Exercise Central Haemodynamics: Physiology and Clinical Relevance in Patients with Type 2 Diabetes Mellitus

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Statements and declarations

Declaration of originality

This thesis contains no material that has previously been accepted for a degree or diploma by the University of Tasmania, or any other institution, except by way of background information and of which is duly acknowledged in the thesis. To the best of my knowledge and belief, this thesis contains no material that has previously been published or written by another person, except where due acknowledgement is made in the text of the thesis, nor does this thesis contain any material that infringes copyright. I have acknowledged, where appropriate, the specific contributions made by my co-authors of published and submitted manuscripts.

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Statement of ethical conduct

All research associated with this thesis abides by the International and Australian codes of human and animal experimentation, and full ethical approval from relevant institutions was obtained for all studies outlined in this thesis. All individual participants provided written informed consent for involvement in the respective research studies.

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Rachel ED Climie July 2015

Publications by the author and statement of co-author contribution to papers contained within this thesis

The following papers are incorporated into the Chapters of this thesis and were either published or submitted for publication in peer reviewed scientific journals during the course of candidature. The papers appear as they were published (or submitted) with minor modifications to fit in context with this thesis.

Chapter 3

Climie RED, Nikolic SB, Otahal P, Keith LJ, Sharman JE. Augmentation index and arterial stiffness in patients with type 2 diabetes mellitus. *Artery Research*, September 2013; 7:194-200.

Author contributions

Climie RED – Data collection, data analysis and interpretation and manuscript preparation
Nikolic SB – Data collection and critical manuscript revision
Otahal P – Data analysis and critical manuscript revision Keith LJ – Data collection and critical manuscript revision
Sharman JE – Study conception and design and critical manuscript revision

Chapter 4

Climie RED*, Moran C*, Callisaya M, Blizzard L, Sharman JE, Venn A, Phan TG, Beare R, Forbes J, Blackburn NB, Srikanth V. Abdominal obesity and brain atrophy in type 2 diabetes mellitus. *PloS One*, November 2015; 10: e0142589. *Joint first authors.

Author contributions

Climie RED – Data analysis and interpretation and manuscript preparation
Moran C – Data analysis and interpretation and manuscript preparation
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Blizzard L – Supervision of statistical analysis and critical manuscript revision
Sharman JE – Data interpretation and critical manuscript revision
Venn A – Study design, data interpretation and critical manuscript revision
Phan TG – Image analysis, data interpretation and critical manuscript revision

Beare R – Development and supervision of image analysis and critical manuscript revision
Forbes J – Study design and critical manuscript revision
Blackburn NB – Genotyping critical manuscript revision
Srikanth V – Study concept, design and supervision, data interpretation and critical manuscript revision

Chapter 5

Climie RED, Srikanth V, Beare R, Keith LJ, Fell J, Davies JE, Sharman JE. Aortic reservoir characteristics and brain structure in people with type 2 diabetes; a cross sectional study. *Cardiovascular Diabetiology*, October, 2014; 2014;13.1:143.

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Climie RED – Data collection, data analysis and interpretation and manuscript preparation
Srikanth V – Study conception and design and critical manuscript revision
Beare R – Data analysis and critical manuscript revision
Keith LJ – Data collection and critical manuscript revision Fell J – Critical manuscript revision
Davies JE – Data analysis and critical manuscript revision
Sharman JE – Study conception and design and critical manuscript revision

Chapter 6

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Davies JE – Data analysis and critical manuscript revision
Sharman JE – Study conception and design and critical manuscript revision

Chapter 7

Climie RED*, Picone DS*, Keske MA, Sharman, JE. Brachial-to-radial systolic blood pressure amplification in patients with type 2 diabetes mellitus. *Journal of Human Hypertension*, October 2015; 10.1038/jhh.2015.101. *Joint first authors.

Author contributions

Climie RED – Study conception and design, data collection, data analysis and interpretation, manuscript preparation

Picone DS – Data collection, data analysis and interpretation and critical manuscript revision

Keske MA – Data analysis and interpretation and critical manuscript revision

Sharman JE – Study conception and design and critical manuscript revision

Appendix I

Climie RED*, Picone DS*, Ahuja KD, Keske MA, Sharman JE. Brachial-to-radial systolic blood pressure amplification: implications of age and estimated central blood pressure from radial tonometry. *Journal of Hypertension*, April 2015; 33.9:1876-1833. *Joint first authors.

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Climie RED – Study conception and design, data collection, data analysis and interpretation and critical manuscript revision

Picone DS – Data collection, data analysis and interpretation and manuscript preparation

Ahuja KD – Data analysis and critical manuscript revision

Keske MA – Data analysis and interpretation and critical manuscript revision

Sharman JE – Study conception and design and critical manuscript revision

Additional publications that do not form part of the thesis

The following publication in a peer reviewed scientific journal arose from the candidature and whilst related, does not form part of the primary thesis.

Climie RED*, Schultz MG*, Sharman JE. Ambulatory and central haemodynamics during progressive ascent to high-altitude and associated hypoxia. Journal of Human Hypertension, January, 2014; 28. *Joint first authors.

Author contributions

Climie RED – Study conception and design, data collection, data analysis and interpretation, manuscript preparation

Schultz MG – Study conception and design, data collection, data analysis and interpretation, manuscript preparation

Sharman JE – Study conception and design and critical manuscript revision

We, the undersigned agree with the above stated contributions for each of the above published peer reviewed manuscripts contained within this thesis:

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Abstracts, presentations and awards at scientific conferences that relate to this thesis

The following abstracts relate specifically to this thesis and were presented at national and/or international scientific conferences during the period of candidature.

Climie RED, Moran C, Callisaya M, Blizzard L, Sharman JE, Venn A, Phan TG, Beare R, Srikanth V. Type Diabetes Mellitus amplifies the adverse impact of abdominal obesity on brain atrophy. European College of Sports Science Conference. **European College of Sports Science-Exercise and Sports Science Australia Research Exchange winner oral presentation**. Amsterdam, Netherlands, July 2014.

Climie RED, Moran C, Callisaya M, Blizzard L, Sharman JE, Venn A, Phan TG, Beare R, Srikanth V. Type Diabetes Mellitus amplifies the adverse impact of abdominal obesity on brain atrophy. International Society of Hypertension Pulse of Asia Symposium **Poster presentation and winner**, Athens, Greece, June 2014.

