (ECG). During the manoeuvre, the heart rate increases sharply, then after exhalation, there is a fall in heart rate and BP leading to an overshoot below the resting BP value which lasts until restored by cardiovagal baroreflexes (Vinik et al., 2003, Therapeutics and Technology Assessment Subcommitee of the American Academy of Neurology, 1996). A Valsalva manoeuvre is considered successful if there is full expansion of the chest upon inspiration, with facial flushing and redness, and external jugular engorgement (Weimer, 2010). In T2DM individuals, the increase in heart rate and BP are expected to be less than that of healthy people, and people with T2DM typically have a slower heart rate recovery. The magnitude of heart rate or BP reduction below typical resting values is indicative of autonomic function. This test provides data for the Valsalva R-R ratio, which is calculated by dividing the longest R-R interval, taken from the recorded ECG, in ms, following breath release by the shortest R-R interval during the manoeuvre (Ziegler et al., 1992). A normal Valsalva ratio for healthy adults (30-65 years) ranges from 1.21 to 1.16, respectively (Ziegler et al., 1992). The ratio decreases with age and autonomic dysfunction (Ziegler et al., 1992).

Tests of responses to postural change

The BP and heart rate response to a sudden change in posture is another test of CAN. People with T2DM frequently have orthostatic hypotension, which is a consequence of CAN and may cause dizziness, blurry vision or syncope upon a change from sitting to

standing (Weimer, 2010). When testing for CAN, passive tilting is preferential over sit-to-stand methods because it removes any exercise reflexes triggered by leg muscle contraction which tend to transiently reduce BP, thus mimicking orthostatic hypotension (Vinik et al., 2003, Weimer, 2010). Typically a 60° to 70° head-up tilt is used with duration between 10 and 60 minutes. However, tilt tests can last as long as it takes to evoke autonomic symptoms, i.e. syncope (Vinik et al., 2003). During a head-up tilt test in healthy individuals, heart rate is increased and is maximal at the ~15th beat post-tilt. This effect is due to vasoconstriction which aims to prevent large reductions in BP as blood suddenly pools in the legs (Vinik et al., 2003). This is followed by reflexive bradycardia, which is intended to restore homeostasis, which is maximal after the ~30th beat post-tilt (Vinik et al., 2003). The BP response to tilting is calculated as the average of two resting brachial BPs minus the lowest BP during the first 3 minutes of tilting (Ziegler et al., 1992). A fall in systolic BP of more than 20 mm Hg and/or a diastolic BP fall of > 10 mm Hg during the first 3 minutes head-up tilt can be indicative of CAN (Ziegler et al., 1992). Autonomic control of heart rate is assessed through the 30:15 tilt ratio which is calculated by dividing the longest R-R interval, in ms, during beats 20-40 by the shortest R-R interval during beats 5-25, all post-tilt (Ziegler et al., 1992). The heart rate response to head-up tilt is typically lower in people with T2DM compared to healthy individuals, with only gradual increases in heart rate. In healthy individuals, the 30:15 tilt ratio reduces with age and is typically between 1.12 to 1.06 in 30 to 65 year olds, respectively (Ziegler et al., 1992). It would be expected that due to the likelihood of some degree of CAN, that people with T2DM would have lower 30:15 tilt ratios than age-matched healthy individuals.

Consequences of cardiovascular autonomic neuropathy

Consequences of CAN include reduced or exaggerated CV responsiveness to challenges of heat, cold and postural change. Over time, microvascular degradation and

demyelination of nerves by chronic hyperglycaemia and AGE products diminishes the noradrenergic response to stressors (Ziegler et al., 1993). Accompanying dysfunction in baroreceptors means that people with CAN tend to have impaired pressor responses to external stressors like sudden heat, cold and changes in posture, such as orthostatic intolerance (Dobretsov et al., 2007, Ziegler et al., 1993). *In vivo* experiments confirm that people with T2DM have reduced expression of, and sensitivity to endogenous vasodilators such as nitric oxide (NO), which leads to impaired reflexive control of thermoregulation via blood redistribution in response to heat (Wick et al., 2006, Williams et al., 1996). Damaged neurons, whether from neuropathies, or other nerve disorders such as multiple sclerosis, tend to conduct optimally at normal physiological temperatures, thus when a person with neuropathy is heated or cooled, nerve conduction blockages occur (Wick et al., 2006, Straver et al., 2011). As a consequence of vasomotor and nerve conduction dysfunction, people with T2DM are more likely to have reduced ability to sense heat and cold (Chao et al., 2007) which can leave them more prone to hyper- (Wick et al., 2006) and hypothermia (Scott et al., 1988, Applebaum and Kim, 2002, Neil et al., 1986).

One study compared the physiological responses during cold exposure to the trunk (by water-perfused suit at 16°C for 45 minutes) in three groups; people with "insulin-treated" diabetes plus diagnosed CAN, people with diabetes but without CAN, and to an age-matched healthy control group (Scott et al., 1988). Given the age range of diabetic participants in that study was 17 to 55 years (Scott et al., 1988), it may be assumed they had type 1 diabetes. Scott *et al.,* (1988) reported that people with diabetes plus CAN had much higher baseline mean BP (although none were classified as hypertensive), and higher baseline heart rate compared to the other groups. However, during cooling, magnitude of increase in mean BP was similar between the three groups, despite the fact that the CAN

group had reduced vasoconstrictor responses; in that skin blood flow did not change significantly during cooling in diabetes plus CAN, compared to the other groups (Scott et al., 1988). Scott *et al.,* (1988) observed that the reasons for the similar increases in mean BP between groups, despite obvious impairments in vasoconstriction in diabetics with CAN during cooling, were not clear. In addition, in that study the group with diabetes plus CAN did not have any change in heart rate during cooling, whereas the other groups had significantly reduced heart rate (Scott et al., 1988), which reflects the impairments in vagal function in people with diabetes plus CAN.

2.5.1.1.3. Prevalence, Incidence & Costs of Managing T2DM

Due to ageing populations and increasingly unhealthy lifestyles, the prevalence, incidence and cost of managing T2DM is rising not only in western countries, but globally (Shaw and Tanamas, 2012, Centers for Disease Control and Prevention, 2011). T2DM is projected to become the leading burden of disease in Australia by the year 2023 (Australian Institute of Health and Welfare, 2012). Incidence of T2DM is notoriously hard to estimate because the onset of T2DM is often unclear, and many people with T2DM remain undiagnosed (Australian Institute of Health and Welfare, 2012). However, there is no doubt that incidence is increasing world-wide.

T2DM is a complex condition that requires frequent medical care and monitoring from a coordinated team of health professionals in order to manage symptoms and to prevent complications and progression of the disease. To this end, people with T2DM are more likely to use hospital and medical services than people without T2DM, and this trend is increasing along with the financial burden of managing T2DM (Shaw and Tanamas, 2012). Indeed, in 2012 the total annual cost of managing T2DM in Australia was $6 billion (Shaw and Tanamas, 2012).

2.5.1.1.4. T2DM-associated mortality

In 2009, T2DM, or its related co-morbidities, caused the deaths of 14,286 Australians, which equates to 10% of deaths from all causes (Australian Institute of Health and Welfare, 2012). Mortality rates from T2DM in the United States of America (USA) are also high (Centers for Disease Control and Prevention, 2011). In the USA, T2DM caused 3% of deaths from all causes in 2007, making it the USA's seventh highest cause of death that year (Xu et al., 2010). Singularly, many of the co-morbidities associated with T2DM will significantly increase risk of mortality. However, frequently a patient will have a cluster of co-morbidities, i.e. hypertension, dyslipidaemia, and CAN. For this reason, persons with T2DM have double the risk of mortality from all causes, than that of a similar-aged peer without T2DM (World Health Organization, 2012b). The morbidities associated with T2DM make patients particularly susceptible to the effects of seasonal illness like influenza (Centers for Disease Control and Prevention, 2012), and the effects of exposure to sudden changes in environment, such as heat (Semenza et al., 1999, Basu, 2009), or cold exposure (Kilgour and Williams, 1998, O'Neill and Ebi, 2009).

2.5.2. Exogenous determinants of altered haemodynamics

2.5.2.1. Effects of medications on haemodynamics

Generally, medications for haemodynamics are designed to minimise fluctuations (typically increases) in BP and treatment goals are set with the optimal BP range in mind (Table 2.1). Ageing and chronic diseases, in particular hypertension and T2DM, cause changes to CV structure and function which result in haemodynamic dysfunction. There are many classes of antihypertensive drugs which target different hypertensive pathways. Some of these drugs work to reduce blood volume, i.e. diuretics, some work in reducing

endogenous hypertensive hormones in the renin-angiotensin pathway, such as angiotensin converting enzyme inhibitors, and angiotensin II receptor antagonists. Other medications work on reducing resistance to blood flow, either indirectly, as with calcium channel blockers or directly with vasodilators, and others still, work on reducing heart rate, i.e. beta blockers (U.S. Department of Health and Human Services, 2004). Medications for control of other components of haemodynamics, such as blood viscosity and cardiac rhythm, include anti-lipid medications, anti-coagulants and anti-platelet medications, and treatments for congestive heart failure. With a number of haemodynamic and glycaemic parameters to manage, people with T2DM frequently take several different medications per day (Webbie and O'Brien, 2006).

2.5.2.2. Effects of nutrition and exercise on haemodynamics

A number of dietary substances are known to alter BP. Some in a detrimental capacity; as with sodium chloride (Fujita, 2010) and alcohol (U.S. Department of Health and Human Services, 2004). However, BP can be affected favourably, as with the plant-based flavonoids, which are bioactive metabolites that are typically found in fruits and vegetables, cocoa, nuts, tea and red wine and have been shown to reduce CVD mortality and aortic PWV (McCullough et al., 2012, Jennings et al., 2012).

Exercise presents a challenge to haemodynamics in many ways. Increased skeletal muscle contractions during exercise cause a "metabolic vasodilation", mediated by increased hyperpolarisation, and lactate, muscular NO, potassium, adenosine and acetylcholine, which work to dilate cutaneous arterioles (Maiorana et al., 2003, Sarelius and Pohl, 2010). Additionally, the increased blood velocity through the arteries during exercise increases mechanical shear stress in the arterioles, which is a trigger for the release of NO, and other vascular relaxing-factors from the vascular endothelium, that in

turn, actively dilate the larger muscular arteries (Sarelius and Pohl, 2010, Sugawara et al., 2007, Buga et al., 1991).

2.5.2.3 Effect of climate (heat, cold and humidity) on haemodynamics

2.5.2.3.1 Adaptive thermoregulation

Human beings are endotherms and regulate their body temperature by neural processes such as adaptive thermogenesis which is controlled by the hypothalamus and sympathetic nervous system via thermosensory proteins (Seebacher, 2009). Healthy individuals maintain a relatively stable core temperature during homeostatic challenges, such as heat exposure or exercise, by adjusting metabolic heat production and dissipating heat via radiation, convection, conduction and evaporation. In contrast, to conserve core heat in the cold, heat generation methods such as peripheral vasoconstriction and shivering are utilised (Seebacher, 2009).

Exposure to sudden changes in climate may occur as part of normal daily climate variation, or when moving from an indoor climate-controlled space to outdoors during winter and summer. Sudden exposure to heat or cold causes circulatory changes throughout the body which aim to maintain optimal core temperature (Seebacher, 2009). Brachial systolic and diastolic BPs are also affected by chronic, seasonal reductions in air temperature, with studies reporting that brachial BPs tend to be higher in winter than summer (Figure 2.10) (Alperovitch et al., 2009, Charach et al., 2004, Halonen et al., 2011).

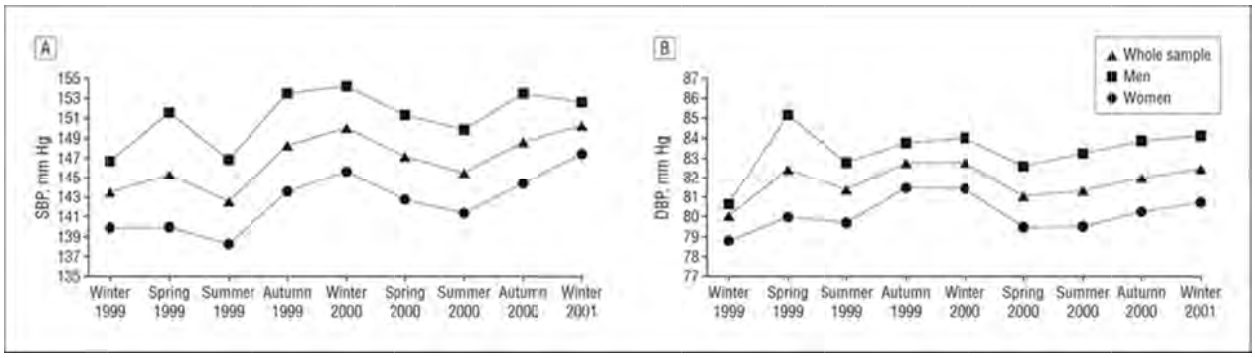


Figure 2.10. Two-year seasonal variation in brachial systolic A) and diastolic B) BPs.

Data from adults > 65 years, *n* = 8,801 in three French cities, from (Alperovitch et al., 2009).

2.5.2.3.2. Climate-associated morbidity and mortality

As a consequence of climate change, overall increases in global temperature of 3 to 4°C are forecast by 2100 that will lead to a greater incidence of, and variability in extreme weather events, particularly in heat waves, and to a lesser extent, cold waves (McMichael, 2013). There is extensive epidemiological evidence to suggest links between environmental heat (Basu, 2009), heat plus humidity (Abrignani et al., 2011), cold (Danet et al., 1999, O'Neill and Ebi, 2009) and haemodynamic perturbations, CV morbidity and mortality. Moreover, CV death rates are higher in winter even after adjustment for the typical peak in respiratory infections and influenza in winter (Bhaskaran et al., 2010). Figure 2.11 shows the typical "J" shaped relationship between highs and lows of environmental temperature and mortality (Ballester et al., 2011).

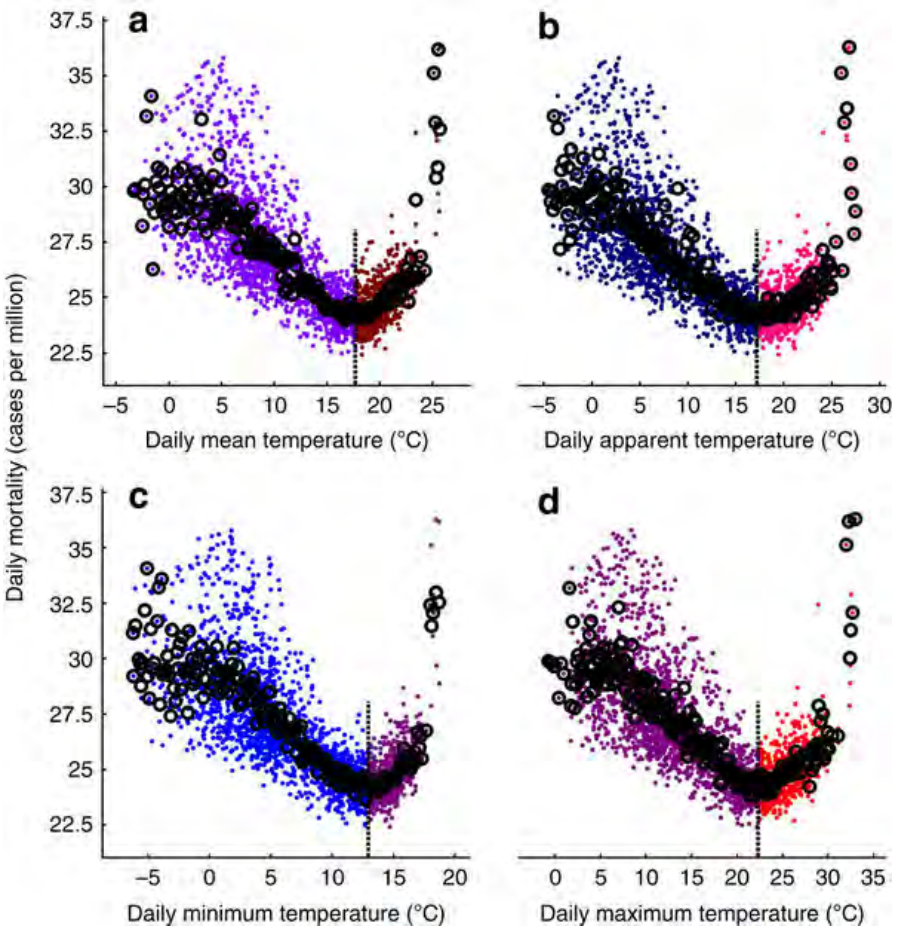


Figure 2.11. Relationships between temperatures and mortality in daily values for A) mean temperatures, B) apparent temperatures, C) lows and D) highs in numbers of deaths daily.

Data are per million inhabitants in 200 European regions from 1998 to 2003 [from (Ballester et al., 2011)].

Differences in global patterns of temperature-associated CV events and mortality have been identified, particularly relating to seasonal cold exposure. While it seems contradictory, temperate regions with milder winters and less climate variability, such as parts of Australia, Greece, Portugal and Ireland, have a larger incidence of cold-associated CV events and mortality than regions with consistently severe-cold climates, such as Finland (Barnett et al., 2005, The Eurowinter Group, 1997). This is thought to be due to a lack of preparedness for sudden large changes in temperature in people living in temperate

regions which is, in turn due to a lack of education on the risks (Curriero et al., 2002). Interestingly, the common pattern of high winter CV morbidity and mortality is absent in warm, subtropical regions of the world that do not have large temperature shifts between seasons (Ku et al., 1998). It is evident then, that CV morbidity and mortality from sudden temperature variations depends more on environmental air temperature, rather than season, and "typical" temperatures depend on latitudinal location. Ultimately, it is more the unusual nature, or suddenness of the heat or cold exposure and not the absolute temperature that has the greatest impact on temperature-related morbidity and mortality (Barnett et al., 2012, Ramon-Medina and Schwartz, 2007, Ku et al., 1998).

2.5.2.3.3. Effects of heat, cold and humidity in healthy and T2DM individuals

Climate-associated mortality rates are highest among the frail, the elderly, and those with chronic disease (Semenza et al., 1999, O'Neill and Ebi, 2009, Makinen and Hassi, 2009, Kenny et al., 2010). Normal aging causes decrements in reflexive cutaneous vasodilation and these effects are exacerbated in people with T2DM (Blatteis, 2011, Petrofsky et al., 2012). Additionally, people with long-standing T2DM are more likely to have autonomic nervous system and vascular dysfunction (Vinik et al., 2003), which impairs thermoregulation and leaves patients more exposed to the risk of heat and cold-related illness (Kenny et al., 2010). The build-up of AGEs which are common in uncontrolled or long-standing T2DM are thought to be one of the mechanisms responsible for the vascular dysfunction and reduced ability to thermoregulate during sudden exposures to heat and cold (Xu et al., 2003, Petrofsky et al., 2012). In an experiment in rabbit arteries, Xu *et al.,* (2003), found that AGEs inhibited the expression of NO-synthase (the enzyme that produces NO) at AGE concentrations that are similar to those found in plasma of T2DM patients (Xu et al., 2003).

The few studies that have investigated physiological challenges posed by heat, cold, or humidity in people with T2DM have not utilised central haemodynamic measures and have largely focussed on variables such as heart rate, brachial BPs and body temperatures. In one study in normal room temperatures, people with T2DM were found to have a significantly larger reduction in brachial systolic BP than healthy, age-matched controls, after a 45° head-up tilt (*n* = 12 T2DM, 15 healthy controls). In the T2DM group brachial systolic BP fell by ~13 ± 3 mm Hg while in the healthy group it fell by ~3 ± 4 mm Hg (Petrofsky et al., 2005a). Then, after ~30 mins heat exposure in a climate chamber at 42°C, the same T2DM group were again tilted to 45° and were reported to have an even greater BP reduction than occurred at room temperature tilting. In the T2DM group, systolic BP was reduced by 27 ± 6 mm Hg during tilt in hot conditions, but data for the healthy group was not reported (Petrofsky et al., 2005a). The difference between pressor responses of T2DM compared to healthy individuals when challenged by heat, cold, or postural change, is likely associated with the following mechanisms: 1) abnormalities in plasma volume redistribution and increased permeability of capillaries to albumin that contributes to orthostatic hypotension (Parving and Munkgaard-Rasmussen, 1973); 2) abnormalities in heart rate variability that diminishes autonomic control of heart rate and cardiac output in the face of external CV challenges; and 3) impaired control of reflexive haemodynamics via decreased vasomotor tone which may be due to neuropathies and vascular dysfunction (Stansberry et al., 1997).

2.5.2.3.4. The role of autonomic function in thermoregulation

Nearly all aspects of human physiology are vulnerable to extremes of temperature, and the nervous system is one of the most sensitive to temperature fluctuations (Rutkove, 2001). People with long-term T2DM frequently have reduced sensation including touch, pain, heat and cold sensation due to damaged peripheral neurons (Chao et al., 2007) and

thus are more vulnerable to hyper- and hypothermia during heat and cold exposure (Neil et al., 1986). One study tested the nerve conduction of individuals with T2DM during localised heating and cooling of the skin surface of the big toe, foot dorsum and index finger of 498 T2DM patients, 76% of whom did not have any neuropathic symptoms (Chao et al., 2007). Chao *et al.,* (2007) reported that participants with the highest HbA_{1c}, i.e. the poorest glycaemic control, were found to have the most impaired thermal sensation compared to healthy age-matched controls (*n* = 434). Additionally, people with neuropathies often have worsening neurological symptoms during cold exposure (Straver et al., 2011). In healthy people, exposure to extremes of heat and cold reduces the biochemical conduction in axons via reduced adenosine triphosphate-ase activity and decreased sodium/potassium pump action, which disrupts saltatory nerve conduction between adjacent nodes (Straver et al., 2011). This detrimental effect of acute temperature changes on biochemical nerve traffic has been observed directly in experimental animal models (Rasminsky, 1973, Rutkove, 2001). For humans with T2DM, the nerve damage from peripheral neuropathy and CAN compounds the nerve conduction decrements caused by heat and cold, and may contribute to the altered stress responses to temperature extremes (Straver et al., 2011, Ziegler et al., 1993). Thus collectively, from the evidence to date, it is clear that in any geographical location, people with T2DM are among the vulnerable during sudden changes in air temperature (O'Neill and Ebi, 2009, Semenza et al., 1999, Ziegler et al., 1993).

2.5.2.3.5. Effects of heat

General physiological effects of heat exposure in healthy individuals

Changing from comfortable, ambient conditions to hot conditions results in increased core and skin temperatures (Rowell et al., 1969). This in turn, causes reflexive vasodilation and blood redistribution to peripheral and cutaneous circulation, with the

purpose of removing heat from the core to maintain homeostasis (Kellogg Jr, 2006). Sudden heat exposure also profoundly affects heart function and brachial BP (Rowell, 1990). In healthy individuals, passive heat stress causes a shift of blood from the core to the cutaneous circulation that can increase skin blood flow to ~7,200 $mL.min^{-1}$ (Crandall and Gonzalez-Alonso, 2010). This redistribution of blood to the periphery, in turn, decreases central blood volume (Crandall et al., 2008). However, cardiac output is typically maintained by an increase in cardiac contraction frequency (Nelson et al., 2010) which stabilises a decline in preload and brachial systolic and diastolic BPs (Truijen et al., 2010). The challenge to haemodynamics and thermoregulation presented by environmental heat exposure is not dissimilar to that induced by exercise. Indeed, the increase in cardiac workload during heat stress via dry sauna has been found to equal that of moderate-to-vigorous exercise (Vuori, 1988).

Several studies have investigated the effects of local and whole-body heating on measures of peripheral haemodynamics in healthy individuals. Skin blood flow is a measure of cutaneous vasodilation and both local (Stansberry et al., 1997) and whole-body (Kellogg Jr et al., 1998, Crandall et al., 2000, Kellogg Jr et al., 2003, Wick et al., 2006) heating have been found to significantly increase skin blood flow. Moreover, localised application of heat via hand water immersion and application of heat packs, has been reported to decrease radial arterial wall stiffness (Bellien et al., 2010), radial smooth muscle tone (Bellien et al., 2010, Huang et al., 2011, Kellogg Jr, 2006), and radial AIx (Huang et al., 2011). Decreases in peripheral smooth muscle tone, and peripheral arterial wall stiffness are potentially due to a sympathetically-mediated increase in vasodilatory hormones such as NO (Kellogg Jr et al., 2003).

Effects of heat exposure on central haemodynamics in healthy individuals

To date, very few studies have used PWA and PWV methods to examine central haemodynamic changes during heating. Indeed, only one study (Ganio et al., 2011) could be found that has measured aortic PWV during heat stress in a group of healthy adults (n = 8) using a water-perfused suit at 49°C, but aortic AIx or BPs were not assessed. Ganio *et al.,* (2011) reported a non-significant increase in aortic PWV from baseline (+0.9 $m.s^{-1,}$ from 6.7 ± 1.6 to 7.6 ± 2.1 $m.s^{-1}$; P = 0.12) during 60 minutes of whole-body heat exposure, compared to control conditions of 34°C water. It is unclear why some heat experiments have appeared to improve (Bellien et al., 2010, Huang et al., 2011), yet others may have worsened arterial stiffness (Ganio et al., 2011). It is apparent that heat affects arterial stiffness via endogenous vasodilation (Kellogg Jr, 2006, Kellogg Jr et al., 2003), but it is possible that some methods of experimental whole-body heating stimulate greater production of vasodilators than others.

Effects of heat and humidity on cardiovascular risk

Prolonged exposure to high environmental temperatures and humidity, such as during heatwaves, is associated with increased incidence of CV morbidity (Abrignani et al., 2011) and mortality (Basu, 2009). Conversely, short-term, exposure to heat via dry-heat sauna has been consistently reported to improve haemodynamic function via enhanced endothelial function, reduced brachial BP, decreased preload and decreased afterload in healthy individuals, and patients with CVDs (Blum and Blum, 2007, Imamura et al., 2001, Crinnion, 2011, Hannuksela and Ellahham, 2001, Tei et al., 1995, Miyata et al., 2008, Kihara et al., 2002). In one study, a group of congestive heart failure patients showed reductions in measures of afterload and peripheral resistance which persisted 30 minutes after single heat exposures in a dry-heat sauna and hot-water bath (Tei et al., 1995). The mechanisms responsible for improved haemodynamics from repeated heat exposure after hot sauna

exposure are thought to relate to an increased cardiac output which triggers increased basal production of endogenous vasodilators (such as endothelial-NO-synthase, NO and prostacyclin) and an increase in heart rate variability (Ikeda et al., 2005, Kellogg Jr et al., 2003, Kihara et al., 2004, Miyata et al., 2008), but precise mechanisms remain unclear.

Physiological effects of heat exposure in people with T2DM

Several reviews have reported the effects of heat exposure on haemodynamic function at rest in healthy populations (Kellogg Jr, 2006, Truijen et al., 2010, Charkoudian, 2010, Crandall and Gonzalez-Alonso, 2010, Crandall et al., 2003) and those with chronic disease such as heart failure and heart disease (Blum and Blum, 2007, Crinnion, 2011, Mussivand et al., 2008, Miyata et al., 2008). However, information on the specific effects of heat exposure on markers of CV risk such as aortic PWV or AIx in individuals with T2DM is virtually non-existent. Previous physiological studies report that people with T2DM often have abnormal responses to heat exposure compared to healthy individuals (Petrofsky et al., 2005b; Stansberry et al., 1997; Wick et al., 2006). In previous studies, heat exposures resulted in a lower sweat rate [32°C for 30 minutes in climate chamber (Petrofsky et al., 2005b)], impaired skin blood flow [45°C exposure via water perfused suit (Stansberry et al., 1997)], and delayed onset of vasodilation [45-50°C exposure via a water perfused suit (Wick et al., 2006)]. Moreover, after maximal exercise, people with T2DM have been reported to have a considerably higher brachial and aortic BP response than healthy controls (Scott et al., 2008). A table of the pertinent literature focussing on the haemodynamic effects of whole-body heat exposure in humans in healthy individuals and people with chronic conditions is in Table 2.2.

Table 2.2. Effects of heat exposure on human haemodynamics

Ref	Population (n, M / F)	Age (years)	Method | Temps used ˚C | Duration of heat exposure (mins)	Study Design/ Protocol	Haemodynamic, and/or relevant physiological/ biochemical measures	Temperature: To, Tty, Tr, Tc, Tbl, Tsk	Summary main findings	Limitations / Future Research Questions
(Rowell et al., 1969)	AH (n = 4M)	24-29	Whole Body: Water Perfused Suit | 47.5 and control @ 33-36 | 40-53	Non-randomised, change x time. Participants had cardiac catheterisation, baseline data collected for 30 mins, then again at 10 mins during and end of heating	↑HR, ↓MP, ↑cardiac output, ↔ stroke volume, ↓ total peripheral resistance, ↑central blood volume	↑ Tsk, ↑ Tr, ↑ Tb	CV responses to just-tolerable heat stress were well below the maximum responses observed for exercise alone, but the large increase in cardiac output exceeds the increase in skin blood flow	Observational. No statistical analysis undertaken.
(Andersen et al., 1976)	AH (n=20M), IHD (n=20 M)	45-59	Whole body: climate chamber | 29 (control @23) | 180	Randomised, controlled, change x time. Effects of increasing temp on haemodynamics and mental performance in patients with IHD vs. AH control (Brachial BPs, HR). Heat and control days were consecutive and randomised.	↑↑ HR in heat AH in first hour ↑IHD then slow decrease over next 2 hrs both gps, ↓brachial SBP in IHD gp in heat, ↔brachial SBP AH heat, ↔ brachial DBP both gps in heat, ↑RPP ctrl, ↔RPP IHD in heat	↑Tr in hot trial equally both gps comp. ctrl (↑Tr higher at baseline in IHD gp), ↓Tsk both grps in ctrl, ↑Tsk both gps heat.	IHD patients had the same sensation of discomfort from temperature shifts as AH. Cognition and work ability was reduced in the IHD group in heat, while work performance was improved in ctrl. IHD patients are more sensitive to heat stress for mental tasks, but heat favourably affected haemodynamics in IHD.	A similar study that included women might expand on these findings with regards to any gender differences.
(Koivisto et al., 1981)	T2DM (n=6M)	29±3	Whole body: climate chamber | 35 (control @20) | 240	Randomised, controlled, change x time. Effects of heat on insulin (injected) absorption on insulin dependent T2DM patients.	↑forearm blood flow in heat	↑Tsk heat,	Heat exposure decreased insulin absorption time in all subjects compared to control trial which was thought be caused by the increased blood flow.	Washout period between tests not reported. A trial comparing effects of heat to exercise on insulin absorption would be warranted to see if results are similar.

Abbreviations: AH (apparently healthy), AIx (augmentation index), AV (atrio-ventricular), CHF (chronic heart failure), CO (cardiac output), DBP (diastolic blood pressure), EF (ejection fraction), ET (exercise test), F (female), HR (heart rate), HRV (heart rate variability), HT (hypertensives), IHD (ischaemic heart disease), LVEDV (left ventricular end diastolic volume), M (male), MP (mean blood pressure), MRI (magnetic resonance imaging), N/A (not applicable/not measured), NO (nitric oxide), NS (non-significant), PWV (pulse wave velocity), RPP (rate-pressure product), RV (right ventricle), SBF (skin blood flow), SBP (systolic blood pressure), SV (stroke volume), T1DM (type 1 diabetes mellitus), Tb (blood temperature), Tc (core temperature – telemetry pill), To (temperature oral – sublingual or oesophageal), Tr (temperature rectal), Tsk (temperature skin), Tty (temperature tympanic), TPR (total peripheral resistance).

Ref	Population (n, M / F)	Age (years)	Method | Temps used ˚C | Duration of heat exposure (mins)	Study Design/ Protocol	Haemodynamic, and/or relevant physiological/ biochemical measures	Temperature: To, Tty, Tr, Tc, Tbl, Tsk	Summary main findings	Limitations / Future Research Questions
(Tei et al., 1995)	AH (n=13, gender not reported), CHF (n=34, 19M)	AH 52±13 / CHF 58±14	Whole body: hot baths and sauna | hot bath @41 / sauna @60, Bath 10 / sauna | 15	Non-randomised, controlled, change x time design. Tested the effects of hot baths & sauna on CV function in CHF patients. Baths completed in all patients, then after 1-2 day washout, sauna test followed. After baseline measures in normothermic air temps, measures were repeated during and 30 mins after each treatment. Results given are for during treatment	↑↑HR in hot bath, ↑ HR sauna, ↑ brachial SBP hot bath, ↔brachial SBP sauna, ↓ brachial DBP hot bath, ↑brachial DBP sauna, ↑cardiac index, stroke index, max 0_2 consumption equally in both trials	↑Tbl equally both trials	Several measures of haemodynamics and LV and RV function of CHF patients were increased during, but reduced after finishing 30 mins of hot water immersion or sauna. Haemodynamic Improvements were attributed to reduced preload and afterload due to heat related systemic vasodilation.	Long term effects of repeated hot water or sauna bathing remains unknown.
(Moses et al., 1997)	AH (n=7M)	24±2	Whole body: climate chamber | 20, 25, 30 & 35 | 150	Randomised temperatures each visit. Resting, seated, 2 hour oral glucose test performed in 4 climate conditions to assess effect of different ambient temperatures on venous blood glucose load. Measures taken at 0, 60, and 120 mins	↑↑HR @ 35˚, ↑ HR@ 30˚, ↑plasma glucose at 30 and 35˚C, ↔plasma insulin any condition	↑Tty & Tsk @35˚	Plasma glucose was increased at 60 mins exposure at high temperatures (30 and 35˚C) with no difference in magnitude of change between the two hot conditions, and was not affected at 20 or 25˚C. Plasma insulin was unaffected by all conditions.	Tests were on young white males , results need confirmation in other ages, genders, racial groups and clinical conditions. Not reported if tests were administered on different days or if there was any washout period.
(Stansberry et al., 1997)	AH (n=20), T2DM (n=40) | gender not reported either group	AH 17-72, T2DM 17-71	Localised: heat pad | 45 | 5	Non-randomised, controlled. Tested skin blood flow (laser Doppler) response to local heat, cold, mental tasks and handgrip. Trials consecutively applied with 5 mins break between each. Results given for heat only	↑↑SBF AH, ↑SBF T2DM	N/A	Application of heat results in impaired skin vasodilation responses in T2DM which is likely related to reduced microvascular reactivity, increased glycosylation of vascular tissue. Negative effect of age on SBF observed in AH group was similar to T2DM response, while there was no effect of age in the T2DM gp which suggests that T2DM effects on vasculature is similar to advanced ageing.	Future study could include neural measures that can determine whether heat-induced decrements in SBF are neural or microvascular in nature for T2DM. Trial order could have been randomised to minimise any potential order effect.
(Kellogg Jr et al., 1998)	AH (n=8, 7M), HT (n=8M)	47±3 / 47±3	Whole body: water perfused suit | 38-42	Non-randomised, controlled, group x time. Effect of heat stress on SBF in hypertenstives.	↓MP in HT, ↔MP in AH, ↑↑SBF HT, ↑SBF AH	To similar both gps	Despite lower SBF in hypertensives than AH at baseline, and there were	Randomisation of trial order may have ensured there was no order effect on results.

Abbreviations: AH (apparently healthy), AIx (augmentation index), AV (atrio-ventricular), CHF (chronic heart failure), CO (cardiac output), DBP (diastolic blood pressure), EF (ejection fraction), ET (exercise test), F (female), HR (heart rate), HRV (heart rate variability), HT (hypertensives), IHD (ischaemic heart disease), LVEDV (left ventricular end diastolic volume), M (male), MP (mean blood pressure), MRI (magnetic resonance imaging), N/A (not applicable/not measured), NO (nitric oxide), NS (non-significant), PWV (pulse wave velocity), RPP (rate-pressure product), RV (right ventricle), SBF (skin blood flow), SBP (systolic blood pressure), SV (stroke volume), T1DM (type 1 diabetes mellitus), Tb (blood temperature), Tc (core temperature – telemetry pill), To (temperature oral – sublingual or oesophageal), Tr (temperature rectal), Tsk (temperature skin), Tty (temperature tympanic), TPR (total peripheral resistance).

Ref	Population (n, M / F)	Age (years)	Method | Temps used ˚C | Duration of heat exposure (mins)	Study Design/ Protocol	Haemodynamic, and/or relevant physiological/ biochemical measures	Temperature: To, Tty, Tr, Tc, Tbl, Tsk	Summary main findings	Limitations / Future Research Questions
			(control @34) | 35-45				differences in MAP during heating, vasodilation threshold was similar between hypertensives and AH gps indicating that the neural control of cutaneous vasodilation is not altered in hypertensives.	
(Minson et al., 1998)	AH young (n=7M), AH older (n=7M)	young gp 23±1 / older gp 70±3	Whole body: water perfused suit | 50 (control @34) | 60	Non-randomised, controlled, group x time. Effect of age on CV variables and SBF.	↑HR similar both gps, ↔MP both gps, ↑↑ brachial SBP young, ↑ brachial SBP older, ↑↑ brachial DBP young, ↑ brachial SBP older, ↑↑CO in younger, ↑older. ↓TPR similar both gps.	↑To and Tsk similar both gps	Haemodynamic and blood flow responses were lower in older compared to younger men, but time to heat tolerance and temps were similar during heating. Amount of CO directed to skin was lower in older vs. younger men. Results have implications for greater CV risk in older men during hot-humid conditions due to their reduced inotropic and increased chronotropic response.	Future work could investigate if younger men have more effective heat dissipation than older men which may account for similar To but different SBF between gps.
(Crandall et al., 2000)	AH (n=14, 10M)	31±5	Whole body: Water Perfused Suit | 48 and control condition with no water perfused | 26-32	Randomised, case-controlled. Fixed and randomised breathing protocols to test baroreflex control of heart rate.	↑HR, ↓MP (NS), ↑ brachial SBP (NS), ↓ brachial DBP (NS), ↑SBF	↑ To, ↑ Tsk	Heat reduced high frequency dynamic baroreflex HR regulation associated with BP reductions	It is possible that alteration in HR was influenced by breath pacing protocols used in this study.
(Kihara et al., 2002)	CHF (n=30, 18M)	63±16	Whole body: sauna | 60 sauna and control at 24 | 15 seated sauna followed by 30 mins warm supine rest	Controlled, group x time. Effect of repeated exposure to heat therapy on cardiac function in CHF patients (sauna 1xday for 5 days over 2 weeks). Tests performed before sauna therapy and on the last day of therapy.	↓brachial SBP ↓LV EDV ↓flow-mediated dilation	N/A	Repeated heat exposure improves endothelial and cardiac function, and improves clinical symptoms of CHF.	Only included stable CHF patients, thus repeated study with acute heart failure of worse CHF is warranted to see if heat therapy is safe and improves symptoms.

Abbreviations: AH (apparently healthy), AIx (augmentation index), AV (atrio-ventricular), CHF (chronic heart failure), CO (cardiac output), DBP (diastolic blood pressure), EF (ejection fraction), ET (exercise test), F (female), HR (heart rate), HRV (heart rate variability), HT (hypertensives), IHD (ischaemic heart disease), LVEDV (left ventricular end diastolic volume), M (male), MP (mean blood pressure), MRI (magnetic resonance imaging), N/A (not applicable/not measured), NO (nitric oxide), NS (non-significant), PWV (pulse wave velocity), RPP (rate-pressure product), RV (right ventricle), SBF (skin blood flow), SBP (systolic blood pressure), SV (stroke volume), T1DM (type 1 diabetes mellitus), Tb (blood temperature), Tc (core temperature – telemetry pill), To (temperature oral – sublingual or oesophageal), Tr (temperature rectal), Tsk (temperature skin), Tty (temperature tympanic), TPR (total peripheral resistance).

Ref	Population (n, M / F)	Age (years)	Method | Temps used ˚C | Duration of heat exposure (mins)	Study Design/ Protocol	Haemodynamic, and/or relevant physiological/ biochemical measures	Temperature: To, Tty, Tr, Tc, Tbl, Tsk	Summary main findings	Limitations / Future Research Questions
(Lietava et al., 2004)	AH (n=21F)	50-60	Whole body: hot water immersion (bath) | 40 | ~20 (until To ↑by~2˚C)	Non-randomised, group x time. Compared effects of maximal exercise test (ET) and hot water bath on CV variables (measured at resting baseline, during, and 5 mins after each test).	↑↑HR ET, ↓HR bath, ↑↑MP ET, ↑MP bath, ↑↑cardiac index ET, ↑ cardiac index bath, ↑↑ cardiac contractility ET, ↔ cardiac contractility bath.	N/A	Hot bath induced lower haemodynamic load compared with a max ET Hot bath decreased LV work contractility and BP compared to ET.	Inclusion of a submax or light-intensity ET and other populations groups in future study would widen applicability of results. Participants were immersed up to the neck which may have increased hydrostatic forces which increase venous return and confounded haemodynamic results. Immersion to clavicular level has been found to minimise hydrostatic pressure.
(Petrofsky et al., 2005a)	AH (n=15), T1DM (n=8), T2DM (n=12) gender not reported any group	51/ 55 / 58	Whole body: climate chamber | 42 (control @ 22) | 30	Non-randomised, controlled. Tested effects of heat on orthostatic intolerance in AH & T1 & T2DM. Measures taken horizontally in room temp, then again during 2.5 mins of 45˚ head up tilt, then again for 2.5 mins when horizontal again. Measures repeated at 10, 20 and 30 mins after room was heated to 42˚C. T1 & T2DM results were found to be similar & were grouped for analysis. Results given are for heat + horizontal position	↑HR similar between gps, ↔ brachial SBP DM, ↓ brachial SBP AH gp, ↔ brachial DBP DM, ↓ brachial DBP AH, ↑cardiac output similar between gps	↑↑Tc and Tsk DM, ↑ Tc and Tsk AH	Combined DM gp showed greater orthostatic intolerance in heat than AH. HR of DM gp was not affected by tilt in warm environment, but HR of AH was increased indicating abnormal CV response in DM gp thought to be due to possible endothelial dysfunction and neuropathies in DM gp.	Given the complex methods, manuscript (results & discussion) are inconsistent and limited data given. Mention of previous studies in area was not cited in text.
(Wilson et al., 2006)	AH (n=15, 7M)	34±2	Whole body: Water Perfused Suit | 44-46 and control at 35 | not reported (heating until Tc increased 0.6-1.0˚C)	Non-randomised, case-controlled. Effect of heat stress on cerebral blood flow and cerebral vascular resistance. 15 participants completed 25 heat stress trials on cerebral variables	↑HR, ↔ MP, ↓cerebral blood velocity, ↑cerebral vascular resistance	↑ Tsk, ↑ Tc	Heat stress reduces cerebral blood flow velocity during orthostatic challenge, and the reduction is even greater when heat stressed. Implications for reduced orthostatic tolerance in heat (i.e. increased syncope)	Exposure duration not reported.

Abbreviations: AH (apparently healthy), AIx (augmentation index), AV (atrio-ventricular), CHF (chronic heart failure), CO (cardiac output), DBP (diastolic blood pressure), EF (ejection fraction), ET (exercise test), F (female), HR (heart rate), HRV (heart rate variability), HT (hypertensives), IHD (ischaemic heart disease), LVEDV (left ventricular end diastolic volume), M (male), MP (mean blood pressure), MRI (magnetic resonance imaging), N/A (not applicable/not measured), NO (nitric oxide), NS (non-significant), PWV (pulse wave velocity), RPP (rate-pressure product), RV (right ventricle), SBF (skin blood flow), SBP (systolic blood pressure), SV (stroke volume), T1DM (type 1 diabetes mellitus), Tb (blood temperature), Tc (core temperature – telemetry pill), To (temperature oral – sublingual or oesophageal), Tr (temperature rectal), Tsk (temperature skin), Tty (temperature tympanic), TPR (total peripheral resistance).

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(Wick et al., 2006)	AH (n=10), T2DM (n=9), gender not reported either group	AH 57±2, T2DM 57±3	Whole Body: Water Perfused Suit | 45-50 | 60-90 (heating until Tc increased by 0.6˚C)	Non-randomised, controlled. Noradrenergic neutransmission was blocked in the L forearm to assess active vasomotion at that site. Measures were taken at baseline with no water perfused, then during cooling (10-12˚C) to assess vasoconstriction at blockaded site, then again during heating.	HR and MP not reported, ↑↑threshold for vasodilation in T2DM compared to control	↑Tor similar between gps	Despite similar increases from baseline in To, the threshold at which cutaneous vasodilation occurred during heating was higher in T2DM group than in control, meaning that the T2DM patients required a higher temp/more heat to induce vasodilation, which is possibly due to altered hypothalamic control of skin blood flow by active vasodilator systems and reduced expression of NO.	A well-controlled T2DM population in this study means that changes observed may be greater (i.e. thermoregulation more impaired) in a population with more co-morbidities. SBF measures only relevant to specific site measured and are dependent on local vascularisation, and thus cannot be generalised to systemic haemodynamics.
(Crandall et al., 2008)	AH (n=10, 7M)	M 29±5, F 32±5	Whole body: Water Perfused Suit | 46-48 and control @34 | ~40	Non-randomised, controlled. Tested effects of heat stress on central blood volume and blood redistribution. Results from heat stress group compared to results from normothermic group.	↑HR, ↓MP, ↓cardiac, thoracic, liver & spleen blood volume, ↓LV end systolic volume, ↔LV end diastolic volume ↑haematocrit, pH, and LV ejection fraction	↑ Tsk, ↑ Tc	Heat stress reduces blood volume from thoracic and splanchnic regions and shits blood to peripheral regions and increases HR. Heat stress increased cardiac performance by increasing EF.	No measure of actual blood distribution between organs (i.e., liver, heart , spleen) therefore possible interference in measures due to redistribution of blood to overlying skin during heating (but unlikely due to relative thinness of skin compared to viscera/heart)
(Miyata et al., 2008)	CHF (n-112, 74M), AH (n=76, 51M)	CHF 63±13, AH 66±14	Whole body: sauna | 60 | 15 seated sauna + 30 mins warm supine rest	Randomised, controlled. Tested effects of thermal therapy (5days X week for 2 weeks) in CHF patients. Results given are for heated group. Control group continued current medical treatment	↑EF ↓brachial SBP ↓brachial DBP	N/A	Repeated heat therapy is safe and improves clinical symptoms and cardiac function in CHF patients.	Effect of heat therapy on other measures such as prognosis in CHF is unknown, future study could assess this

Abbreviations: AH (apparently healthy), AIx (augmentation index), AV (atrio-ventricular), CHF (chronic heart failure), CO (cardiac output), DBP (diastolic blood pressure), EF (ejection fraction), ET (exercise test), F (female), HR (heart rate), HRV (heart rate variability), HT (hypertensives), IHD (ischaemic heart disease), LVEDV (left ventricular end diastolic volume), M (male), MP (mean blood pressure), MRI (magnetic resonance imaging), N/A (not applicable/not measured), NO (nitric oxide), NS (non-significant), PWV (pulse wave velocity), RPP (rate-pressure product), RV (right ventricle), SBF (skin blood flow), SBP (systolic blood pressure), SV (stroke volume), T1DM (type 1 diabetes mellitus), Tb (blood temperature), Tc (core temperature – telemetry pill), To (temperature oral – sublingual or oesophageal), Tr (temperature rectal), Tsk (temperature skin), Tty (temperature tympanic), TPR (total peripheral resistance).