Climie RED, Srikanth V, Keith LJ, Davies JE, Sharman JE. Exercise-induced albuminuria is independently related to exercise aortic reservoir function in patients with type 2 diabetes mellitus. 6th Biennial Scientific Conference, Exercise and Sports Scientist Australia. **Aspire Academy Oral Finalist**, Adelaide, Australia, April 2014.

Climie RED, Moran C, Callisaya M, Blizzard L, Sharman JE, Venn A, Phan TG, Beare R, Srikanth V. Type Diabetes Mellitus amplifies the adverse impact of abdominal obesity on brain atrophy. 6th Biennial Scientific Conference, Exercise and Sports Scientist Australia. **Aspire Academy Young Investigator in Exercise and Health Oral Finalist and winner**, Adelaide, Australia, April 2014.
At this conference the author of this thesis was also awarded the best overall presentation. The prize was an invitation to present at the European College of Sports Science Conference in Amsterdam.

Climie RED, Srikanth V, Keith LJ, Davies JE, Sharman JE. Exercise-induced albuminuria is independently related to exercise aortic reservoir function in patients with type 2 diabetes mellitus. High Blood Pressure Research Council of Australia annual scientific meeting. **Moderated poster student finalist**, Melbourne, Australia, December 2013.

Climie RED*, Picone DS*, Ahuja KD, Keske M, Sharman JE. Brachial-to-radial systolic blood pressure amplification is significantly blunted in patients with type 2 diabetes mellitus: upper limb haemodynamics have an influential role. High Blood Pressure Research Council of Australia annual scientific meeting. Poster presentation, Melbourne, Australia, December 2013. *Joint first authors

Climie RED, Srikanth V, Keith LJ, Davies JE, Sharman JE. Exercise-induced albuminuria is independently related to exercise aortic reservoir function in patients with type 2 diabetes mellitus. ARTERY 13 conference. Moderated poster presentation, London, United Kingdom, October 2013.

Climie RED, Nikolic SB, Keith LJ, Sharman JE. High output, low resistance haemodynamics are associated with augmentation index in patients with type 2 diabetes mellitus. ARTERY 12 conference. Moderated poster presentation, Vienna, Austria, October 2012.

Climie RED, Srikanth V, Beare R, Keith LJ, Davies JE, Fell JW, Sharman JE. Light exercise blood pressure and central haemodynamics are independently related to brain atrophy and white matter lesions. International Society of Hypertension conference. Oral presentation, Sydney Australia, September 2012.

Climie RED, Srikanth V, Beare R, Keith LJ, Davies JE, Fell JW, Sharman JE. Light exercise aortic reservoir function predicts brain atrophy in patients with type 2 diabetes. Australian Diabetes Educators Association conference. Oral presentation, Gold Coast, Australia, August 2012.

Abstract

Patients with type 2 diabetes mellitus (T2DM) are at an increased risk of target organ damage, compared to non-diabetic individuals. The reason for this remains to be fully elucidated, however, high blood pressure (BP) or hypertension (measured in the clinic from the brachial artery) is likely to play a contributory role. Recent evidence suggests that central (or aortic) BP and related haemodynamics may be more closely related to cardiovascular risk than other surrogate markers (including brachial BP). Furthermore, studies have shown that the BP response to light to moderate intensity exercise is predictive of cardiovascular events and mortality, independently of resting brachial BP and other cardiovascular risk factors. Despite this, the association between light to moderate exercise central haemodynamics and target organ damage in patients with T2DM has never been assessed. Therefore, the broad aim of this thesis was to examine the haemodynamic differences and consequent target organ damage between patients with T2DM and non-diabetic controls under resting conditions as well as in response to light to moderate intensity exercise.

In study 1 (*Chapter 2 Part II*) the difference between central and brachial systolic BP in patients with T2DM compared to non-diabetic controls was examined by systematic review and meta-analysis of 17 individual studies (including 2,711 patients with T2DM and 10,460 non-diabetic controls). The main finding from this study was that despite patients with T2DM having elevated central haemodynamics indicative of systolic stress, there was no difference in the level of central to brachial systolic BP or pulse pressure amplification. However, the level of amplification differed throughout T2DM duration. Furthermore, large variation in systolic BP amplification was observed in both patients with T2DM (range= 2.0 – 16.6 mmHg) and non-diabetic controls (range= 1.0 – 16.1 mmHg), suggesting that risk related to central systolic BP cannot be estimated simply from a measure of brachial BP.

Study 2 (*Chapter 3*) examined central haemodynamics in order to determine the association between aortic stiffness and augmentation index (a purported surrogate marker of aortic stiffness) in 53 patients with T2DM and 53 non-diabetic controls. This study showed that despite patients with T2DM having increased aortic stiffness, there was no difference in augmentation index compared to non-diabetic controls ($p=0.184$), and augmentation index was not related to aortic stiffness in either group ($p>0.05$ for both). These findings suggest that augmentation index should not be used as a marker of aortic stiffness in either individuals with

or without T2DM.

Study 3 (*Chapter 4*) sought to determine an explanation as to why patients with T2DM have abnormal brain structure (specifically grey matter atrophy) by examining the effect of potential mediators (including brachial BP, abdominal obesity and physical activity) on the association between T2DM and grey matter atrophy in 258 patients with T2DM and 302 non- diabetic controls. This study found that the association between T2DM and grey matter atrophy was substantially attenuated by abdominal obesity (32%) above and beyond other cardiovascular risk factors including resting brachial BP and, therefore, abdominal obesity may be a target for interventions that aim to maintain brain structure in patients with T2DM. This was an analysis of a convenience sample in which exercise central haemodynamic data was not available and, therefore, the association between these parameters and grey matter atrophy was unable to be determined.

In study 4 (*Chapter 5*) exercise central haemodynamics (including aortic reservoir pressure and excess pressure) were measured in both patients with T2DM (n=37) and non-diabetic controls (n=37) and the association of these variables with grey matter atrophy was examined. This study found that excess pressure integral was significantly elevated in patients with T2DM (compared to non-diabetic controls) both at rest and in response to exercise ($p<0.001$ for both); however, aortic stiffness was the strongest independent predictor of grey matter atrophy ($p=0.036$). In non-diabetic controls, excess pressure integral was independently related to grey matter atrophy ($p=0.043$), thus providing the first evidence that excess pressure may be a novel cardiovascular risk factor related to brain atrophy and a useful clinical marker to identify individuals at risk related to BP in future.

Study 5 (*Chapter 6)* sought to determine the association between exercise central haemodynamics (including excess pressure integral) and kidney function (both at rest and in response to the stress induced by light to moderate intensity exercise) in 39 patients with T2DM compared to 39 non-diabetic controls. In this study, exercise induced-albuminuria was observed in patients with T2DM in response to light to moderate intensity exercise. Importantly, excess pressure measured during exercise was associated with exercise-induced albuminuria in patients with T2DM, independently of resting brachial BP ($p=0.003$), therefore, suggesting that exercise excess pressure may be an important marker to identify individuals at increased risk related to abnormal renal function.

Finally, in study 6 (*Chapter 7*) the effect of abnormal haemodynamics (in particular brachial to radial systolic BP amplification) on the accuracy of central BP estimated using radial applanation tonometry, was examined in 20 patients with T2DM and 20 non-diabetic age-matched controls at rest and in response to light to moderate exercise. During the candidature of this thesis, radial applanation tonometry was the most widely accepted non-invasive method to estimate central BP. This study found that resting radial systolic BP was significantly higher than brachial systolic BP in both patients with T2DM (136 ± 19 vs 127 ± 17 mmHg) and non-diabetic controls (135 ± 12 vs 121 ± 11 mmHg; $p<0.001$ for both). Furthermore, in both groups, brachial to radial systolic BP amplification resulted in significant underestimation in central BP using radial tonometry. The exercising results were similar to the resting data and are presented in *Appendix II,* as they did not form part of the final submitted manuscript (*Chapter 7*). These findings have significant implications for the refinement of methods that determine central BP non-invasively.

Overall, the work contained in this thesis supports that patients with T2DM have abnormal central haemodynamics compared to non-diabetic controls at rest, however, for the first time has shown that these patients have abnormal central haemodynamics in response to light to moderate exercise. Furthermore, this research program has shown that exercise central haemodynamics are related to target organ damage in patients with T2DM, independently of resting brachial BP and other cardiovascular risk factors. Finally, this research highlights the necessity to refine the methods that estimate central BP non-invasively. Taken together, this thesis provides novel information and represents a significant advancement in understanding the relationship between exercise central haemodynamics and target organ damage in patients with T2DM.

Dedication

This thesis is dedicated to my partner Pierre Equipart, for his patience, unwavering support and eternal love; and to my parents, Diana Dahlberg and Richard Climie who have always provided guidance and encouragement throughout my education.

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‘You can never cross the ocean until you have the courage to lose sight of the shore.’

Christopher Columbus

Chapter 1. Introduction

In Australia, type 2 diabetes mellitus (T2DM) is the fastest growing chronic disease and if current trends continue, an estimated 3.3 million Australians will have T2DM by 2031[1]. Patients with T2DM are at an increased risk of target organ damage, that is, they have a greater propensity to develop undesirable changes in the heart and arterial system, brain, eyes and kidneys (target organ damage). The causes for these adverse changes remain to be fully elucidated, however, high blood pressure (BP) or hypertension, is likely to play a contributory role. In clinical practice, BP is typically measured from the brachial artery of the upper arm (brachial BP). Importantly, over the last decade, increasing evidence has emerged suggesting that central (or aortic) BP and associated haemodynamic indices may be more closely related to cardiovascular risk than other surrogate markers (such as brachial BP). Patients with T2DM have generalised vascular irregularities[2-4] that may predispose to abnormal central haemodynamics, which may in turn be associated with accelerated target organ damage.

Although resting BP indices are clinically important, haemodynamic responses to moderate intensity exercise may have stronger prognostic value than resting BP in terms of cardiovascular risk[5], suggesting that pathophysiological insights may be gained from exercise haemodynamics beyond that of resting conditions. This is likely because individuals spend a large proportion of their day ambulatory[6] (doing some form of light to moderate physical activity) and, thus, the BP response to light to moderate intensity exercise may be more akin to the chronic BP loading that occurs during normal daily activity[7]. Currently there is little evidence regarding the association between light to moderate intensity exercise central haemodynamics and target organ damage in patients with T2DM. Understanding this association may lead to better methods for detection and diagnosis as well as more appropriate and targeted treatment strategies in this population. Therefore, the broad aim of this thesis was to examine the haemodynamic differences between patients with T2DM and non- diabetic controls by examining the role of exercise central haemodynamics (in addition to that of rest) and their relation to target organ damage.

Chapter 2 – Review of Literature, Part I of this thesis provides an overview of the physiology and clinical importance of the abnormal central haemodynamics in patients with T2DM, and describes what is known regarding the associations between central haemodynamics and target organ damage in this population. *Part I* of the *Review of Literature* also highlights the importance of light to moderate intensity exercise to unmask haemodynamic abnormalities that may otherwise not be evident under resting conditions. Due to the limited available literature regarding the difference between central and brachial systolic BP in patients with T2DM compared

to non-diabetic controls, *Part II* of the *Review of Literature* is a synthesis of the literature currently available in this field. This systematic review and meta-analysis of 17 original studies (including data from 2,711 patients with T2DM and 10,460 non- diabetic controls) found that there is no difference in the level of central to brachial systolic BP (or pulse pressure) amplification between patients with T2DM and non- diabetic controls, despite patients with T2DM having elevated central haemodynamics indicative of increased systolic stress. Importantly, this study demonstrated that substantial variation in amplification exists in both groups, and that the difference in the magnitude between the groups increased as the duration of T2DM increased. Thus, risk related to BP cannot be definitively determined based on brachial BP alone.

In *Chapters 3* and *7,* central haemodynamic parameters were examined in patients with T2DM compared to non-diabetic controls in order to provide an insight into the potential causative influences of the accelerated cardiovascular-related target organ damage associated with T2DM. The study presented in *Chapter 3* aimed to examine the cardiovascular and clinical determinates of augmentation index (AIx) including arterial stiffness, in 53 patients with T2DM (aged 61±8 years, 51% male) and 53 matched non-diabetic controls (aged 58±6 years, 51% male). The study hypothesis was that arterial stiffness would be significantly elevated in patients with T2DM but would not be related to AIx. AIx is a marker of left ventricular afterload and purported to be a measure of systemic arterial stiffness however, despite patients with T2DM having significantly increased arterial stiffness, there was no difference in AIx compared to non-diabetic controls. This study showed that the factors contributing to AIx differ between patients with T2DM and non-diabetic controls, and that AIx is not related to regional arterial stiffness in patients with T2DM. The results from this study were published in *Artery Research* in 2013.