Ref	Population (n, M / F)	Age (years)	Method | Temps used ˚C | Duration of heat exposure (mins)	Study Design/ Protocol	Haemodynamic, and/or relevant physiological/ biochemical measures	Temperature: To, Tty, Tr, Tc, Tbl, Tsk	Summary main findings	Limitations / Future Research Questions
(Brothers et al., 2009)	AH (n=9M)	38±10	Whole body: Water Perfused Suit | 47-49 and control @35 | Not reported (heating until Tc increased ~1.0˚C)	Non-randomised, case controlled. Tested effects of heat stress on cardiac function. Baseline data collected during normothermia ~30 mins, then again during heating	↑HR, ↓MP (NS), ↑forearm cutaneous vascular conductance, ↓ central blood volume, ↔ measures of cardiac diastolic function, ↑ measures cardiac systolic function	↑ Tsk, ↑ Tc	Despite decreased central blood volume and cardiac filling pressures, heat stress improved atrial and ventricular systolic function and maintained diastolic function in healthy men.	Duration of heating not reported. TPR and afterload were not assessed therefore possible that increases in measures of systolic function were due to reduced afterload.
(Wilson et al., 2009)	AH (n=11M)	28±4	Whole body: water perfused suit | 46 (control @34) | duration not reported (until Tb increased by 1˚C)	Non-randomised, controlled, change x time. Tested effects of consecutive heat and cold and simulated orthostatic tolerance (lower body negative pressure which reduces preload) on CV function compared to thermoneutral. 30 mins recovery between tests. Only heat results presented here.	↑HR, ↓MP, ↓ brachial SBP, ↓ brachial DBP, ↑CO, ↑mixed AV-O_{2sat}% ↑SV, ↑RPP, ↓LVEDV	↑Tbl, ↑Tsk	Heating increased the reduction in SV, CO and LVEDV in response to simulated orthostasis compared to control. Hypotension and decreased PVR during heating did not reduce CO which highlights the importance of increased CO during orthostatic intolerance to overcome this mismatch in up- and downstream pressures.	Symptoms of syncope (severe hypotension) resulted in 3 datasets being excluded from analysis, thus n=8 analysed. Reproduction of trials with plasma volume infusion to stabilise central blood volumes to explore Frank-Starling contractility mechs during heat and orthostatic challenge would be beneficial.
(Bellien et al., 2010)	AH (n=11M)	24±2	Localised: Hot water immersion | 44 | not reported	Non-randomised, controlled. Effect of infusion of NO synthase inhibitor drugs Vs. saline (control). Gradual heating used to minimise effects on systemic haemodynamics. Artery wall stress and blood flow measured via Doppler	↔HR, ↓MP(NS), ↔ brachial SBP, ↔ brachial DBP, ↓ radial mid-wall stress	not reported	Local hand heating with control infusion (saline) resulted in increased shear stress (from increased blood flow) and reduced radial artery wall stress (i.e. smooth muscle relaxation), a decrease in radial artery wall stiffness and an increase in arterial compliance. Inhibition of NO pathways prevented any decrease in smooth muscle tone during heating demonstrating importance of NO to vasodilation via the vascular endothelium during heating	Localised heat used so findings possibly not generalisable to whole body conditions. Moreover, it is still unclear whether NO affects large elastic arterial compliance (i.e. aorta) during whole body heating

Abbreviations: AH (apparently healthy), AIx (augmentation index), AV (atrio-ventricular), CHF (chronic heart failure), CO (cardiac output), DBP (diastolic blood pressure), EF (ejection fraction), ET (exercise test), F (female), HR (heart rate), HRV (heart rate variability), HT (hypertensives), IHD (ischaemic heart disease), LVEDV (left ventricular end diastolic volume), M (male), MP (mean blood pressure), MRI (magnetic resonance imaging), N/A (not applicable/not measured), NO (nitric oxide), NS (non-significant), PWV (pulse wave velocity), RPP (rate-pressure product), RV (right ventricle), SBF (skin blood flow), SBP (systolic blood pressure), SV (stroke volume), T1DM (type 1 diabetes mellitus), Tb (blood temperature), Tc (core temperature – telemetry pill), To (temperature oral – sublingual or oesophageal), Tr (temperature rectal), Tsk (temperature skin), Tty (temperature tympanic), TPR (total peripheral resistance).

Ref	Population (n, M / F)	Age (years)	Method | Temps used ˚C | Duration of heat exposure (mins)	Study Design/ Protocol	Haemodynamic, and/or relevant physiological/ biochemical measures	Temperature: To, Tty, Tr, Tc, Tbl, Tsk	Summary main findings	Limitations / Future Research Questions
(Nelson et al., 2010)	AH (n=8M)	24±2	Whole Body: Water Perfused Suit (plus covered with blanket to minimise any evaporative heat loss) | 50 (control @34) | 60	Effects of passive heat stress (therefore reduced filling pressures) on early diastolic function. Supine resting baseline measures taken during control condition, then again when heat increased. On a separate day (tests 7 days apart) cardiac MRI was performed during identical heating.	↑HR, ↓↓LV stroke volume, ↓RV stroke volume. ↓ RV and LV end diastolic and end systolic, and L atrial volumes, ↑ LV end-systolic elastance, ↓end systolic wall stress ↑ejection fraction, ↑LV untwist rate, ↔ diastolic filling rate	↑Tc, ↑Tsk	Heating reduced bi-ventricular preload and L atrial volume but early diastolic function was maintained due to increased LV twist and untwist rates which augmented diastolic suction. Heating also increased LV contractility (increased L AV valve tissue velocity). As SV did not change, CO augmented solely by increase in HR.	Tc could not be measured on MRI day due to the telemetry pill containing metal therefore Tc changes in MRI test + heating were based on prior results.
(Ganio et al., 2011)	AH (n=8, 3M)	37±11	Whole body: Water Perfused Suit | 49 (control @34 | 36-77	Non-randomised, change x time. Effects of increased Tc on PWV. Baseline data collected during normothermia and at ~30 mins, then repeated during heating	↑HR, ↓MP @36 mins, ↑MP@60-77 mins (NS), ↓ brachial SBP @36 mins (NS) , ↑ brachial SBP @60-77 mins (NS), ↓ brachial DBP @36 mins , ↑ brachial DBP @60-77 mins (NS), ↑aortic and brachial PWV (NS)	↑Tc, ↑Tsk	No significant change in PWV with heat stress but magnitude of change was correlated to a higher baseline PWV	No bloodwork to confirm healthy status.
(Huang et al., 2011)	AH (n=60, 29M)	25±4	Localised: water immersion | 45 | 2	Non-randomised, controlled, change x time. Effects of heat and cold on radial pressure pulse, radial AIx and heart rate variability (HRV). Only heat results presented here.	↔HR, ↔ brachial SBP, ↔ brachial DBP, ↓radial AIx, ↓radial diastolic pressure augmentation	↑Tsk hand	Local heating of hand reduced radial AIx and diastolic augmentation pressure which reflects reduced LV load	The study had good statistical power and gave a lot of data, however, radial AIx is more reflective of peripheral pressure augmentation and may not be an accurate indicator of cardiac function

Abbreviations: AH (apparently healthy), AIx (augmentation index), AV (atrio-ventricular), CHF (chronic heart failure), CO (cardiac output), DBP (diastolic blood pressure), EF (ejection fraction), ET (exercise test), F (female), HR (heart rate), HRV (heart rate variability), HT (hypertensives), IHD (ischaemic heart disease), LVEDV (left ventricular end diastolic volume), M (male), MP (mean blood pressure), MRI (magnetic resonance imaging), N/A (not applicable/not measured), NO (nitric oxide), NS (non-significant), PWV (pulse wave velocity), RPP (rate-pressure product), RV (right ventricle), SBF (skin blood flow), SBP (systolic blood pressure), SV (stroke volume), T1DM (type 1 diabetes mellitus), Tb (blood temperature), Tc (core temperature – telemetry pill), To (temperature oral – sublingual or oesophageal), Tr (temperature rectal), Tsk (temperature skin), Tty (temperature tympanic), TPR (total peripheral resistance).

Passive heating compared to active heating (exercise)

To date, there are no data that demonstrate the effects of whole-body heat exposure on aortic AIx, aortic BP, or on measures of aortic reservoir function in any population. However, it is clear that passive heat exposure causes vasodilatation (Kellogg Jr et al., 2003), as does active heating via exercise in moderately-trained healthy individuals (Munir et al., 2008). While the mechanisms for vasodilation, i.e. increased blood velocity and shear stress, and release of endogenous vasodilators, are thought to be similar for heat and exercise (Brockow et al., 2011, O'Rourke and Hashimoto, 2008) there are distinct biochemical and haemodynamic differences in physiological reactions between the two.

One of the main haemodynamic differences between passive heating and exercise is that exercise causes a larger increase in venous return than heating. This is due to greatly increased pumping of venous blood via skeletal muscle contractions and increased respiration (Powers and Howley, 2009), which leads to an increase in stroke volume via the Frank-Starling mechanism, and an increase in cardiac output of up to ~25 $L.min^{-1}$ during moderate-vigorous exercise in men (Astrand et al., 1964). However, during exercise, increases in cardiac output also increase brachial and aortic BPs (Sharman et al., 2006), while during whole-body passive heating BPs are typically reduced (Crandall and Gonzalez-Alonso, 2010, Rowell et al., 1969). Moreover, the effect of whole-body heating on venous return is typically smaller than that of exercise, and leads to increases in cardiac output of up to ~13 $L.min^{-1}$ in healthy men (Rowell et al., 1969). Thus, changes in cardiac output, and by extension, venous return during heating are more dependent on an increase in heart rate, as stroke volume and BPs do not tend to increase during heat exposure (Rowell et al., 1969).

Vasodilator medications compared to heat and exercise

Along with passive heating and acute exercise, vasodilatory medications are used to study challenges to haemodynamic function. Vasodilatory medications such as nitroglycerin (Munir et al., 2008) and dobutamine (Sharman et al., 2009) have various effects on the heart and blood vessels, and reduce BP as they dilate muscular arteries. However, their effect on central haemodynamic measures such as aortic PWV, aortic AIx, or aortic BP has not been extensively investigated. Two studies have reported that vasodilator drugs decreased AIx by ~23% (Munir et al., 2008) to ~34% (Sharman et al., 2009) and aortic systolic BP by ~10 (Munir et al., 2008) to ~20 mm Hg (Sharman et al., 2009), at peak infusion. The effect of haemodynamic perturbations on components of aortic reservoir function has also been investigated by two studies which used vasodilators [dobutamine in humans (Sharman et al., 2009) and nitroprusside in dogs (Wang et al., 2012)]. These studies each reported decreased P_{res} and P_{ex} (Sharman et al., 2009, Wang et al., 2012) during pharmacological vasodilation.

Table 2.3 presents a comparison of changes from selected studies employing different methods to produce vasodilation and demonstrates that there are marked differences in the magnitude of CV responses resulting from vasodilatory drugs compared to changes via heat exposures and exercise. Indeed, effects of vasodilator medications are dose dependent and different medications will affect vasodilation and heart rate to varying degrees. It could be hypothesised that as moderate exercise and passive heat exposure induce vasodilation by similar mechanisms [i.e. increased shear stress and up-regulation of NO-synthase (Maiorana et al., 2003, Brockow et al., 2011) and result in similar increases to heart rate (Vuori, 1988, Maiorana et al., 2003)], that passive heating may cause similar changes in AIx as moderate exercise. It should be noted, that while the comparison of studies using different methods to produce vasodilation may provide

information one may use to form hypotheses about potential direction of change in CV variables, due to the mixed mechanisms by which medications, compared to heat and exercise, cause vasodilation, any comparisons between studies must be conducted with these differences in mind. Table 2.3 also demonstrates the differences in heat stress on CV variables, most notably on heart rate, between the most commonly used whole-body heating methods. The apparent differences in magnitude of effect between studies using different methodology suggest that of these methods, the water-perfused suit potentially imposes the greatest heat load for a similar exposure time. Water-perfused suits circulate water of any desired temperature through networks of tubes which are directly against the skin (Figure 2.12). Moreover, the suits leave several parts of the body (head, face, forearms, hands and feet) in normothermic temperature, which could be a confounding influence in cool and hot temperatures. These suits are designed to clamp skin temperatures at the experimental target range (Rowell, 1974). Thus, water-perfused suits may diminish the ability of the body to naturally thermoregulate, which may potentially produce a stronger haemodynamic stimulus than would whole-body air exposure at the same temperature.

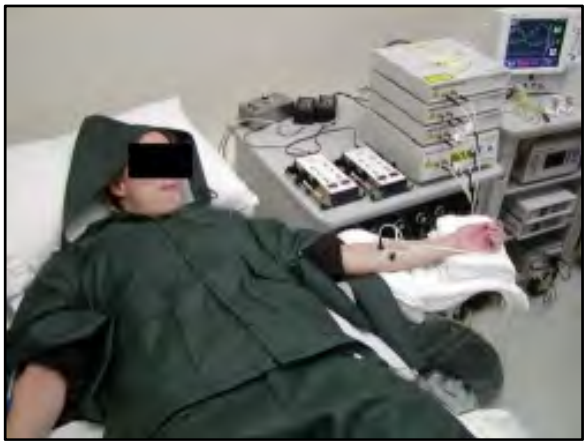

Figure 2.12. A typical experimental water-perfused suit.

From (Kansas State University. Cardiovascular and Thermal Physiology Lab, 2012).

Table 2.3. Example of changes from normothermic baseline of selected cardiovascular variables via different whole-body vasodilation stimuli

Condition Population (Reference)	**Heart rate (beats.min^{-1})**	**Brachial Systolic BP (mm Hg)**	**AIx (%)**	**Aortic PWV (m.s^{-1})**
Medication – dobutamine, peak dose 30-40 μg.kg.min^{-1})				
AH group (Sharman et al., 2009)	↑57	↑5	↓34	↓1.0
Medication –nitroglycerin,				
AH group peak dose 100 μg.min^{-1} (Munir et al., 2008)	↑5	↓8	↓23	↓0.5
AH group peak dose 300 μg.min^{-1} (Kelly et al., 2001)	↑16	↓16	↓17	↓0.7
Heat stress – Water-perfused suit				
AH group - 49°C water, until core temp increased ~1°C (Brothers et al., 2009)	↑27	↓2	_	-
AH group - 34°C, until core temp increased ~1°C (Crandall et al., 2008)	↑40	-	-	-
AH group – 34-36°C water, 30 mins (Rowell et al., 1969)	↑82	-	-	-
AH group - 49°C water, ~77 mins (Ganio et al., 2011)	↑50	↑4	-	↑0.36
Heat stress – head-out water immersion, bath				
CHF group - 41°C, 10 mins (Tei et al., 1995)	↑25	↑3	_	_
AH females - 40°C, 20 mins (Lietava et al., 2004)	↑35			
Heat stress – dry sauna				
CHF group - 60°C, 15 mins (Tei et al., 1995)	↑20	↑1	-	-
Heat stress – climate chamber				
AH group - 42°C / 40% RH, 30 mins (Petrofsky et al., 2005a)	↑8	↓7	-	-
AH group - 35°C / 50% RH, 150 mins (Moses et al., 1997)	↑16	↓3	-	-
T2DM group - 42°C / 40% RH, 30 mins (Petrofsky et al., 2005a)	↑9	-	-	-
IHD group - 29°C / 50% RH, 180 mins (Andersen et al., 1976)	↑7	↓4	-	-
Light exercise (~12 mins)				
AH group (Munir et al., 2008)	↑3	↑2	↓3	↑0.3

Abbreviations: AH (apparently healthy), AIx (augmentation index), BP (blood pressure), CHF (chronic heart failure), PWV (pulse wave velocity), IHD (ischaemic heart disease).

2.5.2.3.6. Effects of humidity

Environmental humid-heat exposure

Prolonged exposure to heat with high humidity may present an additional challenge to thermoregulation over that of dry-heat, as the high water vapour pressure gradient of the air reduces the body's ability to remove heat via evaporative sweat (Holmer, 2006). Prolonged exposure to increased environmental heat plus humidity has been associated with increased hospital admissions for non-fatal acute coronary syndromes (Abrignani et al., 2009). The causes for the association between humid-heat and increased CV events appears to depend somewhat on the geographical location of the population studied. For instance, in one study in subarctic Sweden, increasing environmental heat plus humidity was thought to be associated with increased incidence of acute myocardial infarction due to the unusualness of extreme heat and humidity in that climate (Messner et al., 2003). The population in that study likely had physiological adaptations that are favourable to a cold climate, such as increased blood viscosity and blood clotting factors, and thus lacked acclimatisation to sudden heat and humidity (Messner et al., 2003). In contrast, increased heat plus humidity has also been associated with decreases in hospital admission for angina pectoris in 2,459 patients admitted over 12 years in a study based in Sicily, Italy; a mild, Mediterranean climate (Abrignani et al., 2011). In addition, heat plus humidity was associated with decreases in admission for acute myocardial infarction in a study with 5,458 patients conducted over 8 months in Athens, Greece; a subtropical Mediterranean climate (Panagiotakos et al., 2004), while in another study undertaken in Poland, which has a cool Continental climate, 49 patients with renal failure were found to have atypical decreases in systolic and diastolic BP during exposure to hot-humid conditions (Wystrychowski et al., 2005). Further, in

another epidemiological which focussed on CV mortality, no association was found between CV mortality and humid-heat across 12 cities in the USA (Braga et al., 2002).

Despite the lack of association between CV morbidity, mortality and humid-heat, frequently (Geor et al., 1995, Smolander et al., 1987) but not always (Wenzel, 1978, Moran et al., 1996), heart rates are higher and brachial systolic BPs are lower during exposure to humid compared to dry-heat at rest and during exercise. Thus, mechanisms that may contribute to morbidity and mortality during prolonged exposure to humid-heat include: considerably increased heart rates which can disrupt atheromous plaques and cause myocardial ischemia or infarct in individuals with CHD; inefficient thermoregulation and vasodilation; dehydration; and electrolyte imbalances (Basu, 2009, Panagiotakos et al., 2004, Abrignani et al., 2009, Blatteis, 2011, Petrofsky et al., 2012).

Effects of heat plus humidity on human physiology

Several studies have investigated the effects of short-term exposure to whole-body heat plus humidity on physiology and peripheral haemodynamics during rest and exercise, frequently with the purpose of assessing athletic or work performance during environmental stress. However, none have studied the effects of humidity, with, or without heat, on central haemodynamics at rest. In a rat model under resting conditions, exposure to hot-humid conditions produced lower heart rates, core temperatures, and BPs (measured at the tail) compared to hot-dry conditions (Moran et al., 1996). In resting humans, hot-humid conditions produced lower breathing frequency and higher tidal volume and expiration/inspiration time compared to cool-dry conditions, suggesting improved conditions for gas exchange in hot-humid conditions (Turner et al., 1992). Moreover, Turner *el al.,* (1992) found that similar improvements in respiration in hot-humid conditions were also apparent during low and moderate-intensity exercise, but no

CV variables were measured in that study. The reasons for the apparent improvement in respiratory function in humid-heat are currently unknown, but Turner *et al.,* (1992) suggested it may be due to temperature sensitive receptors that have been found in the larynx in humans, and may also be present in the lungs. It is unclear if the improved respiratory function observed by Turner and colleagues (1992) was a result of heat alone, or of the heat plus humidity, but the results seem to indicate that humidity may be the determinant, as there was no change in breathing frequency or tidal volume in hot-dry conditions. In another study in chronic obstructive pulmonary disease patients, long-term nasal breathing of warm-humid air (1-2 hours every day for 12 months) was shown to reduce symptoms, i.e. reduced sputum volume, cough and dyspnoea, and improve lung function [improved forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC)] (Rea et al., 2010). Further, Rea *et al.,* (2010) found that the magnitude of improvement was similar to that of medication use. However, despite significant symptom improvements, the warm-humid air treatment was not enough to increase FEV_1 or FVC results to "normal" spirometry levels (Rea et al., 2010). In addition, breathing warm-humid air has been shown to reduce incidence of exercise-induced asthma compared to breathing cool-dry air (Tan and Spector, 1998).

Three other studies comparing the effect of light-to-moderate exercise in hot-humid conditions to exercise in hot-dry conditions have reported that hot-humid conditions resulted in: a slightly lower systolic BP, measured plethysmographically (Smolander et al., 1987); lower expression of the stress hormone interleukin-6 (Wright et al., 2012); and a lower heart rate (Wenzel, 1978) than in hot-dry conditions. In contrast, submaximally-exercised horses have been observed to have higher heart rates and lower core temperatures during exercise in hot-humid compared to hot-dry conditions (Geor et

al., 1995). The mixed methodologies and different species used in these studies make definitive conclusions difficult. Nevertheless, taken together, hot-humid conditions appear to improve or at least have little detrimental effect on physiological function.

Independent effects of humidity on physiology

Very few studies have investigated the independent effects of humidity, i.e. high humidity compared to low humidity at the same air temperature, on resting human physiology. One study examined the effects of differing humidity at 'room temperature' of 25°C, on perceived and mucosal comfort, heart rate and brachial BP at rest (Sunwoo et al., 2006). Sunwoo *et. al.,* (2006) reported no change in heart rate, systolic and diastolic BP with exposure to humidity levels at 10, 30 and 50% RH, although no data were presented. Moreover, Sunwoo *et. al.,* (2006) observed decreases in thermal and mucosal comfort; more specifically, increased feelings of coolness and increased eye blinking rate, and decreased skin hydration and temperature with lower humidity exposure at 10 and 30% RH, compared to exposure at 50% RH. However, 10 to 50% RH may be classified as low-to-medium humidity levels (ASHRAE, 2004), thus, the effects of high humidity (i.e. >70% RH) on resting human haemodynamics and other physiological variables remains unknown.

Other studies have also reported that room-temperature conditions with high humidity resulted in no change in skin temperature (Freeman and Lengyel, 1938) and heart rate (Wenzel, 1978), compared to reduced skin temperatures (Freeman and Lengyel, 1938) and slightly increased heart rates (Wenzel, 1978) during exposure to room temperatures with low humidity. Table 2.4 presents the available literature investigating the effects of heat plus humidity, and humidity at room temperature on human haemodynamics and thermoregulation.

Table 2.4. Effects of whole-body exposure to high humidity on human haemodynamics

Reference	Population (n, M / F)	Age (years)	Method | Temps used ˚C | Relative humidity (%) | Duration of Exposure (mins)	Study Design/ Protocol	Haemodynamic, and/or relevant physiological and biochemical measures	Temperature: To, Tty, Tr, Tsk	Summary main findings	Limitations / Future Research Questions
(Freeman and Lengyel, 1938)	AH (n=10M)	not reported	Whole body: climate chamber | 24 and 32 | 20 to 90 | 180	Non-randomised, change x time. Effects of high and low humidity on body temperatures in increasing temperatures.	N/A	@24˚C ↔Tsk @RHs between 50-78%, ↓Tsk @RHs 20-49 and 78-90. At 32˚C ↑Tsk with increasing humidity	Increasing humidity increased Tsk in both 24 and 32˚C compared to 20% RH. In 24˚C, Tsk fell at RH from 20-49% and stabilised (reduction was attenuated) at RHs from 50-78%, from 78-90% RH Tsk began to slowly cool again but stayed well above Tsk reached at 20% RH. At 32˚C increasing humidity caused higher Tsk than heat at low RH.	An early study, but one of the very few that has focussed on direct effects of humidity on human physiology in both "room" temperatures and heat.
(Wenzel, 1978)	AH (n=8M)	"young", age not reported	Whole body: climate chamber | 15 to 57 | 8 to 97 | 240	Non-randomised, change x time. Effects of combinations of temp and humidity during light to moderate treadmill exercise. Comparison of equivalent heat loads but different heat-stress indices.	↔ HR@24°/80%, ↑HR@24°/20%. ↑↑HR @hot-dry (15%), ↑ HR@hot/ humid (88%)	↑↑Tr hot-dry, ↑hot-humid	During light exercise HRs in hot-humid climates of equal WBGT were significantly lower than in hot-dry conditions. A similar but smaller effect was seen for HRs in room temperature with high humidity. At a stable core temperature, RH was only found to influence sweat rate if the workload was increased.	Often results from a single participant were described, thus results may not be generally applicability to wider AH population.
(Smolander et al., 1987)	AH (n=16M, 8 trained / 8 untrained)	28-37	Whole body: climate chamber | 20 / 30 / 40 | 20 / 40 / 80 | 210	Randomised trial order. CV and thermoregulatory variables in response to 3.5 hours of light treadmill exercise in control (20°/40%), hot-dry (40°/20%) and hot-humid (30°/80%) conditions with 1 week washout. Hot-dry and hot-humid had similar WBGT of 28°C. Subjects wore industrial work clothing.	↑HR (NS) in hot/ humid, ↓↓ brachial SBP hot-humid, ↓ brachial SBP hot-dry, ↑oxygen consumption in hot-humid both gps, ↔CO both gps both conditions, ↓SV similar btwn conditions and gps,	↑Tr and Tsk similar during both conditions	Physiological strain was similar between the two conditions. Yet, despite similar increases in core and skin temps in both conditions, HR was NS higher (~5 beats.min^{-1}) in hot-humid compared to hot-dry and systolic BP was lower in hot-humid compared to hot-dry. Results suggest that hot-humid conditions are not more stressful to CV physiology than hot-dry conditions.	Cardiac output was determined by the CO_2 rebreathing method that estimates pulmonary. blood flow, which due to the need to hyperventilate during the procedure, may have influenced HRs and thereby underestimated changes in CO.

Abbreviations: AH (apparently healthy), AIx (augmentation index), CHD (chronic heart disease), CHF (chronic heart failure), CO (cardiac output), COPD (chronic obstructive pulmonary disease), CPAP (continuous positive airway pressure), CV (cardiovascular), DBP (diastolic blood pressure), ET (exercise test), F (female), FEV_1 (forced expiratory volume in 1 second), FVC (forced vital capacity), HR (heart rate), HT (hypertensives), IHD (ischaemic heart disease), IL-6 (interleukin 6), IL-12 (interleukin 12), M (male), MP (mean blood pressure), MRI (magnetic resonance imaging), N/A (not applicable/not measured), NO (nitric oxide), NS (non-significant), OSA (obstructive sleep apnoea), PWV (pulse wave velocity), RH (relative humidity), RPP (rate-pressure product), SBF (skin blood flow), SBP (systolic blood pressure), SV (stroke volume), Tb (blood temperature), Tc (core temperature – telemetry pill), TNFα (tumour necrosis factor alpha), To (temperature oral – sublingual or oesophageal), Tr (temperature rectal), Tsk (temperature skin), Tty (temperature tympanic), TPR (total peripheral resistance), WBGT (wet-bulb globe temperature).

Reference	Population (n, M / F)	Age (years)	Method | Temps used ˚C | Relative humidity (%) | Duration of Exposure (mins)	Study Design/ Protocol	Haemodynamic, and/or relevant physiological and biochemical measures	Temperature: To, Tty, Tr, Tsk	Summary main findings	Limitations / Future Research Questions
(Sunwoo et al., 2006)	AH (n=16M)	23±3	Whole body: climate chamber | 25 | 10 / 30 / 50 | 120	Non-randomised, change x time. Tested effect of different humidity (10, 30, 50%RH) at room temperature. Participants moved from a pre-test room at 25°/50% to either 25°/10%, 25°/30% or 25°/50%.	↔ HR all conditions, ↔ brachial SBP all conditions, ↔ brachial DBP all conditions, ↓saccharine clearance time (indicates a ↓in mucocilliary transport function) in 10%RH, ↑blinking rate (indicating eye dryness) in 10%RH. ↓face skin hydration in 10 and 30%	↓Tsk in 10%RH	No change in CV variables at any of the RH variations, but low RH of 10-30% the skin and mucous membranes become dry and uncomfortable, and the skin temp is reduced. Mucocilliary function is important in clearing allergens and bacteria from the nasal passages. Thus indoor RH should be set above 30% RH to ensure adequate moisture of mucous membranes for optimal respiratory function.	Paper states an aim of observing how low RH caused by ***heating*** affects the body, however no mention of heat in the methodology, which states that the temperature was fixed at 25°. Methods of humidity control not reported. Also, not reported whether all test conditions were on the same or different days. No mention of any washout between conditions. However, the figures suggest 3 separate trials were undertaken. Data for HR and BP is not shown; text reports "no change" in these variables.
(Wright et al., 2012)	AH (n=8M)	23±0.4	Whole body: climate chamber | 46 / 33 | 10% / 60% | 30 mins rest (baseline) + 2 hrs intermittent exercise	Randomised trial order, change x time. Effect of dry and humid heat with equivalent WBGT of 29˚C). Two trials (hot-dry@46/10 and hot-humid@33/ 60) separated by minimum 3 days. After baseline measures, mod cycle exercise performed (6x15min bouts with 5 mins rest in between).	↑HR equally both conditions, ↔plasma cortisol both conditions, ↑↑plasma IL6 hot-dry compared to hot-humid. ↓plasma volume similar both conditions	↑Tr equally both conditions. ↑↑Tsk in hot-dry. During recovery Tsk ↑hot-dry and ↓hot-humid	Despite similar WBGT heat loads, heart rate and body temp responses during exercise, increased IL6 during exercise, and increased Tsk in recovery in hot-dry conditions compared to responses in hot-humid conditions is likely associated with increased thermal strain in hot-dry possibly caused by .	Main effect of IL-6 increase possibly outlier effect, thus future study with greater numbers necessary before making definitive conclusions. Statistical package used not reported. Despite similar heat loads, unknown if the 13˚C difference in temperature between hot-dry and hot-humid conditions was contributing factor of its own. Intermittent exercise protocol may have blunted CV and immune responses, thus a constant exercise protocol could be used in future.

Abbreviations: AH (apparently healthy), AIx (augmentation index), CHD (chronic heart disease), CHF (chronic heart failure), CO (cardiac output), COPD (chronic obstructive pulmonary disease), CPAP (continuous positive airway pressure), CV (cardiovascular), DBP (diastolic blood pressure), ET (exercise test), F (female), FEV_1 (forced expiratory volume in 1 second), FVC (forced vital capacity), HR (heart rate), HT (hypertensives), IHD (ischaemic heart disease), IL-6 (interleukin 6), IL-12 (interleukin 12), M (male), MP (mean blood pressure), MRI (magnetic resonance imaging), N/A (not applicable/not measured), NO (nitric oxide), NS (non-significant), OSA (obstructive sleep apnoea), PWV (pulse wave velocity), RH (relative humidity), RPP (rate-pressure product), SBF (skin blood flow), SBP (systolic blood pressure), SV (stroke volume), Tb (blood temperature), Tc (core temperature – telemetry pill), TNFα (tumour necrosis factor alpha), To (temperature oral – sublingual or oesophageal), Tr (temperature rectal), Tsk (temperature skin), Tty (temperature tympanic), TPR (total peripheral resistance), WBGT (wet-bulb globe temperature).

2.5.2.3.7. Effects of cold

Physiological effects of cold exposure in healthy individuals

Exposure to low environmental temperatures produces inverse haemodynamic reactions to that of heat exposure, the goal of which is to minimise heat loss and prevent a reduction in core temperature (Stocks et al., 2004). Sudden cold exposure causes peripheral and cutaneous vasoconstriction that is sympathetically-mediated and triggered by the release of noradrenaline, adrenaline, and cortisol, and the inhibition of the NO system (Johnson and Kellogg Jr, 2010, Stocks et al., 2004). Thus, peripheral vasoconstriction can be considerable, even in mild-cold conditions (Stocks et al., 2004). The intense peripheral vasoconstriction caused by sudden cold exposure causes an increase in central blood volume, cardiac output, stroke volume, total peripheral resistance, and afterload, with a reduction, or no change in heart rate, and increased arterial pressures measured using the brachial cuff method (Stocks et al., 2004, Kingma et al., 2011).

Cold exposure and cardiovascular mortality

Haemodynamic perturbations, increased incidence of adverse CV events, and increased CV and all-cause mortality are strongly associated with coldwaves and seasonal temperature declines in winter worldwide (Danet et al., 1999). Moreover, the mortality impact from cold days has been found to be independent of season (Thompson et al., 1996, Marchant et al., 1993). Similar to heatwave-associated mortality, the elderly, frail, and individuals with underlying disease are the most at risk (Danet et al., 1999). The mechanisms postulated to account for the increased CV morbidity and mortality from cold exposure include peripheral (Stocks et al., 2004) and coronary vasoconstriction (Nabel et al., 1988), increased myocardial oxygen demand and tendency to ischemia (Meyer et al., 2010), increased LV afterload, increased large artery stiffness, increased

endothelial dysfunction (Danet et al., 1999), and rheological factors, including increased tendency to clotting (Keatinge et al., 1984). However, there is little direct evidence to explain how sudden cold exposure may affect measures of arterial stiffness and central haemodynamics such as PWV, aortic BP, P_{res}, P_{ex} or AIx; which may add to explanations regarding cold-related CV morbidity and mortality.

Physiological effects of cold exposure on individuals with T2DM

To date, no experimental whole-body cold exposure studies using a climate chamber have included humans with T2DM. However, two studies have applied localised cold using a cold pressor test in T2DM groups (Stansberry et al., 1997; Jaryal et al., 2009). The first study reported that people with T2DM had reduced skin blood flow responses during cold stress, compared to healthy individuals (Stansberry et al., 1997) and suggested that this response was due to reduced microvascular reactivity in people with T2DM which in turn, reduced cutaneous vasoconstriction. Further, Stansberry *et al.,* (1997) reported that the vascular responses of people with T2DM, irrespective of age, were similar to responses of older healthy individuals in that study, suggesting that the effect of T2DM on the arteries may be akin to accelerated ageing (Stansberry et al., 1997). The second study (Jaryal et al., 2009) also used a cold pressor test and observed reduced pulse transit time, and reduced amplitude of peak systolic pressure in T2DM compared to healthy participants. Jaryal *et al.,* (2009) suggested that the differences in cold-reactivity between T2DM and healthy individuals was that the endothelial and vascular dysfunction common in T2DM prevents normal smooth muscle responses to cold stress, and thereby prevents normal vasoconstriction. The reduced vasoconstrictive response to cold stress observed in these local-cooling studies (Stansberry et al., 1997, Jaryal et al., 2009) has implications for thermoregulation during cold stress which leave people with T2DM more vulnerable to accidental hypothermia during cold exposure (Neil et al., 1986). Indeed

there have been cases reported of recurrent and fatal hypothermia occurring in people with T2DM (Applebaum and Kim, 2002).

In addition, there have been very few studies that have investigated the effects of cold stress in other chronic disease groups (Nabel et al., 1988, Komulainen et al., 2000). One study used a cold pressor test to examine effects of cold stress on people with increasing degrees of CAD (Nabel et al., 1988). Cold stress was found to increase coronary artery diameter in healthy subjects, but atherosclerotic sections of coronary arteries in patients with CAD were constricted. Thus, coronary perfusion is diminished and tendency to ischemia and potential for acute myocardial infarction is increased during cold stress in people with CAD (Nabel et al., 1988). In people with hypertension, severe whole-body cold stress via a climate chamber at -15°C, was found to provoke greater pressor responses, i.e. higher brachial systolic BP, compared to those of healthy individuals (Komulainen et al., 2000).

Individuals with T2DM are more likely to have autonomic dysfunction (Vinik et al., 2003), increased cold and pain tolerance due to nerve damage (Vinik et al., 2003), impaired skin blood flow (Stansberry et al., 1997), impaired vasomotor tone (Xu et al., 2003), higher incidence of CHD (Hayden and Tyagi, 2003), and hypertension (Salomaa et al., 1995). Thus, based collectively on previous findings, it could be hypothesised that compared to healthy people, individuals with T2DM might have higher baseline haemodynamic measures but either exaggerated, or reduced responses in aortic and brachial PWV, aortic AIx, aortic BP and aortic reservoir parameters. Moreover, due to the higher likelihood of impaired thermoregulation, people with T2DM may also have lower baseline core and skin temperatures in ambient conditions, and larger magnitude with a faster rate of reduction in core and skin temperatures during whole-body cold exposure

than healthy counterparts. Table 2.5 presents the findings from the relevant studies that have previously investigated the effects of cold exposure on human haemodynamics.

Table 2.5. Effects of localised and whole-body cold exposure on human haemodynamics

Reference	Population (n, M / F)	Age (years)	Method | Temp used ˚C | Duration of Exposure (mins)	Study Design/ Protocol	Haemodynamic, and/or relevant physiological/ biochemical measures during cooling	Temperature: To, Tty, Tr, Tc, Tsk	Summary main findings	Limitations / Future Research Questions
(Nabel et al., 1988)	AH (n=8, 5M), minimal CVD (n=9, 6M), advanced CVD (13, 10M)	Gp1. range 17-53 Gp2. 38-66 Gp3. 36-71	Localised: Cold pressor test (hand and forearm) | ~0-4 | 90	Non-randomised, controlled. Change x time, 3 groups: 1. AH (n=8), minimal 2. CAD (n=9), and 3. advanced stenosis (n=13). Coronary angiogram in all subjects, measures taken before (control), peak cold stress, and 5-min post-test.	↑HR all gps, ↑↑HR gp 3, ↑ brachial SBP all gps, ↑↑ brachial SBP gp 3, ↑in coronary vessel diameter in AH gp (dilated). In the CVD gps 2 & 3 irregular & stenotic segments of vessel constricted while smooth sections dilated	N/A	Sympathetic stimulation from exposure to cold pressor test dilates normal (AH) but constricts atherosclerotic coronary arteries, thereby reducing flow. CPT elicits sympathetic release of adrenaline (A) and NA and increases MAP - therefore the 'normal' response to cold is dilation of coronary vessels. Altered response in atherosclerotic vessels may be due to reduced catecholamine sensitivity or reduced NO and endothelial-dependent relaxation factors.	Future work needed to determine if abnormal vasomotion during cold exposure is an indicator of atherosclerosis.
(Scott et al., 1988)	T1DM with CAN (n=12M), T1DM without CAN (n=11M), AH control (n=12M)	T1DM with CAN range 28-55; T1DM no CAN range 17-46; AH control range 23-54	Whole body: water-perfused suit (trunk) | 16 | 45 (or tests terminated if subject shivered)	Non-randomised, healthy control. Tested differences in thermoregulation and CV regulation during cold exposure and looked at presence of CAN as focus.	↔HR T1DM+CAN gp; ↓HR T1DM no CAN and AH gps, ↑MP similar across groups ↔ +CAN gp, ↓ forearm, foot & calf blood flow T1DM no CAN and AH gps	↓ Tc T1DM+CAN gp, ↑Tc T1DM no CAN and AH gps	CV and thermoregulatory responses in T1DM plus CAN group were impaired compared to groups without CAN or T1DM which may be due to a failure to vasoconstrict in people with CAN, which may predispose them to hypothermia.	Exact classification of diabetes was not specified, but were noted as "insulin treated" and the age range of diabetic patients was from 17 to 55 years, thus it is likely that participants were type 1. No studies can be found that have repeated such as study in T2DM, thus similar research is warranted in T2DM, with and without CAN.

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(Stansberry et al., 1997)	AH (n=20), T2DM (n=40), gender not reported either group	AH range 17-72, T2DM 17-71	Localised: cold pressor test | 45 | 5	Non-randomised, controlled. Tested skin blood flow (laser Doppler) response to local heat, cold, mental tasks & handgrip. Trials consecutively applied with 5 mins break between each. Results given for cold only	↓↓SBF AH ↓SBF T2DM	N/A	Cold pressor test results in impaired skin vasoconstriction responses in T2DM which is likely related to reduced microvascular reactivity, increased glycosylation of vessel tissues, depressed reflexive and centrally mediated mechanisms.	Future study could include neural measures that can determine whether cold induced decrements in SBF are neural or microvascular in nature. Trial order could have been randomised to minimise any potential order effect.
(Komulainen et al., 2000)	AH (n=12, 7M), HT (n=10, 8M)	AH. 24±3 HT. 27±8	Whole body: Climate chamber | 18 + wind velocity 3.5m.s^{-1} (equated to -15) | 15	Randomised, double-blind, crossover. 3 tests, 1 week apart. Test 1=familiarisation, test 2 and 3 involved 1 week randomised carvedilol (a combined α and β blocker) or placebo. Measures were taken baseline, then at 3, 9 and 15 minutes of cold exposure. Participants wore winter clothing. Results given are for **placebo** before and after cold.	↔HR in AH, ↓HR in HT, ↑ MP in AH, ↑↑ MP in HT, ↑ brachial SBP AH, ↑↑ brachial SBP HT, ↑ brachial DBP AH, ↑↑ brachial DBP HT	N/A	In placebo, HT group had greater pressor response (MP, SBP, DBP) in cold than AH, but carvedilol reduced pressor response more in AH than in HT gp. BP decreased slowly after cold exposure and was higher 15 mins after cold exposure than immediately after cold exposure. Those with the highest BP increase during placebo and cold also had the greatest pressor reduction with carvedilol and cold. Thus carvedilol did not inhibit normal vasoconstrictive responses in AH or HT groups.	Severe cold conditions, but these are common in the winters of the country of study (Finland) and therefore results may only be generalisable in similar severe cold conditions. Occurrence of shivering was not reported but would be highly likely given the severe cold conditions and with the perceived sensations rated as "cold" and "slightly painful".

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(Geleris et al., 2004)	AH (n=24, 13M)	25-30	Localised: Cold pressor test | 4 and control at "room temp" (˚C not reported) | 3	Partially randomised. Change x time. After 20 mins supine rest, baseline measures taken. Then participants performed handgrip isometric exercise (HIE) for 3 mins. 30 mins rest followed by right hand in CPT. 8 of the 24 were randomly selected to undergo combined testing (HIE on right, at the same time as CPT on left.	↓HR CPT, ↑HR HIE, ↔HR Combo, ↑↑MP CPT, ↑MP HIE, ↑↑ brachial SBP CPT, ↑ brachial SBP HIE, ↑↑ brachial DBP CPT, ↑ brachial DBP HIE, ↑ aortic PWV CPT, ↑↑ aortic PWV HIE, ↑↑↑ aortic PWV in combo, ↑ aortic AIx CPT, ↑↑ aortic AIx HIE. ↑↑↑ aortic AIx in combo	N/A	HIE increased aortic PWV, Aix, SBP, DBP and MAP slightly more than CPT. Greatest increases were in combined CPT and HIE. Authors suggested changes in PWV were possibly due to increased BP in both CPT and HIE which might increase tone in the aortic wall	Several errors in manuscript (e.g. says HR data in table 1, and is not. HR data for subgroup is shown in text, but not full group).
(Komulainen et al., 2004)	HT (n=7) gender not reported	30±9	Whole body: Climate Chamber | -15 (plus wind speed 3.5 m.s-1) | 15	Randomised, cross over, controlled, two trials to test effect of cold on BP and HR after a week of beta blockers (metoprolol) and after a week of placebo	↓HR similar metoprolol & placebo, ↑MP similar metoprolol and placebo, ↑ brachial SBP similar between trials, ↑ brachial DBP similar between trials	not reported	BP and HRs of HT individuals were reduced by beta blockade prior to cold exposure and during cold exposure, were lowered by metoprolol compared to placebo, but metoprolol did not attenuate the magnitude of cold-induced rise in BP which was similar for BP, MP and HR across both conditions.	Effects of other antihypertensive drugs (i.e. ACE inhibitors, angiotensin II receptor blockers) could be explored in future study.
(Edwards et al., 2006)	AH (n=12M)	28±2	Whole body: Climate Chamber | 4 + wind speed 6.1m.s^{-1} and control at 24 | 30	Randomised, controlled, change x time. Participants completed 2 conditions - thermoneutral and cold, trial order randomised and separated by 7 days. Resting seated baseline measures taken in ambient temps then again in climate chamber	↑HR(NS), ↑brachial SBP, ↔brachial DBP, ↑↑aortic SBP, ↔aortic DBP, ↑ aortic AIx, ↓time delay in reflected wave	↓Tsk, ↑Tc	Brachial & aortic SBPs increased more than brachial equivalents indicating the greater load the ventricles must overcome in cold exposure which is not reflected in brachial SBP measures. Cold-induced increases in afterload (increased aortic SBP) and increased coronary vasoconstriction may increase ischemia and risk of MI in cold.	Severe cold stress caused shivering in all participants at ~5 mins exposure. Increased core temp indicative of shivering thermogenesis is potential confounder in haemodynamic findings. Future research needed to determine haemodynamic changes cold in CAD or older populations in response to environmental cold stress in higher risk groups

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(Cui et al., 2007)	AH (n=9, 5M)	32±3	Whole body: Water perfused suit | 16 and control @ 34 | 15	Non-randomised, controlled (normothermic). Measurement of haemodynamics and muscle sympathetic nerve activity taken at baseline, and during infusion of nitroprusside (vasodilator) and phenylephrine (vasoconstrictor) to provide haemodynamic challenge	↓HR (NS), ↑ MP, ↑ brachial SBP, ↑ brachial DBP, ↔ MSNA activity, ↓forearm & chest SBF	↓Tsk, ↔To	Skin surface cooling did not affect baroreflex control of muscle sympathetic nerve activity (MSNA) or heart rate but increased brachial BPs and MAP, and decreased SBF	Findings in contrast to others who have found haemodynamic perturbations during skin cooling does increase MSNA activity, possibly because of methodological differences or low statistical power to detect significant change.
(Mourot et al., 2008)	AH (n=12M)	25±1	Whole body: water immersion | 26/27 (control @35-36) | 20	Non-randomised, controlled (normothermic water and air), change x time. Effects of thermoneutral and cool water immersion compared to responses in neutral air temps, on CV and autonomic function. Trials were consecutive and in head-upright posture (used a tilt table @ 60°). Results presented are cold compared to control air temps	↓HR, ↑ brachial SBP, ↓ brachial DBP, ↓SBP variability ↑brachial PP, ↓CO, ↑TPR, ↑R-R HRV, ↑NA, ↓A, ↔dopamine ↔free fatty acids	↔Tty	Results suggest that both arms of the autonomic nervous system (sympathetic and parasympathetic) are activated at once during cool water immersion. Reduced SBP fluctuations in cool water immersion are possibly due to increased thoracic blood volume. Cool water immersion evoked a pressor response plus shivering in 6 subjects, without changing core temp.	Responses during cool water immersion cannot strictly be compared to cool air exposure as hydrostatic pressure of even neutral-temperature water immersion causes increased central blood volume due to pressure on superficial veins.

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(Edwards et al., 2008)	AH (n=12, 6M)	23±3	Localised: Frozen gel packs on face | ~0 and control (temp not reported) | 7	Randomised, controlled. Two visits, one for PWV and one for PWA. Two visits, participants had two trials 20 minutes apart each visit. A control/ thermoneutral trial and a cold trial. Trial order and experimental condition order were randomised. Measures were taken at baseline then at 2, 5 and 7 mins of each condition.	↑HR (NS), ↑MP, ↑brachial SBP, ↑ brachial DBP, ↑↑aortic SBP, ↑aortic DBP, ↑aortic PWV, ↑aortic AIx, ↑SPTI, ↑DPTI, ↔SEVR	N/A	Large increase in AIx and smaller but significant increase in aortic PWV during superficial facial cooling. Aortic SBPs increased more than brachial SBP. Correlations indicated that MAP not PWV predicted change in AIx suggesting AIx may change independently of aortic PWV. Increase in AIx ascribed to increased wave reflection	Future studies could look at the effects of facial exposure to cold air and wind on arterial stiffness & AIx. Also look at effects of cold on AIx & PWV in CVD or aged populations
(Casey et al., 2008)	AH (n=15, 9M)	21-29	Localised: cold pressor test (hand immersed in ice slush) | 4 and control @ 21-23 | 3	Change x Time, case-controlled. Tested central haemodynamic responses to CPT. Measures taken in normothermia then again during last 15 s of CPT and then 90 and 180 s post CPT.	↑HR (NS), ↑MP, ↑ brachial BSP, ↑ brachial DBP, ↑↑aortic SBP, ↑aortic DBP, ↑aortic AIx ↑aortic AP, ↓time to wave reflection	N/A	Greater change in central BP and PP than in brachial measures. Increase in AIx ascribed to increase in wave reflection speed (based on decreased time to wave reflection). Increased myocardial O2 demand and increased contractile force are required in cold stress to overcome increased afterload, this might contribute to CV-related deaths in the cold	Cold pressor test cannot be used as comparator for whole-body reactions to cold as the cold stress is localised and severe.
(Adamopoulos et al., 2009)	AH whites (n=17M), AH African blacks (n=17M)	white =24±1, blacks=25 ±1	Localised: cold pressor test | 4 (ice) | 2	Group x time, non-randomised. After 10 mins supine rest and baseline measures, participants underwent a cold pressor test while measures were taken.	↑HR similar between gps, ↑MP white, ↑↑ MP black, ↑ brachial SBP whites, ↑↑ brachial SBP blacks, ↑ brachial DBP both gps, ↑aortic PWV whites, ↑↑aortic PWV blacks, ↓SBF whites, ↓↓ SBF blacks	N/A	African black individuals had higher resting aortic PWV, and had a greater increase in BP and aortic PWV in response to the CPT than age-matched whites. Blacks had greater vasoconstrictive response to cold than white individuals.	No information given on whether participants experienced pain from CPT. Remains unknown if there is any racial difference in pressor responses during whole-body cold exposure

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(Hess et al., 2009)	AH Younger (n=12, 6M), AH Older (n=12, 6M)	Y. 25±1 O. 65±2	Whole body: Water perfused suit | 15-18 and control @35 | 20	Randomised trial order, case controlled, change x Time. Mild-cold exposure used to avoid core temp change. Two groups: younger / older. Two consecutive trials control and cold. After 6 minutes of water perfusion, measures taken at baseline in each condition, BP and HR taken every 2 mins. Temps taken every 5 mins. PWV taken between 12-20 mins.	↔HR both groups, ↑MP older, ↑MP (NS) young, ↑ brachial SBP older ↔SBP young, ↑ brachial DBP (NS) both groups, ↑ aortic SBP both groups ↑aortic DBP both groups, ↑brachial PWV older, ↔brachial BPW young ↑aortic PWV older, ↔ aortic PWV younger, ↑ aortic AIx both groups, ↑ RPP older	↓Tsk both groups, ↔To both groups	A divergent central-peripheral result for both groups, but divergent AIx and PWV result between groups. However, magnitude of change for aortic & brachial BP were similar between older & younger groups. Aortic and brachial PWV increased in older group but not in younger group and increase in AIx similar between groups.	Future studies could determine gender differences for older men & women. Hydration status & baseline biochemical variables not accounted for.
(Jaryal et al., 2009)	AH (n=11, 7M), T2DM (n=10, 5M)	AH. 29±6 T2. 47±8	Localised: cold pressor test | 10 (water) | 1	Group x time, non-randomised, case controlled. After 15 mins supine rest, baseline measures were taken for 1 min, then CPT with 1 min data collection, then recovery measures 5 mins post-CPT.	↑HR both gps, ↔ time decay of diastole in T2DM, ↓↓pulse transit time (PTT) T2DM, ↓PTT AH; ↓↓systolic peak amplitude T2DM	N/A	Beat-to-beat systolic peak amplitude and pulse transit time (time delay between R wave and onset of pulse wave) decreased more in T2DM than in AH (likely due to increased HRs) possibly due to smooth muscle abnormalities from T2DM preventing a normal vasoconstrictive response to cold	CPT not comparable to whole body cold as much stronger sympathetic / pain stimulus. AH and T2DM gps not age-matched so not strictly comparable. PPG reflects local perfusion/ haemodynamics thus perhaps not generalisable to systemic haemodynamics

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(Wester et al., 2009)	AH (n=10, 9M)	37±11	Head/whole body immersion: (18-20 (control @23-38 room air) | 1	Non-randomised, case-controlled. Immersion in water filled pool and a water filled head piece. Cold water trials always completed before warm trials with core temp allowed to return to baseline prior to next test. Measures during rest and exercise during immersion in combination of body/facial cold and warm water, plus a control trial in ambient room air.	At rest: ↑↑HR in cold head +body combination compared to other combinations , ↔HR warm water, At rest: ↑MP similar for cold head+ body and warm head+ cold body, ↔MP warm water, At rest: ↑↑ cardiac output in cold heat+body combination	↓Tb	Immersion on its own in thermoneutral water typically causes bradycardia and an increase in CBV and peripheral vasoconstriction (the diving reflex), but in this study, immersion in cool water with cool head temperature caused the greatest rise in haemodynamics over that of combinations of cool body/warm head, warm body/cool head and warm body/warm head and this effect is exacerbated with exercise.	9/10 subjects shivered and one had to discontinue due to shivering. severe discomfort. Thus ↑ haemodynamic stress from shivering may have confounded results.
(Wilson et al., 2009)	AH (n=11M)	28±4	Whole body: water perfused suit | 16 (control @34) | Duration not reported (until Tb increased by 1˚C)	Non-randomised, controlled, change x time. Tested effects of consecutive heat and mild-cold and simulated orthostatic tolerance (lower body negative pressure) on CV function compared to thermoneutral. 30 mins recovery between tests. Only cold results presented here.	↓HR, ↑MP, ↑brachial SBP, ↑brachial DBP, ↔mixed AVO_2sat %, ↔CO, ↓SV, ↔RPP, ↓LVEDV	↑Tbl, ↓Tsk	Cooling attenuated the reduction in SV, CO and LVEDV in response to simulated orthostasis compared to control.	Testing heat and cold on separate days may give different results; 30 mins may not be enough time to physiologically recover from heat stress before being cooled.

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Reference	Population (n, M / F)	Age (years)	Method | Temp used ˚C | Duration of Exposure (mins)	Study Design/ Protocol	Haemodynamic, and/or relevant physiological/ biochemical measures during cooling	Temperature: To, Tty, Tr, Tc, Tsk	Summary main findings	Limitations / Future Research Questions
(Moriyama and Ifuki, 2010)	AH (normal reactors n=15, 7M), (hyper-reactors n= 8, 3M)	20-25	Localised: cold pressor test | 4 (control @24 / 65% RH) | 2	Non-randomised, controlled. CPT normal reactors Vs. hyper-reactors. Resting thermoneutral baseline measures were taken followed by cold pressor test and repeat measures. After at least 3 days protocol was repeated but with a handgrip test in place of CPT	↑HR normal, ↑↑HR hyperreactors, ↑MP normal, ↑↑MP hyperreactors, ↑aortic PWV normal, ↑↑aortic PWV hyperreactors, ↑carotid AIx75 normal, ↑↑ carotid AIx75hyperreactors	N/A	Hyper-reactors had larger increases in arterial stiffness and LV afterload than normal reactors. However, hyper-reactors to CPT did not have a different response than normal reactors to handgrip exercise. Hyper-reactors may have augmented sympathetic response to stressors of CPT (pain and cold)	Used own apparatus for collecting PWV and carotid AIx thus results may not be generalisable to PWV results using other methodology. Further testing warranted to assess if hyper-reactors to CPT also have exaggerated responses to other external stressors such as emotional stress, positional change or whole body exercise.
(Wilson et al., 2010)	AH young (n=11, 8M), AH Older (n=11, 8M)	Y. 20-34 O. 58-76	Whole body: water perfused suit | 15 (control at 35) | 20	Non-randomised, controlled (temperature and group), change x time. Compared effects of mild-cold on LV preload and contractility on young and older adults.	↓HR older, ↑MP young, ↑↑MP older, ↑brachial SBP young, ↑↑brachial SBP older, ↑brachial DBP similar young and older, ↑SV, ↔LVESV, ↔CO older, ↔SV, CO younger, ↑RPP older, ↔RPP younger	↔To, ↓Tsk	Despite increased LV preload and RPP, LV contractility did not increase and diastolic function is impaired during mild-cold stress in older adults compared to younger. This may be due to greater filling pressure in older compared to younger adults which is not mediated by an increase in CO. May be due to increased vasoconstriction in vascular beds other than skin in older compared to younger adults in mild-cold.	A future study to assess baroreceptor contribution to sympathetic outflow and pressor responses would be warranted as this was not measured in this study. Replication of study in climate chamber could test whether effects of whole body cold air has similar results.
(Liu et al., 2011)	AH (n=43M)	21±5	Localised: cold pressor test | 0 (ice) | 1.5	Change x time, randomised. 2 groups: right common carotid artery group and right common femoral artery group. After 15 mins supine rest and baseline measures, participants underwent a CPT while measures were taken at 1 min CPT	↑HR both gps similar btwn gps, ↑MP in RCCA, ↑↑ MP in RCFA, ↑↑ carotid Aix, ↑ femoral AIx	N/A	CPT had differing effects on measures of cerebral (carotid) and peripheral (femoral) resistance (AIx) and mean BP	No information given on whether participants experienced pain from CPT. CPT does not reflect every-day cold exposure, thus remains unknown if the cerebral / peripheral difference persist during whole-body cold exposure

Abbreviations: AH (apparently healthy), AIx (augmentation index), AP (augmented pressure), AV (atrio-ventricular), $AV0_2sat$ (arterio-venous oxygen saturation), CAD (coronary artery disease), CBV (central blood volume), CHF (chronic heart failure), CO (cardiac output), CPT (cold pressor test), CVD (cardiovascular disease), DBP (diastolic blood pressure), DPTI (diastolic pressure-time integral), ET (exercise test), F (female), HIE (handgrip isometric exercise), HR (heart rate), HRV (heart rate variability), HT (hypertensives), IHD (ischaemic heart disease), LVEDV (left ventricular end diastolic volume), M (male), MP (mean pressure), MRI (magnetic resonance imaging), MSNA (muscle sympathetic nerve activity), N/A (not applicable/not measured), NA (noradrenaline), NO (nitric oxide), NS (non-significant), PPG (photoplesythmography), PTT (pulse transit time), PWV (pulse wave velocity), RCCA (right common carotid artery), RCFA (right common femoral artery), RPP (rate-pressure product), RV (right ventricle), SBF (skin blood flow), SBP (systolic blood pressure), SEVR (sub-endocardial viability ratio), SPTI (systolic pressure-time integral), SV (stroke volume), T1DM (type 1 diabetes mellitus), Tb (blood temperature), Tc (core temperature – telemetry pill), To (temperature oral – sublingual or oesophageal), Tr (temperature rectal), Tsk (temperature skin), Tty (temperature tympanic), TPR (total peripheral resistance).

Reference	Population (n, M / F)	Age (years)	Method | Temp used ˚C | Duration of Exposure (mins)	Study Design/ Protocol	Haemodynamic, and/or relevant physiological/ biochemical measures during cooling	Temperature: To, Tty, Tr, Tc, Tsk	Summary main findings	Limitations / Future Research Questions
(Gao et al., 2012)	AH young (n=10), AH Older (n=11), gender not reported both groups	Y. 18-35 / O. 55-79	Whole body: water perfused suit | 15 and control @35 | 20	Randomised, controlled. Trial order randomised, 30 mins between trials, each trial 20 mins. BP and HR taken at baseline then every 2 mins. Temps taken baseline and every 5 mins. Echocardiography taken baseline and at 15 mins of trial.	↔HR young, ↓HR older, ↑MP young, ↑↑MP older, ↑brachial SBP young, ↑↑ brachial SBP older, ↑ brachial DBP both gps, ↑RPP older, ↑LV wall stress both gps, ↓ coronary perfusion time older	↓ Tsk both gps, ↔ To both gps	Older adults have greater pressor response and impaired coronary vasodilatory response while myocardial O2 demand is increasing during cold stress. In contrast, younger adults exhibit coronary vasodilation and increased myocardial O_2 supply during cold stress.	Gender of population not reported. Papers aim was to determine the effects of "aging "on coronary response to cold stress when the comparison is younger group vs. older group. Thus not true "aging" in any longitudinal sense.
(Muller et al., 2011)	AH (n=10, 5M)	25±1	Localised: cold air inhalation, and cold pressor test | Localised: cold air @ 0 to -15, control air @ "room temp" and CPT @ 1 water | 5 (inhaled cold air), 1.5 (CPT)	Group x time, controlled, non-randomised. 3 visits, 1=familiarisation, 2=cold air breathing test, handgrip test, cold + handgrip test, and CPT while continuous measures were taken. Visit 3=repeat tests while measures of myocardial function (tissue velocity via Doppler imaging) were taken. Results are compared to control (resting measures without intervention).	↑HR cold, ↑↑HR grip, ↑↑↑HR cold+grip, ↔MP cold, ↑MP cold+ grip, ↑MP grip), ↔brachial SBP cold, ↑ brachial SBP cold+ grip, ↑ brachial SBP grip, ↔ brachial DBP cold, ↑ brachial DBP cold+ grip, ↑↑ brachial DBP grip, ↑RPP similar btwn cold+grip and grip alone (change from baseline)	N/A	No change in MP in cold alone but identical increases in MAP in cold+grip and grip alone. cold air inhalation increases RPP and decreases coronary blood flow. Cold air breathing plus isometric exercise causes an even greater increase in RPP. But myocardial function was not impaired during cold+grip compared to grip alone. Data indicated a supply-demand mismatch in coronary blood supply after breathing cold air during mild exertion, but that blood flow seems to be redistributed to preserve myocardial function.	Limitations of echocardiography mean that measures of coronary blood flow and tissue velocity cannot be measured simultaneously, thus separate visits were needed, which may have introduced variability and error into the results.
(Zaproudina et al., 2011)	AH (n=15M)	18-28	Localised: ice water immersion | 0 water (foot), bag of ice (face) | 3 (foot), 1 (face)	Non-randomised, group x time, case controlled. Foot cold test was always first with a washout of 2-6 days before face cold test. Baseline measures (digital BP, ECG, skin temp) taken in thermoneutral (22-24˚C) and repeated hen again during testing	↑HR foot, ↔ HR face, ↑ brachial SBP foot, ↑↑ brachial SBP face, ↑ brachial DBP foot, ↔ brachial DBP face,	↓↓Tsk finger, ↓ Tsk toe, ↔ Tsk dorsal foot	Cold exposures at the foot affected haemodynamics more than cold exposure at the face. Systolic BP increases caused by facial cold were in the absence of heart rate changes. The variability of responses between foot and face could be due to the different autonomic reflexes involved. Also, divergent responses between participants suggests that cold reactivity could be partially genetically	Effects of stress and anticipation of pain on HR and BP cannot be discounted in this study as some participants had increases in these parameters prior to cold exposure. Timing of BP and HR results from during cold tests not specified. Presented as "at moment of

Abbreviations: AH (apparently healthy), AIx (augmentation index), AP (augmented pressure), AV (atrio-ventricular), $AV0_2sat$ (arterio-venous oxygen saturation), CAD (coronary artery disease), CBV (central blood volume), CHF (chronic heart failure), CO (cardiac output), CPT (cold pressor test), CVD (cardiovascular disease), DBP (diastolic blood pressure), DPTI (diastolic pressure-time integral), ET (exercise test), F (female), HIE (handgrip isometric exercise), HR (heart rate), HRV (heart rate variability), HT (hypertensives), IHD (ischaemic heart disease), LVEDV (left ventricular end diastolic volume), M (male), MP (mean pressure), MRI (magnetic resonance imaging), MSNA (muscle sympathetic nerve activity), N/A (not applicable/not measured), NA (noradrenaline), NO (nitric oxide), NS (non-significant), PPG (photoplesythmography), PTT (pulse transit time), PWV (pulse wave velocity), RCCA (right common carotid artery), RCFA (right common femoral artery), RPP (rate-pressure product), RV (right ventricle), SBF (skin blood flow), SBP (systolic blood pressure), SEVR (sub-endocardial viability ratio), SPTI (systolic pressure-time integral), SV (stroke volume), T1DM (type 1 diabetes mellitus), Tb (blood temperature), Tc (core temperature – telemetry pill), To (temperature oral – sublingual or oesophageal), Tr (temperature rectal), Tsk (temperature skin), Tty (temperature tympanic), TPR (total peripheral resistance).

Reference	Population (n, M / F)	Age (years)	Method | Temp used ˚C | Duration of Exposure (mins)	Study Design/ Protocol	Haemodynamic, and/or relevant physiological/ biochemical measures during cooling	Temperature: To, Tty, Tr, Tc, Tsk	Summary main findings	Limitations / Future Research Questions
							determined.	cold exposure", but not clear if this means onset of test or sometime during 1-3 mins testing.
(Huang et al., 2011)	AH (n=60, 29M)	25±4	Localised: water immersion | 7 | 2	Non-randomised, controlled, change x time. Effects of heat and cold on radial pressure pulse, radial AIx and heart rate variability (HRV). Cold results presented only.	↔HR, ↑brachial SBP, ↑brachial DBP, ↑ radial Aix, ↑radial diastolic augmentation pressure	↓Tsk hand	Local cooling of the hand increased Radial Aix and diastolic augmentation pressure which reflects increased LV load	The study had good statistical power and provided a lot of data. However, radial AIx possibly more reflective of peripheral pressure augmentation and may not be an accurate indicator of cardiac function
(Muller et al., 2012)	AH (n=9M)	23±2	Lower-body: water immersion | 13 (control @35) | 60	Randomised trials, temp controlled, change x time. Effect of lower body (up to the waist) cool water immersion on body temperatures, and haemodynamics.	↓HR, ↑MP, ↑ brachial SBP, ↑ brachial DBP, ↔oxygen uptake, ↔ SV, ↔ CO	↓Tr, ↓Tsk	Lower body cold water immersion caused a pressor response without changing stroke volume or cardiac output (probably due to reduced heart rates in cold). Cool water immersion seems to damp the usual increase in SV and CO seen with neutral temperature water immersion	The method of measuring SV (impedance cardiography) may not have been sensitive enough to detect changes in CBV during cold water immersion.

Abbreviations: AH (apparently healthy), AIx (augmentation index), AP (augmented pressure), AV (atrio-ventricular), $AV0_2sat$ (arterio-venous oxygen saturation), CAD (coronary artery disease), CBV (central blood volume), CHF (chronic heart failure), CO (cardiac output), CPT (cold pressor test), CVD (cardiovascular disease), DBP (diastolic blood pressure), DPTI (diastolic pressure-time integral), ET (exercise test), F (female), HIE (handgrip isometric exercise), HR (heart rate), HRV (heart rate variability), HT (hypertensives), IHD (ischaemic heart disease), LVEDV (left ventricular end diastolic volume), M (male), MP (mean pressure), MRI (magnetic resonance imaging), MSNA (muscle sympathetic nerve activity), N/A (not applicable/not measured), NA (noradrenaline), NO (nitric oxide), NS (non-significant), PPG (photoplesythmography), PTT (pulse transit time), PWV (pulse wave velocity), RCCA (right common carotid artery), RCFA (right common femoral artery), RPP (rate-pressure product), RV (right ventricle), SBF (skin blood flow), SBP (systolic blood pressure), SEVR (sub-endocardial viability ratio), SPTI (systolic pressure-time integral), SV (stroke volume), T1DM (type 1 diabetes mellitus), Tb (blood temperature), Tc (core temperature – telemetry pill), To (temperature oral – sublingual or oesophageal), Tr (temperature rectal), Tsk (temperature skin), Tty (temperature tympanic), TPR (total peripheral resistance).

Effect of cold exposure on central haemodynamics in healthy individuals

A handful of studies have directly investigated the effects of cold exposure on PWV or AIx in healthy individuals and all have reported increases in these measures (Edwards et al., 2006, Edwards et al., 2008, Casey et al., 2008, Geleris et al., 2004, Moriyama and Ifuki, 2010, Hess et al., 2009). The majority of these studies have applied 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). Gel packs applied to the skin and the cold pressor test are strong sympathetic stimulators which typically induce pain and hypertension (Mitchell et al., 2004), thus, such methods are not a suitable simulation of day-to-day cold exposure. Only two studies have investigated the effects of whole-body cooling on central haemodynamics (Hess et al., 2009, Edwards et al., 2006). The first study used a water-perfused suit with 15-18°C water for 20 minutes, and reported similar magnitude of increase in AIx between a young healthy group (25±1 years; ~7% increase) and an older healthy participant group (65±2 years; ~9% increase), P <0.05 from baseline in each group (Hess et al., 2009). Although in that study, the baseline AIx was significantly higher in the older group, than the younger group (P < 0.05) (Hess et al., 2009). Hess *et al.,* (2009) also measured aortic and brachial PWV (via Doppler flow probes) and reported that aortic PWV was significantly increased during cold-stress in the older age group (~1 $m.s^{-1}$, P < 0.05; results presented in figures only), but not the younger group (~0.05 $m.s^{-1}$). This finding demonstrates that while aortic PWV and AIx are thought to influence each other due to factors such as wave motion and pressure (Nichols and O'Rourke, 2005), they can also change independently of each other during conditions which challenge haemodynamics (Kelly et al., 2001, Edwards et al., 2008).

Edwards *et al.*, (2006) used methods that approximated a more "real-world", environmental whole-body cold exposure model. This study used a climate chamber to

affect whole-body cooling for 30 minutes and reported increased heart rate (5±4 beats.min^{-1}), AIx (16±1.9%), and brachial (3±3 mm Hg) and aortic systolic (13±3 mm Hg) BP during cold exposure, all $P < 0.05$ (Edwards et al., 2006). Aortic BPs were increased to a greater degree than the brachial equivalents during cold stress (Edwards et al., 2006). However, aortic or brachial PWV were not measured in this study, and the cold stimulus used was severe; a 4°C temperature, plus fans to create wind chill of 6.1 m.s^{-1}, resulted in all participants commencing shivering after ~5 minutes of cold exposure (Edwards et al., 2006). As the most intense muscular contractions from shivering are typically in the large, central trunk muscles (Bell et al., 1992), the bodily movement from shivering, coupled with the fact that shivering typically increases heart rate, respiratory rate, oxygen consumption, BP, and other hormonal stress responses (Sessler, 2009), may have added to the physiological and haemodynamic changes observed during cold exposure in that study (Edwards et al., 2006). Indeed the increased heart rates and core temperatures observed in Edwards *et al's.,* (2006) study are in opposition to the decreases in heart rate and core temperature typically observed during whole-body cold exposure (Table 2.6), which demonstrates the extremely stressful nature of the cold exposure in Edwards *et al's*., (2006) study. Moreover, as measures were not recorded prior to the onset of shivering at ~5 mins, the discrete contributions of cold exposure and stress from shivering to the outcomes of Edwards and colleagues (2006) study cannot be separated.

Few other studies have investigated the effect of cold exposure on aortic BPs (Edwards et al., 2008, Casey et al., 2008, Hess et al., 2009). Two studies have reported increases in aortic systolic BP between 22±4 mm Hg (Edwards et al., 2008) to 31±1 mm Hg (Casey et al., 2008) after localised cold exposure using gel packs on the forehead (Edwards et al., 2008), and a cold pressor test (Casey et al., 2008), respectively. One

other study observed an increase in aortic systolic BP of ~16 mm Hg (results given in figure only) during whole-body cold exposure via a water-perfused suit (Hess et al., 2009). However, as effects on physiology are different when localised cold and whole-body cold are applied, results are not strictly comparable in terms of realistic, day-to-day cold exposure. Nonetheless, each study reported a strong pressor response from cold stress, regardless of cooling method (Edwards et al., 2008; Casey et al., 2008; Hess et al., 2009). A comparison of the magnitude of effect, and how different vasoconstrictive methods affect selected CV variables is presented in Table 2.6. Moreover, Table 2.6 demonstrates the more stressful nature of the cold pressor test on heart rate and brachial systolic BP, compared to whole-body cold, and how at a given temperature, hydrostatic pressure from water immersion influences heart rate more so than exposure to similar air temperatures (Arborelius Jr et al., 1972).

There are no studies to date that have measured variables of reservoir function during environmental cold stress. However, the aortic reservoir has recently been found to be responsive to peripheral vasomotor changes via dobutamine (vasodilator) in humans (Sharman et al., 2009) and methoxamine (vasoconstrictor) and nitroprusside (vasodilator) in dogs (Wang et al., 2011). In the single study to use a vasoconstrictor, Wang *et al.,* (2011) reported a 0.44 $m.s^{-1}$ increase in wave velocity (invasively measured PWV) and a 24 mm Hg increase in P_{res} after methoxamine infusion. Methoxamine targets splanchnic and cutaneous vessels and induces peripheral vasoconstriction (DrugBank, 2012). Therefore, while effect magnitude would not likely be similar, it would be reasonable to assume that a similar direction of change in aortic reservoir responses might be observed during cold-induced vasoconstriction.

Table 2.6. Example of changes in selected cardiovascular variables due to different vasoconstrictor stimuli in humans

Condition Population, method (reference)	**Heart rate (beats.min^{-1})**	**Brachial Systolic BP (mm Hg)**	**AIx (%)**	**Aortic PWV (m.s^{-1})**
Cold stress – Localised				
AH group, CPT, 1.5 mins (Nabel et al., 1988)	↑11	↑21	-	-
CVD (severe stenosis) group, CPT, 1.5 mins (Nabel et al., 1988)	↑13	↑42	-	-
AH group, CPT, 3 mins (Geleris et al., 2004)	↓4	↑13	↑0.1	↑0.4
AH group, CPT, 3 mins (Casey et al., 2008)	↑3	↑27	↑16	-
AH group, CPT, 2 mins (Moriyama and Ifuki, 2010)	↑7	-	↑25	↑0.8
AH group, CPT hyper-reactors, 2 mins (Moriyama and Ifuki, 2010)	↑16	-	↑36	↑1.2
AH group, frozen gel packs on forehead, 7mins (Edwards et al., 2008)	↑3	↑17	↑19	↑0.6
Cold stress – Whole-body, water perfused suit				
AH group – younger, ~15°C, 20 mins (Hess et al., 2009)	↓1	↑9	↑7	↑0.5
AH group - younger, 15 °C, 20 mins (Wilson et al., 2010)	↓2	↑4	-	-
AH group – younger, ~15°C, 20 mins (Gao et al., 2012)	↓2	↑7	-	-
AH group – older, ~15 °C, 20 mins (Hess et al., 2009)	↓2	↑25	↑9	↑1.0
AH group – older, ~15 °C, 20 mins (Wilson et al., 2010)	↓2	↑20	-	-
AH group – older, ~15 °C, 20 mins (Gao et al., 2012)	↑1	↑19	-	-
Cold stress – Whole-body, water immersion				
AH group, head-in bath, 18-20°C, 1 min (Wester et al., 2009)	↑24	-	-	-
AH group, head-out bath, 26-27°C, 20 min (Mourot et al., 2008)	↓28	↑7	-	-
Cold stress – Whole-body, climate chamber				
Hypertensive group -15°C, wind 3.5m.s^{-1}, 15 mins (Komulainen et al., 2004)	↓2	↑25	-	-
Hypertensive group -15°C, wind 3.5m.s^{-1}, 15 mins (Komulainen et al., 2000)	↓7	↑20	-	-
AH group - 15°C, wind 3.5m.s^{-1}, 15 mins (Komulainen et al., 2000)	↔0	↑18	-	-
AH group - 4°C , wind 2.5m.s^{-1}, 30 mins (Reed et al., 1991)	↑4	↑3	-	-
AH group- 4°C , wind 6.1m.s^{-1}, 30 mins (Edwards et al., 2006)	↑3	↑3	↑16	-
Medication – Vasoconstrictor, angiotensin II				
AH group – at max dose 300ng.min^{-1} (Kelly et al., 2001)	↔0	↑18	↑9	↑0.7

Abbreviations: AH (apparently healthy), AIx (augmentation index), BP (blood pressure), CPT (cold pressor test), CVD (cardiovascular disease), PWV (pulse wave velocity).

2.6 Chapter Summary

The collective findings of previous research suggest that prolonged exposure to environmental heat has negative effects on CV health, particularly in vulnerable individuals, and these effects are thought to be due to the unrelenting CV and thermoregulatory strain. However, repeated short-term dry-heat exposure has shown beneficial CV effects in improved endothelial and haemodynamic function, and improved perceived quality of life in people with CVD. In healthy individuals, short-term exposure to hot conditions with high humidity may improve or at least have no adverse effects on thermoregulation and haemodynamic function, and it is possible, that short-term exposure to humid-heat may impose less physiological stress compared to similar exposure to dry-heat. Finally, short-term exposure to room temperature with high humidity appears to improve CV function, respiration and thermoregulation compared to room temperatures with low humidity.

In contrast, acute and chronic exposure to cold temperatures causes an increase in brachial systolic BPs which leads to increased cardiac work to overcome higher LV afterload. This increased cardiac work is thought to contribute to excess winter mortality. Moreover, cold-associated mortality is particularly high in temperate regions with less seasonal climate variability. Despite this, very little experimental data are available on the effects of milder-cold temperatures on measures of central haemodynamics. However, such climates may be frequently encountered during winter in a temperate climate, or when moving from a climate-controlled indoor room to outdoors on a cool day in any geographic region.

Localised cold exposures have been shown to cause strong pressor responses (increased brachial and aortic systolic BPs, PPs, and mean pressures) and increased

aortic stiffness in healthy individuals, yet based on the available literature, it is unclear whether responses in people with T2DM are likely to be exaggerated or attenuated compared to responses of healthy individuals. Moreover, localised-cold responses could be partially due to biochemical and emotional pain reactions from skin contact with severe cold. Whole-body cold exposure also causes an increased pressor response, the magnitude of which is dependent on degree of cold stress in healthy individuals; much larger pressor responses are seen above the shivering threshold. There also appears to be a differential central-peripheral haemodynamic response from cold stress that is evident across different cold-simulation methods, such as localised and whole-body, in healthy individuals, which suggests that the potential CV dangers of cold stress, particularly in high-risk individuals, could be masked by the measure of traditional brachial BP alone.

Given the clinical importance of central haemodynamic measures as markers of CV risk, compared to the peripheral equivalents, there is very little information currently available on the effects of heat or cold exposure on specific measures of central haemodynamic function such as aortic PWV, aortic BP, aortic AIx, and aortic reservoir components, in apparently healthy or T2DM individuals. The physiological impairments that result from long-term hyperglycaemia leave patients with T2DM particularly vulnerable during sudden challenges to CV- and thermo-regulation, which may include exercise, postural change, heat and cold exposure. Cold exposure results in impairments in haemodynamic function, and yet conversely, there may be a potential therapeutic CV benefit from short-term heat, and heat-plus-humidity exposures on haemodynamic function in healthy individuals and people with T2DM. With this in mind, the effect of changes in temperature and humidity on the physiology of healthy individuals and those with T2DM is a considerably under-researched area. Thus, this thesis aimed to

determine and compare the how differing temperatures and humidity impact on measures of central haemodynamic function in healthy individuals, and in people with T2DM.

CHAPTER 3 – GENERAL METHODS

3.1 Overview

The thesis comprises two over-arching studies which aimed to determine and compare the effects of changes in temperature and humidity on central haemodynamics in two participant groups, a "Healthy Group" and a "T2DM Group". Measures included aortic and brachial BPs, aortic and brachial PWV, aortic AIx, and components of aortic reservoir function. The presence (or degree) of background cardiovascular autonomic neuropathy (CAN) was determined using three tests in both participant groups. Due to the complexity of the studies, results from the Healthy and T2DM groups were split into two for each group; *mild-cold* and *heat and humidity*, which are presented in the four manuscripts which comprise Chapters 4 to 7 of the thesis.

3.1.1. Ethics

The study designs were approved by the Human Research Ethics Committee (Tasmania) Network. Participants for each study provided written informed consent. The protocols for each study complied with the principles of the Declaration of Helsinki and regular reports for the studies have been provided to the Ethics Committee. The approval number for the Healthy group study is: H0010849 and for the T2DM group, the approval number is: H0011347.

3.1.2 Study design

Trial order was randomised and individually sealed envelopes for each study group were produced by a biostatistician not connected with the study, and delivered prior to any data collection. The experiments were carried out as a cross over-design and every

participant completed each of the five test sessions in random order, with at least 7 to 14 days between each test. For each condition, an estimate of the heat load of each climate condition was calculated (Australian Bureau of Meteorology, 2012) as the wet-bulb globe temperature (WBGT), which is the world-standard measure for estimating heat stress (Parsons, 2006). The five experimental conditions were:

1) 21°C with 40% RH (control; 21/40), WBGT of ~13.3°C

2) 21°C with 80% RH (humid; 21/80), WBGT of ~18.7°C

3) 12°C with 40% RH (mild-cold; 12/40), WBGT of ~6.3°C

4) 36°C with 40% RH (hot-dry; 36/40), WBGT of ~24.9°C

5) 36°C with 80% RH (hot-humid; 36/80), WBGT of ~32.9°C

The conditions of 21°C and 40% RH were chosen as the control, or "room" temperature and RH as they were similar to the usual laboratory conditions where data collection took place. Further, 21/40 was deemed comfortable for people resting in light clothing, and was within the limits recommended for optimum indoor air quality (ASHRAE, 2004) and human comfort (Sunwoo et al., 2006).

3.1.2.1. The Healthy Group study

The Healthy Group study aimed to determine how sudden changes in environmental temperature (heat and mild-cold) and humidity affect central haemodynamics in resting, healthy individuals. Sixteen healthy men and women participated in the study. Participants underwent baseline haemodynamic measures in ambient laboratory temperatures before moving to a climate chamber which was set at one of the five randomised test conditions. Average ambient baseline temperature and RH during the Healthy group study were

22.0±1.9°C, and 38.1±11.0% RH. Time points for data collection were baseline (at least 10 minutes prior to entry into the climate chamber in ambient laboratory conditions), then 10, 30, 60 and 90 minutes post-entry to the climate chamber. Measures included brachial BPs, brachial and aortic PWV, radial pulse wave analysis (PWA), tympanic and skin temperatures, and thermal sensation scale.

3.1.2.2. The T2DM Group study

The T2DM study replicated the Healthy group study, and investigated how sudden exposure to mild-cold, heat and humidity affected central haemodynamics in people with T2DM. Fourteen men and women with T2DM attended five identical climate test sessions to those in the Healthy Group study, except that in order to determine any immediate haemodynamic reactions to the climate changes, there was an extra measure of radial PWA taken at 5 minutes post-entry to the climate chamber. Thus, measures were taken at baseline (-10 minutes in ambient laboratory temperature) then repeated at 5, 10, 30, 60, and 90 minutes inside the climate chamber. Average ambient laboratory temperature and RH for baseline measures during the T2DM study were 22.5±1.4°C, and 37.9±9.7% RH.

3.2 Participant Recruitment

3.2.1. The Healthy Group study

Participant recruitment for the Healthy group commenced in November 2009 with local University volunteers. Inclusion criteria were: male or females aged >18 years with a resting brachial BP ≤ 150/80 mm Hg. Participants were excluded if they had a self-reported clinical history of CV or metabolic disease. A University media release in March 2010 secured one television news interview, two radio interviews (local and National), and several university and local health newsletter articles. Recruitment flyers were distributed around the University and local shopping centres. Thirty-six information packs were sent to

people inquiring about the study. Examples of recruitment questionnaires, participant information, and informed consent forms for the Healthy group are in Appendix 2.

3.2.2. The T2DM Group study

Participant recruitment for the T2DM Group study commenced in October 2011 with a University media release. Resulting from this were two radio interviews (one local and one National), one local newspaper story, and articles in three local health newsletters. Participants over 18 years of age with T2DM were sought. It was specified that blood glucose concentrations must have been stable for the prior six months. Exclusion criteria were: People with known vascular, kidney or liver disease, people taking regular insulin injections, people who were "medically unstable", defined by recent changes, i.e. within last month, in dosage or medication for control of plasma glucose and or BP. People with resting blood pressure ≥160/100 mmHg were ineligible to take part. The higher BP range for participants with T2DM was to allow for the fact that some participants may have hypertension, controlled by medications. Recruitment for this study was slow and in May 2012, letters and flyers were sent to all local health professionals, for instance general practitioners and physiotherapists, and placed in local shopping centres. A total of 40 information packs were sent to people inquiring about the study. Examples of recruitment questionnaires, participant information, and informed consent forms for the T2DM group are in Appendix 2.

3.3 Methodology

3.3.1 Data collection

For the Healthy Group study, where there were two data collectors present on any one day, to reduce inter-observer bias, the same researcher performed all haemodynamic measures on the same participant for all experimental sessions, and the other researcher

performed thermoregulatory measures. For the T2DM Group study, all haemodynamic data collection was undertaken by the author. Data collection forms and questionnaires are in Appendix 3.

3.3.2. Thermoregulatory measures

3.3.2.1. Skin temperature

For the Healthy group, 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. For the T2DM group, skin temperatures were taken at the same four body sites as healthy participants, and averaged, however, new equipment became available for this study and thus, an infra-red dermal thermometer (DermaTemp DT-1001LT, Exergen Corporation, MA, USA) was used for T2DM participants. Skin temperature measurement was not planned to be a major focus of results in this thesis, and if any comparisons of skin temperatures were made, they were made solely within-group. Moreover, dermal infrared thermometers are less invasive and more responsive than thermocouples, and previous studies have found good correlations between skin temperature values measured by thermocouple, compared to values from infra-red thermometry (Takashi et al., 2000, Burnham et al., 2006).

3.3.2.2. Core temperature

For both the Healthy and T2DM studies, core temperatures were estimated using an infrared tympanic thermometer (Genius 3000A, Covidien, MA, USA). Operators were trained in the use of the tympanic thermometer according to accepted techniques (Davie and Amoore, 2010, McCarthy and Heusch, 2006) and the same operator collected data for the same individual participants for every test session.

3.3.2.3. Perceived thermal comfort

For both studies, subjective perception of thermal comfort was taken 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). A copy of the scale is presented in Appendix 4.

3.3.3. Blood sampling

Venous blood samples were collected via a cannula in (usually) the right anticubital vein. Cannula insertion took place at least 30 mins prior to any haemodynamic measures. Bloods were collected at each of the five test sessions at baseline, then again at 30, 60, and 90 minutes inside the climate chamber. Blood analysis results were used to describe the metabolic profiles of the two participant groups and to identify any changes in haematocrit, indicating possible dehydration, particularly during the hot exposures. At the specified time points, bloods were collected into EDTA tubes for haematocrit, serum clot activator tubes for serum cholesterol, and sodium fluoride/potassium oxide tubes for plasma glucose. Samples for cholesterol and glucose 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. Fresh samples were analysed for haematocrit within 30 minutes of the end of a test session on an automated haematology analyser (Sysmex XS-1000i, Roche Diagnostics, Sydney, Australia). Glycosylated haemoglobin (HbA_{1c}) was taken on a single occasion following one of the test sessions, (typically the final session) by

capillary whole blood sample (finger-prick) and analysed with an automated optical immunoassay analyser (DCA Vantage , Siemens Healthcare, Erlangen, Germany).

3.3.4. Haemodynamic measures

Standardised (Van Bortel et al., 2002, Laurent et al., 2006), non-invasive haemodynamic measures were made in duplicate between 1 to 4 minutes apart at each time point, and averaged for analyses.

3.3.4.1. Brachial blood pressure

For both Healthy and T2DM studies, brachial BPs were taken with a semi-automated oscillometric device (Omron T915, Omron Healthcare, Kyoto, Japan) usually on the left arm. However, the right arm was occasionally used for BPs if there were contraindications for constriction of the left arm, such as previously vascular surgery, or if the right arm was not able to be cannulated. BPs were taken on the same arm throughout a single test session and the BP arm did not change between baseline measures and measures inside the climate chamber. Moreover, BPs were always measured on the same arm throughout subsequent test sessions.

3.3.4.2. Pulse wave analysis

The PWA and PWV results for both studies were recorded using applanation tonometry (Figure 3.1, A and B). PWA was performed on radial artery waveform, usually captured from the left radial pulse, using brachial BP as the calibrating value. For Healthy and T2DM studies, all artery waveforms were collected for a minimum of 12 seconds each with a Millar tonometer (SPC-301, Millar Instruments, TX, USA) and processed with dedicated PWA software.

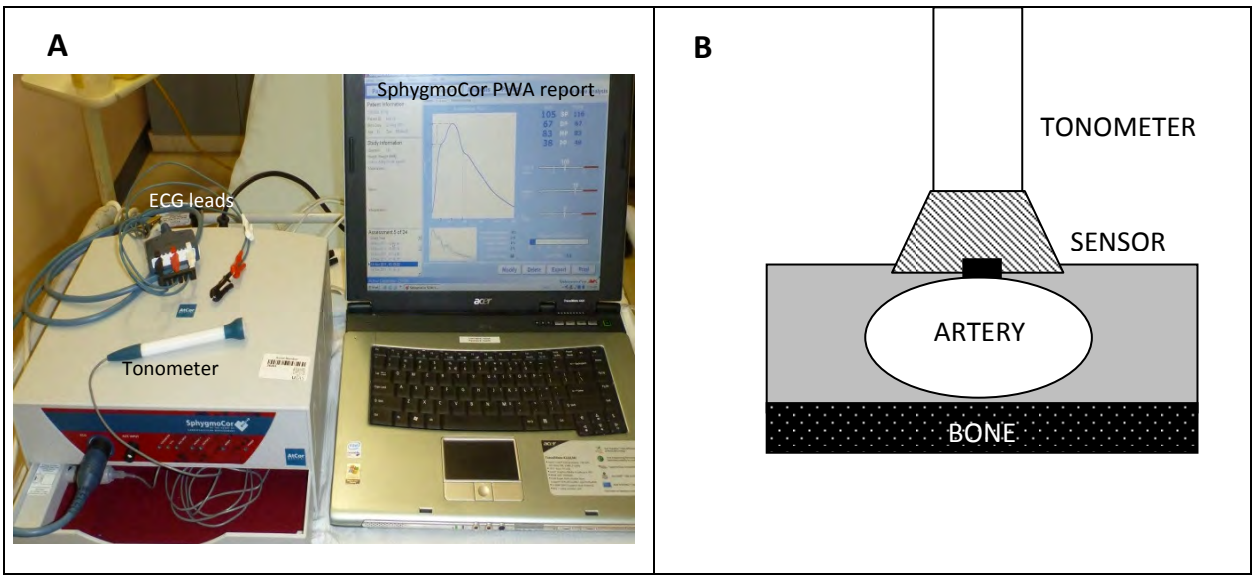

Figure 3.1. A) SphygmoCor apparatus, tonometer and laptop showing an aortic pulse wave synthesised from the measured radial pulse used in both studies, and B) the principle of applanation tonometry.

In applanation tonometry, an artery is compressed against an underlying structure (bone or muscle) and the force of the blood pressure against the artery wall is detected by the sensor.

SphygmoCor software version 8.2 (AtCor Medical, Sydney, Australia) was used for the Healthy Group study, while the subsequent software update, version 9.0 was used for the T2DM Group study. The inbuilt algorithms in the software that calculate central haemodynamic parameters did not change between versions, however, there were some changes to waveform capture methods, i.e. a change from manual to automated capture. These changes did not affect the waveform properties, or values obtained. PWA synthesises the aortic artery pressure from the measured radial artery pressure waveform, and uses a generalised transfer function that has been validated by comparing synthesised results to those measured by direct aortic catheterisation during rest and haemodynamic perturbations including the Valsalva manoeuvre, nitroglycerin, and exercise (Gallagher et al.,

2004, Sharman et al., 2006, Chen et al., 1997). Same-day within-participant measures of AIx can be variable and often have a greater range than aortic PWV or aortic systolic BP (Wilkinson et al., 1998). Despite this, aortic BP, AIx and PWV as measured by the SphymoCor system have previously been shown to be highly reproducible and in accord with measures made by different technology (Wilkinson et al., 1998). Moreover, aortic BP, AIx and PWV data measured by PWA have been shown to have similar reproducibility to that of traditional cuff-measured brachial BPs (Fillipovsky et al., 2000). AIx has previously shown good-to-excellent reproducibility on same-day repeated measures [interclass correlation coefficient (ICC) 0.86] (Papaioannou et al., 2007). The test-retest reproducibility between same-day PWA measures, taken ~3-5 minutes apart, in a subsample of seven healthy participants in the current studies was found to be excellent and comparable to those reported by Papaioannou et al., (2007). The ICC for AIx was 0.94; technical error of the mean (TEM) was 3.16%; 95% confidence interval (CI) of the difference between the first and second measure was -1.5 to 1.4. For aortic systolic BP, ICC was 0.96; TEM was 1.36mm Hg (95% CI: -0.2 to 1.1) and for aortic PWV, ICC was 0.70; TEM was 0.32 $m.s^{-1}$ (95% CI: -0.3 to 0.1).

Besides aortic BP, PWA provides several other estimates of central haemodynamic function including AP. AP is the difference between the first and second systolic pressure peaks. Aortic PP (systolic pressure minus diastolic pressure), mean BP (MBP; the true mean pressure of the integrated averaged radial arterial waveforms), AIx (AP divided by PP expressed as a percentage), time to reflected wave (Tr), systolic (SPTI) and diastolic (DPTI) pressure-time integrals and subendocardial viability ratio (SEVR). SPTI reflects the work of the heart and oxygen consumption, DPTI represents the pressure-time for coronary perfusion and the energy demands of the heart, and SEVR, which is the ratio of SPTI and DPTI, reflects myocardial oxygen supply and demand.

3.3.4.3. Pulse wave velocity

In this thesis, PWV was recorded and calculated by the SphygmoCor system. Aortic PWV was measured by simultaneously recording electrocardiogram (ECG)-gated carotid and femoral artery waveforms, while brachial PWV was calculated from the carotid and radial artery pulse waveforms and simultaneous ECG (O'Rourke et al., 2002, Wilkinson et al., 1998). Similar to PWA, artery waveforms for PWV were collected for a minimum of 12 seconds via applanation tonometry. PWV was calculated as the pulse travel time divided by the artery path length (AtCor Medical, 2009). The pulse travel time was calculated by the foot-to-foot method with the foot of each pulse waveform identified by intersecting tangential algorithms (AtCor Medical, 2009). The aortic artery path length was calculated by deducting the distance between the carotid pulse site and the sternal notch (in mm), from the distance of the sternal notch to the femoral artery pulse; taking a direct path to the femoral pulse site in the upper leg. Similarly, the brachial artery path length was calculated by deducting the distance between the carotid pulse site and the sternal notch from the path length of the sternal notch to the radial pulse, estimating the artery path to the point of the shoulder and directly down to the radial pulse site (AtCor Medical, 2009). The strongest palpated pulse for each arterial site was identified with permanent marker and distances were measured with a non-stretch, flexible tape measure.

3.3.5. Aortic Reservoir Component Calculations

Data for aortic reservoir function were calculated by separating the averaged synthesised aortic pressure waveforms acquired by the SphygmoCor equipment, using methods described previously (Davies et al., 2010, Davies et al., 2007) on customised Matlab software (Mathworks, Inc, Natick, MA). Aortic reservoir components analysed include

maximum reservoir pressure (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), maximum excess pressure (P_{ex}; defined as the amount of pressure relating to aortic in-flow and longitudinal 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:

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

Where P_{∞} is the asymptotic pressure at which flow through the microcirculation is zero, P_d is the measured diastolic pressure at $t = 0$, $b = 1 / RC$, where R = resistance and C = compliance of the system, 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 (Sharman et al., 2009, Aguado-Sierra et al., 2008, Davies et al., 2007). Once P_{res} is calculated, P_{ex} can be calculated as:

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

3.3.6. Daily Protocols

For both the Healthy and the T2DM Group studies, participants fasted overnight (10 to 12 hours, water *ad libitum*) prior to each test session, and were instructed to avoid caffeine, fried/fatty foods, vigorous exercise, and alcohol in the 24 hours prior to a test session. If a participant was on prescription medications, these were not taken on the morning of a test session, but were taken with breakfast, after the completion of the test session. The menstrual cycles of female participants were not taken into account during either 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,

all participants were instructed to wear the same or similar light clothing. For instance a short-sleeved top and light long pants were suggested to be worn to every test session, irrespective of the outdoor weather. If a participant was wearing socks, one, or both of these (if the participant preferred) were removed to allow access to the skin for temperature measurements. To avoid any influence of circadian rhythm, all testing started between 8.00 and 9.30am. To ensure that participants were well hydrated prior to testing, they consumed 150 mL of room temperature filtered water just after arrival at the laboratory and approximately 30 minutes before ambient baseline measures.

After height and weight measures, participants rested supine on a vinyl-covered massage table and had the cannula for blood sampling inserted. Participants had their carotid, femoral and radial pulses marked, and distances between pulse sites measured according to recommendations (AtCor Medical, 2009). Participants were then fitted with a 3-lead ECG and had baseline physiological measures taken in ambient laboratory temperatures. In order of procedure, measures were: 1st brachial BP, 2 x carotid-femoral arterial waveform captures, thermoregulatory measures, 2nd brachial BP, and 2 x carotid-radial arterial waveform captures. PWA was then performed on the two radial waveforms taken during carotid-radial PWV. An operator index of ≥ 80% was required as the minimum for a quality radial pulse wave capture, according to the SphygmoCor guidelines (AtCor Medical, 2009). Following these measures, baseline blood samples were taken via the cannula. After baseline measures, participants walked (~eight steps) into the controlled climate chamber, which was pre-set the night before at one of the randomised experimental conditions (Figure 3.2). Participants were blinded to which condition they were going to have prior to entering the climate chamber.

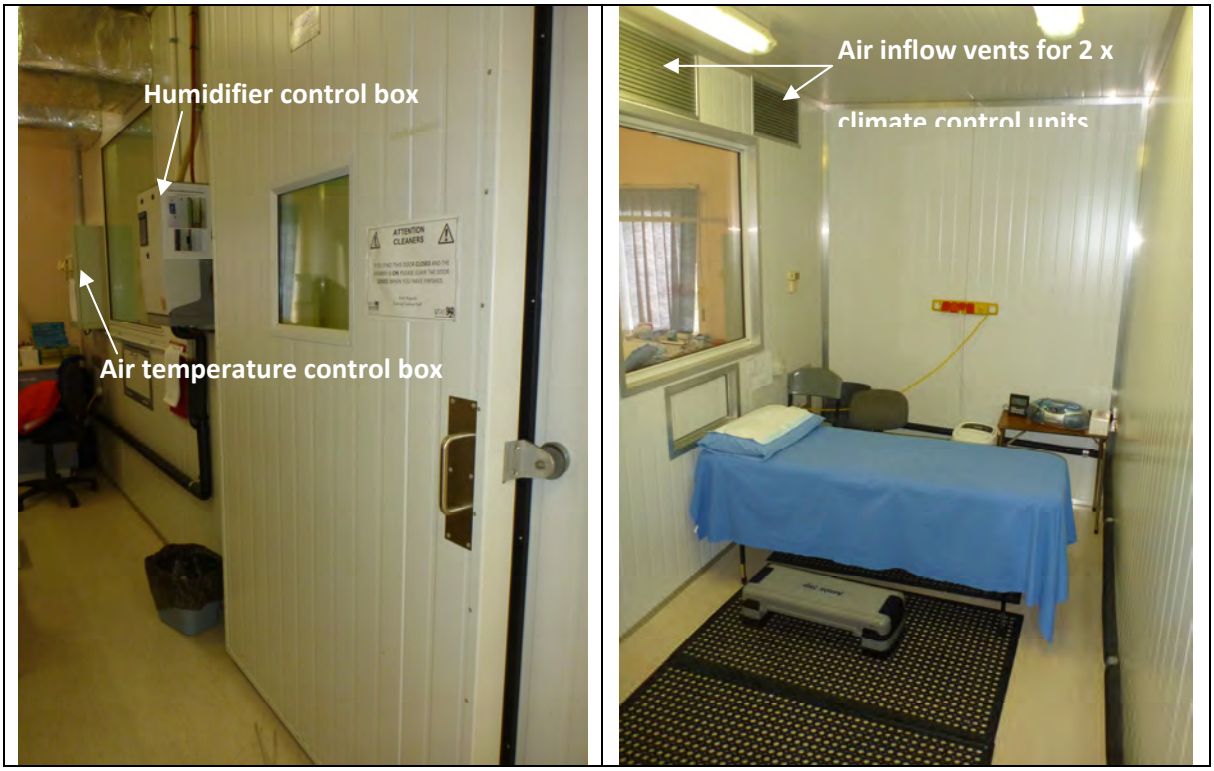

Figure 3.2. A). External view of the climate chamber. B) Internal view of the climate chamber.