In *Chapter 4,* the adverse association between T2DM and target organ damage (namely brain structure assessed via magnetic resonance imaging) was examined in a cross-sectional study of 258 patients with T2DM (aged 67±7 years, 62% male) and 302 non-diabetic control participants (aged 72±7 years, 53% male). The aim of this study was to determine the effect of potential mediators (including abdominal obesity and conventional brachial BP) and the influence of exercise on the T2DM-brain atrophy (grey matter volume) relationship. The hypothesis was that the association between T2DM and grey matter volume would either be modified or mediated by measures of obesity or physical inactivity. This study found that the association between T2DM and grey matter volume was substantially attenuated by increased abdominal obesity alone. The

findings from this study were published in *PLos ONE* in 2015. A limitation of this study however, was that the data used was from an existing dataset in which exercise central haemodynamics were not recorded and, therefore, the association between these parameters and brain atrophy was unable to be determined. Therefore, this line of enquiry was interrogated in the studies presented in *Chapters 5* and *6,* where exercise central haemodynamics were recorded and their relationship to target organ damage (including grey matter volume) in patients with T2DM was assessed.

The aim of the study presented in *Chapter 5* was to determine the associations between central haemodynamics and brain structure at rest and during exercise in 37 patients with T2DM (aged 63±9 years, 47% male) and 37 non-diabetic controls (aged 52±8 years, 51% male). It was hypothesised that central haemodynamics would be significantly elevated in patients with T2DM at rest and during exercise and that exercise central haemodynamics would be associated with adverse brain structural defects in both patients with T2DM and non-diabetic individuals. In this study, aortic reservoir characteristics were also assessed. The aortic reservoir pressure paradigm is a novel approach to analysing arterial pressure waveforms and suggests that the aortic pressure waveform can be separated into an aortic reservoir pressure component, representing proximal aortic volume; and an excess pressure (P_{excess}) component, representing excess left ventricular work that is analogous to left ventricular flow[8, 9]. In patients with T2DM, resting aortic stiffness was inversely related to brain atrophy (grey matter volume), whilst in non-diabetic participants, resting P_{excess} was inversely associated with grey matter atrophy. In opposition to the study hypothesis, the association between exercise central haemodynamics and brain structure was not enhanced compared to resting data. The findings from this study suggest that central vascular mechanisms underlying structural brain changes may differ between individuals with and without T2DM and were published in *Cardiovascular Diabetiology* in 2014.

The study presented in *Chapter 6* is an extension of that presented in *Chapter 5* performed on the same study population but with an expanded exercise protocol focusing on exercise-induced albuminuria. The aim of this study was to determine the associations between resting and exercise central haemodynamics (including the aortic reservoir characteristics) with kidney function assessed at rest and in response to light to moderate exercise in 39 patients with T2DM (aged 63±9 years; 49% male) compared to 39 non-diabetic controls (aged 53±9 years; 51% male). The study hypothesis was that firstly, exercise-induced albuminuria would be more pronounced in patients with T2DM compared with non-diabetic controls and secondly, exercise P_{excess} would be

independently related to exercise-induced albuminuria. Indeed, this study demonstrated that light to moderate intensity exercise (similar to that of normal daily activity) induced albuminuria in patients with T2DM, but not in non-diabetic controls. This finding is extremely novel, as previous studies have only measured exercise-induced albuminuria in response to maximal exercise, however, this study showed that even light to moderate intensity exercise was enough to induce renal abnormalities in patients with T2DM. Importantly, P_{excess} was associated with exercise-induced albuminuria in patients with T2DM, independently of known risk factors associated with albuminuria, including resting brachial BP. For the first time, these novel findings highlight the potential clinical significance of aortic reservoir characteristics in a cohort of patients with T2DM and suggest that P_{excess} could be important for appropriate renal function in this population. The results from this study were published in the *American Journal of Physiology – Heart and Circulatory Physiology* in 2015.

During the candidature of this thesis, the most widely accepted method for estimating central BP non-invasively was via radial applanation tonometry[10]. Using this method, central BP is estimated by applying a validated generalised transfer function[11] to the radial pressure waveform, which is calibrated with brachial systolic and diastolic BP. However, the arterial tree is not uniform in elastic properties and becomes increasingly stiffer further from the heart and results in the pressure waveform being amplified (increase in systolic BP) as it moves towards the periphery. Currently there is no data regarding the degree of systolic BP amplification along the forearm (i.e. from the brachial to radial artery) in patients with T2DM, and any amplification is largely ignored when central systolic BP is estimated via radial applanation tonometry. By failing to account for brachial to radial systolic BP amplification, calibrating the radial pressure waveform with brachial systolic BP may result in systematic underestimation of central systolic BP. Indeed, the vascular irregularities in patients with T2DM may influence this further, but this has never been examined before.

The study presented in *Chapter 7* was undertaken at the same time as the studies presented in *Chapters 5* and *6*, and aimed to determine the magnitude of brachial to radial systolic BP amplification (Bra-Rad-SBP_{Amp}) and the effect of Bra-Rad-SBP_{Amp} on estimated central systolic BP in 20 patients with T2DM (aged 64±8 years, 50% male) and 20 non-diabetic age-matched controls (aged 60±8 years, 50% male). The study hypotheses were firstly, that Bra-Rad-SBP_{Amp} would be elevated in patients with T2DM compared to non-diabetic controls and secondly, Bra-

Rad-SBP_{Amp} would result in underestimation in central systolic BP determined via radial applanation tonometry. In opposition to the first study hypothesis, Bra-Rad-SBP_{Amp} was significantly blunted in patients with T2DM compared to non-diabetic controls. However, regardless of disease status, Bra-Rad-SBP_{Amp} resulted in significant underestimation of central systolic BP. These findings have significance for how central BP is estimated non-invasively and for the implementation of central BP into clinical practice. This manuscript was published in the *Journal of Human Hypertension* in 2015.