Laboratory temperature and RH were recorded at the participant's arrival time each test day (Vantage VUE weather station console 6351, Davis Instruments, CA, U.S.A). There was minimal air velocity in the climate chamber and the air inflow vent was at the opposite end of the chamber and directed away from the resting participant. Climate chamber temperature and RH was recorded in close proximity to the participant at 10, 30, 60 and 90 minutes during each test session (Perception II weather station, Davis Instruments, CA, USA). Dimensions of the chamber are 5m L x 2.5m W x 4m H (Figure 3.2).

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 to 20 minutes of exposure and pulled up to the neck if participants indicated 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, humid, hot, or hot-humid conditions in either the Healthy or T2DM studies.

To account for the possible confounding influence of dehydration on haemodynamics during the test conditions, particularly in the heat (Armstrong et al., 1997), on every test day, healthy participants consumed 300 mL and T2DM had 250 mL of filtered, room temperature water immediately after 60 minute measures during every trial condition. The slightly smaller water volume for T2DM participants was to minimise bladder urgency over the 90 minutes of testing for this T2DM group, in whom bladder dysfunction is more common (Watanabe and Miyagawa, 1999). However, if required, all participants were permitted to void their bladder between the 60-90 minute time points and there was at least 5 minutes quiet supine rest prior to haemodynamic measures at the next time point.

3.3.6.1. The Healthy Group study

Procedures for the Healthy Group study were identical between all five climate trials.

3.3.6.2. The T2DM Group study

Study procedures for the T2DM Group study were identical to the Healthy Group study, except that in order to detect immediate haemodynamic changes after moving from ambient baseline temperatures to the trial conditions, a 5 minute time point was added after entry to the climate chamber. At 5 minutes post-entry to the climate chamber, an additional 2 x brachial BPs and 2 x radial pulse wave readings were taken. Figure 3.3 presents a protocol timeline for the studies.

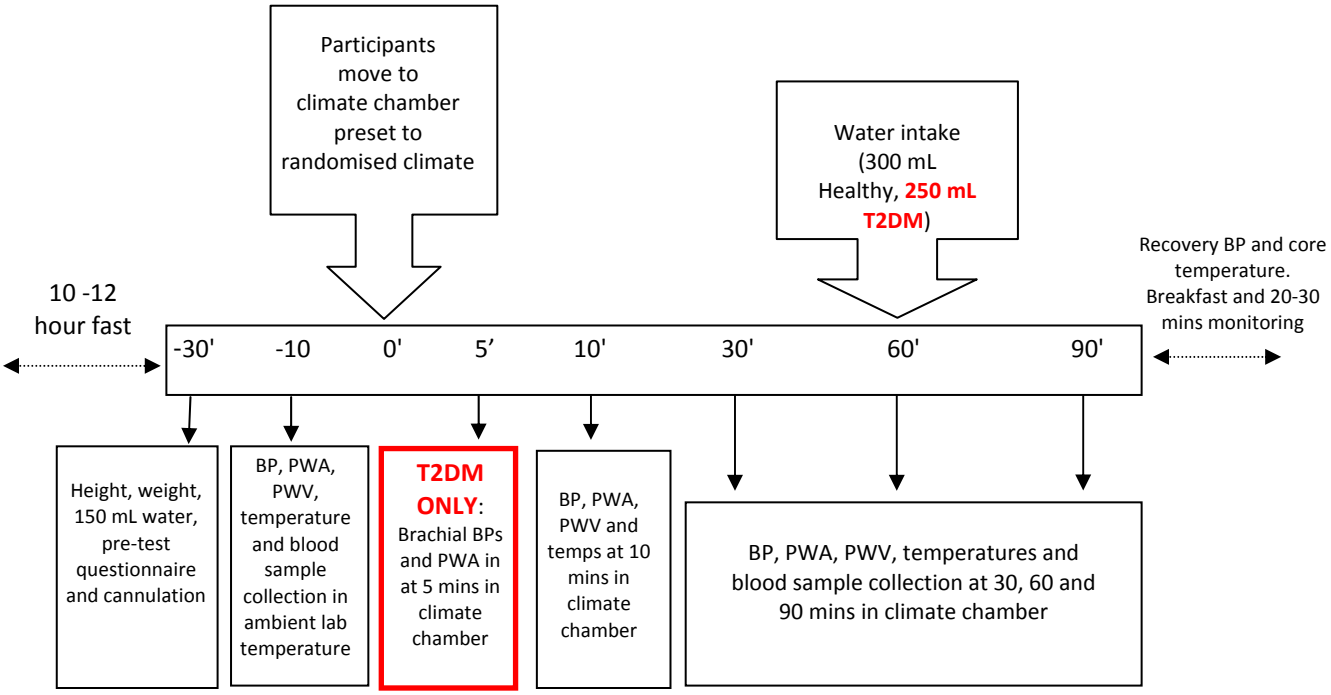

Figure 3.3. Daily protocol for the Healthy and T2DM Group studies.

Differences between the two groups' protocols are highlighted in red.

3.3.7. Cardiovascular autonomic function testing

Three valid and reliable (Ziegler et al., 1992, Vinik et al., 2003, Weimer, 2010) tests of cardiovascular autonomic neuropathy (CAN) were undertaken to assess the presence or degree of CAN in both Healthy and T2DM groups. Tests were the heart rate response to Valsalva manoeuvre, the heart rate response to head-up tilting and the brachial BP response to head-up tilting. Abnormal results from CAN testing, together with signs of CAN in a patient, provide a strong indication of prognosis and mortality risk (Ewing et al., 1976).

CAN was not an exclusion criteria for T2DM recruitment, and these tests were not intended to be a diagnosis of CAN. However, a positive response in more than one CAN test was used as criteria for the likely presence of CAN in a participant (Spallone et al., 2011).

CAN tests were performed only once, either on the day of the participants final visit, or on a separate day at their convenience. Tests were completed between 8.30 am and 3.30 pm. Medications that affect the autonomic nervous system, for example, antidepressants, antihistamines, over-the-counter cough or cold medications, diuretics, and aspirin were not taken within ~18 hours of CAN testing (Khan, 1992) and where necessary, were taken with food after testing.

CAN test procedures and data collection sheets used are in Appendix 3. In brief, participants from Studies 1 and 2 arrived after a two to three hour fast (two hours post food, three hours post caffeine, water *ad libitum*) and had the study protocols explained. Participants were fitted with a three-lead ECG and then rested in a supine position on an automated, padded, vinyl-covered tilt table (Dual Action Tilt Table, ABCO Health Care, VIC, Australia) for at least five minutes. Baseline resting brachial BP and heart rates were taken (with the same semi-automated machine used during the climate tests) in duplicate, at least two minutes apart, then participants had two to three practice runs at the full Valsalva manoeuvre. Two full Valsalva tests were recorded, spaced five minutes apart. Following the recording of two full Valsalva tests, there was a break of at least 10 minutes in which participants could use the bathroom, or change positions, i.e. sit upright or walk around, before lying supine again in preparation for the tilt test. Upon completion of the tilt test, participants were offered a cold or hot drink and a light snack, observed for any adverse signs or symptoms from the tests, e.g. pallor, dizziness, nausea, chest pain, for at least 10 minutes, then cleared to leave the laboratory.

3.3.7.1. Valsalva manoeuvre test

The Valsalva test reflects the autonomic control of heart rate and has been used for many years to detect abnormal responses to haemodynamic challenge in diverse chronic

disease groups (Vinik et al., 2003). A Valsalva manoeuvre involves straining, or breathing out, against a closed glottis, which results in a sharp increase in brachial BP from mechanical compression of the vena cava and other large vessels, followed by a BP fall as a result of vagal stimulation. During the manoeuvre, heart rate increases, then after the release of pressure, or exhalation, there is a reduction in heart rate and BP leading to an 'overshoot' to below the resting BP value, until restored by baroreflexes (Vinik et al., 2003). The magnitude of heart rate or BP reduction below typical resting values is indicative of autonomic function. Participants blew at a fixed pressure (40 mm Hg) into an individually-sterilised mouthpiece, approximately 10 mm internal diameter, for 15 seconds. The mouthpiece was attached to a modified BP manometer (Figure 3.4). Airflow and ECG data were acquired and processed with LabChart (Vs. 5, ADInstruments, NSW, Australia) and a PowerLab system (Model 4/25T, ADInstruments, NSW, Australia). The Valsalva R-R ratio was calculated by dividing the longest R-R interval (from the recorded ECG, in seconds) following breath release by the shortest R-R interval during the manoeuvre (Ziegler et al., 1992). The ratio decreases with age and autonomic dysfunction (Ziegler et al., 1992). The beginning and end of each Valsalva manoeuvre was detected visually by a sudden increase and decrease (respectively), in airflow detected by the PowerLab.

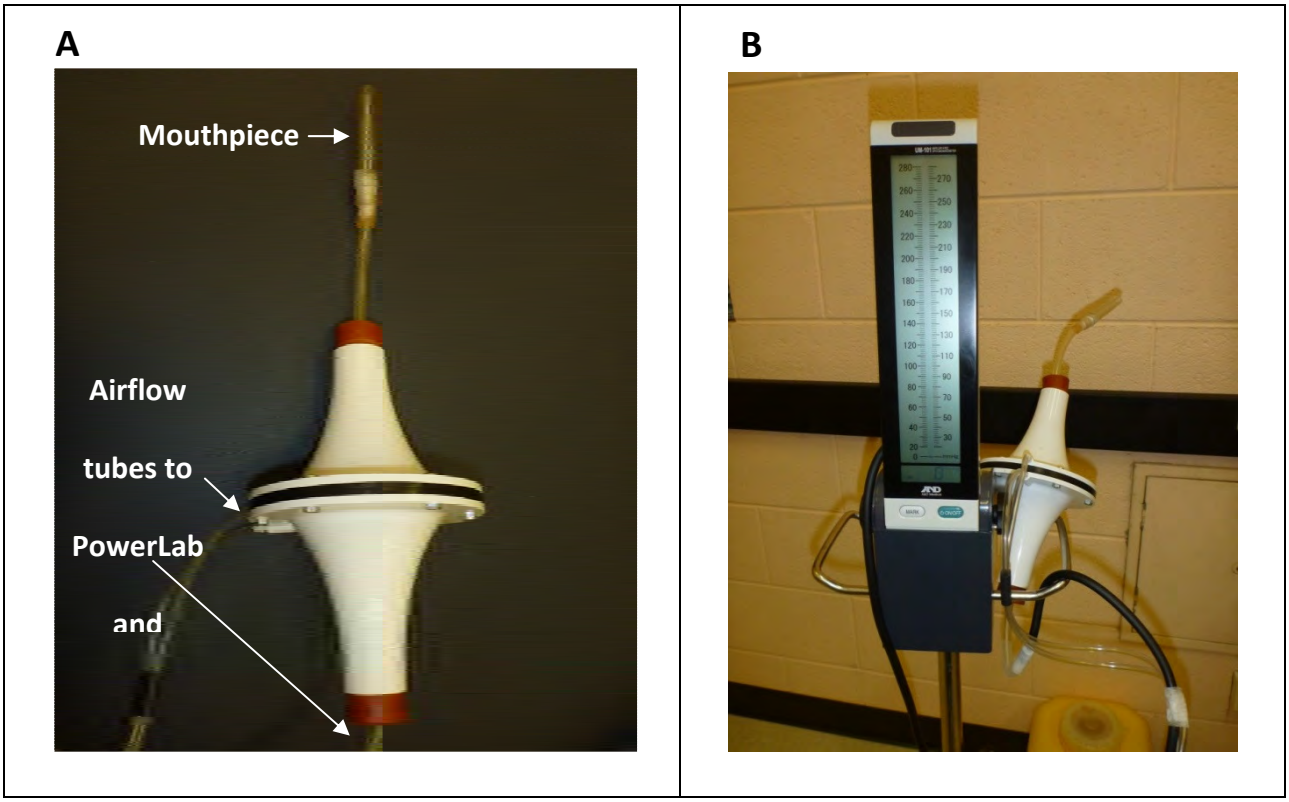

Figure 3.4. A) Apparatus for Valsalva test. B) Valsalva apparatus with manometer.

After at least five minutes quiet, supine rest, the ECG was inspected for rhythm disturbances for 30 seconds, and then participants performed the Valsalva manoeuvre while watching the manometer to keep the pressure at 40 mm Hg (Figure 3.5). Participants were observed for correct Valsalva technique, including full chest expansion, facial flushing, and jugular engorgement, and asked to repeat the test after a further five minute rest. If a Valsalva manoeuvre or test recording was deemed below the acceptable standard, the test was repeated in full after five minutes rest. Criteria for repeat testing were movement artefact in the ECG obscuring R-waves, incorrect technique, and inability to achieve, or hold the pressure at 40 mm Hg. Participants were closely observed for adverse effects during every Valsalva manoeuvre, including eye or chest pain, syncope, arrhythmias, severe hyper-

or hypotensive response. However, no test was interrupted or terminated due to adverse signs or symptoms of CAN testing.

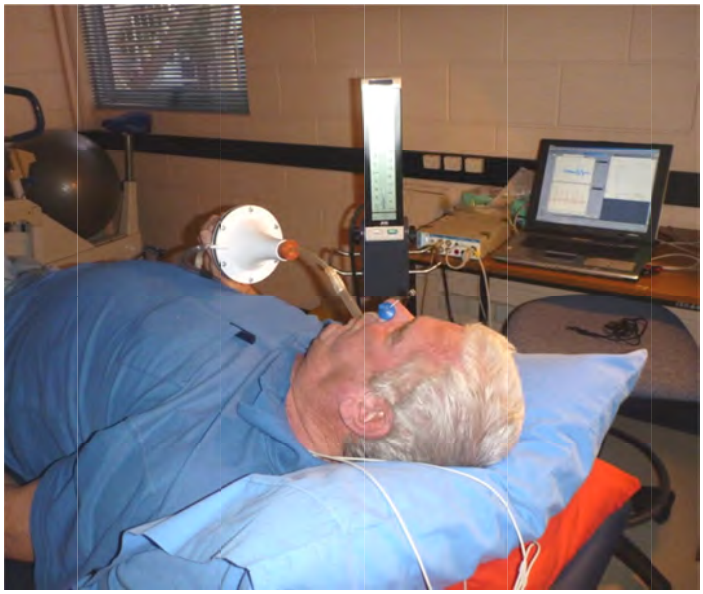

Figure 3.5. Participant from the T2DM Group study performing the Valsalva test

(Image used with permission of participant)

3.3.7.2. Tilt test

The purpose of the head-up tilt test is to assess the effects of postural, or orthostatic hypotension, which in people with CAN may result in dizziness, blurry vision and syncope upon standing up. The BP response to tilting was calculated as the average of two resting brachial BPs minus the lowest BP during the first three minutes of tilting. BPs were recorded up to 10 minutes because some individuals have been found to have a delayed hypotensive response to head-up tilt which can be missed if data are recorded for only two to three minutes (Weimer, 2010). A systolic BP fall of more than 20 mm Hg and/or diastolic BP fall of more than 10 mm Hg after two to three minutes head-up tilt is indicative of CAN

(Vinik et al., 2003). The heart rate response to tilting in people with T2DM is typically less than in healthy individuals, with a gradual increase in heart rate (Vinik et al., 2003). To assess autonomic control of heart rate during the tilt, the 30:15 tilt Ratio was calculated by dividing the longest R-R interval (in ms) during beats 20-40 by the shortest R-R interval during beats 5-25, all post-tilt (Ziegler et al., 1992).

For the tilt test, all participants rested their feet directly on the foot-board of the table and were strapped securely with a large Velcro straps. Straps were placed over the chest (over the pectorals in men, but either above or below the breasts in women, depending on their height), and there were individual straps for each lower leg, below the knee joint (Figure 3.6). Participants were inclined to 60° measured by an inbuilt slope gauge on the table. BP was usually measured on the right arm unless medically contraindicated for that participant, and was taken immediately upon reaching 60° for a 30-second post-tilt measure, then again every minute for 10 minutes. ECG was recorded continuously throughout testing, and time points for "upright" and "end tilt" were marked on the LabChart ECG recording. Participants were kept under observation and asked regularly throughout the test if they were experiencing any adverse signs or symptoms of head-up tilt, for instance any dizziness, blurry vision, or nausea. Participants were brought back to horizontal immediately after the deflation of the BP cuff after the final 10 minute measure. No participant asked to terminate a test early due to adverse symptoms from tilting.

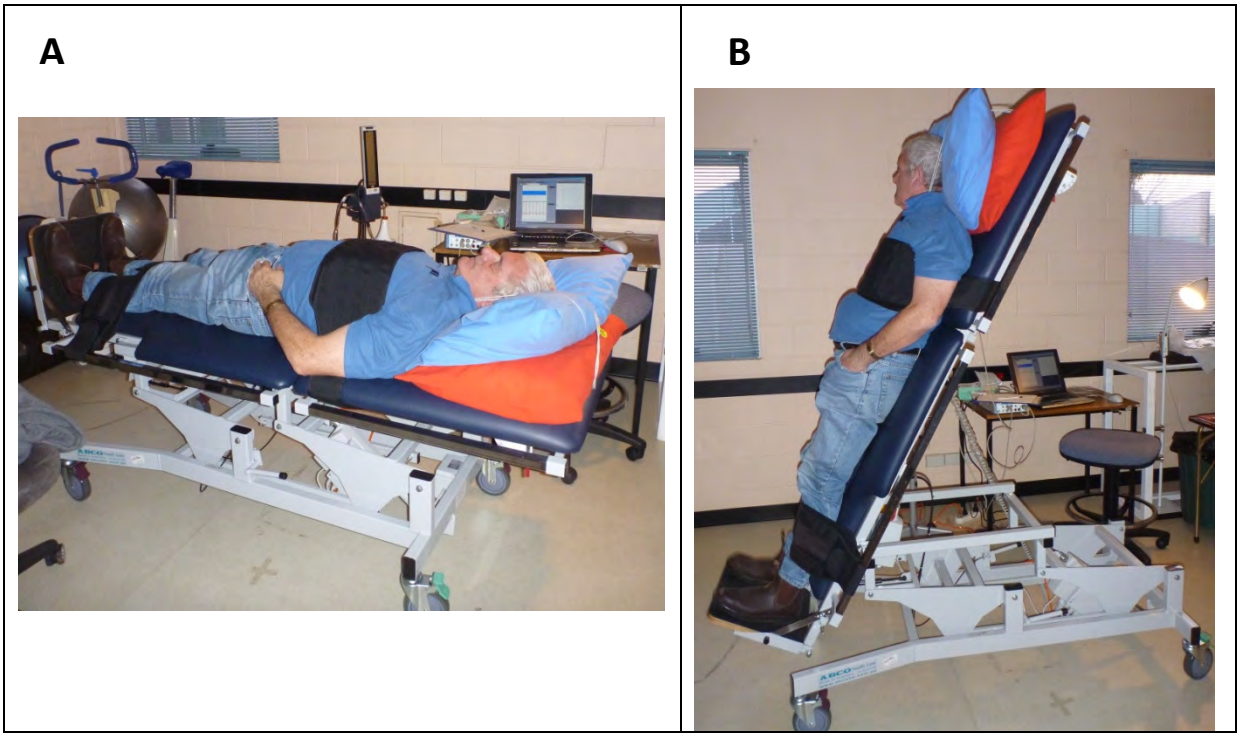

Figure 3.6. Participant from the T2DM Group study performing the tilt test. A) supine and B) head-up tilt to 60°.

(Image used with permission of participant)

3.3.8. Statistical Analysis

Power calculations were undertaken prior to commencement of the Healthy Group study. A sample size of 20 was determined on the basis of an expected change in AIx in mild-cold conditions. 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 the current studies were considerably milder, it was 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 of test session and visit number, in order to account for any learning effect of repeat testing. For both Healthy and T2DM groups, data were split into *mild-cold vs. control* results, which included data from 21°C / 40% RH (control) and 12°C / 40% RH (mild-cold) trials, and into and *heat and humidity vs. control* results which included data from 21°C / 40% RH (control), 21°C / 80% RH (humid), 36°C / 40% RH (hot-dry), and 36°C / 80% RH (hot-humid) trials. Results were corrected for multiple comparisons by the Holm method (Holm, 1979). Regression residuals were calculated and used to test the assumptions of linear regression using decomposition tests of heteroskedascity, skewness & 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. In-text and demographic tabular results are presented as mean ± standard deviation (SD), figure data are presented at mean and standard error of the mean (SEM), and comparative tabular data are presented as mean difference and 95% confidence intervals. Regression coefficients, and 95% CI's 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. *P* values of ≤ 0.05 were considered significant.

CAN was not among the exclusion criteria for T2DM participants, nonetheless, it is acknowledged that the presence of CAN in the T2DM group may be considered a potential confounder of results. Accordingly, a sub-analysis of the main variables in the *mild-cold* and *heat and humidity* results was undertaken which accounted for the influence of the three CAN tests. Variables analysed in these sub-analyses included aortic PWV, brachial systolic BP, aortic systolic BP, MBP, AIx and core temperature. Each variable was re-analysed to remove the influence of CAN, using the mean Valsalva R-R ratio, 30:15 tilt ratio, and the brachial systolic BP response to tilt results as co-variates.

3.3.8.1. Mild-cold – Specific analyses

Means for baseline values and individual time points in the climate chamber (at 10, 30, and 60 minutes for the Healthy Group study, and at 5, 10, 30, and 60 minutes for the T2DM Group study) were calculated for each variable in both experimental conditions. For within-condition change, difference from baseline values to the average of all time points in the climate chamber up to 60 minutes was determined for all variables in mild-cold and control conditions, and results for each condition were then compared for between-condition changes. The data to provide within-condition changes were averaged to give an overview of change in each condition. However, it is acknowledged that this approach may have provided a conservative view of within-condition changes and may have masked acute changes which occurred during the early minutes of exposure. An area-under-the-curve approach may have been preferable, but the studies in this thesis were not designed with the number of the time points required for area-under-the-curve analyses. Ninety minute measures were recorded in all cold trials, however, the 90 minute data were omitted from the analysis for mild-cold results for both the Healthy and T2DM Groups, as dehydration was not considered a confounder during the cold exposures.

3.3.8.2. Heat and humidity – Specifics analyses

For heat and humidity results, within-condition change (change from baseline to the average of all time points up to 60 minutes) was determined and used in main analysis. For between-condition differences, change from baseline to the average of all time points up to 60 minutes for each condition was compared to the change from baseline in all other conditions. Additionally, measures collected at 90 minutes of exposure were analysed separately for within- and between-condition changes for both the Healthy and T2DM groups. This separate analysis determined any influence of dehydration and rehydration,

and took into account the water intake after the 60 minute measures during heat and/or humidity exposure.

CHAPTER 4 –HEALTHY GROUP, MANUSCRIPT 1

EFFECTS OF WHOLE-BODY MILD-COLD EXPOSURE ON CENTRAL HAEMODYNAMICS: A RANDOMISED, CROSS-OVER TRIAL IN HEALTHY ADULTS

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An edited version of this chapter has been published in the European Journal of Applied Physiology as an original research investigation (Submitted May 2012, accepted October 2012, published May 2013: *Eur J Appl Physiol*, 113 (5), 1257-1269. DOI 10.1007/s00421-012-2543-1). The methods section, in particular, has been reduced to avoid duplication of Chapter 3, General Methods.

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. This 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] and AIx), and aortic reservoir components (including reservoir and excess pressures). 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 minutes 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 mm Hg, 95% confidence interval (CI) 0.36 to 4.36; $P = 0.01$] and AIx (+6%, 95% CI 1.24 to 10.1; $P = 0.02$) increased, and time to maximum excess pressure (a component of reservoir function related to timing of peak aortic in-flow) decreased (-7ms, 95% CI -15.4 to 2.03; $P = 0.01$) compared to control. Yet there was no significant change in aortic PWV (+0.04 $m.s^{-1}$, 95% CI - 0.47 to 0.55, $P = 0.87$) or brachial PWV (+0.36 $m.s^{-1}$; -0.41 to 1.12; $P = 0.35$) between conditions. Mild-cold exposure increases central haemodynamic stress and alters timing of peak aortic in-flow without differentially affecting arterial stiffness in healthy adults.

INTRODUCTION

Acute cold exposure results in peripheral vasoconstriction, accompanied by increased arterial pressures measured using the brachial cuff method (Stocks et al., 2004, Kingma et al., 2011). 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 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 (Wilson et al., 2010, Gao et al., 2012) 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, Vlachopoulos et al., 2010b). 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 (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 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 (Kelly et al., 2001, Edwards et al., 2008, Hess et al., 2009).

While the few studies that have directly investigated the effects of cold exposure on PWV or AIx have reported increases in these measures (Edwards et al., 2006, Edwards et al., 2008, Casey et al., 2008, Geleris et al., 2004, Moriyama and Ifuki, 2010, Hess et al., 2009), 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 date, 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 minutes (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 minutes 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 PWV, and the cold stimulus used was severe (4^{o}C

plus fans to create wind chill of 6.1 $m.s^{-1}$). All participants in that study commenced shivering after ~5 minutes 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, 2011). By controlling for the systemic effects of shivering and using a whole-body mild-cold exposure in a controlled climate chamber, this study was designed to more realistically reflect haemodynamic changes from exposure to a cool environment. The aim of this study was to test the hypothesis that compared to a control condition (21°C), 60 minutes exposure to mild-cold (12°C) would increase central haemodynamic stress in a healthy adult population. To this end, regional arterial stiffness (aortic and brachial PWV) and measures of central haemodynamics including augmented pressure, AIx, BPs, and aortic reservoir components including reservoir pressure, excess pressure, and timing of excess pressure were taken in each condition.

MATERIALS AND METHODS

Details of materials and methods for physiological data collection, and statistical analysis procedures are in Chapter 3, General Methods.

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 mm Hg; brachial diastolic BP, 72 ±7 mm Hg; fasting plasma glucose, 4.7 ± 0.3 $mmol.L^{-1}$; and fasting serum total cholesterol, 4.3 ± 0.8 $mmol.L^{-1}$. 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. 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 ($P = 0.007$; Figure 4.4 and Table 4.1). 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 ~10-30 minutes of exposure. For those 7 participants, the blanket was used 2 ± 0.4 times per session for 4 ± 1 minutes per time. 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 to 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" (-0.1 ± 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" (-0.1 ± 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 $m.s^{-1}$; *P* = 0.56) or control (6.9 ± 2.0 to 7.0 ± 2.2 $m.s^{-1}$; *P* = 0.72; Figure 4.1, A), and between conditions, there was no difference in change in aortic PWV in mild-cold compared to change in control (+0.04 $m.s^{-1}$; 95% CI -0.47 to 0.55; *P* = 0.87; Figure 4.1, A).

Brachial pulse wave velocity. Brachial PWV increased from baseline in mild-cold (8.7 ± 1.4 to 9.4 ± 1.7 $m.s^{-1}$; *P* = 0.02, Figure 4.1, B) with no significant change in control (8.7 ± 1.4 to 8.8 ± 1.4 $m.s^{-1}$; *P* = 0.32), yet between conditions, there was no difference in change in brachial PWV in mild-cold compared to change in control (+0.4 $m.s^{-1}$; 95% CI -0.41 to 1.12; *P* = 0.35; Figure 4.1, B).

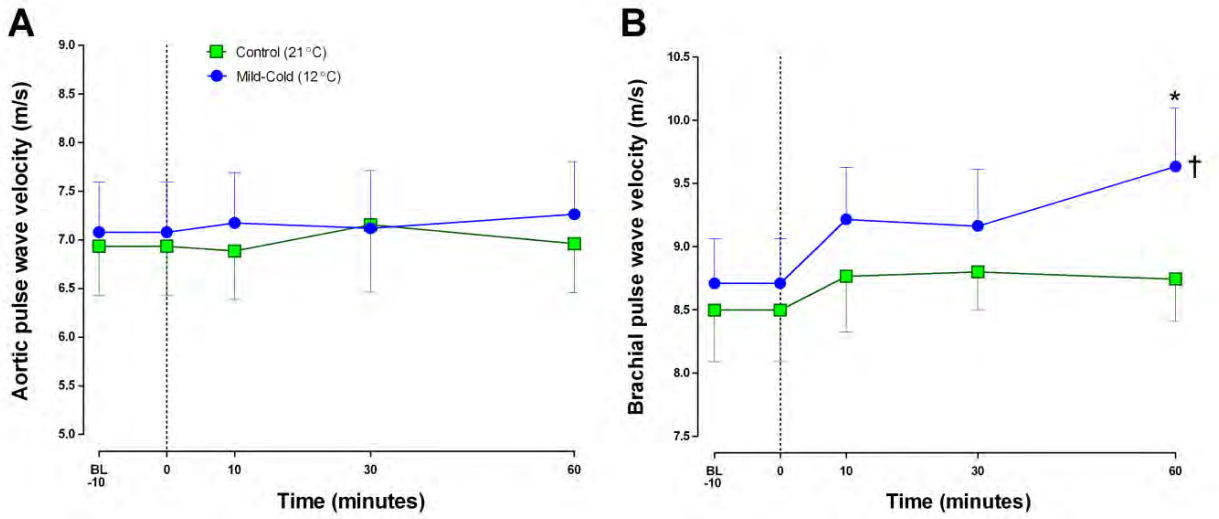

Figure 4.1. A) Aortic pulse wave velocity, and B) brachial pulse wave velocity in mild-cold (12°C) and control (21°C) in healthy individuals.

Data presented as mean ± SE; n =16; baseline data (BL / -10 minutes at ambient laboratory temperature) was obtained ~10 minutes before entry to climate chamber (0 minutes; dashed vertical line; replicates data from baseline); *significantly different from control at specific time point; †significant change within condition (i.e. between baseline and average of data during 60 minutes in test condition)

Haemodynamic Responses

Augmented pressure. AP increased from baseline in mild-cold (6 ± 7 to 9 ± 8 mm Hg, P < 0.001) with no change in control (6 ± 5 to 6 ± 7 mm Hg, P = 0.47; Figure 4.2, A and between conditions, AP increased in mild-cold compared to change in control (+2 mm Hg, 95% CI -0.36 to 4.36, P = 0.01; 4.2, A).

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 control trials (15 ± 11 to 15 ± 14 %; P = 0.72; Figure 4.2, B). Between conditions, AIx increased in mild-cold compared to change in control (+6%; 95% CI 1.25 to 10.1; P = 0.02; Figure 4.2, B).

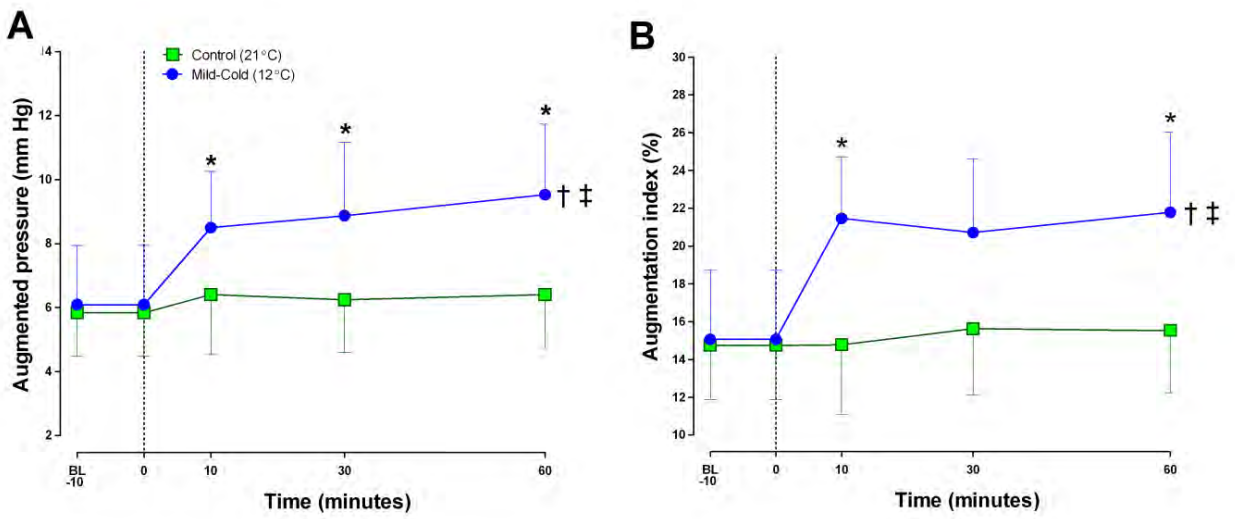

Figure 4.2. A) Augmented pressure, and B) augmentation index in mild-cold (12°C) and control (21°C) in healthy individuals.

Data presented as mean ± SE; *n* =16; baseline data (BL / -10 minutes at ambient laboratory temperature) was obtained ~10 minutes before entry to climate chamber (0 minutes; dashed vertical line; replicates data from baseline); * 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)

Aortic reservoir function components. P_{res} increased from baseline in mild-cold (103 ± 10 to 107 ± 12 mm Hg; *P* = 0.01) with no change in control (100 ± 10 to 100 ± 9 mm Hg; *P* = 0.93; Figure 4.3, A), but between conditions, there was no difference in change in P_{res} in mild-cold compared to change in control (+3.73 mm Hg; 95% CI -0.72 to 8.18; *P* = 0.33; Figure 4.3, A).

There was no change in P_{ex} from baseline in mild-cold (*P* = 0.90; Table 4.1), or control (*P* = 0.90; Table 4.1), and there was no difference in change between conditions (*P* = 0.65; Table 4.1). 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; Figure 4.3, B), 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; Figure 4.3, B).

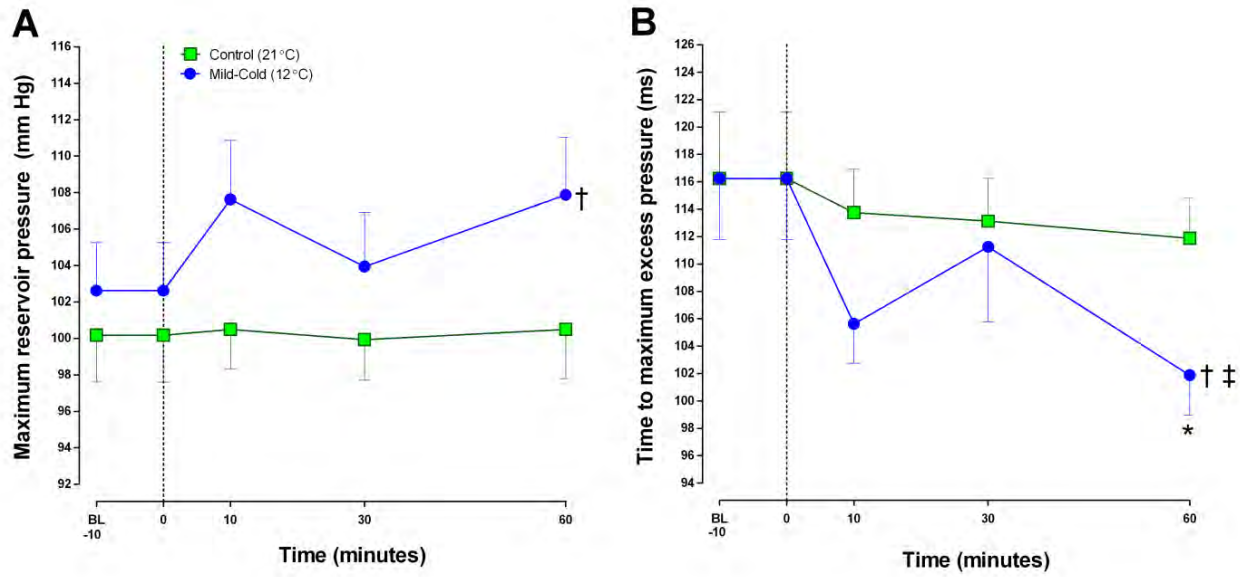

Figure 4.3. A) Maximum reservoir pressure and B) time to maximum excess pressure in mild-cold (12°C) and control (21°C) in healthy individuals.

Data presented as mean ± SE; n =16; baseline data (BL / -10 at ambient laboratory temperature) was obtained ~10 minutes before entry to climate chamber (0 minutes; dashed vertical line; replicates data from baseline); * significantly different from control at specific time point; † significant change within condition; ‡ significant difference between conditions

Blood pressure. Brachial systolic BP trended higher from baseline in mild-cold (123 ± 17 to 126 ± 17 mm Hg; $P = 0.06$) with no change in control condition (121 ± 15 to 121 ± 15 mm Hg; $P = 0.97$; Figure 4.4, A). 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 (Figure 4.4, A and B). Aortic systolic BP increased from baseline in mild-cold (109 ± 18 to 113 ± 19 mm Hg; $P = 0.004$), with no significant change in control (106 ± 15 to 107 ± 16 mm Hg; $P = 0.79$; Figure 4.4, C). However, between interventions there was no difference in change in aortic systolic ($P = 0.14$; Figure 4.4, C) or diastolic BP ($P = 0.52$; Table 4.1) 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 4.1) yet there was no significant change in brachial PP in either mild-cold ($P = 0.57$) or control from baseline ($P = 0.66$; Table 4.1). However, between interventions, neither aortic ($P = 0.24$) nor brachial PP ($P = 0.38$) changed in mild-cold, compared to change in control (Table 4.1).

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 4.1).

Mean blood pressure. MBP increased from baseline in mild-cold ($P = 0.03$) with no significant change in control ($P = 0.71$; Table 4.1). Yet between interventions there was no difference in change in MBP in mild-cold compared to change in control ($P = 0.45$; Table 4.1).

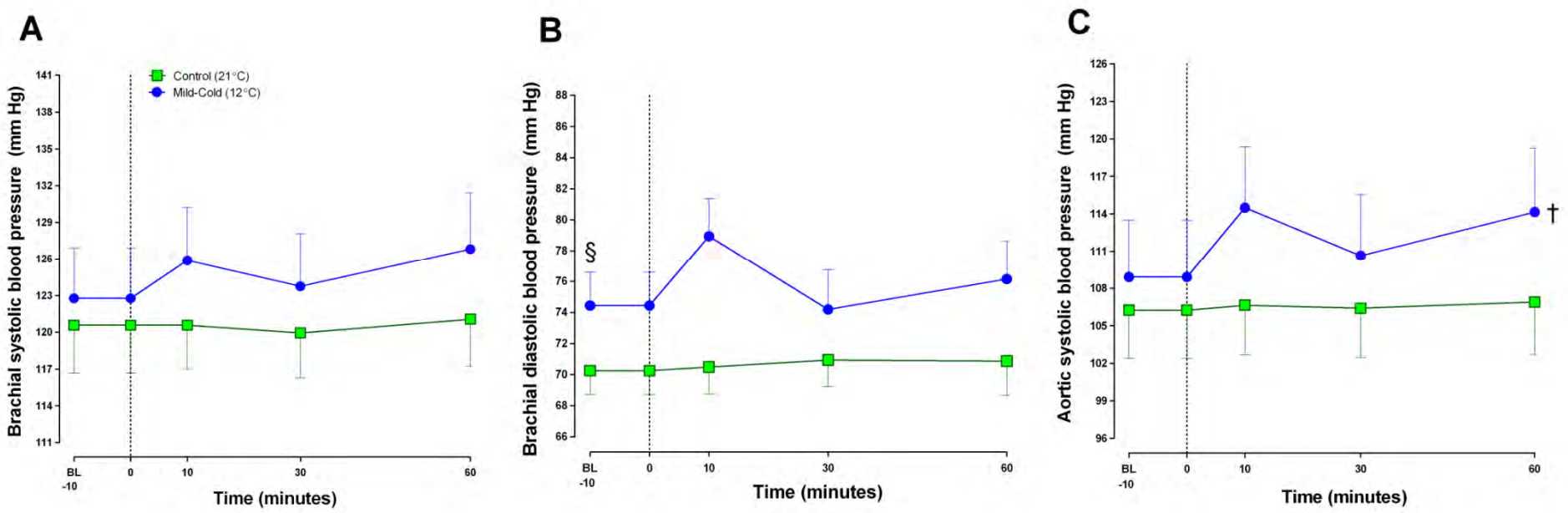

Figure 4.4. A) Brachial systolic, B) brachial diastolic, and C) aortic systolic blood pressures in mild-cold (12°C) and control (21°C) in healthy individuals.

Data presented as mean ± SE; *n* =16; baseline data (BL / -10; ambient laboratory temperature) was obtained ~10 minutes before entry to climate chamber (0 minutes; dashed vertical line); * significantly different from control at specific time point; † significant change within condition; **§** significantly different from control at baseline.

Table 4.1. Comparison of selected haemodynamic measures in mild-cold (12˚C) and control (21˚ C) conditions in healthy individuals

Condition	Baseline (-10 minutes)	10 minutes	30 minutes	60 minutes	Average (10-60 minutes) [a]	*P* (within condition) †	Δ mild-cold Vs. Δ control [b]	*P* (between conditions) ‡
Brachial pulse pressure (mm Hg)								
Control	50 ± 12	50 ± 11	49 ± 11	50 ± 10	50 ± 10	0.66	1.3 (-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 (mm Hg)								
Control	71 ± 6	71 ± 7	71 ± 7	71 ± 9	72 ± 7	0.74	1.4 (-2.16 to 4.96)	0.52
Mild-Cold	75 ± 9 **§**	79 ± 10	75 ± 10	77 ± 10	77 ± 10	0.15		
Aortic pulse pressure (mm Hg)								
Control	35 ± 10	35 ± 11	35 ± 11	35 ± 10	35 ± 10	0.81	2.7 (-0.45 to 5.92)	0.24
Mild-Cold	34 ± 11	35 ± 11	36 ± 12	38 ± 13	36 ± 12	**0.03**		
Mean blood pressure (mm Hg)								
Control	86 ± 10	87 ± 10	87 ± 10	87 ± 11	87 ± 10	0.71	2.2 (-1.39 to 5.77)	0.45
Mild-Cold	90 ± 11	95 ± 13	90 ± 13	92 ± 13	92 ± 13	**0.03**		

Table 4.1. Continued...

Condition	Baseline (-10 minutes)	10 minutes	30 minutes	60 minutes	Average (10-60 minutes) [a]	*P* (within condition) †	Δ mild-cold Vs. Δ control [b]	*P* (between conditions) ‡
Mean heart rate (beats.min^{-1})								
Control	59 ± 11	57 ± 10	57 ± 10	56 ± 10	57 ± 10	**0.01**	0.2 (-2.26 to 2.66)	0.90
Mild-Cold	57 ± 9	55 ± 8	55 ± 9	54 ± 8	54 ± 8	**0.01**		
Rate pressure product (mm Hg.beats.min-1)								
Control	7121 ± 1751	6887 ± 1697	6840 ± 1583	6820 ± 1624	6839 ± 1584	0.05	144 (-250 to 538)	0.47
Mild-Cold	6985 ± 1701	6963 ± 1755	6825 ± 1700	6836 ± 1779	6844 ± 1757	0.32		
Maximum Excess Pressure (mm Hg)								
Control	33 ± 11	32 ± 11	33 ± 11	33 ± 10	33 ± 11	0.90	-0.3 (-15.4 to 2.03)	0.41
Mild-Cold	32 ± 9	31 ± 10	32 ± 9	33 ± 12	32 ± 10	0.90		

Note: *n* = 16; data presented as mean ± standard deviation; **Δ**, mean change; [a], data collected at 10, 30 and 60 minutes in climate chamber were averaged for comparison against baseline data (baseline was at -10 minutes in ambient laboratory conditions); [b], **Δ** mild-cold compared to **Δ** control condition (95% confidence interval); **§**, significantly different from control at baseline; †, *P* value of **Δ** within condition (i.e. from baseline to the average of 10, 30, and 60 minute 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.

DISCUSSION

The novel findings of this study were first, that compared to a control condition (at 21° C), exposure to whole-body mild-cold (12° C) for 60 minutes leads to increased central haemodynamic stress and LV systolic afterload (AP and AIx). Second, this study shows for the first time, that mild-cold exposure alters time to P_{ex} (peak aortic in-flow timing) without changing P_{ex} (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, it was 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.

The results demonstrating a higher AIx in response to cold exposure are in agreement with previous studies that have investigated the effects of cold stress on measures of central haemodynamics (Edwards et al., 2006, Edwards et al., 2008, Casey et al., 2008, Geleris et al., 2004, Moriyama and Ifuki, 2010, Hess et al., 2009). However, this is the first study to my 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 and colleagues (2006) where a climate chamber was used at 4°C (with fans to create 6 $m.s^{-1}$ wind chill) for 30 minutes, 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 the current study's cold and control

conditions (+6% absolute value; Figure 4.2, B) than that observed by Edwards *et al.,* (2006).

The results for AIx are more comparable to a whole-body cooling study by Hess and colleagues (2009), where measures from a mild-cold trial using 15 to 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 and O'Rourke, 2005, Nichols et al., 2008). 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 (Nichols and O'Rourke, 2005), has recently been challenged by an emerging Reservoir-wave paradigm (Wang et al., 2003, Davies et al., 2010, Tyberg et al., 2008). 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 (Westerhof et al., 2009, Davies et al., 2007), 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 reservoir pressure (P_{res}) plus a smaller pressure component (related to aortic in-flow and travelling waves) named excess pressure (P_{ex})(Wang et al., 2003, Davies et al., 2010, Davies et al., 2007).

It has recently been proposed (Wang et al., 2003), and supported by human clinical trials (Davies et al., 2010, Sharman et al., 2009, Heffernan et al., 2010), 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 and colleagues (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 the best of my knowledge this is the first data available on the effect of cold exposure 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 was observed during mild-cold exposure in this study 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 reservoir pressures 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 temporarily exceeds aortic out-flow capacity and this

imbalance may have increased aortic P_{res} and altered timing of P_{ex} during mild-cold exposure 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 (Wilson et al., 2010, Muller et al., 2011).

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, Wang et al., 2011). Further, it has been shown mathematically, that P_{ex} is the additional pressure needed to overcome reservoir pressure, 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 P_{res}; Table 4.1). 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.

No previous studies have as yet 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 minutes (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 no significant differences were observed in aortic or brachial PWV between the mild-cold and control conditions in the current study, within condition, brachial PWV increased (+8% from baseline, Figure 4.1, B) with little change in aortic PWV (+1% from baseline; Figure 4.1, A) 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 this group of healthy adults.

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 (Edwards et al., 2006, Edwards et al., 2008, Casey et al., 2008), but not all (Hess et al., 2009) previous cooling studies. Additionally, a peak was observed in certain variables at the 10 minute time point during the mild-cold trials (i.e. AIx, P_{res}, time to P_{ex}, and BPs; Figures 4.2, B; 4.3; and 4.4) in the current study. Taken together, these results suggest that even short-term mild-cold stimulus places strain on the CV system of healthy individuals, 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 ischemia, combined with increased LV afterload could be potential contributory factors adding to the increased risk of CV events in older individuals with underlying atherosclerosis.

In summary, whole-body mild-cold exposure for 60 minutes 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).

CHAPTER 5 – HEALTHY GROUP, MANUSCRIPT 2

EFFECTS OF HUMIDITY AND HEAT ON CENTRAL HAEMODYNAMIC FUNCTION IN HEALTHY ADULTS

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An edited version of this chapter is currently under peer-review for publication as an original research investigation. The methods section has been reduced to avoid duplication with Chapter 3, General Methods.

ABSTRACT

Heat exposure causes vasodilation which reduces peripheral arterial tone in order to shunt blood to the skin to regulate body temperature. However, very little is known about how humidity impacts human physiology, particularly indicators of cardiovascular risk, such as augmentation index (AIx) and aortic pulse wave velocity (PWV). This study tested the hypothesis that whole-body heat exposure, at low and high relative humidity (RH), would reduce measures of left ventricular (LV) afterload, including AIx, aortic systolic blood pressure (BP) and mean BP (MBP), and aortic stiffness (aortic PWV). To this end, the study determined the effects of temperature and humidity, combined and separately to locate the source of the effect, on central haemodynamics in resting healthy adults. Sixteen volunteers (age 43 ± 19 years; 10 men) were randomised to receive each of the four experimental climates on separate days (7-14 days apart). Conditions were: 21°C/40% relative humidity (RH; control), 21°C/80% RH (humid), 36°C/40% RH (hot-dry), and 36°C/80% RH (hot-humid). Supine, resting measures were taken at baseline (ambient temperature) and after 10, 30, and 60 minutes exposure to each condition in a climate chamber. Aortic PWV was reduced in 36°C/80% vs. 21˚C/40% (-0.8 $m.s^{-1}$, $P = 0.009$) and 36°C/80% vs. 21 /80% (-0.7 $m.s^{-1}$, $P = 0.01$) but there was no significant difference in 36°C /40 RH vs. 21°C /40 RH (-0.4 $m.s^{-1}$, $P = 0.28$). However, there was no difference in MBP or AIx under any combination of conditions. In healthy adults, high humidity appears to drive a reduction in aortic stiffness in the heat, all without affecting measures of LV load (MBP and AIx). High humidity may potentiate the effects of heat on the arteries by increasing the heat load which may augment vasodilation.

INTRODUCTION

Heat exposure profoundly affects haemodynamics (Rowell, 1990, Abrignani et al., 2011, Basu, 2009). Blood redistribution from the core to the periphery during heat exposure causes an increase in left ventricular (LV) contraction rate (Nelson et al., 2010) to stabilise declines in preload, i.e. diastolic/right atrial filling pressures, and afterload, i.e. mean blood pressure (MBP) (Truijen et al., 2010). Cardiac output is typically maintained or slightly increased during heating due to increased heart rates, but increases in cardiac output are somewhat dependent on magnitude of heat load (Crandall and Gonzalez-Alonso, 2010). Repeated, short-exposure to dry-heat has been shown to improve haemodynamics, specifically endothelial function and skin blood flow, in people with cardiovascular (CV) diseases, including heart failure (Blum and Blum, 2007). Conversely, prolonged exposure to heat combined with high humidity can present a particular challenge to the CV system as the high water vapour content of the air may diminish the body's ability to dissipate heat via evaporative cooling (Holmer, 2006). The resultant CV stress is thought to contribute to morbidity and mortality during heatwaves (Basu, 2009). It is well accepted that central haemodynamic measures are a stronger indicator of CV risk and better reflect the workload of the LV than brachial BPs (Roman et al., 2007, Willum-Hansen et al., 2006). However, there is no information about the effect of short-term whole-body heat exposure with or without humidity, on central haemodynamic measures such as aortic pulse wave velocity (PWV) or augmentation index (AIx) in any population.

Localised application of heat via hand water immersion and the use of heat packs, decreases smooth muscle tone (Bellien et al., 2010, Huang et al., 2011, Kellogg Jr, 2006), AIx at the radial artery (Huang et al., 2011), and brachial arterial wall stiffness (Bellien et al., 2010). A single study has investigated changes in PWV during whole-body heat stress

using a water-perfused suit at 49°C (Ganio et al., 2011). The authors reported non-significant increases in aortic and brachial PWV, as well as heart rate during 60 minutes of heating, compared to control conditions perfusing 34°C water, with no change in mean BP or brachial systolic BP (Ganio et al., 2011). Few studies have specifically investigated the effects of humidity with or without heat on CV physiology. In a rat model at rest, exposure to humid-heat produced lower heart rates, lower core temperatures, and lower BPs than exposure to dry-heat (Moran et al., 1996). However, as rats are a non-sweating species, results of that study may not strictly be extrapolated to humans. Humans undergoing low-intensity exercise in humid-heat had lower systolic BP (Smolander et al., 1987), lower expression of stress hormones (Wright et al., 2012), lower heart rate (Wenzel, 1978), and higher cardiac output (Smolander et al., 1987) than exercise in dry-heat. One study examined the effects of increasing humidity from 10 to 50% RH at comfortable 'room temperature' of 25°C in resting humans, and reported no change in heart rate, systolic and diastolic BP with exposure to humidity at 10, 30 and 50% RH, although no data were provided (Sunwoo et al., 2006). However, Sunwoo *et al.,* (2006) observed improvements in perceived and mucosal comfort, i.e. decreased feelings of coolness and reduced eye blinking rate, and higher skin hydration and body temperature with humidity from 30 to 50% compared to 10% RH. There are currently no data on exposure to differing humidity with or without heat, on any measure of central haemodynamics in humans.

To maintain adequate tissue perfusion during rest and challenges to haemodynamics such as heat exposure, the elastic aorta acts as a buffer and a reservoir that stores blood in systole and expels it during diastole, effectively smoothing the pulsatile flow of the LV into a smoother, consistent flow, which then becomes a steady flow that perfuses the microcirculation (Westerhof et al., 2009). The emerging Reservoir-wave theory states that total measured pressure is composed of a reservoir pressure (P_{res}),

produced by the buffering effect of the aorta and is the pressure required to overcome net arterial resistance, plus an excess pressure (P_{ex}) which is closely related to aortic in-flow and wave motion in the aorta (Parker, 2009). "Wave reflections" are said to be represented by the AIx (Nichols and O'Rourke, 2005). AIx and wave reflections are reported to decrease during vasodilation via medications and exercise (Munir et al., 2008). However, recent research suggests that it is P_{res}, rather than wave reflections, that are the major determinant of AIx (Davies et al., 2010). To date, there is no information available on the effects of sudden whole-body environmental climate changes on components of reservoir function, or on AIx. However, it could be expected that as vasodilatory medications cause AIx (Munir et al., 2008) and P_{res} to reduce (Sharman et al., 2009, Wang et al., 2011, Wang et al., 2012), vasodilation via heating may also cause AIx and P_{res} to reduce.

Collectively, previous findings suggest that short-term heat on its own (Huang et al., 2011, Bellien et al., 2010, Kellogg Jr, 2006), and humidity with or without heat (Moran et al., 1996, Smolander et al., 1987, Wright et al., 2012, Wenzel, 1978, Sunwoo et al., 2006) may improve haemodynamic function via improved endogenous vasodilation and hydration of skin and mucous membranes. The present study aimed to explore, in resting healthy adults, how central haemodynamic measures, including aortic PWV, aortic, brachial and mean BPs, aortic AIx and aortic reservoir components including P_{res}, and P_{ex}, were affected by 60 minutes exposure to different combinations of temperature (21°C and 36°C) and relative humidity (RH) at 40% and 80% in a climate chamber. Based on previous work, it was hypothesised that whole-body heat exposure, at low and high RH, would reduce LV afterload (AIx, aortic systolic BP and MBP), aortic and brachial PWV and P_{res}.

MATERIALS AND METHODS

Details of materials and methods for physiological data collection and statistical analysis procedures for this study are in Chapter 3, General Methods.

RESULTS

Nineteen volunteers met the inclusion criteria. However, three withdrew prior to commencement of data collection for personal reasons. Sixteen participants (10 men, 6 women) each completed all four experimental sessions. Participant demographics and baseline data are shown in Table 5.1 (mean ± SD). Mean ambient laboratory conditions during the study were 22.0 ± 1.9°C and 38 ± 11% RH. Mean climate chamber temperature and RH for each trial were, control 20 ± 1°C, 44 ± 9% RH; humid 21 ± 0°C, 83 ± 4% RH; hot-dry 36 ± 1°C, 38 ± 6% RH; and hot-humid 36 ± 1°C, 78 ± 8% RH. No participant terminated any test due to discomfort or adverse effects of climate.

There were no significant differences in baseline measures between trials (all $P > 0.26$) except for brachial diastolic (-3 mm Hg in 36/80 vs. 21/40; $P = 0.01$) and aortic diastolic BP (-4 mm Hg in 36/80 vs. 21/40; $P = 0.01$). All participants were non-smokers and complied with pre-test instructions.

Table 5.1. Healthy participant demographics and baseline results

	Mean ± SD
Age (years)	43 ± 19
Brachial systolic blood pressure (mm Hg)	122 ± 16
Brachial diastolic blood pressure (mm Hg)	72 ± 7
Aortic systolic blood pressure (mm Hg)	106 ± 16
Mean blood pressure (mm Hg)	86 ± 11
Aortic pulse wave velocity ($m.s^{-1}$)	7 ± 2
Brachial pulse wave velocity ($m.s^{-1}$)	9 ± 2
Augmentation index (%)	16 ± 13
Fasting plasma glucose ($mmol.L^{-1}$)	4.7 ± 0.3
Fasting total cholesterol ($mmol.L^{-1}$)	4.3 ± 0.8

Absolute and mean changes from baseline for main haemodynamic variables are presented in Table 5.2.

Table 5.2. Absolute and mean change from baseline to 60 minutes in selected haemodynamic variables in control (21° C / 40% RH), humid (21° C, 80% RH), hot-dry (36° C, 40% RH), and hot-humid (36° C, 80% RH) conditions in healthy individuals

Condition	Time point (minutes)				Mean Δ †	*P* (within condition)‡
	Baseline	10	30	60		
Aortic pulse wave velocity (m.s^{-1})						
Control	6.9 ± 2.1	6.9 ± 1.9	7.1 ± 2.8	6.9 ± 2.1	6.9 ± 2.2	0.74
Humid	7.2 ± 2.3	7.1 ± 2.4	6.9 ± 2.4	7.1 ± 2.5	7.0 ± 2.4	0.65
Hot-dry	6.9 ± 2.1	6.7 ± 1.9	6.6 ± 2.1	6.4 ± 1.8	6.6 ± 1.9	0.15
Hot-humid	6.9 ± 2.2	6.2 ± 1.6 a	6.3 ± 1.7	6.1 ± 1.6 a	6.2 ± 1.6	**<0.001**
Brachial pulse wave velocity (m.s^{-1})						
Control	8.5 ± 1.7	8.8 ± 1.8	8.8 ± 1.2	8.7 ± 1.3	8.8 ± 1.5	0.20
Humid	8.5 ± 1.5	9.1 ± 1.4	8.8 ± 1.2	8.9 ± 1.6	8.9 ± 1.4	**0.02**
Hot-dry	8.7 ± 1.1	8.4 ± 1.4	8.3 ± 1.6	8.2 ± 1.6	8.3 ± 1.6	**0.01**
Hot-humid	8.4 ± 1.1	8.4 ± 1.4	8.2 ± 1.3	8.3 ± 1.4	8.3 ± 1.3	0.10
Brachial systolic blood pressure (mm Hg)						
Control	121 ± 15	120 ± 15	120 ± 14	121 ± 15	121 ± 15	0.87
Humid	123 ± 16	119 ± 16 a	116 ± 17 a	119 ± 16	118 ± 16	**0.006**
Hot-dry	118 ± 17	114 ± 13	116 ± 16	115 ± 11	115 ± 13	0.06
Hot-humid	117 ± 13	116 ± 13	113 ± 12	111 ± 14	113 ± 13	**0.03**
Brachial diastolic blood pressure (mm Hg)						
Control	70 ± 6	71 ± 7	71 ± 7	71 ± 9	71 ± 7	0.41
Humid	72 ± 10	70 ± 8	70 ± 8	71 ± 8	71 ± 8	0.40
Hot-dry	69 ± 9	66 ± 9	66 ± 10	64 ± 8 a	65 ± 9	**<0.001**
Hot-humid	67 ± 7 b	68 ± 8	65 ± 7	61 ± 8 a	65 ± 8	0.09
Aortic systolic blood pressure (mm Hg)						
Control	106 ± 15	107 ± 16	106 ± 16	107 ± 17	107 ± 16	0.82
Humid	110 ± 19	106 ± 17	104 ± 18	106 ± 18 a	106 ± 17	**0.009**
Hot-dry	105 ± 18	102 ± 14	103 ± 17	101 ± 11	102 ± 14	0.11
Hot-humid	103 ± 14	103 ± 13	100 ± 11	98 ± 12	100 ± 11	0.10
Augmentation index (%)						
Control	15 ± 11	15 ± 15	15 ± 14	15 ± 13	15 ± 14	0.72
Humid	17 ± 13	17 ± 12	18 ± 12	17 ± 12	17 ± 12	0.95
Hot-dry	15 ± 13	18 ± 11	18 ±13	19 ± 12	19 ± 12	**0.04**
Hot-humid	15 ± 13	17 ± 10	16 ± 9	17 ± 10	17 ± 10	0.51

Table 5.2. Continued...

Condition	Time point (minutes)				Mean Δ †	*P* (within condition)‡
	Baseline	10	30	60		
Heart rate (beats. min^{-1})						
Control	58.8	56.8	56.8	56.1	56.1	**0.01**
Humid	58.4	56.1	55.5	54.3	55.0	**0.001**
Hot-dry	57.3	60.2	60.9	64.2	62.5	**< 0.001**
Hot-humid	59.4	61.9	63.7	68.2	65.9	**< 0.001**
Maximum reservoir pressure (mm Hg)						
Humid	103 ± 12	100 ± 11	99 ± 12 [a]	100 ± 10	99 ± 11	0.10
Hot-dry	99 ± 12	95 ± 11	97 ± 12	93 ± 9	95 ± 10	**0.01**
Hot-humid	96 ± 9	96 ± 10	92 ± 8	88 ± 11 [a]	92 ± 10	**0.01**
Maximum excess pressure (mm Hg)						
Control	33 ± 11	32 ± 11	33 ± 11	33 ± 10	33 ± 11	0.78
Humid	33 ± 10	31 ± 12	30 ± 10	32 ± 10	31 ± 11	0.26
Hot-dry	32 ± 10	32 ± 6	32 ± 9	34 ± 7	32 ± 7	0.51
Hot-humid	34 ± 9	32 ± 8	32 ± 6	34 ± 6	33 ± 7	0.73

Note: *n* = 16; data were adjusted for order and period effect and presented as mean ± standard deviation; Δ= mean change; †= average of data collected at 10, 30, and 60 minutes in climate chamber; ‡= *P* value is Δ from baseline (baseline was -10 min in ambient laboratory conditions) to average of 10, 30, and 60 minute data; [a]= significantly different compared to control at specific time point, $P \leq 0.05$; [b]=significantly different from control at baseline, $P \leq 0.05$.

Effect of increased heat at 40% RH (21/40 vs. 36/40.)

Brachial PWV (Figure 5.1, A), brachial diastolic BP (Figure 5.2, B) and P_{res} (Figure 5.3, C) were significantly lower in 36/40 than in 21/40 (Table 5.3, column A). RPP (Table 5.3, A), heart rate (+6.5 beats.min^{-1}, 95% CI 3.8 to 9.26, $P < 0.001$), core temperature (+1.0°C, $P < 0.001$, Table 5.3, A), skin temperature (+3.5°C, 95% CI 2.10 to 4.86, $P < 0.001$), and perceived thermal sensation (+1.8 arbitrary units, 95% CI 1.37 to 2.14, $P < 0.001$) were significantly higher in 36/40 than 21/40. There was no significant difference for the changes in any other measure (Table 5.3, A).

Effect of increased heat at 80% RH (21/80 vs. 36/80)

Aortic PWV (Figure 5.1, A) and brachial PWV (Figure 5.1, B) were significantly lower in 36/80 than in 21/80 (Table 5.3, column B). While heart rate (+8.3 beats.min^{-1}, 95% CI 5.57 to 11, $P < 0.001$), core temperature (+0.9°C, $P < 0.001$, Table 5.3, B), skin temperature (+2.7°C, 95% CI 1.35 to 4.11, $P < 0.001$), and perceived thermal sensation (+2.0 arbitrary units, 95% CI 1.60 to 2.38, $P < 0.001$) were all significantly higher in 36/80 compared to 21/80. There was no significant difference for the changes in any other measure (Table 5.3, B).

Table 5.3. Effect of 60 minutes exposure to heat (36°C) at two different humidity levels (40% and 80% RH) on selected haemodynamic measures in healthy individuals

Variable	A. Effect of increased heat at 40% RH Δ 36/40 vs. Δ 21/40		B. Effect of increased heat at 80% RH Δ 36/80 vs. Δ 21/80	
	Difference (95% CI)	*P* value	Difference (95% CI)	*P* value
Heart rate (beats.min^{-1})	6.5 (3.8 to 9.3)	**< 0.001**	8.3 (5.6 to 11.0)	**< 0.001**
Brachial pulse wave velocity (m.s^{-1})	-0.8 (-1.40 to -0.20)	**0.04**	-0.8 (-1.42 to -0.22)	**0.007**
Aortic pulse wave velocity (m.s^{-1})	-0.4 (-0.91 to 0.20)	0.28	-0.7 (-1.22 to -0.10)	**0.01**
Brachial systolic blood pressure (mm Hg)	-3.1 (-8.10 to 1.83)	0.61	1.2 (-3.78 to 6.15)	0.64
Brachial diastolic blood pressure (mm Hg)	-4.9 (-8.15 to -1.66)	**0.02**	-1.0 (-4.23 to 2.25)	0.55
Aortic systolic blood pressure (mm Hg)	-3.3 (-8.31 to 1.73)	0.56	1.8 (-3.27 to 6.78)	0.49
Aortic diastolic blood pressure (mm Hg)	-4.4 (-7.71 to -1.10)	0.34	-0.2 (-3.55 to 3.06)	0.88
Brachial pulse pressure (mm Hg)	1.8 (-2.66 to 6.20)	0.42	2.2 (-2.27 to 6.60)	0.34
Aortic pulse pressure (mm Hg)	1.1 (-2.73 to 4.96)	0.61	2.0 (-1.85 to 5.85)	0.30
Mean blood pressure (mm Hg)	-3.6 (-7.36 to 0.17)	0.20	1.2 (-2.61 to 4.91)	0.54
Rate pressure product (mm Hg.beats.min^{-1})	562 (115 to 1010)	**0.003**	15.2 (-519 to 550)	0.96
Augmentation index (%)	2.8 (-1.75 to 7.24)	0.42	1.4 (-3.05 to 5.94)	0.52
Reservoir pressure (mm Hg)	-4.3 (-9.27 to 0.60)	**0.03 ***	0.0 (-4.93 to 4.93)	1.00
Excess pressure (mm Hg)	0.3 (-3.66 to 4.18)	0.89 *	0.4 (-3.56 to 4.27)	0.85
Core (tympanic) temperature (˚C)	0.99 (0.72 to 1.27)	**<0.001 ***	0.94 (0.67 to 1.22)	**<0.001 ***

Note: *n* = 16; RH, relative humidity; Δ, change in. control (21°/40%); humid (21°/80%); hot-dry (36°/40%); and hot-humid (36°/80%). Unless indicated * data violated assumptions of general linear modelling, *P* values taken from ordinal logistic regression. Results are adjusted for order and period effect.

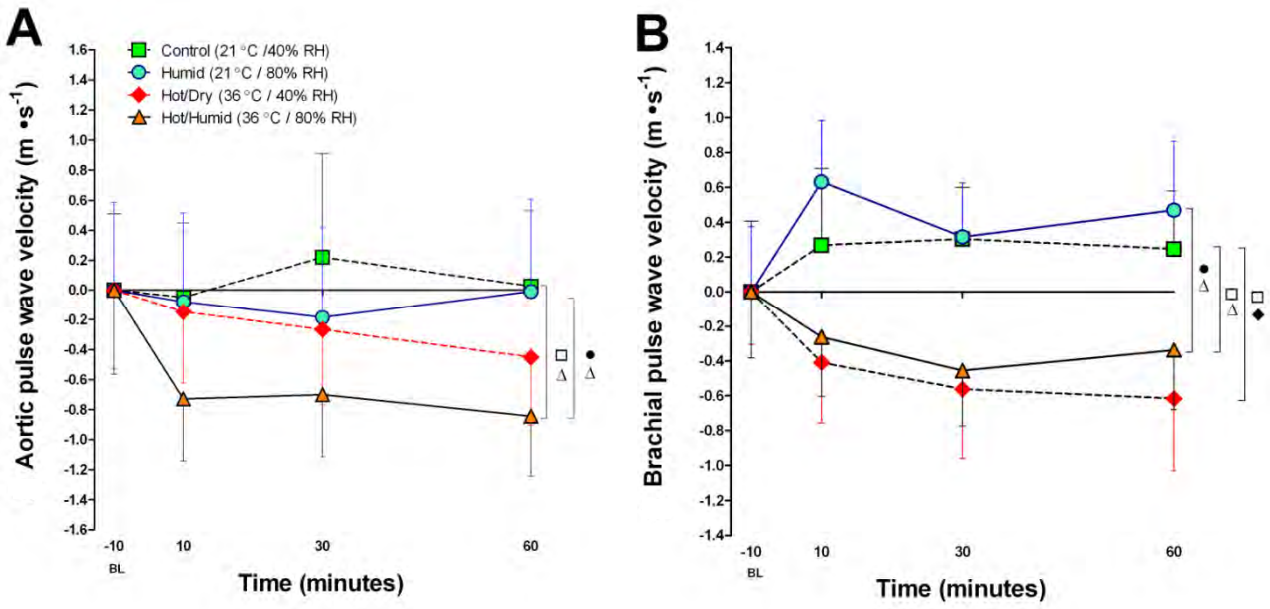

Figure 5.1. Change in A) aortic pulse wave velocity, and B) brachial pulse wave velocity from baseline to 60 minutes in control (21°C/40% RH), humid (21°C/80% RH), hot-dry (36°C/40% RH), and hot-humid (36°C/80% RH) in healthy individuals.

Data presented as mean ± SEM; *n* =16. Baseline data (-10) was obtained in ambient laboratory conditions ~10 minutes before entry to climate chamber. Vertical square bracket indicates significant change between specified conditions ($P < 0.05$).

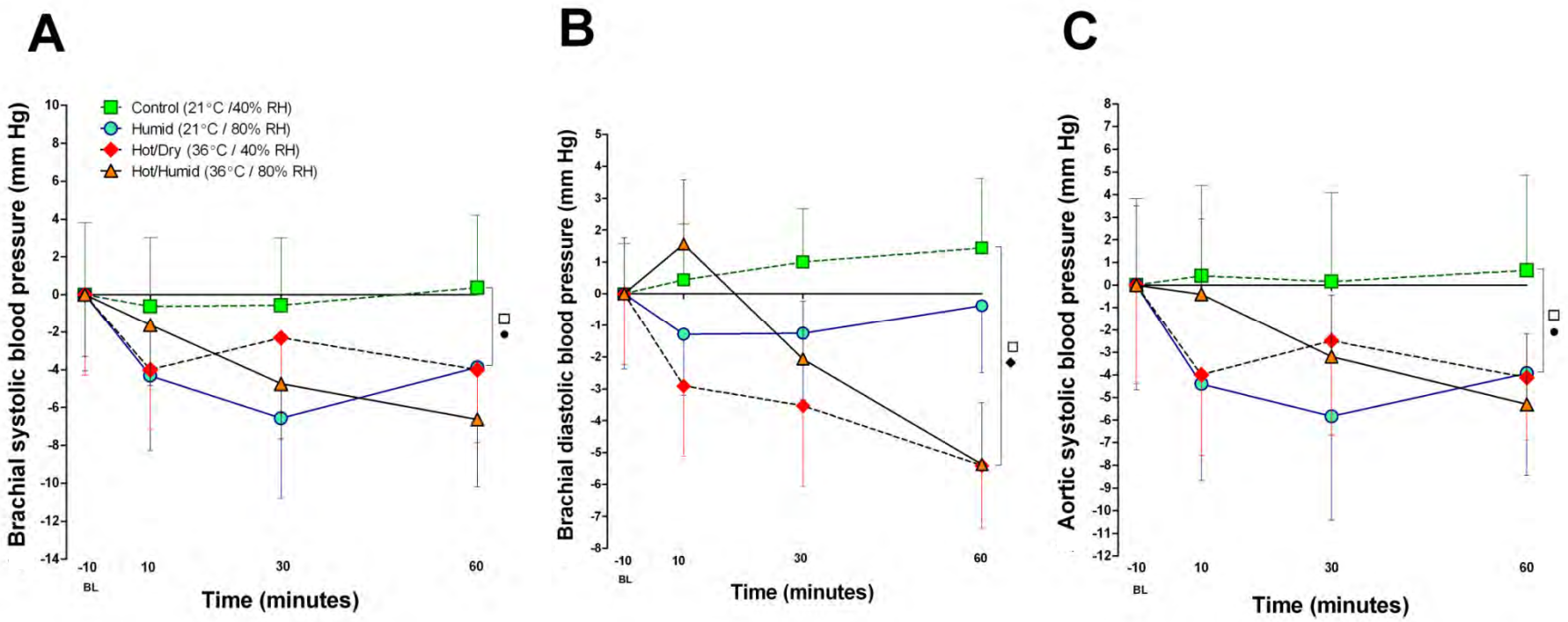

Figure 5.2. Change in A) brachial systolic, B) brachial diastolic, and C) aortic systolic blood pressure in each condition in healthy individuals.

Data presented as mean ± SEM; *n* =16. Baseline data (-10) was obtained in ambient laboratory conditions ~10 minutes before entry to climate chamber. Vertical square bracket indicates significant change between specified conditions ($P < 0.05$).

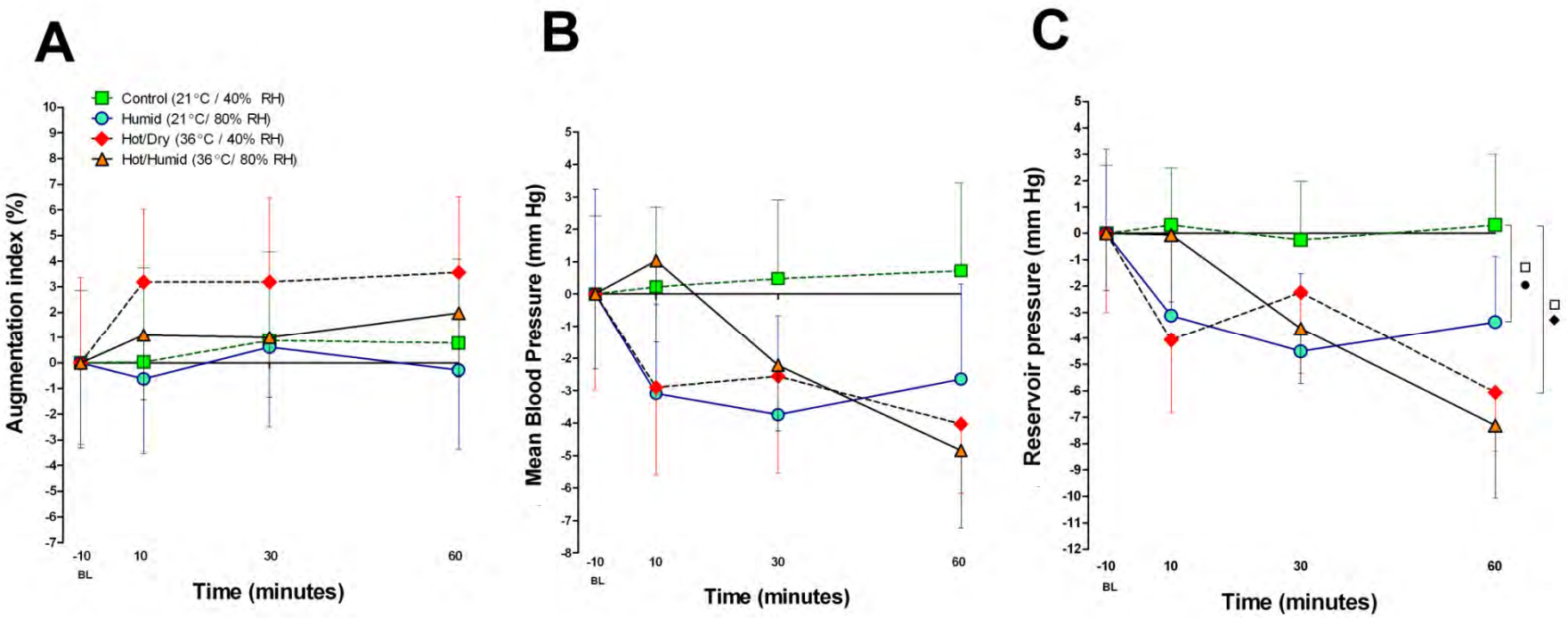

Figure 5.3. Change in A) augmentation index, B) mean blood pressure, and C) reservoir pressure in each condition in healthy individuals.

Vertical square bracket indicates significant change between specified conditions ($P < 0.05$).

Effect of increased humidity at 21°C (21/40 vs. 21/80)

Brachial systolic BP (Figure 5.2, A), aortic systolic BP (Figure 5.2, C), RPP (Table 5.4 A), and P_{res} (Figure 5.3, C) were significantly lower in 21/80 compared to 21/40. Additionally, reductions in brachial (P = 0.06) and aortic PP (P = 0.07) were of borderline significance (Table 5.4, A) in 21/80 compared to 21/40. There were no significant differences for the changes in any other measure (Table 5.4, A).

Effect of increased humidity at 36°C (36/40 vs. 36/80)

There were no significant differences for the changes in any measure between 36/80 and 36/40 (Table 5.4, column B).

Table 5.4. Effect of 60 minutes exposure to humidity (80% RH) at two different temperatures (21°C and 36°C) on selected haemodynamic measures in healthy individuals

Variable	A. Effect of increased humidity at 21◦C Δ 21/80 vs. Δ 21/40		B. Effect of increased humidity at 36◦C Δ 36/80 vs. Δ 36/40	
	Difference (95% CI)	*P* value	Difference (95% CI)	*P* value
Heart rate (beats.min^{-1})	-0.9 (-3.6 to 1.9)	0.49	0.9 (-1.8 to 3.6)	0.51
Brachial pulse wave velocity (m.s^{-1})	0.2 (-0.39 to 0.79)	0.75	0.2 (-0.42 to 0.77)	0.56
Aortic pulse wave velocity (m.s^{-1})	-0.2 (-0.71 to 0.40)	0.83	-0.5 (-1.02 to 0.08)	0.10
Brachial systolic blood pressure (mm Hg)	-4.6 (-9.61 to 0.32)	**0.04 ***	-0.3 (-5.29 to 4.64)	0.89
Brachial diastolic blood pressure (mm Hg)	-1.9 (-5.17 to 1.31)	0.47	2.0 (-1.25 to 5.23)	0.22
Aortic systolic blood pressure (mm Hg)	-5.1 (-10.1 to -0.10)	**0.02**	-0.1 (-5.11 to 4.94)	0.97
Aortic diastolic blood pressure (mm Hg)	-1.9 (-5.20 to 1.40)	0.99	2.3 (-1.04 to 5.56)	0.18
Brachial pulse pressure (mm Hg)	-2.7 (-7.14 to 1.72)	0.06	-2.3 (-6.74 to 2.12)	0.30
Aortic pulse pressure (mm Hg)	-3.2 (-7.07 to 0.62)	0.07	-2.3 (-6.19 to 1.50)	0.23
Mean blood pressure (mm Hg)	-3.6 (-7.39 to 0.13)	0.17	1.1 (-2.65 to 4.88)	0.56
Rate pressure product (mm Hg.beats.min^{-1})	-37 (-812 to 75.9)	**0.02 ***	-68.1 (-603 to 466)	0.80
Augmentation index (%)	-0.7 (-5.15 to 3.84)	0.97	-2.0 (-6.45 to 2.54)	0.39
Reservoir pressure (mm Hg)	-3.8 (-8.72 to 1.14)	**0.03 ***	-0.5 (-4.39 to 5.48)	0.82
Excess pressure (mm Hg)	-2.1 (-5.94 to 1.89)	0.31 *	-1.9 (-5.85 to 2.00)	0.33
Core (tympanic) temperature (˚C)	-0.14 (-0.13 to 0.41)	0.47	0.09 (-0.19 to 0.37)	0.22

Note: *n* = 16; control (21°/40%); humid (21°/80%); hot-dry (36°/40%); and hot-humid (36°/80%); RH, relative humidity; Δ, change in. Unless indicated * data violated assumptions of general linear modelling, *P* values taken from ordinal logistic regression. Results are adjusted for order and period effect.

Effect of increased heat plus humidity (21/40 vs. 36/80)

Aortic PWV (Figure 5.1, A) and brachial PWV (Figure 5.1, B) were significantly lower and RPP was significantly higher ($P = 0.03$) in 36/80 compared to 21/40 (Table 5.5). Additionally, heart rate (+7.4 beats.min^{-1}, 95% CI 4.69 to 10.2, $P < 0.001$), core temperature ($P < 0.001$, Table 5.5), skin temperature (+2.8°C, 95% CI 1.4 to 4.18, $P < 0.001$), and perceived thermal sensation (+2.1 arbitrary units, 95% CI 1.72 to 2.50, $P < 0.001$) were all significantly higher in 36/80 compared to 21/40 (Table 5.5). There were no significant differences for the changes in any other measure (Table 5.5).

Table 5.5. Effect of 60 minutes exposure to heat plus humidity on selected haemodynamic measures in healthy individuals

Condition	Δ 36/80 RH vs. Δ 21/40 Effect of increased heat + high humidity	
	Difference (95% CI)	***P* value**
Heart rate (beats.min^{-1})	7.4 (4.7 to 10.2)	**<0.001**
Aortic pulse wave velocity (m.s^{-1})	-0.8 (-1.38 to -0.26)	**0.009**
Brachial systolic blood pressure (mm Hg)	-3.5 (-8.42 to 1.51)	0.90
Brachial diastolic blood pressure (mm Hg)	-2.9 (-6.16 to 0.33)	0.16
Aortic systolic blood pressure (mm Hg)	-3.4 (-8.40 to 1.65)	0.53
Aortic diastolic blood pressure (mm Hg)	-2.1 (-5.45 to 1.16)	0.34
Brachial pulse pressure (mm Hg)	-0.5 (-4.97 to 3.89)	0.91
Aortic pulse pressure (mm Hg)	-1.2 (-5.07 to 2.62)	0.99
Mean blood pressure (mm Hg)	-3 (-6.24 to 1.28)	0.50
Rate pressure product (mm Hg.beats.min^{-1})	659 (215 to 1104)	**0.03**
Augmentation index (%)	0.8 (-3.71 to 5.29)	0.82
Maximum reservoir pressure (mm Hg)	-3.8 (-8.72 to 1.14)	0.06
Maximum excess pressure (mm Hg)	-1.7 (-5.58 to 3.2)	0.40 *
Core (tympanic) temperature (°C)	1.09 (0.81 to 1.36)	**<0.001**

Note: *n* = 16; RH, relative humidity; control (21°/40%); humid (21°/80%); hot-dry (36°/40%); and hot-humid (36°/80%); Δ, change in. Unless indicated * data violated assumptions of general linear modelling, *P* values taken from ordinal logistic regression. Results are adjusted for order and period effect.

Data from 60 to 90 minutes were analysed separately to determine any effects of hydration on haemodynamics which may have occurred after ingestion of 300 mL water which occurred immediately post-60 minute measures. At 90 minutes, AIx was increased in 21/40 vs. 36/40 (+5%, $P = 0.01$) and also in 21/40 vs. 36/80 (+6%, $P = 0.03$) compared to 60 minutes. Additionally heart rate was reduced in 21/40 vs. 36/80 (-3.0 beats.min^{-1}; $P = 0.03$) and also in 21/80 vs. 36/80 (-2.1 beats.min^{-1}; $P = 0.05$). Finally hydration reduced tympanic core temperature in 21/40 vs. 36/80 (-0.2°C; $P = 0.04$) and in 21/80 vs. 36/80 (-0.2°C; $P = 0.05$). There was no significant effect of water consumption on any other measure from 60 to 90 minutes.

Haematocrit was unchanged in 21/80 vs. 21/40 (+0.5%; $P = 0.86$), unchanged in 36/40 vs. 21/40 (-0.5%; $P = 0.45$), unchanged in 36/40 vs. 36/80 (-0.9%; $P = 0.65$), but haematocrit was decreased in 21/80 vs. 36/80 (-2%; $P = 0.01$), and (36/80 vs. 21/40 (-1.5%; $P = 0.01$).

DISCUSSION

The primary findings of this study were that heat at either high or low humidity reduced brachial PWV, but aortic PWV was reduced only in hot conditions with high humidity. Importantly, the decrease in aortic and brachial PWV in hot-dry and hot-humid conditions occurred in the absence of any significant change in traditional measures of peripheral brachial systolic or MBP. At room temperature, brachial and aortic systolic BPs, aortic PP and P_{res} were reduced in conditions with high humidity compared to low humidity, but this reducing effect of humidity did not occur when

conditions were already hot. Contrary to the hypothesis, AIx was not affected by any combination of conditions in this study.

Previous investigations into the effects of climate on central haemodynamics have found differential central–peripheral haemodynamic responses during varying degrees of cold stress, i.e. greater increases in central measures of BP and PP than in peripheral measures (Casey et al., 2008, Edwards et al., 2006, Edwards et al., 2008). However, there is currently little information on the effects of hot-dry and hot-humid conditions on central haemodynamic measures such as PWV, AIx, or aortic BPs. Many people deal with such climate transitions on a regular basis, especially in summer, or while visiting tropical climates and moving from an air-conditioned room to outdoor hot-humid weather. Given there is evidence that short-term dry-heat exposure in people with CVD improves haemodynamic function (Blum and Blum, 2007), it is important to determine the potential haemodynamic effects of short-term exposure to heat, with and without humidity, initially in a healthy population.

Effects on pulse wave velocity. Hot-dry (36/40) and hot-humid (36/80) conditions had differential effects on measures of regional arterial stiffness in this study. Separately, it was found that increased heat or humidity did not significantly affect aortic PWV compared to control. However, after exposure to hot-humid conditions, aortic PWV was lower compared to control and also compared to humid-room temperature conditions (21/80). High humidity may amplify the potential of heat to reduce aortic PWV, possibly due to the increased heat load, which in turn produces a passive relaxation of the elastic aorta (Kelly et al., 2001, Nichols et al., 2008). This reduction in aortic stiffness may occur via flow-mediated increases in shear stress (Kellogg Jr et al., 2003, Sugawara et al., 2007, Buga et al., 1991), which triggers release of nitric oxide (NO)

and other endogenous vasodilators that decrease large artery stiffness (Wilkinson et al., 2002) independently of changes in BP (Fitch et al., 2001). Conversely, in the current study, heat alone appeared to be the primary determinant of reduced brachial PWV. Brachial arterial stiffness was reduced by heat at 40% and 80% RH. This effect of heat on the brachial artery has previously been shown to occur via sympathetically-stimulated peripheral vasodilation which actively reduces smooth muscle tone (Bellien et al., 2010, Nichols et al., 2008). However, when conditions were already hot, (36/40 vs. 36/80 conditions; Table 5.4, column B), increasing humidity had no additional influence on PWV or on any other measure, which could mean a maximal response had already occurred.

In contrast to the current results for PWV, one study has observed non-significant higher aortic and brachial PWV during whole-body heat exposure using a water-perfused suit at 49°C compared to 34°C control conditions (Ganio et al., 2011), which is an intervention that could be likened to humid-heat stress. The difference in results for Ganio *et al's.,* (2011) study and the present study may be explained by methodological differences, as PWV was measured via Doppler flow probes, or by the possibility that the water-perfused suit may have led to higher physiological stress by reducing any potential for heat loss through evaporative cooling (Holmer, 2006). This suggestion is somewhat supported by the much larger increase in heart rate (+50 beats.min^{-1}) in Ganio *et al's.,* (2011) study compared to the present study's results (Tables 5.2 to 5.5).

Effects on markers of LV afterload. AIx is sometimes regarded as a surrogate marker of systemic arterial stiffness (Nichols and O'Rourke, 2005) and is also a marker of LV systolic load (Saba et al., 1993). Therefore, given the reductions observed in regional arterial stiffness in hot-dry and hot-humid conditions in the current study, and

that LV afterload is typically reduced by heat (Truijen et al., 2010), it is surprising that AIx and other markers of afterload (aortic systolic BP and PP) were unaffected by all conditions of increased heat in the present study. Humidity by itself tended to reduce these same measures of LV afterload (21/80; Table 5.4, column A). There are currently no data available on AIx during heat exposure. However, heat exposure causes vasodilation (Kellogg Jr et al., 2003) and AIx has been shown previously to decrease during vasoactive interventions such as exercise and vasodilatory drugs (Munir et al., 2008, Kelly et al., 2001, Sharman et al., 2009). The difference between previous findings (Sharman et al., 2009, Kelly et al., 2001, Munir et al., 2008) and the current results may be related to the milder effects of heat on heart rate and cardiac contractility (Kellogg Jr et al., 2003), compared to exercise or vasodilatory medications (Overgaard and Dzavik, 2008). Additionally, it is possible for aortic PWV and AIx to change independently of one another during vasoactive interventions (Kelly et al., 2001). This is likely because AIx is derived from brachial BP and is therefore more immediately dependent on changes in heart rate, contractility and ejection duration (Wilkinson et al., 2000) than aortic PWV. The results of the current study tend to agree with others that have suggested AIx is not an accurate indicator of systemic arterial stiffness (Khoshdel, 2007, Gurovich et al., 2009). As aortic PWV is the current gold standard non-invasive measure of large artery stiffness (Van Bortel et al., 2002), the present results indicate that humid-heat reduces aortic stiffness, without significantly affecting LV afterload (MBP, AIx, aortic systolic BP or PP). Despite the lack of comparative change in AIx in our study, it is noteworthy that individually, heat and humidity had inverse effects on AIx and aortic PP; heat caused increases, and humidity caused decreases in this group of healthy adults (Tables 5.3 and 5.4, respectively).

The exact mechanisms of how high humidity affects haemodynamic function are unknown at present. It is possible that the present results are due to an increased heat load which accompanies increasing humidity at any temperature (Australian Bureau of Meteorology, 2012). Further, in previous studies high humidity at room temperature were found to improve thermoregulation (Freeman and Lengyel, 1938) and improve respiratory function (Turner et al., 1992) compared to the same temperatures at low humidity, and these effects were possibly due to the increased water vapour content of the air preventing drying of mucous membranes (Sunwoo et al., 2006), and reducing heat loss via evaporation. This provides further evidence that short-term exposure to humid conditions at a given air temperature may be no more physiologically stressful than exposure to dry conditions at the same air temperature, as others have inferred (Moran et al., 1996, Wright et al., 2012, Wenzel, 1978, Sunwoo et al., 2006).

Environmental exposure to heat and humidity are important factors in human health and disease (Basu, 2009). The current study has provided new data on the effects of heat and humidity on central haemodynamic function that would be overlooked if measuring traditional brachial BPs alone. The results are consistent with findings from previous work which suggests that short-term heat therapy improves haemodynamic function (Imamura et al., 2001, Blum and Blum, 2007).

In conclusion, this study has shown for the first time that in the absence of any significant change in traditional brachial systolic or MBP, high humidity reduces aortic stiffness in hot conditions, while heat reduces brachial artery stiffness irrespective of humidity in resting, healthy adults. Humidity at room temperature, on the other hand, tends to reduce measures of central haemodynamic afterload (aortic systolic BP and PP), as well as P_{res}, all without affecting AIx. These responses are potentially due to

peripheral vasodilation which up-regulates NO release and is in turn triggered by increased blood flow and shear stress, and also possibly by improved hydration of skin, mucous, and respiratory membranes caused by the effects of high humidity.

CHAPTER 6 –T2DM GROUP, MANUSCRIPT 3

WHOLE-BODY MILD-COOLING IN INDIVIDUALS WITH TYPE 2 DIABETES MELLITUS: IMPACT ON ARTERIAL STIFFNESS AND CENTRAL HAEMODYNAMICS

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An edited version of this chapter will soon be submitted for publication as an original research investigation. The methods section has been reduced to avoid duplication with Chapter 3, General Methods.

ABSTRACT

People with type 2 diabetes mellitus (T2DM) are prone to autonomic and vascular dysfunction which impacts blood flow and thermoregulation. However, little is known about how sudden cold exposure affects central haemodynamics and arterial stiffness in people with T2DM. This study aimed to determine the effect of whole-body mild-cold exposure (12°C) compared to a control condition (21°C) on aortic and brachial pulse wave velocity (PWV), aortic systolic blood pressure (BP) and mean BP (MBP) in a group of adults (n = 14) with T2DM (8 men, age 63 ± 7 years; mean ± SD). Participants attended two randomised conditions on separate days. Supine resting measures were taken at baseline (ambient temperature) and after 5, 10, 30, and 60 minutes exposure to each condition in a climate chamber. There was a greater increase in aortic PWV in mild-cold compared to control (+0.5 $m.s^{-1}$; 95% CI -0.2 to 1.2; $P = 0.03$) but brachial PWV (+0.3 $m.s^{-1}$; 95% CI -0.3 to 0.9; $P = 0.27$) was unaffected. While both aortic systolic BP and MBP increased within-condition in mild-cold, these were not significantly different compared to control (aortic systolic BP, +2 mm Hg; 95% CI -4.2 to 8.8; $P = 0.80$; MBP, +2 mm Hg; 95% CI -2.8 to 6.2; $P = 0.46$). In adults with T2DM mild-cold exposure increases aortic stiffness and elicits a modest pressor response, when compared to ambient conditions of 21°C, which may be associated with impaired autonomic and vasomotor function that is common in people with T2DM. These findings have implications for increased CV risk and tendency to hypothermia in people with T2DM during cold exposures.

INTRODUCTION

Sudden cold exposure causes peripheral and cutaneous vasoconstriction that is sympathetically-mediated and often precedes any reduction in core temperature (Stocks et al., 2004). Thus, peripheral vasoconstriction can be substantial, even in mild-cold conditions (Johnson and Kellogg Jr, 2010, Stocks et al., 2004). Peripheral vasoconstriction increases brachial blood pressures (BPs) (Stocks et al., 2004, Kingma et al., 2011). Brachial BPs are also observed to increase for the duration of winter (Alperovitch et al., 2009, Charach et al., 2004, Halonen et al., 2011), with an associated increase in cardiovascular (CV) morbidity and mortality (Bhaskaran et al., 2010) that is particularly prevalent in the elderly, and those with chronic disease (Semenza et al., 1999, Makinen and Hassi, 2009).

Type 2 diabetes mellitus (T2DM) is a chronic disease that, over time, causes autonomic and vascular dysfunction (Vinik et al., 2003) which impairs thermoregulation and thermal-perception, and leaves T2DM patients more vulnerable to heat and cold-related morbidity and mortality (Kenny et al., 2010, Chao et al., 2007, Scott et al., 1988). Aortic pulse wave velocity (PWV) and augmentation index (AIx) are independent predictors of CV risk and mortality (Vlachopoulos et al., 2010a, Vlachopoulos et al., 2010b) yet no studies to date have investigated the effect of whole-body cold exposure on central haemodynamic measures including aortic PWV and AIx in people with T2DM. However, previously, one study investigated whole-body cooling effects on central haemodynamics using a water-perfused suit at 15-18°C, and compared responses of an older healthy group of 65±2 years to a younger healthy group of 25±1 years (Hess et al., 2009). These authors reported similar magnitude of increase in aortic AIx between the younger and older age group, but increased aortic PWV only in the older group (Hess et

al., 2009). Given that T2DM has similar, but accelerated effects on the arteries as normal ageing (Stansberry et al., 1997), it is feasible that individuals with T2DM might have greater responses in AIx and aortic PWV to mild-cold exposure than similar-aged, healthy individuals.

Aortic PWV is typically higher at rest in people with T2DM, compared to healthy, similar-aged individuals, which is thought to be due to loss of vasomotor tone and elasticity due to endothelial dysfunction, which is in turn caused by the effects of chronic hyperglycaemia (Stehouwer et al., 2008). The elastic aorta is a reservoir and a buffer that stores blood in systole and expels it during diastole, effectively smoothing an intermittent flow from the LV into a more continuous flow in the smaller arterioles before becoming a steady flow in the microcirculation (Westerhof et al., 2009). In healthy adults, whole-body mild-cold increased aortic reservoir pressure (P_{res}) without affecting excess pressure (P_{ex}) compared to a control condition (Chapter 4). Experimental research into reservoir function is in its infancy, however, and to date no studies have investigated aortic reservoir function in response to environmental temperature changes in people with T2DM.

It is indisputable that acute environmental cold exposure has a negative influence on CV health, particularly in individuals with chronic disease. Given that considerable peripheral vasoconstriction is present during mild-cold conditions, and people with T2DM are more prone to abnormal vasomotor and haemodynamic responses to cold stress, the hypothesis that 60 minutes whole-body, mild-cold exposure would increase markers of CV risk and LV load was tested in a group of individuals with T2DM. To this end, the effects of a mild-cold condition (12°C) were compared to a control condition (21°C) on measures of regional arterial stiffness (aortic and brachial PWV) and measures

of central haemodynamics including aortic AIx, aortic BPs, and MBP, and on measures of aortic reservoir function, including P_{res} and P_{ex} in a group of people with T2DM.

MATERIALS AND METHODS

Details of materials and methods for physiological data collection and statistical analysis procedures for this study are in Chapter 3, General Methods.