The study presented in *Chapter 7* formed part of a larger study that was performed in a healthy ageing population (*Appendix I*). In *Appendix I*, Bra-Rad-SBP_{Amp} was examined in 40 healthy younger (aged 28±5 years, 50% male) and 20 healthy older individuals (aged 60±8 years, 50% male) to determine the magnitude and effect of ageing on Bra-Rad-SBP_{Amp} and the effect of Bra-Rad-SBP_{Amp} on estimated central systolic BP. The findings from the study presented in *Appendix I* were published in the *Journal of Hypertension* in 2015 by the author of this thesis. In this larger study, the effect of light to moderate intensity exercise on Bra-Rad-SBP_{Amp} was also examined. The findings from the comparison between patients with T2DM and non- diabetic controls were similar to those at rest and are presented in *Appendix II*, as they did not form part of the final submitted manuscript presented in *Chapter 7*.

Overall, this series of original research projects has made several novel contributions to the literature. Firstly, this research has shown that there is large variation in central to brachial systolic BP amplification in patients with T2DM and that the magnitude of amplification differs substantially throughout disease progression. Thus, risk related to elevated central BP may not be adequately assessed based on a measure of brachial BP in patients with T2DM. Secondly, the research contained in this thesis has confirmed that patients with T2DM have abnormal central haemodynamics, and for the first time has shown that these adverse changes contribute to altered systolic BP amplification down the forearm. Moreover, systolic BP amplification contributes significantly to underestimation of central BP via radial applanation tonometry, therefore, highlighting the necessity to refine the methods that determine central BP non-invasively whereby the influence of Bra-Rad-SBP_{Amp} is minimised. Additionally, this research has shown that the central haemodynamic response to exercise is altered in patients with T2DM and that exercise central haemodynamics are related to exercise-induced albuminuria, independently of resting haemodynamic measures in this population. Finally, this research program highlights the potential

clinical significance of aortic reservoir characteristics in relation to target organ damage in patients with T2DM.

Thesis aim

The broad aim of this thesis was to examine the relation of resting and exercise central haemodynamics with target organ damage among patients with type 2 diabetes mellitus compared with non-diabetic healthy controls.

Chapter 2 Part I. Review of literature

This chapter includes two sections; *Part I* reviews the physiology and clinical importance of central haemodynamics and their relation to target organ damage in patients with type 2 diabetes mellitus; *Part II* is a systemic review and meta-analysis of the currently available literature on central to brachial systolic blood pressure amplification in patients with type 2 diabetes mellitus compared to non-diabetic controls.

2.1.1 Type 2 diabetes mellitus in Australia

Type 2 diabetes mellitus (T2DM) is the fastest growing chronic disease in Australia and everyday, 280 people develop the disease. T2DM currently affects an estimated 1.5 million people and if trends continue an estimated 3.3 million people will have T2DM by 2031[1]. Patients with T2DM have greater propensity to develop adverse structural and functional changes in the heart and arterial system, brain, kidneys and eyes (termed target organ damage), which predisposes these individuals to increased risk of cardiovascular disease, dementia, nephropathy and retinopathy. Indeed, cardiovascular disease is the leading cause of mortality in patients with T2DM[12] and the risk of an acute myocardial infarction is five times higher in this population compared to non-diabetic individuals[13]. T2DM is a strong independent predictor of cerebro-vascular disease and stroke[14, 15], and the risk of developing dementia is increased by two-three fold in these patients[16]. The number of diabetics requiring dialysis or a kidney transplant has dramatically risen in Australia over the last few decades, largely due to the increased prevalence of T2DM[12]. Moreover, the prevalence of diabetic retinopathy is nearly four times higher in patients with established T2DM compared to those with newly diagnosed diabetes[17]. Although the pathogenesis of these adverse changes is largely influenced by genetic, metabolic and lifestyle factors, the underlying pathophysiological causes remain to be elucidated.

2.1.2 Hypertension in patients with type 2 diabetes mellitus

Cardiovascular disease accounts for 80% of all deaths in patients with T2DM[18], with high blood pressure (BP), or hypertension, affecting a reported 70% of this population[19]. The development of T2DM is nearly 2.5 times more likely in individuals with pre-existing hypertension[20] and hypertension is more prevalent in patients with T2DM compared to non-diabetic individuals[21], suggesting that these two conditions commonly coexist. Moreover, hypertension is associated with an increased risk of myocardial infarction, stroke, kidney disease and mortality[22, 23] (figure 2.1.1), which can lead to an increased cardiovascular burden in hypertensive patients with T2DM. The prevalence of masked hypertension (normal resting office BP but elevated 'out-of-office' BP)[24, 25] and white coat hypertension (elevated office BP but normal 'out-of-office' BP)[26] are higher in patients with T2DM compared to non-diabetic individuals. Similarly, the prevalence of a hypertensive response to exercise, which is a known risk factor for cardiovascular events and future onset hypertension[27], is greater in patients with T2DM compared to those without[28, 29]. Altogether these observations suggest that an individual's risk related to hypertension is elevated in the presence of T2DM. Although hypertension is largely influenced by genetic, lifestyle and

environmental factors, the underlying pathophysiological mechanisms for the development of hypertension remain unclear.

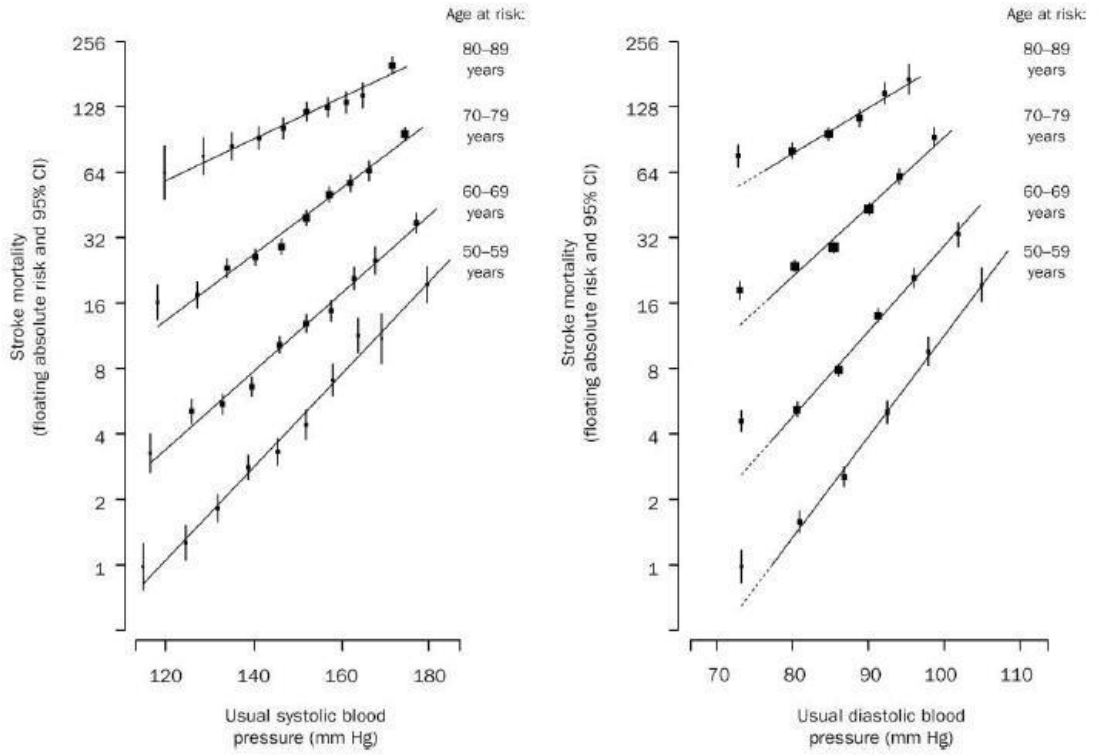


Figure 2.1.1. The association between systolic (left) and diastolic blood pressure (right) and mortality due to stroke for each age category, increasing by decade[22].

In clinical practice, BP is typically measured from the brachial artery of the upper arm (brachial BP)[30] and although raised brachial systolic BP is a strong risk factor for cardiovascular disease[22], over the last decade increasing evidence[31-34] has emerged to suggest that central (aortic) BP and related haemodynamics may be more closely related to cardiovascular risk than other surrogate markers such as brachial BP. The structure of the arterial tree supports a significant amplification in systolic BP as the forward travelling pressure wave generated by left ventricular contraction propagates from the central elastic vessels towards the smaller and more muscular peripheral vessels. Indeed, central systolic BP is generally lower than brachial systolic BP but may vary considerably between individuals (up to 33 mmHg)[2, 4] and depends on a number of variables including age, sex, height and heart rate[35-37]. Since the left ventricle encounters aortic pressures with each cardiac ejection and the aortic pressures are the primary determinants of coronary perfusion, central BP may be better correlated with the chronic loading occurring in the heart, aorta and central arteries (coronary and cerebral) and the central organs (brain, kidneys and the eyes) than brachial BP[34, 38, 39]. Central BP indices are predictive of mortality in

high-risk individuals independently of brachial BP[33, 34] and central BP responds differently compared to brachial BP to certain BP lowering (hypotensive) medications[40, 41]. Indeed the clinical importance of elevated central systolic BP has recently been highlighted and cut off values that denote 'central hypertension' (≥130 mmHg) have been developed[42]. However, central BP may be influenced by individual physiological factors including left ventricular ejection, pulse transit time[43] and medications[40]. Importantly, patients with T2DM elicit vascular irregularities including increased arterial stiffness (both centrally[44, 45] and peripherally[46]), impaired endothelial mediated vasodilation[47], loss of myogenic responsiveness[48] and small vessel hypertrophy and remodeling[49] compared to non-diabetic individuals and emerging research has suggested that these adverse changes in arterial structure and function may contribute (via increased central systolic stress) to accelerated target organ damage in this population. However, this remains to be elucidated fully.

The evidence for the superiority of central BP beyond brachial BP is, however, not universally accepted, mainly due to issues surrounding the methods used to estimate central BP[50]. The accuracy and reproducibility of central BP measurement using non- invasive methods, requires confirmation before central BP can be established as a clinically useful tool. Standardised treatment strategies which incorporate central BP readings must also be developed and universally accepted[51]. Indeed, the 2013 European Society of Cardiology Guidelines for the management of arterial hypertension suggests that further investigation is required before central BP can be recommended for routine clinical use[52]. Nonetheless, evidence does exist to support the use of central BP in clinical practice (outlined in table 2.1.1), and highlights that although some methodological and technical issues require refinement, the use of central BP measurement may significantly aid decision making for doctors and enhance patient care, above and beyond conventional measures of brachial BP.

Table 2.1. 1. Evidence to support the use of central blood pressure (BP) in clinical practice.

Evidence	Strength of evidenc	Clinical advantage beyond brachial blood pressure
Major differences in central BP occur in people with similar brachial BP	+++	Improved accuracy of assessmer of risk related to BP
The response to antihypertensive medicatior differs between central and brachial BP	+++	Improved accuracy of assessmer of BP response to treatment
Central BP indices independently relate to target organ damage	++	
Changes in target organ damage in response to therapy independently relate to central BP	++	
Central BP indices independently relate to cardiovascular events and mortality	++	Enhanced discrimination of cardiovascular risk
Measurement of central BP improves the predictive accuracy of future cardiovascula events beyond brachial BP and other cardiovascular risk factors	+	
Central BP has superior diagnostic accuracy over brachial BP	+	Increased probability of clinicians making relevant treatment and management decisions
Central BP measurement results in different management decisions compared to usual care	+	Improved patient care

Adapted from Sharman et al.[51] + indicates minimal evidence, +++ indicates substantial evidence.