RESULTS

Demographics

Seventeen adults volunteered for the study and met the inclusion criteria. Three volunteers withdrew prior to commencement of data collection for personal reasons. Therefore, 14 participants (8 men) completed both experimental sessions in random order. Baseline means were: age 62.5 ± 6.9 years; brachial systolic BP, 136 ± 15 mm Hg; brachial diastolic BP, 75 ± 7 mm Hg; HbA_{1C}, 6.3 ± 0.8 %; fasting plasma glucose, 7.2 ± 1.5 $mmol.L^{-1}$; and fasting serum total cholesterol, 3.8 ± 0.5 $mmol.L^{-1}$. Average duration of T2DM was 6.3 ± 4.3 years. Of the 14 participants, 8 completed the control condition first (4 men, 4 women), and 6 completed the mild-cold condition first (2 men, 4 women). After a washout of approximately 7-14 days between trials, participants returned to complete their remaining test session.

One participant was a regular smoker and 11 of the 14 participants took regular prescription medications. Of the 11 who took medications, 73% were on one or more medication per day for hypertension and glycaemic control, and 82% were on one or more medications for hyperlipidaemia. Medications for hypertension used in the group included angiotensin converting enzyme inhibitors (3 individuals), calcium channel blockers (2 individuals), angiotensin II receptor blockers (2 individuals), direct vessel

dilators (2 individuals), beta blocker (1 individual), and a diuretic (1 individual). Other medications included aspirin (55%), medications for gastrointestinal disorders (36%) and antidepressants (27%). Medications were not taken on the morning of a test session, and no changes in medication dose or prescription were made during the study period. All participants complied with pre-test instructions. Mean ambient laboratory environmental temperature was 22.5 ± 1.4 °C. Climate chamber mean temperature for control condition was 21.1 ± 0.9 °C, and 12.4 ± 0.5 °C for the mild-cold condition. To avoid shivering in the mild-cold condition, a blanket was requested by 7 of the 14 participants between ~5 - 30 minutes of exposure. For those 7 participants, the blanket was used ~3 times per session for ~3 - 4 minutes per time. 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 (34.8±0.7 to 33.7±1.2 °C; $P < 0.001$), but there was no significant decrease from baseline in control condition (35.2±0.6 to 34.9±0.6 °C; $P = 0.22$). Between conditions the change in core temperature was significantly lower in mild-cold compared to change in control (-0.8 °C; 95% CI -1.3 to -0.2; $P < 0.001$).

Skin temperature. Skin temperature was reduced from baseline in mild-cold (30.1±1.4 to 28.7±1.8 °C; $P < 0.001$) and control (30.8±0.9 to 30.2±1.1°C; $P = 0.05$) but between conditions there was no significant difference in change in skin temperature compared to change in control (-0.7 °C; 95% CI -1.6 to 0.2; $P = 0.41$).

Perceived thermal comfort. There was a decline in self-reported thermal comfort from baseline in mild-cold, from feeling "comfortable" to "cool" (0.1±0.7 to -1.3±0.9 arbitrary units; $P < 0.001$) and a smaller decrease in the control condition, from

"comfortable" to just below "neutral" thermal comfort (0.2±0.4to -0.3±0.9 arbitrary units; *P* = 0.03). Between conditions, thermal comfort was reduced in mild-cold condition compared to change in control (-0.9 arbitrary units; 95% CI -1.44 to -0.29; *P* = 0.003).

Regional arterial stiffness responses

Aortic pulse wave velocity. There was an increase in aortic PWV from baseline in mild-cold (9.7±2.4 to 10.3±2.6 $m.s^{-1}$; *P* = 0.01) with no significant change in control (10.3±2.7 to 9.2±1.9 $m.s^{-1}$; *P* = 0.69; Figure 6.1, A). Between conditions, the change in aortic PWV was significantly greater in mild-cold compared to change in control (+0.5 $m.s^{-1}$; 95% CI -0.2 to 1.2; *P* = 0.03; Figure 6.1, A).

Brachial pulse wave velocity. There was no significant increase in brachial PWV from baseline in mild-cold (8.9±0.9 to 9.3±0.9 $m.s^{-1}$; *P* = 0.10, Figure 6.1, B) or in control (8.5±0.9 to 9.2±1.9 $m.s^{-1}$; *P* = 0.95), nor was there any significant difference in change between conditions (+0.3 $m.s^{-1}$; 95% CI -0.3 to 0.9; *P* = 0.27; Figure 6.1, B).

Haemodynamic Responses

Blood pressures. Brachial systolic BP increased from baseline in mild-cold (138±15 to 143±17 mm Hg; *P* = 0.02) while there was no significant change in control condition (133±15 to 134±14 mm Hg; *P* = 0.55; Figure 6.2, A). Between conditions there was no significant difference in change in brachial systolic (+4mm Hg; *P* = 0.24) in mild-cold compared to change in control (Figure 6.2, A).

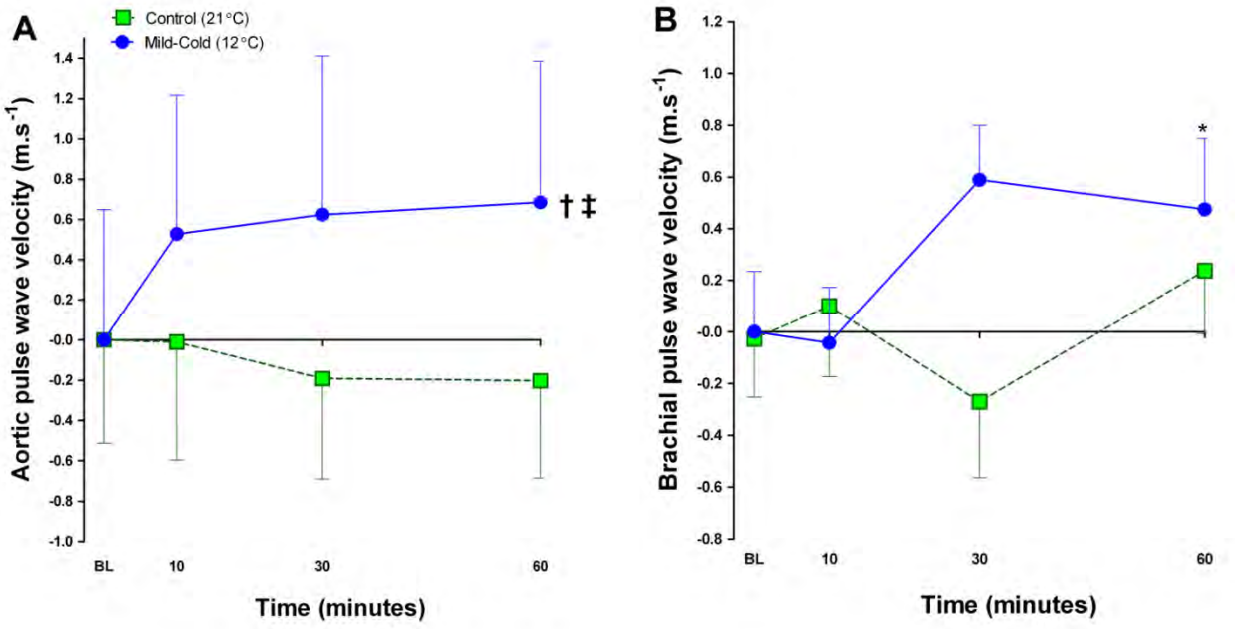

Figure 6.1. A) Aortic pulse wave velocity, and B) brachial pulse wave velocity in mild-cold (12°C) and control (21°C) in individuals with T2DM.

Data presented as mean ± SEM; n =14; baseline data (BL / -10 minutes at ambient laboratory temperature) was obtained ~10 minutes before entry to climate chamber; *significantly different from control at specific time point; †significant change within condition (i.e. between baseline and average of data during 60 minutes in test condition), ‡ significant change between conditions.

Aortic systolic BP increased from baseline in mild-cold (130±16 to 135±18 mm Hg; *P* = 0.04), while there was no significant increase in control (123±15 to 125±14 mm Hg; *P* = 0.31; Figure 6.2, C). Between conditions there was no significant difference in change in aortic systolic BP (+2 mm Hg; *P* = 0.80; Figure 6.2, C). Additionally, there was no significant change in brachial diastolic (Figure 6.2, B) or aortic diastolic pressure (Table 6.1) from baseline, or between conditions.

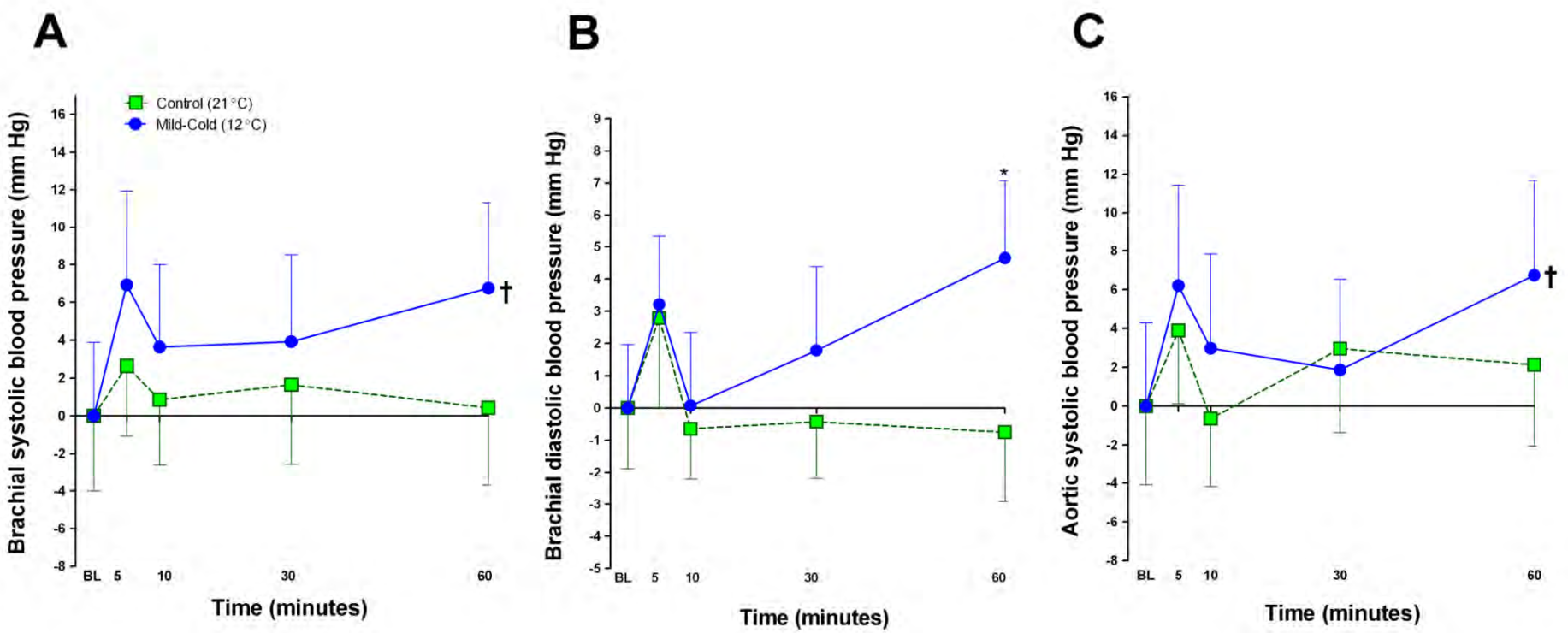

Figure 6.2. A) Brachial systolic and B) brachial diastolic, and C) aortic systolic blood pressures in mild-cold (12°C) and control (21°C) in individuals with T2DM.

Data presented as mean ± SE; *n* =14; baseline data (BL / -10; ambient laboratory temperature) was obtained ~10 minutes before entry to climate chamber; * significantly different from control at specific time point; † significant change within condition.

Augmented pressure. There was no significant increase in AP from baseline in mild-cold (*P* =0.52) while there was a trend towards an increase in control (*P* = 0.07). There was no significant difference in change between conditions (1 mm Hg; *P* = 0.42, Table 6.1).

Augmentation index. There was no significant change in AIx from baseline in mild-cold (33.5±11.7 to 32.5±10%; *P* =0.45) but AIx increased in control (27.6±9.2 to 30.4±9.4%; *P* = 0.02; Figure 6.3, A). Between conditions, AIx was significantly lower in mild-cold compared to change in control (-3.8%; 95% CI -7.3 to -0.3; *P* = 0.03; Figure 6.3, A).

Aortic reservoir components. P_{res} increased from baseline in mild-cold (112±11 to 116±13mm Hg; *P* = 0.05) with no significant increase in control (106±13 to 107±11 mm Hg; *P* = 0.46; Figure 6.3, B), but between conditions, there was no significant difference in change in P_{res} in mild-cold compared to change in control (+2.5 mm Hg; 95% CI -3.3 to 8.3; *P* = 0.40; Figure 6.3, B). There was no significant change in P_{ex} from baseline in mild-cold (45±10 to 45±12 mm Hg; *P* = 0.83), or control (42.±11 to 44±11 mm Hg; *P* = 0.42; Figure 6.3, C), and there was no significant difference in change between conditions (-1.1 mm Hg; 95% CI -6.2 to 4.0; *P* = 0.67; Figure 6.3, C). There was a trend towards a decrease in time to P_{ex} from baseline in mild-cold (*P* = 0.07, Table 6.1) with no significant change in control (*P* = 0.61, Table 6.1), and there was no difference in change between conditions (-5.7 ms; *P* = 0.33, Table 6.1).

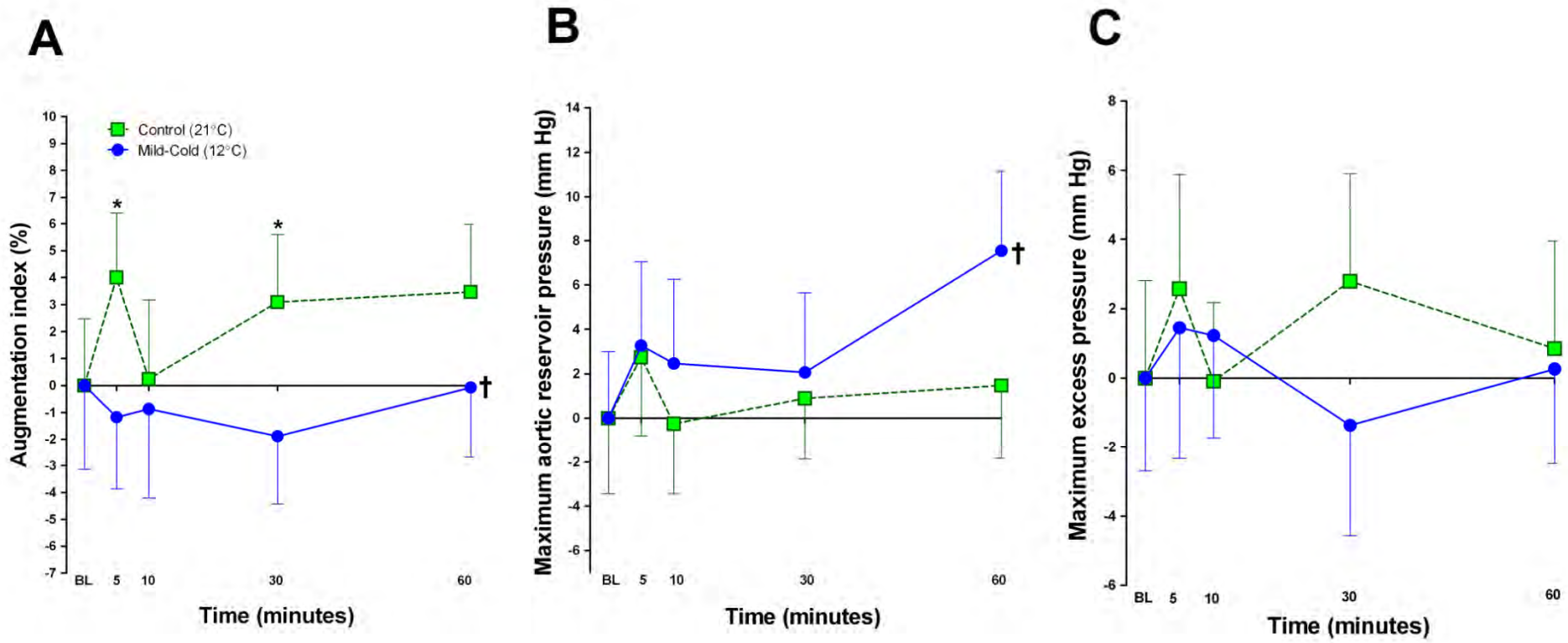

Figure 6.3. A) Augmentation index, B) maximum reservoir pressure and C) maximum excess pressure in mild-cold (12°C) and control (21°C) in individuals with T2DM.

Data presented as mean ± SE; *n* =14; baseline data (BL / -10 minutes at ambient laboratory temperature) was obtained ~10 minutes before entry to climate chamber; * significantly different from control at specific time point; † significant change within condition.

Table 6.1. Change from baseline, and comparison of selected haemodynamic measures between mild-cold (12°C) and control (21° C) conditions in individuals with T2DM.

Variable / Condition	Time point (minutes)					Average (10-60 minutes) *	*P* (within condition) †	Δ mild-cold Vs. Δ control ‡	*P* (between conditions) §
	Baseline (-10)	5	10	30	60				
Aortic diastolic blood pressure (mm Hg)									
Control	74±7	77±10	73±6	74±7	73±8	74±8	0.92	2.3 (-2.12 to 6.65)	0.31
Mild-Cold	78±8 [a]	81±8	78±9	79±10	82±9 [b]	80±9	0.12		
Brachial pulse pressure (mm Hg)									
Control	59±13	59±14	61±10	62±12	61±13	61±12	0.64	1.7 (-5.25 to 8.71)	0.63
Mild-Cold	61±12	65±16	65±13	63±16	63±13	64±14	0.25		
Aortic pulse pressure (mm Hg)									
Control	49±13	49±13	49±11	52±13	51±13	51±12	0.34	0.4 (-6.40 to 6.49)	0.98
Mild-Cold	52±13	55±17	55±15	53±17	55±14	54±15	0.33		
Mean blood pressure (mm Hg)									
Control	94±10	98±11	93±8	94±10	94±10	95±10	0.45	1.7 (-2.81 to 6.15)	0.46
Mild-Cold	100±10	104±11	101±12	101±11	104±12	102±11	0.07		
Augmented pressure (mm Hg)									
Control	14±7	16±6	14±6	17±7	17±7	16±7	0.07	-1.3 (-4.44 to 1.88)	0.42
Mild-Cold	18±9 [a]	19±9	19±10	17±9	20±9	19±9	0.52		
Rate-pressure product (mm Hg.beats.min^{-1})									
Control	7972±1355	-	7869±891	7697±1093	7467±1025	7682±992	0.22	195 (-217 to 608)	0.35
Mild-Cold	8171±1439	-	8167±1369	8256±1576	8133±1405	8185±1418	0.92		

Table 6.1. Continued…

Variable / Condition	Time point (minutes)					Average (10-60 minutes) *	P (within condition) †	Δ mild-cold Vs. Δ control ‡	P (between conditions) §
	Baseline (-10)	5	10	30	60				
Heart rate (beats.min^{-1})									
Control	60.1±8.1	--	59.1±6.9	58.4±7.1	56.9±6.7	58.2±6.8	**0.05**	-0.41 (-2.61 to 1.79)	0.71
Mild-Cold	59.3±9.3	--	57.7±8.3	58.1±8.7	56.2±7.8	57.3±8.1	**0.01**		
Time to maximum excess pressure (ms)									
Control	132±1.93	133±33.7	131±12.1	130±13.6	127±11.8	130±19.4	0.61	-5.7 (-17.3 to 5.89)	0.33
Mild-Cold	131±22.8	120±13.1	124±17.4	13.0±15.8	120±19.4	123±16.5	0.06		
Heart rate (beats.min^{-1})									
Control	60.1±8.04	-	59.1±6.93	58.4±7.10	56.9±6.67	58.2±6.79	**0.05**	-0.4 (-2.61 to 1.79)	0.71
Mild-Cold	59.3±9.32	-	57.7±8.28	58.1±8.71	56.2±7.81	57.3±8.11	**0.01**		

Note: *n* = 14; Data presented as mean ± standard deviation; **Δ**, mean change; ***,** Data collected at 5, 10, 30 and 60 minutes in climate chamber were averaged for comparison against baseline data (baseline was at -10 minutes in ambient laboratory conditions); **†**, *P* value is **Δ** within condition (i.e. from baseline to the average of 10, 30, and 60 minute data); **‡**, **Δ** mild-cold compared to **Δ** control condition (95% confidence interval); §**, *P*** value between conditions (i.e. comparison of **Δ** mild-cold and **Δ** control condition); **, data violated the assumptions of linear regression, *P* value taken from ordinal logistic regression; [a], significantly different from control baseline; [b], significantly different from control at specific time point; Results are adjusted for order and period effects.

Pulse pressure. There was no significant change from baseline in aortic PP in mild-cold (P = 0.33) or control (P = 0.92) nor any significant difference in change between conditions (0.5 mm Hg; P = 0.31; Table 6.1). Similarly, changes in brachial PP in mild-cold (P = 0.25) and control (P = 0.64) were not significantly different from baseline, nor was there any significant difference in change between conditions (+1.7 mm Hg; P = 0.63; Table 6.1).

Mean blood pressure. There was a trend towards increased MBP from baseline in mild-cold (P = 0.07) with no significant change in control (P = 0.45; Table 6.1) and between conditions there was no significant difference in change (+2 mm Hg; P = 0.46; Table 6.1).

Rate pressure product. There were no significant changes in RPP from baseline in mild-cold (P = 0.92) or control condition (P = 0.22), and there was no significant difference in change between conditions (+195 mm Hg.min-1; P = 0.35; Table 6.1).

Cardiovascular autonomic neuropathy tests

Eight of the 14 participants tested positive to one CAN test and three of these participants had abnormal results to more than one CAN test. The mean Valsalva R-R ratio for the group was 1.67±0.24 and considered normal, however, the range was 1.19 to 2.07 with one abnormal response (i.e. < 1.21). The mean 30:15 tilt ratio was considered normal at 1.21±0.18 but the range was 1.01 to 1.57, and 5 of the 14 participants had an abnormal response (i.e. < 1.10). The mean brachial systolic BP response to tilting was normal with a fall of -16±10 (range -2 to -32) mm Hg, however, six of the 14 participants had an abnormal systolic BP response of > 20 mm Hg. The

mean brachial diastolic pressure fell by -2±7 (range -18 to +9) mm Hg in 3 minutes of tilting.

In a sub-analysis of data which accounted for the results of the three CAN tests on the main variables, it was found that aortic PWV was the only variable influenced by any of the CAN results during mild-cold exposure. For every 1 SD decrease in Valsalva R-R ratio, aortic PWV was significantly increased by 0.25m.s^{-1} (P = 0.001). Moreover, the difference in change in aortic PWV between conditions was smaller after adjustment for CAN results and became non-significant in all three CAN tests. Significance went from P = 0.03 in unadjusted results, to P = 0.13 after adjustment for Valsalva R-R ratio, and to P = 0.23 after adjustment for 30:15 tilt ratio, and to P = 0.18 after adjustment for difference in change in systolic BP response to tilting. In addition, reduction of AIx in mild-cold was no longer significant after adjustment for CAN results in 30:15 tilt ratio and systolic BP response to tilting tests, each went from P = 0.03 to P = 0.08. Results for brachial systolic BP were also larger after adjustment for CAN results, and the difference in change in brachial systolic BP between conditions was no longer significant after adjustment for the systolic BP response to tilting test (from P = 0.03 to P = 0.08).

DISCUSSION

To date, no experimental whole-body cold exposure studies have included humans with T2DM. The novel findings of this study are that in a group of adults with T2DM, whole-body exposure to mild-cold (12° C) for 60 minutes increased aortic stiffness without increasing brachial artery stiffness, and produced a modest pressor

response in MBP, brachial and aortic systolic BP, compared to a control condition (21° C).

Typically, increased aortic PWV is influenced by factors such as ageing, increased heart rate, MBP, and aortic PP (Van Bortel et al., 2002, Laurent et al., 2006). However, as heart rate was reduced and aortic PP did not change it is possible the increase in aortic PWV is associated with the small increase in MBP, aortic systolic BP, and P_{res} observed in mild-cold exposure in this study. The T2DM group in the current study had reasonable glycaemic control, as demonstrated by their mean fasting plasma glucose and HbA_{1c} results (World Health Organization and the International Diabetes Federation, 2006), but eight individuals had positive results for CAN in a single test and three had positive results to two CAN tests, indicating a moderate degree of CAN in the study population. Moreover, the mean baseline brachial BPs of the group could be classified as pre-hypertensive (U.S. Department of Health and Human Services, 2004). In a sub-analysis of results which accounted for CAN results, higher aortic PWV was found to be positively associated with greater degree of CAN, which has been observed previously (Kim et al., 2011). Thus, it appears that autonomic dysfunction may be a confounding factor in the current study's results. The presence of three participants with high likelihood of CAN in the T2DM group has potentially increased aortic stiffness, and decreased the pressor responses (i.e. brachial systolic BP) to mild-cold. Haemodynamic impairments including a small pressor response during cold exposure in T2DM is consistent with findings in rats and humans (Kilgour and Williams, 1998, Stansberry et al., 1997). Despite this, these three CAN responders were included in the final analysis as it was deemed invalid to separately analyse data from such a small number of participants.

In comparison to the increased aortic PWV in T2DM individuals (+0.5 $m.s^{-1}$) observed in the current study, healthy individuals exposed to conditions identical to the present study (12˚C in a climate chamber for 60 minutes; Chapter 4) showed no change in aortic (+0.04 $m.s^{-1}$, $P = 0.87$) PWV compared to control. However, MBP increased significantly (+2mm Hg, $P = 0.03$) in the healthy group (Chapter 4). Due to the toxic effects of long term hyperglycaemia (Stehouwer et al., 2008, Bansal et al., 2006), individuals with T2DM are predisposed to autonomic dysfunction (Vinik et al., 2003) which leads to altered cold and pain tolerance due to nerve degradation (Vinik et al., 2003, Ziegler et al., 1993), impaired skin blood flow (Stansberry et al., 1997), and impaired vasomotor tone (Xu et al., 2003). Moreover, the effects of T2DM on arterial stiffness have been found to be similar to an advanced ageing effect, such that a younger person with T2DM may have arterial stiffness equivalent to a much older, healthy person (Stansberry et al., 1997). The reason for the differences between groups is likely due to the higher degree of vascular dysfunction in a T2DM group, compared to a healthy group (Stansberry et al., 1997).

In the current study, exposure to mild-cold in T2DM individuals caused only a modest pressor response, i.e. small increases in MBP, aortic systolic BP and P_{res}, but this may be a normal response to cold exposure for people with T2DM. However, as no other studies have yet investigated the effects of cold exposure on central haemodynamics in a human T2DM population, it is difficult to speculate on the mechanisms responsible for these results. In an animal model, streptozotocin-induced T2DM rats cooled to 10°C for two hours did not exhibit any pressor response, compared to increased MBP in similarly-cooled healthy control rats, despite similar baseline MBPs between the two groups (Kilgour and Williams, 1998). Moreover the T2DM rats in that study had a faster rate of reduction in core temperature during cooling, compared to control rats (Kilgour

and Williams, 1998). However, rats have a larger body surface area: mass ratio and different thermoregulatory abilities to humans (Gordon, 2007), and for this reason, results may not be strictly comparable. Despite this, it is possible that the modest pressor response and lack of change in AIx observed in the present study are not abnormal responses to cold exposure in a T2DM population and may be explained by the presence of peripheral small-fibre sensory nerve degradation in this participant group. Indeed, diabetic neuropathy has been shown to impair thermoregulation, (Ziegler et al., 1993, Semenza et al., 1999, Scott et al., 1988), reduce cold discrimination (Chao et al., 2007), prevent normal sympathetically-mediated vasoconstrictive responses via nerve blockages that occur during extremes of temperature (Straver et al., 2011, Jaryal et al., 2009), and reduce heart rate variability (Kilgour and Williams, 1998), all of which reduce reflexive control of haemodynamics in response to external stressors such environmental cold (Ziegler et al., 1993, Chao et al., 2007, Petrofsky et al., 2005a). A diminished pressor response to mild-cold exposure, such as observed in the current study, has implications for increased vulnerability to hypothermia in people with T2DM (Applebaum and Kim, 2002, Neil et al., 1986), as the core temperature continues to fall in the absence of peripheral blood flow diversion to warm the body's core (Kilgour and Williams, 1998).

These are currently no data available on aortic reservoir components during cold exposure in people with T2DM. In the current study, P_{res}; the pressure required to overcome arterial resistance, increased significantly from baseline which suggests increased cardiac work requirements during mild-cold exposure. However this was not supported by RPP, a traditional measure of myocardial workload, which did not increase in the current study (Table 6.1). It is possible RPP did not increase due to the significantly decreased heart rates in this study. Moreover, P_{ex} did not increase, which,

coupled with no change in "time to the reflected wave" from baseline in mild-cold (135±17.6 to 138±10 ms, $P = 0.33$), suggests that wave reflection was not increased by cold exposure in this study population, which is contrary to the suggestions of previous cooling studies in healthy people (Edwards et al., 2006, Edwards et al., 2008, Casey et al., 2008). Despite this, the reason that wave reflections may not have been increased in the systolic period during mild-cold exposure in this study may be explained by results from a recent study by Wang *et al.,* (2012) in anaesthetised dogs. Wang *et al*., (2012) found that while P_{res} was significantly increased during infusion of vasoconstrictor medications, wave reflections, i.e. backwards compression waves, were not increased in the systolic period as is typically reported, but in the diastolic period, which does not augment systolic pressure. Wang *et al.,* (2012) suggested that the increase in aortic systolic pressure in their study was due to increased P_{res}, and not due to wave reflections, which, by extension could also be the case in the present study.

The results of the current study may add evidence to the current explanations for the higher incidence of cold-related CV morbidity and mortality (Danet et al., 1999), which is particularly prevalent in people with CV risk factors (O'Neill and Ebi, 2009). People with T2DM have higher incidence of CHD (Hayden and Tyagi, 2003) and atherosclerotic coronary arteries are known to constrict *in vivo* during cold stimulus, rather than dilate as healthy coronary arteries do when exposed to cold (Nabel et al., 1988). Thus, taken together, the increased potential for cold-induced myocardial ischemia in people with T2DM, combined with increased aortic stiffness, i.e. increased resistance to LV ejection (Laurent et al., 2006), and higher aortic pressures could contribute to the increased CV risk in individuals with T2DM during mild-cold exposure.

In summary, this study shows for the first time, that in a group of individuals with reasonably well-controlled T2DM and moderate degree of CAN, exposure to a mild-cold climate for 60 minutes increases stiffness in the large elastic aorta but not the smaller muscular arteries as evidenced by an increase in aortic PWV, but no significant change in brachial PWV. Moreover, mild-cold exposure elicited only a modest pressor response in brachial and aortic systolic BPs, and P_{res} in this T2DM population. These responses may be associated with impaired autonomic, endothelial, and vasomotor function, and may have implications for increased CV risk and tendency to hypothermia during even a mild-cold stimulus in people with T2DM.

CHAPTER 7 - T2DM GROUP, MANUSCRIPT 4

HUMIDITY AND HEAT: EFFECTS ON CENTRAL HAEMODYNAMICS IN INDIVIDUALS WITH TYPE 2 DIABETES MELLITUS

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An edited version of this chapter will shortly be submitted for publication as an original research investigation. The methods section has been reduced to avoid duplication with Chapter 3, General Methods.

ABSTRACT

Dry-heat therapy via sauna has been consistently found to improve haemodynamic function in people with heart failure, who have similar impairments in microvascular function as people with type 2 diabetes (T2DM). However little is known about the effects of heat and / or humidity on central haemodynamics in people with T2DM. This study aimed to determine the effects of short-term whole-body heat and humidity (combined and separately to locate the source of the effect) on haemodynamic measures including aortic pulse wave velocity (PWV), augmentation index (AIx) and mean blood pressure (MBP) in adults with T2DM. On separate days, 14 volunteers (8 men, age 63 ± 7 years; mean ± SD) were randomised into four experimental conditions: 21°C / 40% relative humidity (RH), 21°C / 80% RH, 36°C / 40% RH, and 36°C / 80% RH. Supine, resting measures were taken at baseline (-10 minutes; ambient temperature) and after 5, 10, 30, and 60 minutes exposure to each condition in a climate chamber. Aortic PWV was reduced by heat only when humidity was high (-0.8 $m.s^{-1}$, $P = 0.05$ for 36˚/80% vs. 21˚/80%; and -0.8 $m.s^{-1}$, $P = 0.03$ for 36˚/80% vs. 21˚/40%). AIx was reduced by humid-heat (-4.1%, $P = 0.03$ for 36˚/80% vs. 21˚/40%) but not dry-heat (-0.5%, $P = 0.31$), and AIx was the only measure reduced by humidity at room temperature (21˚/80% vs. 21˚/40%, -3.9%, $P = 0.04$). MBP was reduced similarly in all hot comparisons (-9 mm Hg, $P < 0.001$, 36˚/40% vs. 21˚/40%; -8 mm Hg, $P = 0.005$ for 36˚/80% vs. 21˚/40%; and -7 mm Hg, $P = 0.003$ for 36˚/80% vs. 21˚/80%). High humidity reduces aortic PWV during heat exposure in individuals with T2DM, which is likely associated with reduced AIx and MBP and is potentially due to the increased heat load which promotes vasodilation.

INTRODUCTION

During heat exposure, humans maintain a relatively stable core temperature by adjusting metabolic heat production and dissipating heat via radiation, convection, conduction and evaporation (Sessler, 2009). However, over time, people with type 2 diabetes mellitus (T2DM), experience microvascular degradation and demyelination of nerves by chronic hyperglycaemia and advanced glycation end-products which impairs the noradrenergic response to stressors (Ziegler et al., 1993). The accompanying dysfunction in baroreceptors means that people with T2DM tend to have either exaggerated or diminished vascular responses to external stressors like sudden heat, cold, and changes in posture (Dobretsov et al., 2007, Ziegler et al., 1993). People with T2DM also have reduced expression of, and sensitivity to endogenous vasodilators such as nitric oxide (NO), which leads to impaired reflexive control of thermoregulation via blood redistribution, in response to heat (Wick et al., 2006, Williams et al., 1996). Moreover, as a consequence of vasomotor and nerve conduction dysfunction, people with T2DM are more likely to have reduced ability to perceive heat and cold (Chao et al., 2007) which, together with impaired neural and vascular control of thermoregulation, can leave them highly prone to hyperthermia, illness and death from environmental prolonged heat exposure (Wick et al., 2006, Ziegler et al., 1993, Semenza et al., 1999).

Conversely, repeated short-term exposure to dry-heat via sauna has been reported to improve haemodynamic function via enhanced endothelial function, reduced brachial BPs, decreased cardiac preload and afterload in healthy individuals and patients with cardiovascular disease (CVD), including heart failure (Blum and Blum, 2007, Imamura et al., 2001, Crinnion, 2011, Hannuksela and Ellahham, 2001, Miyata et al., 2008, Kihara et al., 2002). However, while patients with heart failure are also known

to have down-regulated NO-synthase and impaired thermoregulation (Blum and Blum, 2007), it is unknown whether the effects of short-term heat and/or humidity on central haemodynamic function are similar in a T2DM population. To this end, the current study aimed to explore, in resting adults with T2DM, how central haemodynamic measures, including aortic and brachial PWV, aortic, brachial and mean BPs, and aortic AIx, were affected by 60 minutes exposure to different combinations of temperature (21°C and 36°C) and relative humidity (RH) at 40% and 80% in a climate chamber. Based on previous findings that have reported improved haemodynamics after short-term heat exposure in people with CVD (Crinnion, 2011, Blum and Blum, 2007, Miyata et al., 2008), it was hypothesised that whole-body heat exposure at low and high RH would reduce aortic and brachial PWV and measures of left ventricular (LV) afterload (AIx, aortic systolic BP and MBP) in adults with T2DM.

MATERIALS AND METHODS

Details of materials and methods for physiological data collection and statistical analysis procedures for this study are in Chapter 3, General Methods.

RESULTS

Seventeen adults volunteered for the study and met the inclusion criteria. Three volunteers withdrew prior to commencement of data collection for personal reasons. Therefore, 14 participants (8 men) completed all four experimental sessions in random order with a washout period of 7-14 days between each trial. Mean baseline data and demographics are presented in Table 7.1 and raw change in selected haemodynamic variables is presented in Table 7.2.

Table 7.1. T2DM participant demographics and baseline results

	Mean ± SD
Age (years)	62.5 ± 6.9
Duration of T2DM (years)	6.3 ± 4.3
Brachial systolic BP (mm Hg)	136 ± 17
Brachial diastolic BP (mm Hg)	76 ± 9
Aortic systolic BP (mm Hg)	126 ± 17
Aortic PWV ($m.s^{-1}$)	9.4 ± 2.0
Brachial PWV ($m.s^{-1}$)	8.7 ± 0.9
Augmentation index (%)	30.4 ± 10.3
HbA_{1C} (%)	6.3 ± 0.8
Fasting plasma glucose ($mmol.L^{-1}$)	7.2 ± 1.5
Fasting total cholesterol ($mmol.L^{-1}$)	3.8 ± 0.5

One participant was a regular smoker and 11 of the 14 participants took regular prescription medications. Of the 11 who took medications, 73% were on one or more medication per day for hypertension and glycaemic control, and 82% were on one or more medication for hyperlipidaemia, which is likely why the group results for fasting total cholesterol were lower in this T2DM group than the healthy group reported in Chapters 4 and 5. Other regular medications in this T2DM group included aspirin (55%), medications for gastrointestinal disorders (36%) and antidepressants (27%). No medication prescriptions or doses were altered during the study period. All participants complied with pre-test instructions and no climate tests were terminated early due to adverse reactions or discomfort. Mean laboratory temperatures and RH over the data collection period were 22.7 ±1.4 °C and 41.6±9.6 % RH. Mean climate chamber temperature and RH for each condition were: *control* 21.2±0.8°C / 42.4±5.0% RH; *humid* 21.1±0.5°C / 84.6±2.9% RH; *hot-dry* 35.4±0.6°C / 40.6±5.0% RH; *hot-humid* 35.4±0.8°C / 80.5±6.0% RH.

Table 7.2. Absolute change from baseline to 60 minutes in selected haemodynamic variables in individuals with T2DM

	Time point (minutes)						*P* (within condition)‡
Variable / Condition	**Baseline (-10)**	**5**	**10**	**30**	**60**	**Mean Δ 5-60**	
Aortic pulse wave velocity (m.s^{-1})							
Control	9.3 ± 1.9	-	9.3 ± 2.2	9.1 ± 1.9	9.1 ± 1.8	9.2 ± 1.9	0.50
Humid	9.6 ± 2.6	-	9.9 ± 2.9	9.7 ± 2.1	9.5 ± 2.7	9.8 ± 2.5	0.52
Hot-dry	9.2 ± 2.0	-	9.1 ± 2.2	8.9 ± 2.7	8.7 ± 2.3	8.9 ± 2.4	0.25
Hot-humid	9.6 ± 1.7	-	8.7 ± 1.6	9.0 ± 1.6	9.0 ± 2.2	8.9 ± 1.8	**0.01**
Brachial pulse wave velocity (m.s^{-1})							
Control	8.6 ± 1.1	-	7.4 ± 3.2	7.6 ± 2.4	7.6 ± 3.3	8.5 ± 0.9	0.95
Humid	9.01 ± 0.8	-	8.1 ± 2.4	8.2 ± 2.5	8.3 ± 2.5	8.9 ± 0.6	0.34
Hot-dry	8.4 ± 1.1	-	8.2 ± 0.8	8.6 ± 0.8 [a]	8.0 ± 2.5	8.3 ± 0.9	0.49
Hot-humid	8.8 ± 1.1	-	7.8 ± 2.4	7.1 ± 3.1	8.1 ± 2.4	8.5 ± 0.8	0.17
Brachial systolic blood pressure (mm Hg)							
Control	133 ± 15	136 ± 14	134 ± 13	135 ± 16	133 ± 15	134 ± 14	0.58
Humid	137 ± 17	140 ± 17	136 ± 16	138 ± 16	138 ±15	138 ± 16	0.57
Hot-dry	135 ± 18	134 ± 20	125 ± 16 [a]	123 ± 16 [a]	121 ±17 [a]	126 ± 18	**< 0.001**
Hot-humid	137 ± 19	132 ± 17	126 ± 16 [a]	126 ± 15 [a]	125 ±17 [a]	127 ± 16	**< 0.001**
Brachial diastolic blood pressure (mm Hg)							
Control	73 ± 7	76 ± 10	73 ± 6	73 ± 7	73 ± 8	74 ± 8	0.87
Humid	77 ± 8	78 ± 8	76 ± 9	76 ± 10	75 ± 9	77 ± 9	0.96
Hot-dry	78 ± 12	74 ± 9	72 ± 9	71 ± 10 [a]	67 ± 9 [a]	71 ± 9	**< 0.001**
Hot-humid	76 ± 9	75 ± 9	71 ± 7	71 ± 8 [a]	69 ± 8 [a]	72 ± 8	**0.005**
Aortic systolic blood pressure (mm Hg)							
Control	122 ± 15	127 ± 14	122 ± 13	126 ± 16	125 ± 16	125 ± 14	0.28
Humid	127 ± 16	131 ± 17	127 ± 17	129 ± 17	130 ± 16	129 ± 16	0.36
Hot-dry	127 ± 16	126 ± 18	117 ± 15 [a]	115 ± 16 [a]	112 ± 16 [a]	117 ± 17	**< 0.001**
Hot-humid	128 ± 20	125 ± 17	118 ± 17 [a]	117 ± 16 [a]	115 ± 15 [a]	119 ± 16	**< 0.001**
Augmentation index (%)							
Control	27.8 ± 9.2	31.8 ± 8.9	26.0 ± 12.7	31.0 ± 9.3	31.3 ± 9.4	30.6 ± 9.4	**0.02**
Humid	32.4 ± 12.3	31.0 ± 9.6	31.1 ± 10.4	31.7 ± 12.7	32.2 ± 8.3	31.5 ± 10.1	0.51
Hot-dry	30.1 ± 8.1	31.7 ± 8.3	29.2 ± 10.5	30.2 ± 8.9	27.1 ± 8.9 [a]	29.5 ± 9.1	0.72
Hot-humid	30.9 ± 11.7	3.31.5 ± 9.6 [a]	30.9 ± 10.7	29.2 ± 11.7 [a]	27.0 ± 8.7 [a]	29.6 ± 9.4	0.42
Maximum reservoir pressure (mm Hg)							
Control	106 ± 13	109 ± 13	106 ± 11	107 ± 10	107 ± 12	107 ± 11	0.51
Humid	111 ± 12	113 ± 13	111 ± 14	112 ± 14	114 ± 15	112 ± 14	0.75
Hot-dry	112 ± 15	109 ± 19	102 ± 16 [a]	100 ± 15 [a]	97 ± 13 [a]	102 ± 16	**< 0.001**
Hot-humid	110 ± 16	108 ± 14	102 ± 15 [a]	101 ± 16 [a]	99 ± 14 [a]	102 ± 15	**< 0.001**
Maximum excess pressure (mm Hg)							
Control	42 ± 11	45 ± 12	42 ± 8	45 ± 12	43 ± 12	44 ± 11	0.40
Humid	42 ± 11	45 ± 12	43 ± 11	44 ± 9	43 ± 9	44 ± 10	0.26
Hot-dry	38 ± 10	41 ± 12	38 ± 10	36 ± 9	38 ± 11	38 ± 11	0.99
Hot-humid	43 ± 12	41 ± 12	39 ± 8	39 ± 11 [a]	40 ± 11	39 ± 10	**0.03**

Note: *n*=16; data were adjusted for order and period effect and presented as mean ± standard deviation; Δ= mean change; †= average of data collected at 10, 30, and 60 minutes in climate chamber; ‡= *P* value is Δ from baseline (baseline was -10 min in ambient laboratory conditions) to average of 10, 30, and 60 minute data; [a]= significantly different compared to control at specific time point, $P \leq 0.05$.

Effect of increased heat at 40% RH (21/40 vs. 36/40)

Brachial (-11 mm Hg, 95% CI -18 to -3.7, *P* = 0.005, Figure 7.3, A) and aortic (-12 mm Hg, 95% CI -18.5 to -5.2, *P* = 0.001, Figure 7.3, C) systolic BP, brachial diastolic BP (-7 mm Hg, 95% CI -11.6 to -3.3, *P* = 0.001, Figure 7.3, B), MBP (P < 0.001, Table 7.3, A), and P_{res} (-11 mmHg, 95% CI -17.6 to -5.1, *P* = 0.001, Figure 7.4, A) were significantly lower in 36/80 than in 21/40. Heart rate (+4.7 beats.min^{-1}; *P* < 0.001), core temperature (+1.7 °C; *P* < 0.001), skin temperature (+3.6 °C; *P* < 0.001), and perceived thermal sensation (+2.4 arbitrary units; *P* < 0.001) were all significantly higher in 36/40 than 21/40 (Table 7.3, A). There was no significant difference in change in any other measure (Table 7.3, A).

Effect of increased heat at 80% RH (21/80 vs. 36/80)

Aortic PWV (-0.8 m.s^{-1}, 95% CI -1.61 to -0.05, *P* = 0.05; Figure 7.1, A), brachial systolic BP (-11 mm Hg; 95% CI -17.6 to -3.8; *P* = 0.002; Figure 7.3, A), aortic systolic BP (-11 mm Hg; 95% CI -17.9 to -4.6; *P* = 0.001; Figure 7.2, C), brachial diastolic (-4 mm Hg; 95% CI -8.4 to -0.1; *P* = 0.04; Figure 7.2, B), MBP (-7 mm Hg; *P* = 0.003; Table 7.3, B), brachial PP (-7 mm Hg; *P* = 0.03; Table 7.3, B), aortic PP (-8 mm Hg; *P* = 0.02; ; Table 7.3, B), P_{res} (-9 mm Hg; 95% CI -15.2 to -2.7; *P* = 0.005; Figure 7.4, A) and P_{ex} (-6 mm Hg; 95% CI -10.9 to -0.7; *P* = 0.02; Figure 7.4, B) were all significantly lower in 36/80 than in 21/80. Heart rate (+4.6 beats.min^{-1}; *P* < 0.001), core temperature (+1.6 °C; *P* < 0.001), skin temperature (+3.1 °C; *P* < 0.001), and perceived thermal sensation (+1.2 arbitrary units; *P* = 0.01) were all significantly higher in 36/80 compared to 21/80 (Table 7.3, B). There was no significant difference in change in any other measure (Table 7.3, B).

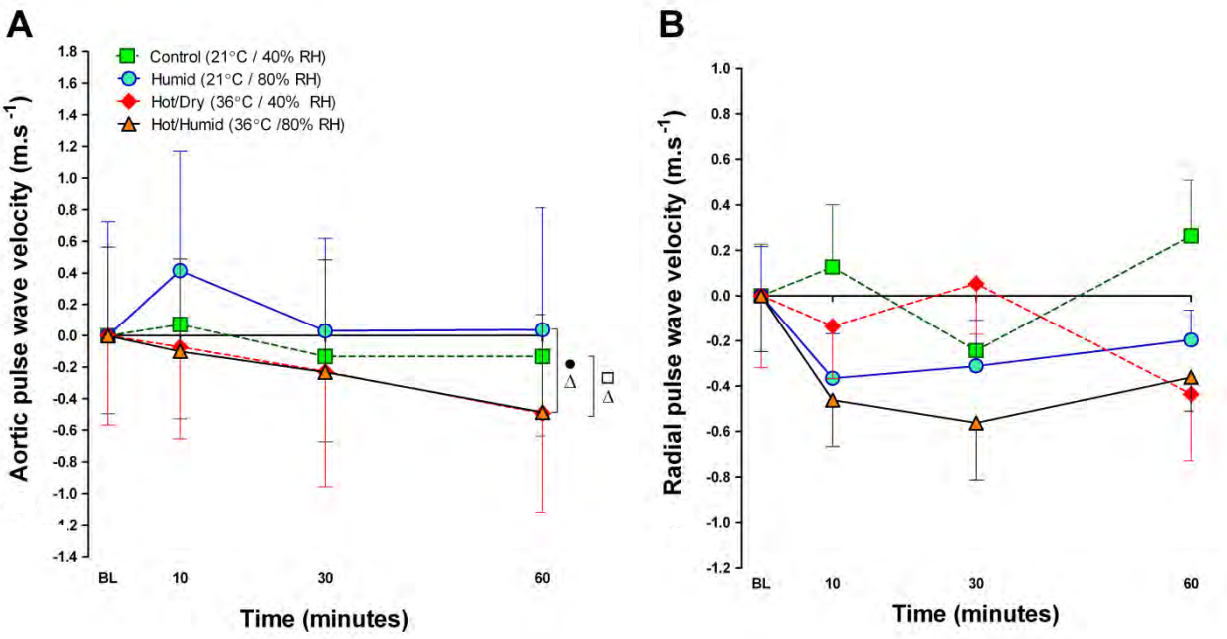

Figure 7.1. Changes in A) aortic pulse wave velocity, and B) brachial pulse wave velocity from baseline in 21°C/40% relative humidity (RH; control), 21°C/80% RH (humid), 36°C/40% RH (hot-dry), and 36°C/80% RH (hot-humid) conditions in individuals with T2DM.

Raw mean data presented as mean ± SEM; n =14. Baseline data (-10) were obtained in ambient laboratory conditions ~10 minutes before entry to climate chamber. Vertical bracket indicates significant difference in change (P ≤0.05) between identified conditions (from baseline to average of 60 minute data).

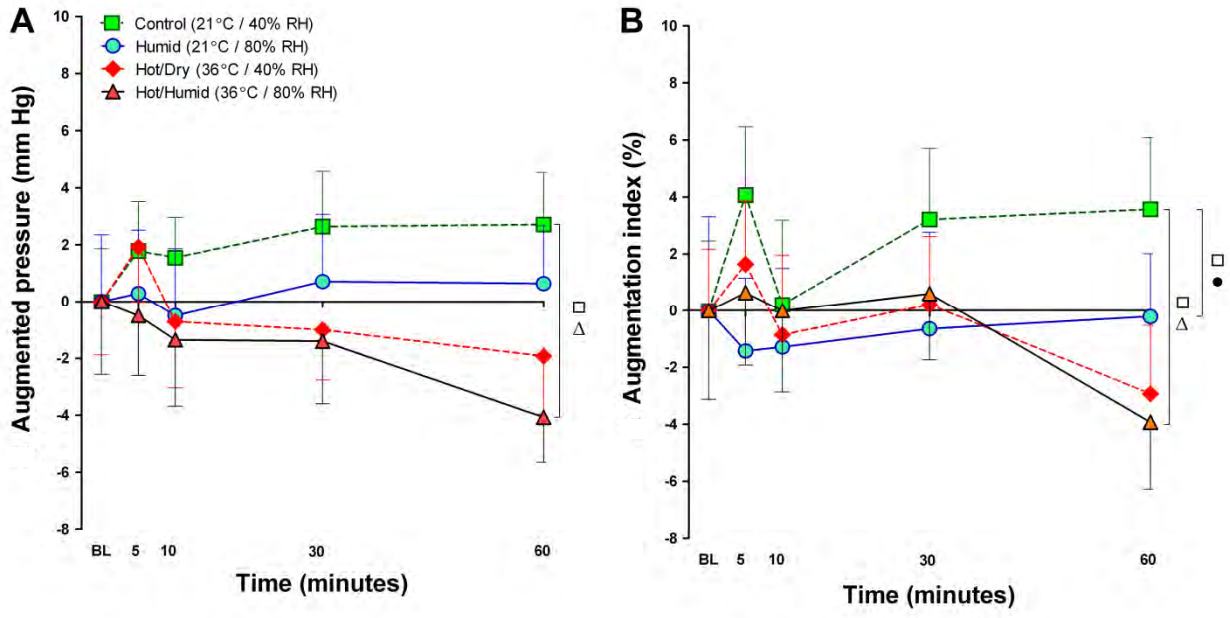

Figure 7.2. Changes in A) Augmented pressure, and B) augmentation index from baseline to 60 minutes in each condition in individuals with T2DM.

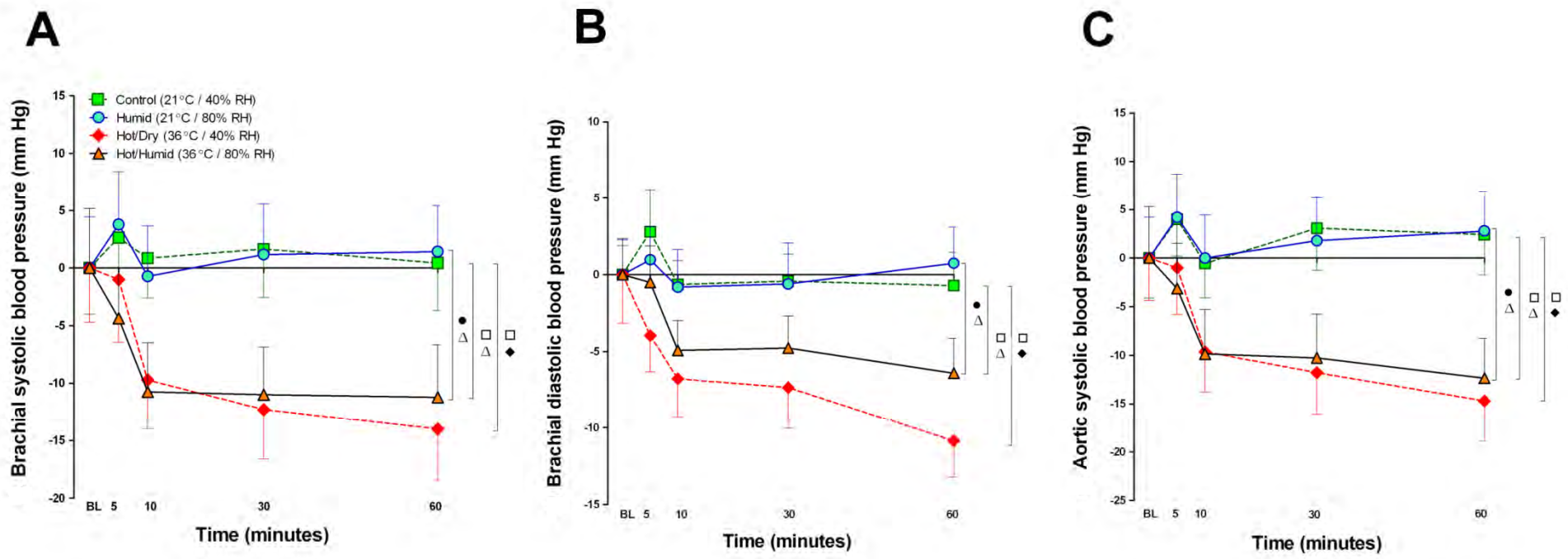

Figure 7.3. Changes in A) brachial systolic, B) brachial diastolic, and C) aortic systolic blood pressures from baseline to 60 minutes in each condition in individuals with T2DM.

n =14. Vertical bracket indicates significant difference in change ($P \leq 0.05$) between identified conditions (from baseline to average of 60 minute data).

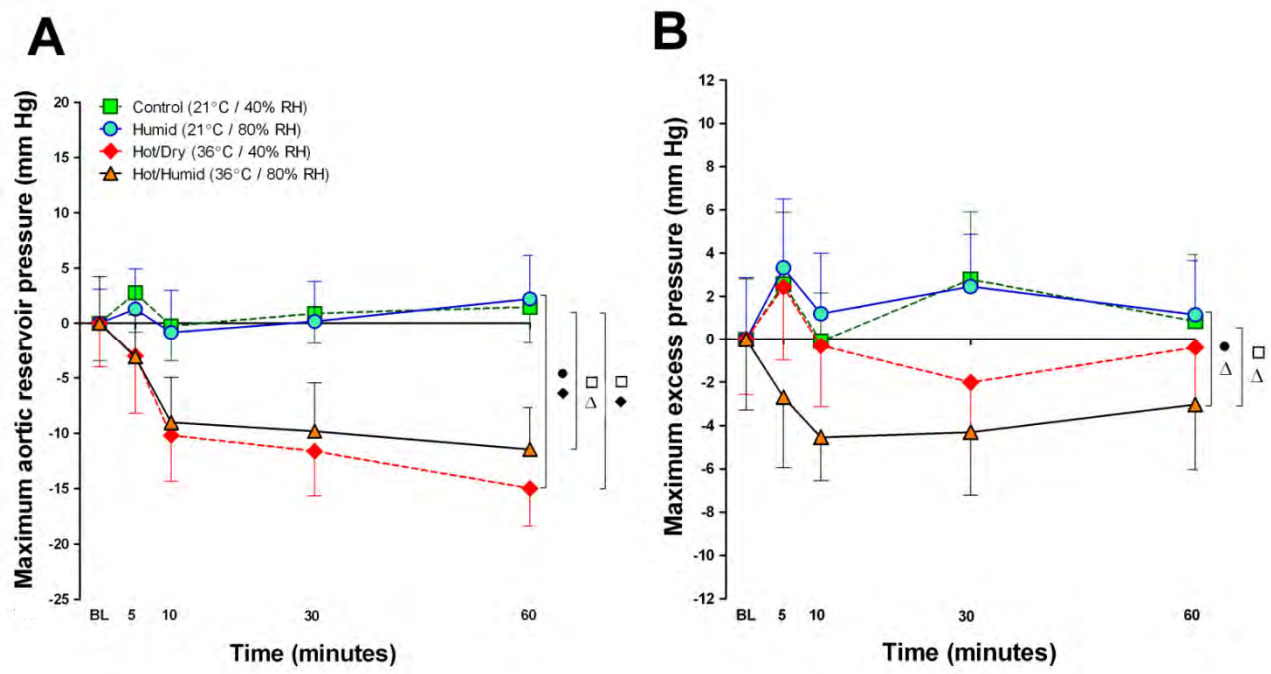

Figure 7.4. Changes in A) maximum reservoir pressure, and B) maximum excess pressure from baseline to 60 minutes in each condition in individuals with T2DM.

n =14. Vertical bracket indicates significant difference in change ($P \leq 0.05$) between identified conditions (from baseline to average of 60 minute data).

Table 7.3. Effect of 60 minutes exposure to heat at two different humidity levels (40% and 80% RH) on selected central haemodynamic measures in individuals with T2DM

Condition	A. Effect of increased heat at 40% RH Δ 21°C/40% RH vs. Δ 36°C/40% RH		B. Effect of increased heat at 80% RH Δ 21°C/80%RH vs. Δ 36°C/80% RH	
	Δ (95% CI)	**P value**	**Δ (95% CI)**	**P value**
Aortic diastolic blood pressure (mm Hg)	-7 (-11.5 to -3.2)	**0.001**	-4 (-8.3 to 0.1)	**0.05**
Mean blood pressure (mm Hg)	-9 (-13.6 to -4.3)	**<0.001**	-7 (-11.7 to -2.4)	**0.003**
Brachial pulse pressure (mm Hg)	-3 (-9.3 to 3.0)	0.63	-7 (-12.6 to -0.4)	**0.03**
Aortic pulse pressure (mm Hg)	-3 (-10.0 to 3.3)	0.64	-8 (-14.2 to -0.8)	**0.02**
Rate pressure product (mm Hg.beats.min^{-1})	-161 (-673 to 351)	1.00	20.6 (-502 to 543)	0.93
Time to excess pressure (ms)	9.3 (0.7 to 23.4)	0.22	15.2 (3.8 to 26.6)	**0.009**
Heart rate (beats.min^{-1})	4.7 (1.8 to 7.6)	**< 0.001 ***	4.6 (1.7 to 7.5)	**< 0.001 ***
Core temperature (°C)	1.7 (1.1 to 2.3)	**< 0.001 ***	1.6 (1.0 to 2.3)	**< 0.001 ***
Skin temperature (°C)	3.6 (2.4 to 4.8)	**< 0.001 ***	3.1 (1.9 to 4.2)	**< 0.001 ***
Perceived thermal comfort (AU)	2.4 (1.2 to 3.6)	**< 0.001 ***	1.2 (-0.2 to 2.5)	**0.01 ***

Note: *n* = 14; * data violated assumptions of general linear modelling, *P* value from ordinal logistic regression. All results are adjusted for order and period effect. Δ, change; RH, relative humidity; AU, arbitrary units

Effect of increased humidity at 21°C (21/40 vs. 21/80)

AIx was significantly lower in 21/80 compared to 21/40 (-3.9 %; 95% CI -7.7 to -0.1; *P* = 0.04; Figure 7.2, B) and skin temperature (+0.3 °C; *P* = 0.05) and perceived thermal comfort (+1.6 arbitrary units; *P* = 0.009) were significantly higher in 21/80 than in 21/40 (Table 7.4, A). There was no significant difference in change in any other measures (Table 7.4, A).

Effect of increased humidity at 36°C (36/40 vs. 36/80)

There was a trend towards reduced skin temperature in 36/80 compared to 36/40 (-0.2 ° C; *P* = 0.09; Table 7.4, B), but no significant difference in changes in any other measure between 36/80 and 36/40 (Table 7.4, B).

Table 7.4. Effect of 60 minutes exposure to humidity (80% RH) at two different temperatures (21°C and 36°C) on selected central haemodynamic measures in individuals with T2DM

Condition	A. Effect of increased humidity at 21˚C Δ 21°C/40%RH vs. Δ 21°C/80%RH		B. Effect of increased humidity at 36˚C Δ 36°C/40%RH vs. Δ 36°C/80%RH	
	Δ (95% CI)	***P* value**	**Δ (95% CI)**	***P* value**
Aortic diastolic blood pressure (mm Hg)	-0.2 (-4.4 to 3.9)	0.90	3 (-1.1 to 7.2)	0.15
Mean blood pressure (mm Hg)	-0.9 (-5.5 to 3.8)	0.71	1 (-3.7 to 5.7)	0.66
Brachial pulse pressure (mm Hg)	0.2 (-5.9 to 6.4)	0.95	-3 (-9.3 to 3.0)	0.31
Aortic pulse pressure (mm Hg)	0.9 (-5.8 to 7.6)	0.79	-3 (-9.9 to 3.5)	0.34
Rate pressure product (mm Hg.beats.min^{-1})	-107 (-629 to 414)	1.00	74.2 (-439 to 587)	0.77
Time to excess pressure (ms)	-3 (-14.4 to 8.3)	0.58	3 (-8.6 to 14)	0.62
Heart rate (beats.min^{-1})	0.08 (-2.8 to 3.0)	0.81 *	-0.01 (-2.9 to 2.9)	0.85 *
Core temperature (°C)	0.03 (-0.6 to 0.7)	0.78 *	-0.01 (-0.6 to 0.6)	0.74 *
Skin temperature (°C)	0.3 (-0.9 to 1.5)	**0.05 ***	-0.2 (-1.4 to 0.9)	0.09
Perceived thermal comfort (AU)	1.6 (0.2 to 2.9)	**0.009 ***	0.3 (-0.9 to 1.6)	0.88

Note: *n* = 14; * data violated assumptions of general linear modelling, *P* value from ordinal logistic regression. All results are adjusted for order and period effect. Δ, change; RH, relative humidity; AU, arbitrary units

Effects of increased heat plus humidity (21/40 vs. 36/80)

Aortic PWV was lower (-0.8 $m.s^{-1}$; 95% CI -1.6 to -0.7, $P = 0.03$), brachial systolic BP (-11 mm Hg; 95% CI -17.6 to -3.8; $P = 0.004$), aortic systolic BP (-12 mm Hg; 95% CI -18.3 to -4.9; $P = 0.002$), brachial diastolic (-4 mm Hg; 95% CI -8 .6 to -0.2; $P = 0.05$), MBP (-8 mm Hg; $P = 0.005$), brachial PP (-6 mm Hg; $P = 0.04$), AP (-4 mm Hg; 95% CI -7.3 to -1.2; $P = 0.006$), AIx (-4 %; 95% CI -7.9 to -0.3; $P = 0.03$), P_{res} (-9 mm Hg; 95% CI -15.9 to -3.5; $P = 0.006$) and P_{ex} (-5 mm Hg, 95% CI -10.4 to 0.2; $P = 0.04$) were all lower in 36/80 than in 21/40 (Figures 7.1 to 7.4, and Table 7.5). Whereas, time to P_{ex} (+12 ms; $P = 0.04$), heart rate (+4.7 $beats.min^{-1}$; $P = 0.006$), core temperature (+1.7 °C; $P < 0.001$), skin temperature (+3.4 °C ; $P < 0.001$), and perceived thermal sensation (+2.7 arbitrary units; $P = 0.001$) were all higher in 36/80 compared to 21/40 (Table 7.5). There was no significant difference in change in any other measure (Table 7.5).

Table 7.5. Effect of 60 minutes exposure to heat plus humidity (21° C/40% vs. 36° C/80% RH) on selected central haemodynamic measures in individuals with T2DM

Condition	Effect of increased heat + humidity Δ 21°C/40% RH vs. Δ 36°C/80% RH	
	(95% CI)	***P* value**
Aortic diastolic blood pressure (mm Hg)	-4 (-8.6 to -0.2)	**0.04**
Mean blood pressure (mm Hg)	-8 (-12.5 to -3.2)	**0.005**
Brachial pulse pressure (mm Hg)	-6 (-12.4 to -0.2)	**0.04**
Aortic pulse pressure (mm Hg)	-7 (-13.3 to 0.1)	0.10
Rate pressure product (mm Hg.beats.min^{-1})	-86 (-602 to 428)	1.00
Time to excess pressure (ms)	12.0 (0.7 to 23.4)	**0.04**
Heart rate (beats.min^{-1})	4.7 (1.8 to 7.6)	**0.006**
Core temperature (°C)	1.7 (1.1 to 2.3)	**< 0.001 ***
Skin temperature (°C)	3.4 (2.2 to 4.5)	**< 0.001 ***
Perceived thermal comfort (AU)	2.7 (1.5 to 3.9)	**0.001**

Note: *n* = 14; * data violated assumptions of general linear modelling, *P* value from ordinal logistic regression. All results are adjusted for order and period effect. Δ, change; RH, relative humidity; AU, arbitrary units

Effect of hydration post-60 minute time point

In a separate analysis of data from 60 to 90 minute time points (data not shown), there were no significant effects of hydration after consuming water at 60 minutes on any haemodynamic measure at 90 minutes. However, tympanic core temperature was lower after water ingestion in both room temperature conditions (-0.48˚C in 21/40 and -0.45˚C, in 21/80, both $P < 0.001$) compared to both hot conditions (36/40 and 36/80), respectively. Additionally, there was no change in haematocrit from baseline in any condition, but there was a trend towards a decrease in haematocrit 36/40 vs. 21/40 (-2%, 95% CI -0.04 to -0.01, $P = 0.09$).

Cardiovascular autonomic neuropathy

Results of CAN testing have been previously reported in Chapter 6 (Page 181). In an analysis of data which accounted for the potential influence of CAN on the main study variables, it was found that change in aortic PWV was significantly and negatively associated with baseline Valsalva R-R ratio ($P = 0.01$), and AIx was significantly and negatively associated with baseline Tilt 30:15 ratio ($P = 0.03$). There were no other significant associations between CAN test results and any other variable. When CAN was accounted for, the differences in change in the main pressor variables (brachial systolic BP, aortic systolic BP and MBP) during exposure to the hot-dry and hot-humid conditions, compared to control, were larger than in the unadjusted mean data; the difference in change in MBP in hot-dry compared to control was -11 mm Hg after adjustment, vs. -8 mm Hg in the unadjusted data which included CAN responders, but the significance of these variables did not change. Moreover, when CAN was adjusted for,

core temperature increases in hot-dry and hot-humid conditions, compared to control were attenuated and no longer significant; from $P < 0.001$ in both hot-dry and hot-humid vs. control in unadjusted data, to $P = 0.98$ in hot-dry, and $P = 0.50$ in hot-humid vs. control in the adjusted data.

DISCUSSION

The novel findings of this study are first, that aortic PWV was reduced by heat only when humidity was high (i.e. in 36/80 vs. 21/80 and 36/80 vs. 21/40), but brachial PWV was not affected by any combination of condition. Second, pressor responses were varied between conditions, with brachial and aortic PPs, and P_{ex} reduced only by heat with high humidity, but aortic and brachial systolic BPs were reduced equally by dry (36/40) and humid-heat (36/80). However, diastolic pressures, MBP and P_{res} were reduced slightly more in dry-heat than in humid-heat. Finally, AIx was not affected in dry-heat, but was reduced in humid-heat compared to control (36/80 vs. 21/40). Moreover, AIx was the only haemodynamic variable to be reduced by high humidity, on its own, in normal room temperatures (21/80 vs. 21/40).

Adults with T2DM in this study had a similar magnitude of reduction in aortic PWV to during humid-heat exposure to healthy participants exposed to identical climate conditions (Chapter 5). Aortic PWV is dependent on changes in MBP, heart rate and AIx, and wave reflections (Van Bortel et al., 2002, Laurent et al., 2006). However, given that MBP was reduced and heart rates were increased similarly across all hot comparisons in the present study but aortic PWV was only reduced in the hot-humid condition, it is possible that the decreased aortic stiffness in hot-humid conditions in patients with

T2DM may be related to reduced wave motion (P_{ex}), P_{res}, and AIx. This is the first time that P_{ex}, which is a measure of wave-related pressure and longitudinal wave reflections (Davies et al., 2010, Wang et al., 2003), has been shown to reduce in response to environmental heat exposure in humans. P_{ex} was significantly reduced only in hot-humid conditions (36/80 vs. 21/40 and 36/80 vs. 21/80). Taken together, reduced P_{res}, P_{ex}, and AIx in hot-humid-conditions suggest that aortic reservoir function may be involved in reducing aortic stiffness in humid-heat exposure in individuals with T2DM. However, further research is required to clarify this supposition. It is not inconceivable that the observed reduction in aortic PWV could potentially be a result of haemoconcentration and reduced total blood volume after fluid loss via sweating during exposure to hot and hot-humid conditions (Jimenez et al., 2002). Unfortunately due to the complicated data collection procedures for each test session, it was not possible to measure sweat loss as well as the principal haemodynamic and thermoregulatory variables in the studies of this thesis. However, we found no increase in haematocrit in any climate condition, which suggests that the reductions in aortic PWV were likely due to reduced mean, aortic and reservoir pressures, rather than reduced total blood volume.

AIx was the only haemodynamic variable to be significantly reduced by the independent effects of humidity in the current study (i.e. in 21/80 vs. 21/40, Figure 7.2, B). There are few human studies that have focussed on humidity as a dependent variable in resting conditions [for example (Freeman and Lengyel, 1938, Wenzel, 1978, Sunwoo et al., 2006)]. However, based on previous data in healthy individuals, high humidity at room temperatures may affect haemodynamics through reduced heat dissipation via evaporation, which due to the higher water vapour gradient, is unable to occur at room temperatures with high humidity (Freeman and Lengyel, 1938, Sunwoo et

al., 2006). Moreover, the additional heat load that accompanies increasing humidity at a given temperature (Parsons, 2006, Ramanathan and Belding, 1973) may be involved in regulating heart rate (Wenzel, 1978), and improving thermal (Freeman and Lengyel, 1938) and mucosal comfort (Sunwoo et al., 2006) compared to low humidity. In the current study, the wet-bulb globe temperature (WBGT) of the humid condition was 18.7˚C compared to 13.3˚C for the control condition (Section 3.1.2., Chapter 3, General Methods). Thus, high humidity at room temperature may promote vasodilation due to the higher heat load, which in this study, may have reduced AIx. Further research is warranted to determine the repeatability of, and the specific mechanisms responsible for these findings.

Brachial PWV was not significantly changed by any condition in the current study. In contrast, healthy participants exposed to identical conditions (Chapter 5) had a significant decrease in brachial PWV in both humid (36/80 vs. 21/80) and dry-heat 36/40 vs. 21/40). In healthy individuals, a reduction in brachial PWV during heating is likely due to NO-mediated vasodilation (Kellogg Jr, 2006, Wilkinson et al., 2002). Therefore, the lack of change in brachial PWV in the current T2DM population may potentially be due to peripheral vascular dysfunction caused by reduced expression of NO-synthase and diminished basal NO (Xu et al., 2003, Petrofsky et al., 2012).

The few previous studies that have investigated the physiological challenges posed by heat in people with T2DM have largely focussed on variables such as skin blood flow and NO availability (Wick et al., 2006, Stansberry et al., 1997, Koivisto et al., 1981). One study measured brachial BPs during orthostatic challenge, before and after heating in T2DM and healthy individuals (Petrofsky et al., 2005a). Petrofsky *et al.,* (2005a) reported that despite similar heart rate increases in healthy and T2DM groups during

supine heating (30 minutes at 42°C with RH between 35 and 40% in a climate chamber), people with T2DM had a smaller reduction in brachial systolic BP than that experienced by the healthy group, ~3 mm Hg vs. ~7 mm Hg, respectively (Petrofsky et al., 2005a). Conversely, the T2DM population in the current study had a greater reduction in brachial and aortic BPs during heating than the healthy participants previously exposed to identical climate conditions as in the current study (Chapter 5). The reasons for a larger fall in BP in a T2DM compared to a healthy population during heating in the current study may be explained by the reduced ability of a dysfunctional sympathetic nervous system to stabilise pressor responses during heat exposure (Ziegler et al., 1993). Despite this, a reduction of any magnitude in usual BP equates to a proportional reduction in CV risk (McInnes, 2005). Given that there were no adverse reactions to any heating protocol in the current study, there is potential for repeated humid-heat exposure to be used as a therapy to reduce aortic stiffness and aortic BPs in a T2DM population.

The slightly lower core and skin temperatures, coupled with the reduced aortic stiffness and markers of LV systolic load recorded during hot-humid exposure compared to hot-dry in the current study, seem to indicate that humid-heat may have beneficial lowering effects on measures of central haemodynamics. Indeed, results suggest that despite the greater WBGT in hot-humid vs. hot-dry conditions in this study, a hot-humid climate may impose a smaller heat-stress than dry-heat in this T2DM population. Several previous studies have investigated the therapeutic potential of whole-body dry-heat sauna exposure in patients with CVD (Crinnion, 2011, Blum and Blum, 2007, Kihara et al., 2004, Imamura et al., 2001, Tei et al., 1995). One such study reported reductions in measures of afterload and peripheral resistance which persisted 30 minutes after a single heat exposure of 15 minutes in a dry-heat sauna at 60 °C and 10 minute hot-water

baths at 41 °C) in people with congestive heart failure (Tei et al., 1995). The mechanisms for improved haemodynamics from repeated short-term dry-heat exposure were thought to be due to increased production of endogenous vasodilators such as NO synthase, NO and prostacyclin (Ikeda et al., 2005, Kellogg Jr et al., 2003). People with heart failure have been shown to have similar decrements in endothelial function and basal NO production as people with T2DM (Drexler and Burkhard, 1999). However, precise mechanisms of how heat improves endothelial function remain unclear, and it is unknown whether people with T2DM would benefit from repeated heat exposure in the same way as people with heart failure. Thus, future research could determine any lasting CV benefits of repeated exposure to short-term heat plus humidity, compared to dry-heat, and further define the potential benefits of humidity *per se*, on central haemodynamic function in people with T2DM.

An analysis of data which accounted for the potential confounding influence of CAN on the main variables in this study, found that a baseline Valsalva R-R ratio or 30:15 tilt ratio was negatively associated with aortic PWV and aortic AIx. A lower Valsalva R-R or 30:15 tilt ratio is indicative of a worsening degree of CAN (Ziegler et al., 1992), and higher aortic PWV and AIx are indicators of increased CV risk (Vlachopoulos et al., 2010a, Vlachopoulos et al., 2010b). Thus, this finding agrees with prior research which reported a higher degree of CAN was associated with greater aortic stiffness (Kim et al., 2011). Despite these results, unadjusted data were used in this study as it was not possible to analyse the data based on only three participants who may have had CAN. However, the potential confounding influence of CAN on haemodynamics should be taken into account when designing future studies.

In conclusion, this study has shown for the first time, that in a group of T2DM individuals with reasonable glycaemic control and moderate degree of CAN, high humidity determines a reduction in aortic stiffness during heating, which is potentially associated with reduced MBP, brachial and aortic PPs, arterial wave reflections (P_{ex}), P_{res}, and AIx. However, peripheral arterial stiffness was not affected by heat or humidity in any combination, which is possibly due to impaired peripheral vasodilation, and down-regulated basal endothelial NO due to the toxic effects of chronic hyperglycaemia. This study has provided the first data on the independent effects of humidity on central haemodynamics in a T2DM group, and has shown AIx was the only variable significantly reduced by humidity *per se*. This effect of humidity at room temperatures (thus, independent of heat) on AIx could be related to the greater heat load causing vasodilation, compared to control conditions, and improving thermoregulation and mucosal irritation in the humid conditions. Collectively, the findings of the current study may have important implications for the use of humidity, with or without heat, as a therapy to improve central haemodynamic function, reduce LV load and aortic stiffness, in people with T2DM.

CHAPTER 8 – EVALUATION OF RESULTS: HEALTHY AND T2DM STUDIES

8.1. Introduction

This chapter presents a general discussion of results from the two studies that aimed to determine the effects of sudden changes in environmental climate on central haemodynamic function in healthy individuals and in people with type 2 diabetes mellitus (T2DM). The studies were not planned for comparison and participant groups were not matched for age, therefore, no statistical comparisons were performed between the two groups. However, Tables 8.1, 8.2 and 8.3 present the results for cardiovascular autonomic neuropathy (CAN) testing, and the mean differences in change in the main variables in the *mild-cold* and *heat and humidity* conditions, respectively, for the two groups. This information is presented in order to provide a contrast of the responses between healthy individuals, and those with T2DM during sudden climate changes.

8.2. Results contrast and discussion

8.2.1 Findings of studies in mild-cold

The arterial stiffness and pressor responses for the T2DM group were in opposition to those observed in the healthy group when exposed to mild-cold (Table 8.1). Results showed that a change from a comfortable ambient climate to a mild-cold climate, as commonly happens when moving from indoors to outdoors on a cool day, significantly increased haemodynamic stress and measures of left ventricular (LV) load such as augmentation pressure (AP) and AIx, but there was very little response in aortic PWV (i.e. aortic stiffness) during cooling in healthy individuals (Chapter 4 and Table 8.1). Conversely, mild-cold significantly increased aortic PWV in people with T2DM (P = 0.03)

and elicited only a modest pressor response in this T2DM group (Chapters 4 and 6, and Table 8.1).

In T2DM individuals there was a rapid rise in aortic PWV to the first peak at 10 minutes with a further slow rise to the 60 minute time point (Figure 6.1, Chapter 6). Not only were the pressor responses of people with T2DM attenuated during mild-cold exposure compared to the larger magnitude in pressor responses observed in the healthy group, T2DM participants had a slightly greater core temperature reduction in mild-cold compared to control (Table 8.1), and did not subjectively perceive the cold as much as the healthy participant group (-0.9 arbitrary units, T2DM felt "cool" vs. -1.1 arbitrary units, and Healthy group felt "cold"). It is likely that the differences in responses in aortic stiffness, haemodynamics, thermoregulation, and thermal perception in T2DM compared to the healthy group during mild-cold exposure are due to impaired neurologic and vascular reactivity to external stressors (Vinik et al., 2003, Chao et al., 2007), although the age difference between the groups cannot be discounted as a potential confounder (London et al., 2001).

Baseline CAN results for the healthy and T2DM groups, and thresholds for normal responses are presented in Table 8.2. No healthy participants tested positive to CAN tests. However, while mean values for T2DM were normal, three T2DM participants were positive to more than one CAN tests, while eight were positive to a single test, suggesting a moderate degree of CAN in this T2DM population.

Table 8.1. Change in selected variables during mild-cold (12°C) and control (21°C) in healthy and T2DM participants

Healthy Group	T2DM Group
Difference in change in mild-cold compared to change in control (95% CI)	Difference in change in mild-cold compared to change in control (95% CI)
Aortic Pulse Wave Velocity ($m.s^{-1}$)	
+0.04 (- 0.47 to 0.55)	+0.5 (-0.2 to 1.2) *†
Brachial Systolic Blood Pressure (mm Hg)	
+4 (-1.3 to 8.4)	+4 (-2.7 to 10.5)
Brachial Diastolic Blood Pressure (mm Hg)	
+4 (0.1 to 7.2) *	+2 (-2.1 to 6.43)
Aortic Systolic Blood Pressure (mm Hg)	
+4 (-0.9 to 8.2)	+2 (-4.2 to 8.8)
Augmentation Index (%)	
+6 (1.2 to 10.1) *	-4 (-7.3 to -0.3) *
Mean Blood Pressure (mm Hg)	
+2 (-1.4 to 5.8)	+2 (-2.8 to 6.2)
Maximum Reservoir Pressure (mm Hg)	
+4 (-0.7 to 8.2)	+2.5 (-3.3 to 8.3)
Maximum Excess Pressure (mm Hg)	
-0.3 (-15.4 to 2.1)	-1.1 (-6.2 to 4.0)
Core (tympanic) Temperature (°C)	
-0.7 (-1.9 to 0.5) * †	-0.8 (-1.3 to -0.2) *
Heart Rate ($beats.min^{-1}$)	
-0.2 (-2.3 to 2.7)	-0.4 (-2.6 to 1.8)

Notes: Healthy group n = 16, age 43±19 years, T2DM (type 2 diabetes mellitus) group n = 14, age 63±7 years, 95% CI = 95% confidence interval, * significant difference in particular group's change between mild-cold and control conditions ($P \leq 0.05$). † data abnormally distributed and P value taken from ordinal logistic regression post-hoc testing.

In a sub-analysis of the T2DM group's *mild-cold* results which accounted for the effects of CAN on the main variables, it was found that aortic PWV was the only variable significantly influenced by any of the CAN test results during cold exposure (Chapter 6). Mean Valsalva R-R ratio was found to be negatively associated with aortic PWV. A lower Valsalva R-R ratio is indicative of worsening CAN (Ziegler et al., 1992), thus this finding supports others who have found a higher degree of baseline CAN is associated with greater resting arterial stiffness (Kim et al., 2011). The sub-analysis also showed that the change in aortic PWV between mild-cold and control conditions was smaller after adjustment for CAN, implying that people with CAN may have greater aortic reactivity to cold exposure compared to people with T2DM but without CAN. Further, this sub-analysis also showed that the change in AIx in mild-cold compared to control was larger after adjustment for CAN results, which suggests that the presence of CAN-responders in the overall group data attenuates the AIx response to mild-cold exposure. This is in agreement with Table 8.1 where AIx responses were smaller in the T2DM group, which included CAN-responders, compared to the Healthy group. Taken together, results of this sub-analysis suggest that people with T2DM plus CAN have exaggerated aortic stiffness responses, but attenuated pressor responses to cold exposure compared to people with T2DM but without CAN, and healthy individuals.

Indeed, similar divergent haemodynamic responses have been previously reported during trunk cooling in people with diabetes plus CAN compared to people with diabetes without CAN, and to healthy controls (Scott et al., 1988). Scott *et al.,* (1988) reported increased mean BP across all groups during cooling, but reduced vasoconstrictor responses only in the diabetes plus CAN group. Scott *et al.,* (1988) did not speculate on the reasons for the divergent response between those with, and without CAN. However,

the vascular endothelium of people with T2DM is degraded over time (Ziegler et al., 1993, Xu et al., 2003, Aronson, 2003), and thus, endothelial, arterial, haemodynamic and thermoregulatory function are more likely to be impaired during cold exposure in a T2DM population (Stansberry et al., 1997).

Table 8.2. Results of cardiovascular autonomic neuropathy (CAN) testing for healthy and T2DM groups

CAN Test	Healthy Group Mean ± SD	T2DM Group Mean ± SD	Threshold for normal response*
Valsalva R-R ratio	1.74 ± 0.38	1.67 ± 0.25	≥ 1.21
Tilt 30:15 ratio	1.44 ± 0.44	1.21 ± 0.18	≥ 1.10
Change in brachial systolic blood pressure post-tilt (mm Hg)	+7 ± 9	-16 ± 10	< 20

Notes: Data presented as mean ± standard deviation, CAN, cardiovascular autonomic neuropathy, Healthy group *n* = 13, age 43±9 years; T2DM, type 2 diabetes mellitus group n = 14, age 63±7 years. Three of the healthy participants were unable to return for CAN testing as they had moved interstate/overseas. *Threshold values from (Ziegler et al., 1992).

Of note, P_{ex}, did not change during mild-cold exposure in either the healthy or the T2DM group (Table 8.1), which suggests there was no increase in wave motion during mild-cold exposure in either group. This is supported by a lack of change in time to reflected wave, which did not change during mild-cold exposure in either group (Healthy Group 145±16 to 139±10ms, $P = 0.62$; and T2DM Group 135±13 to 139±10ms, $P = 0.33$). Traditional wave-impedance theory states that when AIx, AP, or aortic systolic BP are increased, it is likely as a result of increased intensity in both magnitude and velocity, of wave reflections returning from peripheral reflecting sites (Nichols and O'Rourke, 2005). However, the augmented pressor responses which were observed in both groups during

mild-cold exposure can potentially be explained by the within-condition increase in P_{res} in healthy and T2DM groups (Table 8.1), as P_{res} has previously been found to be the major determinant of AIx (Davies et al., 2010).

8.2.1.1. Implications of results: Mild-cold

The studies into mild-cold exposure in healthy individuals and those with T2DM presented in this thesis substantially add to the research literature on the physiological effects of cold exposure in humans. They show for the first time that mild-cold exposure has opposing effects on measures of aortic PWV and of LV load (AIx, and aortic systolic pressures) in healthy individuals compared to people with T2DM. Most importantly, findings from this thesis show that a mild-cold condition, designed to avoid shivering, is capable of causing significant haemodynamic stress in both healthy individuals and people with T2DM. Increased aortic stiffness increases resistance to LV ejection which, in turn, increases brachial and aortic BPs, and thus, myocardial work (London and Pannier, 2010). In individuals with increased CV risk, such as people with long-term T2DM and more severe degree of CAN, a reactive and stiff aorta during cold exposure increases cardiac work and may increase tendency to myocardial ischaemia and risk of acute myocardial infarction, or death. These findings may help to explain the greater excess winter mortality in vulnerable chronic disease populations, such as people with T2DM.

8.2.2. Findings of studies into heat and humidity

Despite higher baseline values for aortic PWV in T2DM compared to healthy participants (Tables 7.2 and 5.2 in Chapters 7 and 5, respectively), both groups demonstrated that hot-humid conditions caused significant reductions in aortic PWV, while hot-dry conditions did not (Table 8.3). During humid-heating, aortic PWV reached

its nadir faster in the Healthy group who had the greatest slope of decline at 10 minutes, then a slower fall to 60 minutes (Figure 5.1, A, Chapter 5), while in the T2DM group, aortic PWV had a slow, steady decline to reach a maximal reduction at 60 minutes (Figure 7.1, A, Chapter 7). The more rapid reduction in aortic PWV in healthy people, compared to those with T2DM may be due to the greater basal availability of NO in the healthy endothelium which triggers vasodilation (Kellogg Jr, 2006), compared to the likely impairments in vascular reactivity that are typical in T2DM (Xu et al., 2003).

Brachial PWV was reduced in response to heat at either low or high RH in healthy individuals, but did not significantly change in T2DM individuals (Chapters 5 and 7). The difference in brachial PWV responses between the healthy and T2DM groups during heating is likely related to the differences in vascular physiology in healthy individuals and T2DM patients. Due to neuropathies, T2DM patients often have abnormal vasodilator responses which predominantly affect the muscular peripheral arteries (Ziegler et al., 1993, Stansberry et al., 1997, Xu et al., 2003).

In previous studies in healthy individuals, high humidity at room temperatures improved thermoregulation (Freeman and Lengyel, 1938). This was possibly due to the increased water vapour content of the air reducing heat loss via evaporation, which thereby maintained skin and core temperatures (Freeman and Lengyel, 1938). It is also possible that high humidity favourably affects physiology by preventing drying of skin and mucous membranes (Sunwoo et al., 2006). When combined with heat, high humidity may improve respiratory function (Rea et al., 2010; Turner et al., 1992) and promote a passive relaxation of the elastic aorta which decreases PWV.

Table 8.3. Change in selected variables in humid (21°C/80%RH), hot-dry (36°C/40%RH), and hot-humid (36°C/80%RH) compared to control (21°C/40%RH) in healthy and T2DM participants

Condition	Healthy Group	T2DM Group
	Difference in change compared to control (95% CI)	Difference in change compared to control (95% CI)
	Aortic Pulse Wave Velocity ($m.s^{-1}$)	
Humid	-0.15 (-0.71 to 0.40)	-0.01 (-0.78 to 0.38)
Hot-Dry	-0.35 (-0.91 to 0.20)	-0.49 (-1.26 to 0.27)
Hot-Humid	-0.82 (-1.38 to -0.26) *	-0.84 (-1.60 to -0.67) *
	Brachial Systolic Blood Pressure (mm Hg)	
Humid	-5 (-9.6 to 0.3) *	+0.1 (-6.9 to 6.9)
Hot-Dry	-3 (-8.1 to 1.8)	-11 (-17.5 to -3.7) *
Hot-Humid	-4 (-8.4 to 1.5)	-11 (-17.6 to -3.79) *
	Brachial Diastolic Blood Pressure (mm Hg)	
Humid	-2 (-5.2 to 1.3)	-0.2 (-4.3 to 3.9)
Hot-Dry	-5 (-8.2 to -1.7) *	-7 (-11.6 to -3.3) *
Hot-Humid	-3 (-6.2 to 0.3)	-4 (-8 .6 to -0.2) *
	Aortic Systolic Blood Pressure (mm Hg)	
Humid	-5 (-10.1 to -0.1) *	-0.4 (-7.1 to 6.3)
Hot-Dry	-3 (-8.3 to 1.7)	-12 (-18.5 to -5.2) *
Hot-Humid	-3 (-8.4 to 1.7)	-12 (-18.3 to -4.9) *
	Augmentation Index (%)	
Humid	-0.7 (-5.2 to 3.8)	-3.9 (-7.7 to -0.1) *
Hot-Dry	+2.8 (-1.8 to 7.2)	-3.5 (-7.3 to 0.3)
Hot-Humid	+0.8 (-3.7 to 5.3)	-4.1 (-7.9 to -0.3) *
	Mean Blood Pressure (mm Hg)	
Humid	-4 (-7.4 to 0.1)	-0.9 (-5.5 to 3.8)
Hot-Dry	-4 (-7.4 to 0.2)	-9 (-13.6 to -4.3) *
Hot-Humid	-3 (-6.2 to 1.3)	-8 (-12.5 to -3.2) *
	Maximum Reservoir Pressure (mm Hg)	
Humid	-4 (-8.7 to 1.1) * †	-0.8 (-7.0 to 5.5)
Hot-Dry	-4 (-9.3 to 0.6) * †	-11 (-17.6 to -5.1) *
Hot-Humid	-4 (-8.7 to 1.1)	-9 (-15.9 to -3.5) *

Table 8.3. Continued...

Condition	Healthy Group	T2DM Group
	Difference in change compared to control (95% CI)	Difference in change compared to control (95% CI)
	Maximum Excess Pressure (mm Hg)	
Humid	-2 (-5.9 to 1.9)	+0.50 (-4.6 to 5.6)
Hot-Dry	+0.3 (-3.7 to 4.2)	-2 (-6.6 to 3.6)
Hot-Humid	-2 (-5.6 to 3.2)	-5 (-10.4 to -0.2) *
	Heart Rate (beats.min^{-1})	
Humid	-0.87 (-3.61 to 1.86)	+0.1 (-2.8 to 3.0)
Hot-Dry	+6.5 (3.8 to 9.3) *	+4.7 (1.8 to 7.6) *
Hot-Humid	+7.4 (4.7 to 10.2) *	+4.7 (1.8 to 7.6) *
	Core (tympanic) Temperature (˚C)	
Humid	-0.14 (-0.13 to 0.41)	+0.03 (-0.60 to 0.67)
Hot-Dry	+0.99 (0.72 to 1.27) *	+1.68 (1.04 to 2.32) *
Hot-Humid	+1.09 (0.81 to 1.36) *	+1.67 (1.03 to 2.32) *

Note: Healthy group *n* = 16, age 43±19 years, T2DM (type 2 diabetes mellitus) group *n* = 14, age 63±7 years, 95% CI = 95% confidence interval, * significant change between specified condition and control in specific group ($P \leq 0.05$), † data abnormally distributed and *P* value taken from ordinal logistic regression post-hoc testing.

The mechanisms behind a reduction in aortic PWV during humid-heating are potentially due to higher cardiac output which increases shear stress and up-regulates release of NO and endothelium-derived relaxing factors that, in turn, decrease large artery stiffness (Wilkinson et al., 2002, Sugawara et al., 2007, Buga et al., 1991, Pohl et al., 1986). However, while the precise mechanisms responsible for the effects of humidity *per se* on haemodynamic function are currently unknown, increasing humidity at any temperature increases the heat load as measured by WBGT (Parsons, 2006, Australian Bureau of Meteorology, 2012, Ramanathan and Belding, 1973). The results of the present studies into *heat and humidity* demonstrate that high humidity appears to drive reductions in certain measures of haemodynamics, both with, and without heat. However, questions remain: Are there specific and direct physiological effects of high humidity which may beneficially influence haemodynamic function, i.e. improved thermoregulatory and respiratory function, or mucosal hydration? Alternatively, are the apparent benefits of humidity associated with the increased heat load which accompanies an increase in humidity, and as a consequence, elicits vasodilation? Comparative studies which measure central haemodynamic and biochemical markers under conditions of equivalent WBGTs are needed to clarify these questions.

Despite higher baseline measures for haemodynamic in the T2DM group, compared to the healthy group, several haemodynamic parameters, for example BPs, MBP, AIx, and P_{res} were changed to a greater degree by heat, both dry and humid, in people with T2DM compared to healthy individuals (Table 8.3). For instance, brachial systolic BP and aortic systolic BP were reduced by 8 and 9mm Hg more, respectively, in T2DM than in healthy participants in hot-dry conditions (36/40 vs. 21/40), and by 7 mm Hg (brachial) and by 9 mm Hg (aortic) more in hot-humid conditions (36/80 vs. 21/40, Table 8.3). Moreover, T2DM participants experienced a smaller magnitude of heart rate

increase compared to the larger rise in heart rate observed in healthy participants in the same hot-humid conditions; heart rate was 1.8 and 2.7 beats.min^{-1} less in T2DM than healthy participants during exposures to 36/80 vs. 21/40 and 36/80 vs. 21/40, respectively (Table 8.3). However, the core (tympanic) temperatures of this T2DM group were nearly a degree C higher (+0.6 and +0.7˚C higher in dry-heat, 36/40, and humid-heat, 36/80, respectively, vs. control) than the core temperatures of the healthy group in these conditions. Moreover, the perceived thermal comfort was variable, being +0.65 higher in dry-heat (21/40 vs. 36/40), and -0.83 lower in humid-heat (21/80 vs. 36/80) in the T2DM compared to the healthy group (Chapters 5 and 7). Collectively, these results indicate that the T2DM group had dysfunctional BP, heart rate, and core temperature responses to heat stress compared to the "normal" responses of the Healthy group. The reasons for these differences between individuals with T2DM and healthy people may potentially be explained by the reduced ability of an impaired sympathetic nervous system to stabilise haemodynamic and thermoregulatory responses during challenge by heat (Ziegler et al., 1993).

In a sub-analysis of the T2DM group's *heat and humidity* results which took into account the potential confounding influence of CAN on the main study variables, it was found that aortic PWV was significantly and negatively associated with mean Valsalva R-R ratio (P = 0.01), and AIx was significantly and negatively associated with the mean tilt 30:15 ratio (P = 0.03). There were no other significant associations between CAN results and any other variable. Similar to the *mild-cold* results for the CAN sub-analysis, a lower Valsalva R-R ratio and 30:15 tilt ratio indicates worsening CAN (Ziegler et al., 1992). Thus, these results suggest that there is positive relationship between worsening CAN and higher aortic stiffness (Kim et al., 2011) and AIx. Moreover, the sub-analysis accounting for CAN results showed that the reductions in the main pressor variables; brachial

systolic BP, aortic systolic BP and MBP, were slightly greater during heating after adjustment for CAN results. This indicates that people with T2DM plus CAN may have a smaller response in pressor variables during heating, than people with T2DM but without CAN. This difference may be due to greater impairments in peripheral vasodilation due to neuropathies (Petrofsky et al., 2005a, Stansberry et al., 1997) which prevents normal haemodynamic responses in the CAN responders in this thesis. In addition, when data were adjusted for CAN in the T2DM *heat and humidity* studies, core temperature increases were attenuated; i.e. there were larger increases in core temperature in the hot-dry and hot-humid conditions in the original data which included the CAN-responders. This finding is consistent with the differences observed between T2DM and healthy groups (Table 8.3), and may be due to a reduced ability to thermoregulate during sudden climate changes in people with T2DM (Xu et al., 2003, Petrofsky et al., 2012).