2 1.3 Abnormal haemodynamics in patients with type diabetes mellitus

T2DM is associated with classic cardiovascular risk factors (including hypertension, smoking and hyperlipidaemia) as well as diabetes specific risk factors (including hyperglycaemia, hyperinsulinaemia, obesity and inflammation), all of which can influence the normal functioning of the cardiovascular system. Hypertension exerts an increased load on the chamber wall of the heart and vessels by increasing wall tension. This increased ventricular wall tension may cause changes in the structure of the wall of the left ventricle in an attempt to normalise the increased myocardial stress. Additionally, an increase in diameter and wall thickness of the large elastic arteries occurs due to passive distension and to minimise intima media stress[53]. Both smoking and hyperlipidaemia reduce the availability of nitric oxide (a potent vasodilator) and contribute to endothelial dysfunction[54, 55]. Hyperglycaemia is a major metabolic alteration that contributes to vascular impairment early on in the progression of T2DM, and may even contribute to vascular abnormalities prior to the diagnosis of T2DM[56]. The mechanisms of hyperglycaemia driven vascular impairment include increased reactive oxygen species and advanced glycation end product concentrations, impaired vasodilatory processes due to nitric oxide inhibition, accumulation of endothelial growth factors and vascular smooth muscle cell dysfunction[56]. Adrenergic activity is stimulated by hyperinsulinaemia[57] and it has been postulated that chronic hyperinsulinaemia may lead to enhanced sympathetic activity and functional overload of the heart and vasculature[58, 59]. Both hyperglycaemia and hyperinsulinaemia have a direct toxic effect on cardiomyocytes which can lead to adverse changes in cardiac structure and function[60]. Increased adipose tissue is associated with greater arterial stiffness possibly via elevated oxidative stress and inflammation[61, 62]. Finally, inflammation itself reduces the bioavailability and increases the inactivation of nitric oxide as well as releasing vasoconstrictor prostanoids, which can lead to endothelial dysfunction and also increased arterial stiffness[61]. Collectively, these aforementioned risk factors can have an unfavourable and often deleterious effect on the normal functioning of the heart and vasculature, predisposing individuals with T2DM to haemodynamic abnormalities as summarised in table 2.1.2.

Table 2.1.2. Summary of the change in central and peripheral haemodynamics in patients with type 2 diabetes mellitus compared to non-diabetic individuals.

Central haemodynamics	
Heart rate	↑
Stroke volume	↑
Cardiac output	↑
Blood pressure	↑
Pulse pressure	↑
Augmentation index	-
Augmentation pressure	↑
Arterial stiffness	↑
Peripheral haemodynamics	
Systemic vascular resistance	↓
Blood pressure	↑
Pulse pressure	↑
Pulse pressure amplification	↓
Arterial stiffness	↑

Central haemodynamics

Heart rate, stroke volume and cardiac output

Increased heart rate[63] and impaired heart rate variability[64] are common in patients with T2DM, possibly as a result of autonomic nervous system dysfunction[65, 66] and hyperinsulinaemia[58, 59, 67]. Damage to autonomic nerve fibers can lead to sympathetic dominance and a resultant higher heart rate[65]. Additionally, hyperinsulinaemia alters the variability of sinoatrial node activity in response to both sympathetic and parasympathetic influences thereby enhancing sympathetic outflow whilst at the same time withdrawing vagal tone[66]. Furthermore, in patients with T2DM[64] and also obese individuals[66] stroke volume is increased, most likely due to the enhanced sympathetic outflow to the heart[66], causing an increase in the strength of myocardial contraction. Elevated cardiac output has been observed in patients with T2DM compared to non-diabetic individuals[67] and also prior to the development of T2DM in individuals with insulin resistance[60] and is most likely due to the

aforementioned elevated heart rate, but also stroke volume in these patients. Furthermore, any increase in body mass, whether it is due to an expansion of adipose or muscle tissue, requires an increase in cardiac output and blood volume in order to meet the increased metabolic demands[68, 69].

Central BP

Typically central systolic BP is elevated in patients with T2DM compared to non- diabetic individuals[2, 4, 67, 70-72] and is most accurately determined invasively using a high-fidelity pressure transducer in the ascending aorta[39]. However, this method is not feasible for routine clinical use and, therefore, alternate methods that estimate central BP non-invasively have been developed. During the candidature of this thesis, the most widely accepted non-invasive method to determine central BP was applanation tonometry at the superficial arteries (most commonly the radial) and the use of commercially available devices such as the SphygmoCor (AtCor Medical, Sydney, Australia)[10]. Using this method, a central (ascending aorta) waveform and BP are estimated by applying a validated transfer function[11, 73] to the radial pressure waveform, which is calibrated with brachial systolic and diastolic BP. While mean arterial pressure and diastolic BP remain fairly constant throughout the arterial system, it is generally accepted that systolic BP is amplified as it moves towards the periphery (as shown in figure 2.1.2)[74]. However, radial applanation tonometry relies on negligent brachial to radial systolic BP amplification (Bra-Rad-SBP_{Amp}) and thus, due to systolic BP amplification, calibrating the radial waveform with brachial systolic and diastolic BP may result in central systolic BP being consistently underestimated. The use of oscillometric mean arterial pressure (which is less dependent on brachial systolic BP) and diastolic BP to calibrate the radial waveform may improve the precision of waveform calibration and central systolic BP estimation[75]. However, the magnitude and effect of Bra-Rad-SBP_{Amp} on the estimated central systolic BP remains unknown in healthy ageing individuals and also in patients with T2DM, in whom vascular irregularities may influence the level of Bra- Rad-SBP_{Amp}. Therefore, this was investigated in *Chapter 7* and *Appendix I* of this thesis. In a cohort of patients with T2DM and non-diabetic controls, *Chapter 7* examines the effect of Bra-Rad-SBP_{Amp} on the estimated central systolic BP, and explores some of the underlying physiology that may influence Bra-Rad-SBP_{Amp}. The findings presented in *Appendix I* suggest that there is significant Bra-Rad-SBP_{Amp} that occurs in both young healthy individuals and also older healthy individuals, and that Bra-Rad-SBP_{Amp} contributes significantly to underestimation of central systolic BP via radial applanation tonometry.

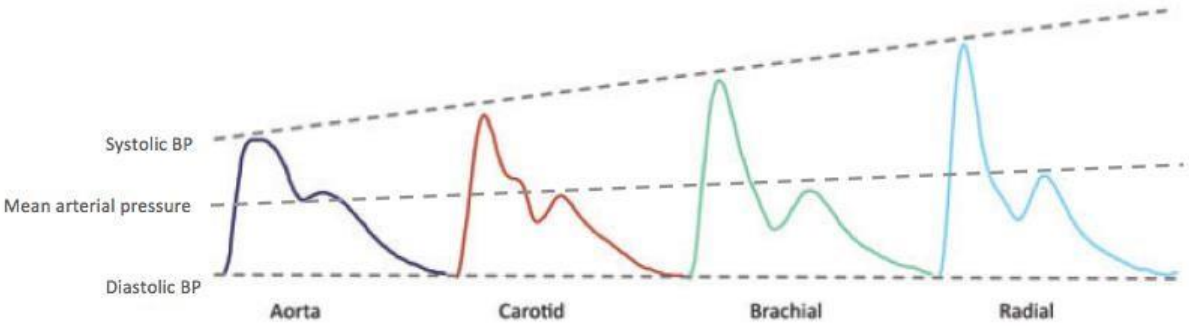


Figure 2.1.2. Pressure wave amplification. Systolic blood pressure (BP) is amplified as it moves from the central arteries (aorta) to the peripheral arteries (brachial and radial), while mean arterial pressure and diastolic BP remain relatively constant throughout the arterial system[76]. Whether systolic BP is amplified from the brachial to radial artery is yet to be definitively determined.