8.2.2.1. Implications of results: Heat and humidity

Long-term heat exposure increases the potential for heat-related morbidity and mortality in individuals with T2DM due to impaired thermoregulation which stresses the CV system. In line with the differences observed in core temperatures and thermal perception in the two study groups in this thesis, previous studies have shown that people with T2DM have impaired peripheral vascular reactivity to heat exposure, which results in a smaller-than-usual increase in skin blood flow during heating (Stansberry et al., 1997). Thus, in T2DM, heated blood is not effectively redirected away from the core to the cutaneous circulation to achieve heat dissipation (Stansberry et al., 1997, Wick et al., 2006). Compounding this, people with T2DM have an increased heat-perception threshold (Chao et al., 2007). An increasing but un-perceived heat load has implications for progressively increasing core temperatures and increased risk of hyperthermia, heat illness, dehydration, and death during prolonged heat exposure in people with T2DM

(Wick et al., 2006, Semenza et al., 1999). Education of the potential risks, and behavioural conditioning to avoid prolonged exposure to heatwave conditions is crucial for people with T2DM to avoid complications of heat illness (Curriero et al., 2002).

In contrast, short-term heat exposure has potentially beneficial effects on arterial stiffness and central haemodynamics as shown in the studies presented in Chapters 5 and 7, and in previous studies in people with heart failure (Tei et al., 1995, Imamura et al., 2001, Ikeda et al., 2005, Miyata et al., 2008). The studies into *heat and humidity* presented in this thesis demonstrate that individuals with T2DM have greater reductions in haemodynamic measures when exposed to humid-heat than dry-heat, and that these reductions are larger in people with T2DM than those observed in healthy individuals exposed to identical climate conditions. These differences are likely due to impaired microvascular reactivity and vasomotor tone that causes abnormal pressor responses to external stressors in people with T2DM (Ziegler et al., 1993). The results presented in this thesis provide evidence that with, or without heat, exposure to humid conditions for ~1 hour is no more physiologically stressful than dry conditions. Indeed in some cases, for example aortic PWV in the heat, high humidity may be beneficial for CV function in both healthy and T2DM individuals. For people with T2DM, humid-heat therapy may represent a safe and cost-effective non-pharmacological alternative for treatment of hypertension and arterial stiffness.

8.3. Possible mechanisms

It is possible that the mechanisms behind the changes observed in haemodynamics during cooling and heating in this thesis are the result of sympathetically-mediated vasoconstriction and vasodilation, respectively (Sessler, 2009). Profound haemodynamic

and vascular changes during cooling and heating are caused by alterations in core temperature (Seebacher, 2009). In an effort to maintain homeostasis during core temperature changes, heart rate, cardiac output, and skin blood flow are altered, which increase mechanical shear stress. Increased shear stress is detected by baroceptors, which, in turn, trigger modulations in expression of NO to induce vasomotion (Bellien et al., 2010, Sugawara et al., 2007, Buga et al., 1991).

The differences between responses to whole-body passive cooling and heating in people with T2DM and healthy individuals are potentially related to the down-regulation of endogenous metabolites such as NO which are caused by the detrimental effects of hyperglycaemia on endothelial function in people with T2DM. The reduced availability of endothelial NO which is common in people with T2DM likely impairs vasomotor function and thermoregulation, and thus, alters normal, healthy responses to external heating and cooling.

CHAPTER 9 – THESIS CONCLUSIONS AND FUTURE DIRECTIONS

9.1. Thesis summary

The most important findings arising from the studies undertaken in this thesis are that a change from a comfortable ambient climate to a mild-cold climate, as commonly happens in day-to-day life, significantly increases haemodynamic stress and measures of LV load without differentially affecting arterial stiffness in healthy individuals. Conversely however, mild-cold significantly increases aortic stiffness in people with T2DM and elicits only a modest pressor response in brachial and aortic BPs, and MBP, compared to responses observed in healthy individuals. As such, even a brief exposure, as little as 5 to 10 minutes, to a non-shiver-inducing cold temperature of 12°C can potentially increase aortic stiffness and augment LV load enough to contribute to morbidity and mortality in high-risk individuals.

This thesis also demonstrates for the first time, that humidity appears to be the factor that reduces aortic stiffness during whole-body heating in both healthy and T2DM individuals; a result that was not apparent when both groups were exposed to dry-heat. The findings presented in this thesis have shown that haemodynamic reductions caused by humid-heat were greater in people with T2DM, than in healthy individuals. Furthermore, this thesis presents the first data on the haemodynamic effects of humidity, independent of heat, which demonstrate that exposure to humid-room temperature conditions significantly reduced aortic systolic BP, RPP and P_{res} in healthy individuals, and reduced AIx in people with T2DM. The potential of high humidity, with or without heat, to reduce measures of aortic stiffness and LV load may have clinical relevance for reduction of CV risk in T2DM individuals.

9.2 Limitations and future directions

9.2.1 Age matching and sample size

Sample sizes of 20 were sought for each study group. However, due to prolonged challenges with participant recruitment and participant withdrawals during the data collection phase of each study, final sample sizes were smaller than planned. Further, the two study groups were not planned to be matched for age. Thus, comprehensive statistical associations to compare the Healthy and T2DM data, and determine correlations were not performed. Future studies with greater participant numbers, where groups are matched for age and body composition, and data adjusted for effects of gender and baseline CAN will build on the current results and further define the reasons for the differences between haemodynamic responses in healthy individuals and those with T2DM.

9.2.2. Effect of medications on haemodynamics in T2DM participants

All efforts were made to control for the potential effects of medications on haemodynamic measures in the T2DM studies, particularly anti-hypertensive medications. Moreover, for individual participants, all test sessions were undertaken at the same hour of the morning for all five test sessions in order to minimise diurnal fluctuations on BP. It is unknown whether the ~18 hour time period between cessation of usual medications the evening before and the next morning's test session was sufficient to allow for the effect of anti-hypertensive medications to fully subside. However, it was deemed unethical to withhold prescription medications from T2DM participants for a longer time period.

9.2.3 Menstrual cycles of female participants

The menstrual cycles of female participants were not taken into account during either study as the effect of ovarian hormones on reflexive BP control during haemodynamic perturbations has been found to be negligible (Hayashi et al., 2006, Rahman et al., 1991). However, it is acknowledged that others dispute (Hassan et al., 1990), or have found conflicting (van Beek et al., 1996) results regarding the influence of the different phases of the menstrual cycle on haemodynamics. In future studies, female participants might be studied immediately post-menstruation to avoid any potential influence of female reproductive hormones on haemodynamics or thermodynamics (Inoue et al., 2005).

9.2.4. CAN as a potential confounder of haemodynamics

Given that in this thesis, degree of CAN was found to be associated with two of the main haemodynamic variables, aortic PWV and AIx, it is essential that in future studies with T2DM groups, presence of CAN is tested for at baseline and participants grouped according to test results. Further, it is important that CAN is listed as an inclusion or exclusion criteria for recruitment purposes, depending on the focus of the study.

9.2.5. Control condition temperature

Some variables were significantly affected during exposure to the control condition compared to mild-cold in the T2DM group, for example AIx (Figure 6.3, A). These changes were contrary to the expected negligible changes which are typical during supine rest in neutral temperatures and indicate that the planned control temperature of 21°C may have been as cooler than thermoneutral. The Healthy group perceived the control condition on average as "cool" and thermal perception was reduced ($P = 0.001$) from baseline, but core ($P = 0.14$) and skin ($P = 0.29$) did not change from baseline.

However, the T2DM group perceived the control condition as "neutral/comfortable" on average, and thermal perception ($P = 0.03$) and skin temperature ($P = 0.05$) were reduced from ambient baseline measures, but core temperature did not change from baseline in T2DM participants ($P = 0.22$). However, 21°C, was similar to our usual laboratory temperature which was deemed comfortable for people resting in light clothing. The possible coolness of the control condition may have underestimated the differences in haemodynamic parameters between a thermoneutral and a mild-cold condition or over-estimated differences between hot conditions and control. Future studies could use a control condition between 22-24˚C, as others have suggested as thermoneutral (Laurent et al., 2006).

9.2.6. Core temperature estimations

Baseline core temperatures of the T2DM group were lower than the normal range for the participant age group (Sund-Levander et al., 2002). Core temperatures in both studies 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, 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 control for any inconsistencies in tympanic temperature collection, data collectors were trained in the correct use of the thermometer according to accepted techniques (Davie and Amoore, 2010, McCarthy and Heusch, 2006). Notwithstanding this, individuals with T2DM could be expected to have lower baseline core temperatures due to impaired thermoregulation (Scott et al., 1988). In this thesis, core and skin temperatures were collected purely to detect shifts in temperature in the two conditions, and this remains valid despite low baseline values.

9.2.7. Effects of dehydration and rehydration during heating

A possible confounder in the methodology of the studies was the cumulative effects of dehydration on measures of haemodynamic function, particularly during heat exposure (Armstrong et al., 1997). To control for this, healthy participants drank 300 mL, and T2DM participants drank 250 mL of water immediately post-60 minute measures in all five climate trials and data from 60 to 90 minutes was analysed separately. There were no changes in any haemodynamic variable from 60 to 90 minutes in any combination of hot conditions in both healthy and T2DM groups (Chapters 5 and 7). This, together with the fact that haematocrit did not increase in either healthy and T2DM groups during heat and/or humidity exposures, supports the conclusion that dehydration or haemoconcentration were not significant factors in haemodynamic changes observed in these studies. Due to the complex nature of the haemodynamic measures, sweat loss was not measured in these studies. Future research should measure sweat loss and compare results to haematocrit changes to clarify whether central haemodynamics are affected by dehydration during hot and hot-humid conditions.

9.2.8. Mechanistic measures

A potential limitation of the thesis is the limited ability to explain the physiological mechanisms responsible for the study findings. The complexity and long duration required to undertake data collection in the studies meant that it was not feasible to add further measures of haemodynamics and CV function. However, given the evident lowering effects of humid-heat on arterial stiffness, and of humidity *per se* on measures of LV afterload, determining the mechanisms behind these changes is necessary to understand these novel findings. Future studies could compare what effects humidity, with and without heat, has on traditional mechanistic measures of vascular function, such as cardiac output, stroke volume, total peripheral resistance, and skin blood flow.

Moreover, measurement of biomarkers of vasodilation (i.e. endothelial-derived relaxing factor, endothelial NO, NO-synthase, and cytochrome-related hyperpolarising factors) and sympathetic nervous system activation (catecholamine concentrations), and thermal discrimination in healthy and T2DM populations may provide the information to explain the clear differences observed in vascular responses between humid and dry-heat in this thesis.

9.2.9. Future studies arising from thesis results

Based on the results of this thesis, the following future research is proposed:

9.2.9.1 Potential cold studies

A smaller pressor response than is typically observed in healthy individuals is potentially a normal response for a T2DM population during cold exposure. In the current T2DM population, the smaller pressor response observed in the T2DM group than in the healthy group may be due to the moderate degree of CAN in the T2DM group that impairs normal vascular reactivity to cold stress and prevents a robust pressor and thermoregulatory response to external homeostatic challenge. However, very few studies into cold exposure have included individuals with T2DM, and even fewer have focussed on central haemodynamics. Moreover, as people with T2DM are known to have diminished sensory perception, it may be that the mild-cold protocol was insufficient to cause a significant pressor response in this group. It would be warranted to undertake further study in people with stable T2DM to assess the effects of a colder protocol, with a shorter-duration, between 20 to 30 minutes, to avoid hypothermia, on arterial stiffness and central haemodynamics. Inclusion of thermal perception and baseline CAN testing would clarify the magnitude of sensory loss and how this may affect thermoregulation and haemodynamics in a T2DM group.

9.2.9.2 Potential heat and humidity studies

Possible haemodynamic benefits of high humidity, with or without heat

There is great potential for further study into the possible CV health benefits of repeated exposure to hot-humid or room temperature humid-air in T2DM and other chronic disease populations. In particular, reduced aortic PWV during humid-heating is potentially of interest. Although a transient reduction in aortic PWV is not yet known to have any significant clinical benefits, aortic PWV is an independent predictor for CV mortality (Vlachopoulos et al., 2010b), and a sustained reduction of ~0.9 $m.s^{-1}$ is considered clinically relevant for minimising CV risk, i.e. from a high-normal aortic PWV of 7.9 $m.s^{-1}$ to an optimal 7.0 $m.s^{-1}$ (Boutouyrie and Vermeersch, 2010). In this thesis, transient reductions in aortic arterial stiffness were observed during short-term exposure to hot-humid conditions in healthy individuals and people with T2DM, which did not reach significance in hot-dry conditions. Prior studies have demonstrated a favourable effect of repeated dry-heat sauna on improving haemodynamics in healthy people and those with CVDs (Crinnion, 2011, Miyata et al., 2008). Thus, future study is warranted that further investigates the safety and clinical potential of repeated, short-term exposure to humid-heat, of between 10 and 30 minutes, as a therapy to reduce aortic stiffness and improve central haemodynamic function in T2DM, and other chronic vascular conditions. Ambulatory BP monitoring for 24 hours post-exposure, or next-day repeat testing in ambient conditions may determine the duration of any haemodynamic benefits after an acute exposure, and after repeated short-term humid-heat exposures. Further, findings from the *heat and humidity* studies in this thesis could form the basis of future studies to determine the effects of short-term heat and humidity exposure in people who are acclimatised to heat and/or humidity, or for people living in climates with little seasonal variation.

Emphasis on standard heat indices

The current studies were not planned to compare equivalent heat-loads or to use different heat exposure methods. However, based on the review of literature, it seems likely that some methods of experimental heating and cooling stimulate greater vasomotion than others (Tables 2.3 and 2.6, Chapter 2). Comparative experiments are required to measure vasomotor responses during heating and cooling using localised, i.e. heat or ice packs and water immersion, and whole body heating or cooling using a water-perfused suit and a climate chamber at equal WGBT heat loads to determine any differences between different heating and cooling methods and heat-loads.

Aortic reservoir function and environmental climate

Results from heat and/or humidity exposures in this thesis suggest that reductions in aortic P_{res} may be a determinant of reduced aortic stiffness during humid-heat exposure in healthy individuals and people with T2DM. Research is required to clarify this supposition, either in an animal model, or in humans undergoing scheduled angiography, to compare invasive pressure and flow measures with calculated non-invasive measures of reservoir function. Furthermore, invasive studies employing a Reservoir-wave approach and wave intensity analysis would be beneficial to determine and compare reservoir responses between vasomotion caused by medications, and those caused by heating and cooling. Findings may clarify the reasons for any differences between calculated and *in vivo* haemodynamic measures during interventions that cause vasomotion.

9.3 Concluding Comments

The results from this thesis show divergent haemodynamic responses between cooling and heating in people with T2DM and healthy individuals. During cooling, some haemodynamic responses to mild-cold were exaggerated in T2DM (i.e. increased aortic stiffness), and some were attenuated (i.e. pressor responses) compared to responses of healthy individuals. Conversely, during humid-heating, people with T2DM had exaggerated pressor reductions and similar magnitude, but slower rate of reduction in aortic stiffness compared to healthy individuals. Results of this thesis highlight the differences and similarities between responses of healthy individuals and people with T2DM during sudden climate changes, and show that cold exposure is detrimental to central haemodynamic function, while short-term heating (particularly with high humidity) improves central haemodynamic function in healthy individuals and those with T2DM.

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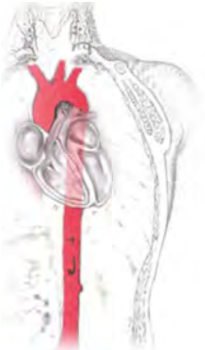
APPENDICES

1. Copy of Poster Presentation, Healthy Group, Mild-Cold Exposure
2. Participant Information, Informed Consent and Eligibility Questionnaires; Healthy and T2DM Groups
3. Example of Data Collection Forms, Questionnaires and CAN Protocols
4. Thermal Sensation Scale

APPENDIX 1 –POSTER PRESENTATION, HEALTHY GROUP; MILD-COLD STUDY



Effect of Acute Mild Cold Exposure on Central Haemodynamics

UTAS FACULTY OF HEALTH SCIENCE School of Human Life Sciences

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Background and Aims

- Acute exposure to cold increases risk of cardiovascular (CV) events and mortality[1]. Paradoxically, cold-associated CV events and deaths are particularly prevalent in regions of the world with temperate climates, such as Australia[2].
- Our primary aim was to determine the effect of exposure to acute mild cold and to a thermoneutral control condition on two independent predictors of CV risk and mortality[3,4]: carotid-femoral pulse wave velocity (PWV_{cf}) and augmentation index (AIx), and on secondary measures including central blood pressures (BPs), augmentation pressure (AP), and aortic reservoir function in healthy adults.

Methods

Design and Participants:

- Randomised, controlled, cross-over design
- Two climatic test conditions, each separated by ~7 days: 21°C with 40% relative humidity [RH]; control), and 12°C with 40% RH (mild cold).
- For each test, baseline measures were taken in ambient lab temperatures, then at 10, 30, 60 & 90 minutes post entry to controlled climate chamber.
- 16 participants (10 M, 6 F, age 43±19 years; mean ± SD).
- Inclusion criteria: >18 years, no known CV or metabolic disease, & a resting peripheral BP of <150/80 mm Hg.

Non-invasive Measures:

- Pulse wave velocity, AIx, central BPs, & AP (via standardised procedures[5] using integrated software [v8.2] and a Sphygmocor system[6].
- Aortic reservoir function (calculated from pulse wave data as per previous recommendations[7,8].
- Peripheral BPs.
- Core & skin temperatures, perceived rating of thermal comfort.

Participant in the climate chamber having a pulse wave reading taken of the carotid artery

Results

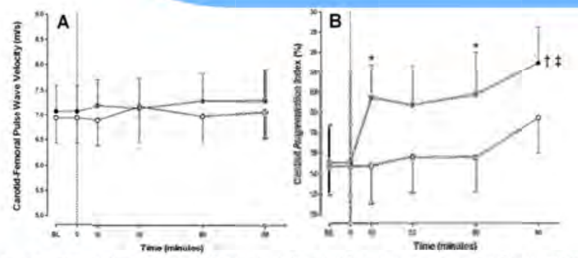

Figure 1. Change in (Δ) carotid-femoral pulse wave velocity (A) augmentation index (B) in mild cold (●; closed circles; 12°C 40% RH) and control (○; open circles; 21°C 40% RH) conditions. Timepoints represent baseline (BL; ambient laboratory temperature) and at 0 minutes (dashed vertical line; entry to climate chamber), & 10, 30, 60 and 90 mins at supine rest in the climate chamber. * indicates value in mild cold condition different from value in control at specific timepoint, p ≤ 0.05. † indicates Δ over 90 minutes significant from baseline, p ≤ 0.05. ‡ indicates mean of values from timepoints 10, 30, 60, and 90 minutes in mild cold significantly different from mean Δ control values, p ≤ 0.05.

In mild cold compared to control:

- PWV_{cf} was not different (Fig. 1, panel A)
- AIx increased +6% (95% CI 0.8 to 10.3; *p* = 0.02; Fig. 1, panel B)
- Central systolic & diastolic BPs were not different
- AP increased +2 mm Hg (95% CI -0.1 to 4.2; *p* = 0.02)
- Time to peak excess pressure (reflects timing of aortic inflow) decreased -6.7 ms (95% CI -14.8 to 1.4; *p* = 0.02)

*Data table available upon request

Conclusion

The differential effects of mild cold on PWV_{cf} and AIx are likely related to cold-associated peripheral vasoconstriction (a thermoregulatory mechanism) affecting peripheral rather than central vessels[9], thereby increasing AIx (which is influenced by the reservoir pressure in the aorta[8]), yet leaving PWV_{cf} unaffected.

Increased AIx and AP indicate increased left ventricular afterload[10], while decreased time to excess pressure demonstrates reduced timing of aortic inflow during cold exposure[7]. Taken together, results suggest that in healthy adults, mild cold exposure increases central haemodynamic stress (workload on the heart per beat), without altering aortic stiffness.

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Phone: 03 6324 3688

APPENDIX 2 – INFORMATION SHEETS AND ELIGIBILITY QUESTIONNAIRES

PARTICIPANT INFORMATION SHEET – HEALTHY GROUP

Aim: To determine the body's ability to regulate blood pressure in response to sudden changes in environmental temperature and humidity.

Scientific Title: Effect of Acute Changes in Air Temperature and Humidity on Central Blood Pressure and Arterial Compliance

Research Team: Ms. Sibella King (PhD Candidate), Dr. Kiran Ahuja, Dr. Andrew Williams, Ms. Jezreel Kay and Ms. Melissa Williams

This consent form may contain information that you do not understand. Please ask one of the research team members to explain anything that you do not clearly understand.

INTRODUCTION AND AIM OF STUDY

Traditionally, blood pressure is measured at the upper-arm (at the brachial artery) using an inflatable cuff. High blood pressure is considered a major risk factor for cardiovascular disease and death as it increases the work that the heart must perform to maintain adequate blood flow to the body. Recent research suggests that the measurement of aortic (central) blood pressure may be a better indicator of cardiovascular outcomes than the traditional upper-arm blood pressure method.

Exposure to heat or cold temperatures cause changes to the circulation with the aim to maintain the body's blood pressure and core temperature. During exposure to the heat, blood vessels under the skin dilate, resulting in increased blood flow to the limbs (periphery) and increased heart rate to maintain blood pressure, while exposure to cold results in constriction of peripheral blood vessels which is accompanied by increased blood pressure when measured using the traditional upper-arm blood pressure method. World-wide evidence suggests that environmental temperatures may also affect the incidence of cardiovascular events (such as a heart attack) with an increased number of events occurring during winter and during heatwave conditions.

STUDY PURPOSE

Very little is known about the effect of acute changes in air temperature and humidity on central blood pressures. Therefore, this study aims to provide insight into how acute environmental stress affects the cardiovascular system. The outcomes of this study will provide information for the health and safety of people most at risk (ie. those with chronic disease, or the elderly) during extreme weather events, or even when moving from indoor to outdoor environments, as happens often during winter and summer. Additionally, the study outcomes will provide information for the safety of workers in extreme temperatures (eg. military personnel, municipal workers, food handlers, foundry workers, etc).

INCLUSION AND EXCLUSION

To participate in this study you must be over 18 years. People with diagnosed hypertension or resting blood pressure ≥ 150 / 80 mmHg or with known vascular disease may be excluded.

IF YOU WOULD LIKE TO PARTICIPATE IN THE STUDY, THE REQUIREMENTS ARE:

Attend the Exercise Physiology Laboratory, in the School of Human Life Sciences, University of Tasmania at the Launceston campus, five times over a period of four to five weeks.

Each visit will last for approximately two and a half hours and there will be at least a 4-7 day gap between any two consecutive tests.

Each visit will be in the morning after an overnight fast of 10-12 hours, ie. *Before breakfast* (water is allowed and the exact date and time for testing will be discussed with you and will be at your convenience). Breakfast will be provided after your testing session.

Each of the five testing days will investigate the effect of a different temperature and humidity on blood pressures and blood coagulation. The different conditions will be randomly allocated to you on each visit. The different test conditions are:

21°C with 40% humidity (normal room temperature and humidity)

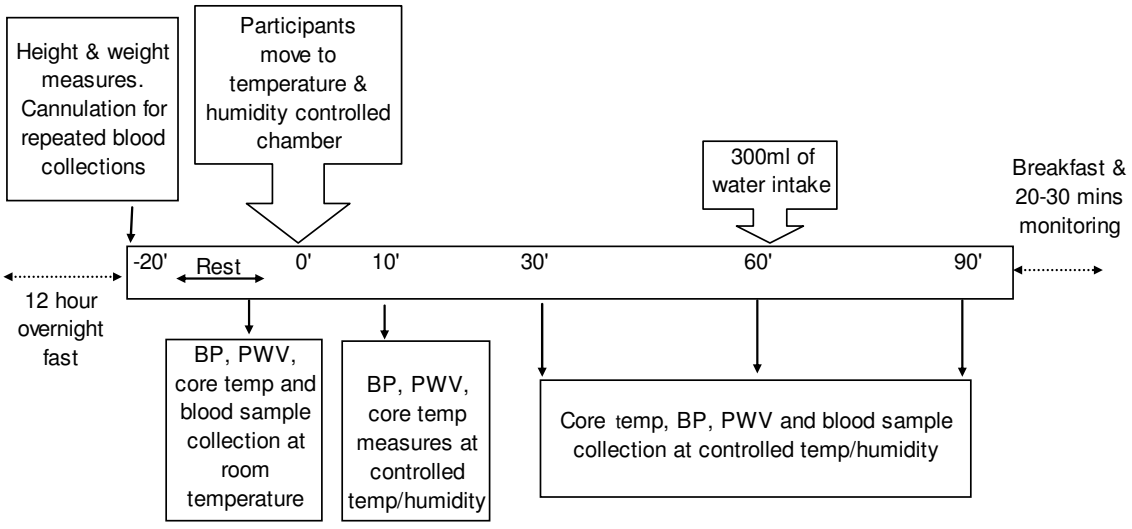
21°C with 80% humidity (normal room temperature and high humidity)

12°C with 40% humidity (cold temperature and normal humidity)

36°C with 40% humidity (hot temperature and normal humidity)

36°C with 80% humidity (hot temperature and high humidity)

The following figure shows a time-line representation of a typical testing day:



TEST DAY PROTOCOL (refer to the Figure shown above).

You will arrive after an overnight fast of 10-12 hours (water allowed) before the arranged test time. We will ask that you refrain from vigorous exercise, alcohol and fried foods in the 24 hours before your test time. We will also advise you to wear light long pants and bring a jumper or jacket regardless of the air temperature on the day of your testing.

Test procedures will be explained and you will have the opportunity to ask any questions you may have (you may also ask questions at any time during test procedures).

You will be weighed, and have your height measured.

You will then consume a glass of water (room temperature).

A cannula (a soft plastic tube that keeps the vein open for repeated sampling) will then be inserted in your anticubital vein (front of the elbow). This is the *one and only* needle you will receive during any testing day.

You will rest lying down for 10 minutes at normal room temperature then your body's core temperature will be measured, followed by blood pressure (using the traditional upper-arm method) and pulse wave velocity, followed by a blood sample collection via the cannula (*more details on these measures later*).

Upon completion of these initial tests, you will move to the climate controlled chamber. To determine your body's reaction to the change in conditions, core temperature, blood pressure and pulse wave velocity will be measured at 10, 30, 60 and 90 minutes. Blood samples will be taken via the cannula at 30, 60 and 90 minutes.

To prevent dehydration, you will be asked to consume 300ml of water at 60 minutes (room temperature), followed by the final round of testing (temperature, blood pressure, pulse wave velocity and blood samples) at 90 minutes.

After completion of testing at 90 minutes, you will be taken out of the climate controlled chamber and re-acclimatised.

Breakfast will then be provided to you and you will be monitored for any adverse signs and symptoms to the testing protocols for 20 to 30 minutes before leaving the lab.

WHAT MEASURES WILL WE USE?

All test procedures will be undertaken by trained, competent university researchers.

Central and peripheral blood pressures and an estimation of the flexibility (compliance) of your blood vessels will be measured. Peripheral blood pressure will be measured by the traditional upper-arm cuff technique, and central blood pressure by a painless, non-invasive technique called *Pulse Wave Velocity*, in which a pen-like device will be placed over the pulses in your wrist (radial artery pulse), neck (carotid artery pulse) and upper leg (femoral artery pulse). These measures take approximately 10 minutes per session.

Body core temperature will be measured with a tympanic (ear) thermometer using a new, disposable probe for each participant. Skin temperature will be measured at four sites (forehead, trunk, hand and foot) using sensors taped to the skin.

Blood Sampling. A total of 40mL of blood will be collected (approximately equivalent to 2 tablespoons) over the four blood collection points.

POTENTIAL RISKS AND DISCOMFORTS

Exposure to extremes of temperature and humidity:

Participants may experience some discomfort upon exposure to the test conditions of high heat, humidity, and cold. Blanket(s) will be provided to prevent shivering (while at 12°C condition). If participants show any signs of dizziness, hyper or hypothermia they will be removed from the climate controlled chamber immediately. Additionally, some participants prone to severe claustrophobia may feel uncomfortable in the climate controlled chamber. Its dimensions are approximately 2.5 x 5 metres with a high ceiling. If you believe this may be an issue for you, please discuss it with research team member.

Pulse Wave Velocity Measures:

This procedure requires the tonometer (pen-like device) to be placed on the wrist, neck, and the femoral artery at the top of the leg near the groin. It will be necessary to ask you to remove jewellery or adjust your garments to gain access to the three pulse sites.

Blood sampling:

Blood sampling is a low risk activity but participants should be aware that there are a number of minor complications that can result from cannulation and blood sampling. In order for participants to give informed consent to blood sampling they should read and understand the following:

Fainting: This is not common in healthy volunteers but can more commonly occur if participants are unwell or suffering from a viral infection such as a cold or 'flu. Participants who are extremely apprehensive about the procedure or the sight of blood may also be prone to fainting. If this does occur the participant will be laid down, and adequate ventilation with fresh air provided. A glass of cold water or cool cloth on the back of the neck often helps to alleviate the symptoms.

Nausea and Vomiting: Whilst a feeling of nausea is a relatively common response (especially in first time participants), vomiting is rare in adults as a response to blood sampling.

Bruising and Haematoma formation: Bruising is the most common post-procedure complication. The likelihood of bruising can be greatly diminished by applying pressure to the puncture site for 5 minutes after the completion of the procedure. Haematoma (bleeding under the skin to form a raised swelling) can also occasionally occur and is minimised with prolonged application of pressure to the site.

Embolism: this is very rare but can be caused by air, a thrombus, or fragment of a cannula breaking off and entering the venous system. To avoid air embolus, every effort will be made to have the system free of air. Needles will not be reinserted.

Phlebitis: an inflammation of the vein resulting from mechanical or chemical irritation or from an infection. Phlebitis will be avoided by carefully choosing the site for cannulation and using careful technique.

Infiltration: when fluid enters the tissue instead of the vein. Infiltration will be avoided by fixing the cannula firmly to the site with tape. If infiltration should occur, the cannula will be immediately removed and a cold compress applied.

Convulsions: Rare and usually only seen in participants who faint. These are normally minor in nature and last less than a minute. The procedures for this are the same as for fainting although, of course, care is taken to ensure that the participant does not harm themselves.

BENEFITS

The findings of this study will increase our understanding of the effects of acute changes in temperature and humidity on central and peripheral blood pressures and arterial compliance that may be associated with increased incidence of cardiovascular events such as heart attack and stroke in high-risk individuals. You will be provided with your baseline results (and your test results if you wish) that will give you an understanding of your vascular function at rest in normal room temperatures, and during test conditions.

CONFIDENTIALITY

All data will be treated in the strictest of confidence. The information that will be collected will only be used for the purpose of this project. Records identifying your identity will only be accessible by the principal investigators and will not be made publicly available. If the results of the trial are published, they will be presented as group data and your identity will remain confidential. If reference to a single individual is made, this will only be done using code numbers.

COST

Participation in this trial will not result in any costs for you and there is no payment for participation in this study.

PARTICIPATION

Your participation is entirely voluntary and you may withdraw at any time without prejudice.

WITHDRAWAL

If you choose to withdraw at any stage, you may request that any, or all, of your data collected for the purpose of this project be destroyed. Any such request will be complied with without prejudice.

RESULTS

The results of this study will be analysed and presented as group data only. A summary of results will be available at the end of the study and these can be mailed to you upon request.

CONTACTS

If you have a question about this study, or would like more information kindly contact:

Ms. Sibella King on (03) 6324 3688, email Sibella.King@utas.edu.au

The study (application number H0010849*) has been approved by the Tasmania Health and Medical Human Research Ethics Committee in accordance with the National Health and Medical Research Council's guidelines.*

If you have any concerns of an ethical nature or complaints about the manner in which the project is conducted, you may contact the Executive Officer of the Tasmanian Human Research Ethics Committee (Tasmania) Network on 62267479 or human.ethics@utas.edu.au. You will need to quote ethics reference number H0010849*.*

If you would like to participate in this study please complete and sign the accompanying consent form and return it to:

Ms. Sibella King

Locked Bag 1320

School of Human Life Sciences

University of Tasmania, LAUNCESTON TAS 7250

We look forward to the opportunity of working with you on this project

Thanking you in anticipation

PARTICIPANT INFORMATION SHEET – T2DM GROUP

The information sheet used for T2DM participants was identical to the Healthy Group sheet, except for the following changes:

INTRODUCTION AND AIM OF STUDY

Sudden exposure to hot or cold temperatures causes changes to the circulation with the aim to maintain the body's core temperature. Scientific literature suggests that this ability may be compromised in people living with chronic diseases such as type-2 diabetes mellitus (T2DM) – a condition associated with high blood sugar concentrations. Patients with T2DM are often at higher risk of developing hypertension (high blood pressure), cardiovascular disease and also impaired nervous system function (neuropathies). Cardiovascular autonomic neuropathy is one of the most common diabetes associated complications and results in impaired ability of the cardiovascular system to deal with challenges to the body such as exposure to environmental extremes.

Hypertension is considered a major risk factor for cardiovascular disease and death as it increases the work that the heart must perform to maintain adequate blood flow to the body. Traditionally, blood pressure is measured at the upper-arm using an inflatable cuff. However, recent research suggests that the measurement of aortic (central) blood pressure may be a better indicator of cardiovascular outcomes than the traditional upper-arm blood pressure method.

STUDY PURPOSE

Very little is known about the effect of sudden changes in climate (air temperature and humidity) on central blood pressure and blood coagulation in T2DM. Therefore, this study aims to determine the effect of heat, humidity and cold on cardiovascular function and blood coagulation, and to explore their association with the degree of neuropathy (if any) in people with stable T2DM.

INCLUSION AND EXCLUSION

To participate in this study you must be over 18 years of age living with type-2 diabetes. Your blood glucose concentrations should have been stable for the last 6 months. You should have no known vascular, kidney or liver disease. People taking regular insulin injections will not be eligible for this study. If you are medically unstable, as defined by recent (i.e. within last month) changes in dosage or medication for control of blood glucose and or blood pressure, you will not be eligible. People with resting blood pressure ≥160/100mmHg will be ineligible to take part in this study.

CARDIOVASCULAR AUTONOMIC NEUROPATHY TESTS

Two 2 brief tests will be performed only once; either at your final visit, or on a separate day after your final session. These tests do not need to be in the morning but do need to be performed 2 hours after eating. CAN tests assess the ability of your cardiovascular system to adjust rapidly to changes in posture and breathing patterns (which is controlled by your autonomic nervous system) and are conducted as follows:

- Firstly, your heart rate will be monitored for 30 seconds after lying down for five minutes. Then you will be asked to take a deep breath and hold your breath (the Valsalva manoeuvre) for a period of about 15 seconds. The amount of change in heart rate during these two conditions provides an indication of the presence or degree of autonomic dysfunction.

- For the second test, you will be required to lie on a special bed called a tilt table. Blood pressure and heart rate will be monitored while lying down and then immediately after the table is moved to a standing position. You will be secured to the table with straps to avoid any chance of falling. The degree of variation between the measurements taken while lying down and in a standing position gives an indication of autonomic nervous system function.

Overnight fast: 10 hours for T2DM participants.

Data collection time points: a 5 minute time point for data collection was included after entry to the climate chamber for each condition.

Water consumption to avoid dehydration: 250mL of water was consumed after 60 minute measures

Skin temperature measurement: Was taken using a dermal infrared thermometer

POTENTIAL RISKS AND DISCOMFORTS

Cardiovascular Autonomic Neuropathy (CAN) tests:
Risks associated with standardised non-invasive measures of CAN are minimal and testing is considered safe for people with diabetes. Some tests do, however, carry a small risk of an adverse event. While undertaking the Valsalva (breath holding) manoeuvre for 15 seconds, the pressure in your chest, eyes and head increases, then decreases to normal as you relax, therefore there is a small risk of eye damage. However, the risk is considered minimal as this pressure is similar to that experienced during many tasks of everyday life. During the postural CAN tests you will be secured to a tilt table with straps to avoid any chance of your moving or falling down. You will be monitored for comfort throughout all tests and you may stop any tests at any time you wish.

BENEFITS

The findings of this study will increase our understanding of the effects of sudden climate change (temperature and humidity) on central and peripheral blood pressures, arterial stiffness, and blood coagulation that may be associated with increased incidence of cardiovascular events such as heart attack and stroke in high risk people. All study participants will gain information about their, blood pressure, heart and nervous system function, and also their blood sugar and cholesterol status. Additionally at the end of the study, participants are eligible to participate in a free "lifestyle make-over" program, run by accredited Exercise Physiologists and Nutritionists, including exercise and nutritional counselling, a take-home exercise prescription and the opportunity to attend group exercise classes at UTAS.

ETHICS

The study (application number H0011347) has been approved by the Tasmania Health and Medical Human Research Ethics Committee in accordance with the National Health and Medical Research Council's guidelines.

INFORMED CONSENT FORM USED FOR HEALTHY AND T2DM PARTICIPANTS

1. I, _____ have read and understand the above information and I agree to take part in the study investigating the effects of acute changes in air temperature and humidity on vascular function and blood coagulation.

2. I have received an explanation of the nature, purpose, duration and foreseeable effects of the study and what is expected of me. The possible risks and benefits of the study have been explained to me. I was given time and opportunity to inquire about the trial and all my questions were answered to my satisfaction.

3. I am aware that the Tasmania Health and Medical Human Research Ethics Committee have subjected this study for review and have granted approval.

4. I understand that I will be required to visit the university on five occasions over a period of four to five weeks for approximately two and a half hours each time.

5. I understand that at each visit a cannula will be inserted in my forearm for repeated blood collection over each testing session and a total of about 40ml (approx. 2 tablespoons) of blood will be collected.

6. I understand that I am free to withdraw from the study at any time, without the need to justify my decision.

7. I agree that the results of the study may be published or presented, however my name and contact details will be kept entirely confidential.

8. I understand that this research will be conducted in accordance with the Declaration of Helsinki, NH&MRC Guidelines, and applicable privacy laws.

9. I voluntarily consent to participate in this study

_____ _____/_____/_____

Participant's Signature *Date*

Investigator Statement

I _____ have explained this study and the implications of participating in it to this volunteer and I believe that the consent is informed and that he understands the implications of participating in the study. The participant has consented to participate by his personally dated signature

_____ _____/_____/_____

Investigator's Signature *Date*

_____ _____/_____/_____

Witness *Date*

ELIGIBILITY QUESTIONNAIRE – HEALTHY GROUP

1. Name: _____

2. Date of birth: _____/_____/_____

3. Do you have any travel or holiday plans for the next 2 months ☐ yes ☐ no

3a. If so, when and for how long _____

4. Do you smoke ☐ yes ☐ no

5. Do you have a history of any of the following medical conditions?

Diabetes ☐ yes ☐ no

Hypertension ☐ yes ☐ no

CVD/ Heart disease ☐ yes ☐ no

Kidney or Renal ☐ yes ☐ no

Gastrointestinal ☐ yes ☐ no

Hepatic/Liver disease ☐ yes ☐ no

Any other condition(s)_____

6. Have you or other family members had/have a history of any of the above medical conditions? ☐ yes ☐ no

If yes, please complete the table below:-

Family member	**Condition**
Eg. Maternal grandmother	Hypertension ,maturity onset diabetes

7. Are you currently taking any antibiotics or other medication(s)? ☐ yes ☐ no

If yes, please complete the following table:-

Medication	**Dose**	**Start date**

7a. Are you currently taking any anticoagulant / antiplatelet medication ☐ yes ☐ no

(eg. Clopidogrel, aspirin, disprin, warfarin, clexane, ibuprofen)

If yes, please complete the table below:-

Medication	Dose	Start date

7b. Are you currently taking any vitamin or mineral supplements? ☐ yes ☐ no

e.g. Primrose oil, Echinacea, Berrocca, multivitamin, fish oil etc.

If yes, please complete the table below:-

Supplement	Dose	Reason for Supplement	Taken How Often?		
			once	occasionally	regularly

7c. If not currently taking any supplements, have you ever taken vitamin or mineral supplements? ☐ yes ☐ no

If yes, when did you stop? ☐ less than 4 weeks ago.

☐ more than 4 weeks ago.

8. Have you had blood samples taken before? ☐ yes ☐ no

If yes, did you had any problems i.e.: severe bruising, fainting ☐ yes ☐ no

9. Do you exercise regularly ☐ yes ☐ no

If **yes**, please complete the table below;-

Exercise Mode	Duration	Frequency
Eg. walking	30 minutes	3 times per week

10. Do you consume alcohol on a regular basis? ☐ yes ☐ no

If yes what type:

Beer ☐
Wine – Red or white ☐
Basic spirits ☐

10a. If yes, how often do you consume alcohol? _____

10b. If yes, how many drinks do you have when you drink?_____

11. What is your usual occupation/ working routine? *e.g. nurse/shift worker* or *bar attendant/8 hours, varied shifts*

12. Are you interested in participating in this study? ☐ yes ☐ no

13. Postal address:-

_____

14. Contact details. Phone BH _____

Phone AH _____

Mobile _____

Email _____

Preferred option for contact : phone / mobile / email (please circle)

if phone / mobile, best time to call: _____

Thank you for taking the time to complete this questionnaire,

please return it to us in the enclosed envelope.

Sibella King, PhD Candidate

Study Coordinator

ELIGIBILITY QUESTIONNAIRE - T2DM GROUP

The eligibility questionnaire for the T2DM group was identical to the Healthy questionnaire, except that the following questions were added:

- When were you diagnosed with Type 2 Diabetes? (years/months ago) _____
- How do you control your blood glucose? *eg. diet, medication*_____
- When did you last check your blood glucose? _____
- Do you know your usual blood pressure? ☐ yes ☐ no

  If so, what is it? _____

APPENDIX 3 – EXAMPLES OF DATA COLLECTION FORMS AND PROTOCOLS

An example of a test session data collection form from the T2DM study.

DATE: _____/_____/_____ ID#: _____

	OUTSIDE	LAB	CLIMATE CHAMBER
TEMPERATURE			
RELATIVE HUMIDITY			

TEST SESSION: 1 2 3 4 5

INTERVENTION PROTOCOL: **1** (21/40) **2** (21/80) **3** (12/40) **4** (36/40) **5** (36/80)

PW Op:_____ PWV PROXIMAL **mm** (CAROTID): _____ DISTAL RAD):_____ DISTAL (FEM):_____

BATHROOM VISIT? Y N

START TIMER WHEN ENTERED CLIMATE CHAMBER: TIME_____

	T1 / 0' (AMBIENT)	(10') +5 (start)	T2 (30') + 20 (start)	T3 (60') (+50 start)	T4 (90') (+1.20 start)	Recovery (after 5' rest)
$T_{Tympanic}$						
$T_{Forehead}$						
T_{Trunk}						
T_{Hand}						
T_{Foot}						
TSS						
C.C. TEMP/RH	**N / A**					
BLOODS TIME		**N / A**				
OBSERVATIONS			*Get glass water @~50'*			
AT 1:10' (70min) 2nd GLASS OF WATER (250mL)			**Time start**		**Time to Consume (s)**	

TIME EXIT CLIMATE CHAMBER: _____

POST RECOVERY MEASURES- END TIME: _____

An example of a pre-test questionnaire administered to all participants prior to every test session (this is the T2DM version):

Pre-test Questionnaire

DATE: _____/_____/_____ START TIME: _____

NAME: _____ ID#: _____ GENDER: M F

DOB: _____/_____/_____ HEIGHT: _____ MASS: _____ 1st glass of water (150 mL) ☐

Arrival Questions:-

1. When did you last have something to eat _____
(NB: if not in fasted state, cannot continue with data collection – make apt for another day)

2. When did you last have something to drink _____ (besides the 150mL)

3. What did you eat for dinner last night? _____

4. What was your last blood glucose reading (if applicable) _____

5. Did you have any alcohol last night? Y N

5.a. If yes, how many drinks & what type? _____

6. How did you get to today's session? _____

7. Have you done any unusual vigorous exercise in the past 24 hours? Y N

8. Did you take any medications this morning? Y N

8.a. If yes, what? _____

9. Did you bring any medications with you to take after the study? Y N

NOTES: _____

An example of a pre-test questionnaire administered to all participants prior to the CAN testing session, and a CAN data collection sheet (same format was used for both groups):

CARDIOVASCULAR AUTONOMIC TESTING

Name: _____ Id#_____ Date: _____

(*circle*) AH T2DM

When did you last have something to eat?_____

When did you last have something to drink?_____

How did you get to today's session? _____

Resting HR: 1)_____ 2)_____ AV) _____

Valsalva R-R Ratio Longest R-R (s) following breath release / shortest R-R (s) during 15s manoeuvre (Ziegler, 1991)

_____ms / _____ms = _____ (1st)

_____ms / _____ms = _____ (2nd)

_____ms / _____ms = _____ (AV)

Comments: _____

Postural Tilt R-R Ratio Longest R-R during beats 20-40 / Shortest R-R during beats 5-25 (all POST-tilt)(Ziegler, 1991)

_____ / _____ = _____ (1st)

BP Response to Tilting

PRE TILT (SUPINE) BP1 _____/_____ (1st)

BP2 _____/_____ (2nd)

BP _____/_____ (avg)

DURING TILT BP1 _____/_____ Immediately @60° (for 30s post tilt)

BP2 _____/_____ 1.30s (for 2min)

BP3 _____/_____ 2.30s (for 3min)

BP4 _____/_____ 3.30s (for 4min)

BP5 _____/_____ 4.30s (for 5min)

BP6 _____/_____ 5.30s (for 6min)

BP2 _____/_____ 6.30s (for 7min)

BP3 _____/_____ 7.30s (for 8min)

BP4 _____/_____ 8.30s (for 9min)

BP5 _____/_____ 9.30s (for 10min)

Avg BP (supine) – Lowest BP (tilted) (Ziegler 1991)

_____/_____ - _____/_____ = _____/_____

Δ systolic / Δ diastolic

"normal" = Systolic BP falls <10 mmHg after 30s

"abnormal" if falls ≥ 20 - 30 SBP OR ≥10 DBP mmHg 2-3mins after tilt (Wiemer, 2010)

Comment: _____

CAN TESTING PROTOCOLS

Valsalva R-R procedures:	Done
Suggest bathroom visit	
Ask if participant has any known eye (retinopathy) or heart conditions	
Explain procedures to participant – ensure they report any eye or chest pain, dizziness, or heart arrhythmias (heart 'skipping-a-beat' feeling)	
Supine rest 5-10 mins (with pillow)	
>5 min quiet rest - record resting HR with pulse oximeter and baseline BP x 2	
Attach ECG - ensure leads are taped and steady	
Show participant how to do Valsalva with own mouthpiece.	
Attach nose clip	
2 x Practice runs of maintaining 40mmHg for 15s for participant (watch for noise from flow meter leads). Take a full inspiration – expand chest cavity, then immediate forced expiration with open glottis; keep a tight seal on tubing with lips so air cannot escape; try not to allow cheeks to expand; must watch gauge to maintain pressure on 40mmHg for 15s, give 10s alert; 5 seconds to go, encourage where necessary At 15 s tell them to "release" strain suddenly and resume normal respiration, preferably without gasping	
Start recording baseline ECG ~40s-60s prior to inhale	
Make LabChart comment with participant code & test #. E.g. "05BW Valsalva 1"	
When ready – participant begins manoeuvre:	
Full inspiration *When reached maximum inspiration t*ell them to blow - Start timer – 15s!	
10 s alert: "5 s to go…" At 15 s tell them to "release" breath - Keep timer going	
At 35s on timer make comment on Chart "end test" for 20 s post-exhale, record ECG until 45-65s post exhale then stop test and save data.	
Remove nose clip, wait ~3-5 mins (HR must reach steady state, within 5bpm resting value) and repeat test	
Pack away flow meter & cords in prep for tilt tests	

Tilt R-R & blood pressure procedures:	Done
5-10 mins supine rest with pillow – Feet touching baseboard	
Attach BP cuff L arm, attach BP monitor to bed	
Explain procedures – ensure participant reports any dizziness, nausea, arrhythmias etc	
Securely & comfortably attach Velcro straps	
Record baseline ECG 30 – 60 s	
Take/record baseline BP -(rest ~1 min)- take/record another baseline BP (average)	
Write in LabChart comment "upright"	
Perform tilt to ~60° – START TIMER!	
Enter comment "upright" when at 60° (to mark the beginning of the test - for analysis)	
SIMULTANEOUSLY Take BP (for 30s post-tilt reading) + record	
Take further BPs at 1 min intervals for 10 mins (up to 9.30s on timer) + record	
Make comment "end test" on LabChart when finished last BP record	
Record tilted EGC further 30s and stop recording	
Return bed to supine position	
Test finished – Save data	
Check participant feeling well – observe 10-20 mins, give snack / drink if required - allow participant to leave	

APPENDIX 4 – THERMAL SENSATION SCALE

-4	Unbearably cold
-3.5	
-3	Very cold
-2.5	
-2	Cold
-1.5	
-1	Cool
-0.5	
0	Neutral (Comfortable)
+0.5	
+1	Warm
+1.5	
+2	Hot
+2.5	
+3	Very hot
+3.5	
+4	Unbearably hot

From (ASHRAE, 2010)