Central pulse pressure

Analysis of the central pressure waveform permits the derivation of other haemodynamic indices such as central pulse pressure (the difference between systolic and diastolic BP; see figure 2.1.3). Several studies have shown that central pulse pressure is elevated in patients with T2DM[67, 77, 78] and other populations with increased cardiovascular risk[79, 80], despite having similar brachial BP compared to healthy individuals. Indeed, Schultz et al.[67] noted that patients with T2DM have an abnormal haemodynamic response when moving from a seated to standing posture and that central pulse pressure did not differ between the two positions, the authors suggesting that this persistent elevation in central pulse pressure may adversely affect cardiovascular health.

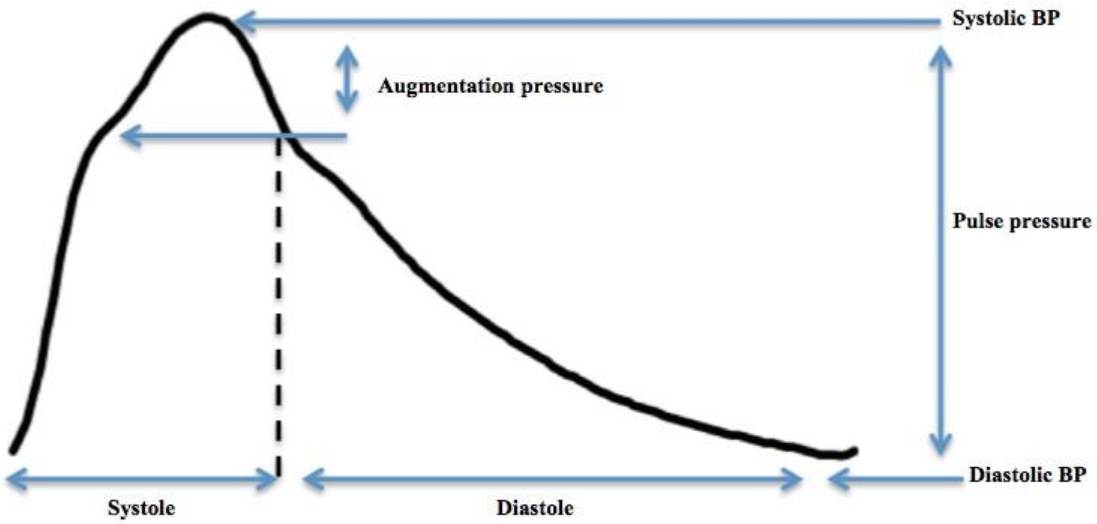


Figure 2.1.3. The central (aortic) pressure waveform. Important parameters can be derived from this waveform including; systolic blood pressure (BP), central pulse pressure (systolic – diastolic BP), augmentation pressure (second - first systolic peak) and augmentation index (augmentation pressure expressed as a percentage of the pulse pressure).

Augmentation pressure

Augmentation pressure is defined as the difference between the second and first central systolic peaks and is elevated in patients with T2DM compared to non- diabetic individuals[81]. The conventional explanation of waveform morphology and pressure transmission through the arterial system, the wave reflection theory, would suggest that when the forward traveling pressure wave generated by left ventricular contraction meets sites of impedance mismatch (i.e. arterial bifurcations), it is reflected back towards the heart where it is believed to augment, or increase, systolic BP in the central arteries[43]. However, a recent study showed that reflected waves contribute minimally to a rise in the systolic peak and that the elastic properties of the arteries may be the principle determinants of elevated augmentation pressure[82], and this hypothesis is discussed in more detail later in this chapter. The degree of central pressure augmentation directly relates to the duration of diabetes[83] and is influenced by a number of factors (including increasing age, a history of smoking, hypertension and hyperlipidaemia[81]).

Augmentation index

Augmentation index (AIx), which quantifies the degree of augmentation pressure relative to central pulse pressure, has been purported to be a marker of systemic arterial stiffness. This is because an increase in arterial stiffness results in a faster traveling pressure wave and an earlier return in the reflected wave (according to wave reflection theory), which may arrive back at the heart during systole causing a rise in the systolic peak[43]. Some studies have reported that AIx is elevated in patients with T2DM[44, 70, 84], however, on the other hand, a number of studies[85-89] have reported that AIx is not elevated, despite these patients having increased arterial stiffness compared to non-diabetic individuals. Importantly, AIx is a composite measure that is influenced by a number of factors including the strength of left ventricular ejection, heart rate and pulse wave transit time[90-92], which may explain the discrepancies between previous studies. Nonetheless, the association between AIx and arterial stiffness in patients with T2DM remains inconclusive and further studies are required to clarify this relationship. In *Chapter 3* of this thesis, the association between AIx and arterial stiffness has been examined in order to determine the cardiovascular and clinical determinants of AIx in patients with T2DM compared to non-diabetic individuals.

Central arterial stiffness

A reliable marker of aortic and large vessel function is central arterial stiffness, which is typically determined via aortic pulse wave velocity (carotid-femoral pulse wave velocity [PWV])[10]. Using this method arterial stiffness is estimated by measuring PWV via applanation tonometry sequentially at the carotid and femoral artery in combination with a three-lead electrocardiogram to determine the timing between the foot of the waveform at each site (as shown in figure 2.1.4). The surface distance between the two measurement sites is taken as the distance travelled by the pressure waves.

Central arterial stiffening is a common feature of ageing and is exacerbated by T2DM[45, 93] and is evident even before the onset of T2DM in individuals with impaired glucose tolerance[94]. In patients with T2DM arterial stiffness is a systemic change, however, regional differences exist with the aorta and carotid arteries being preferentially affected (beyond the peripheral arteries)[95, 96]. Cruickshank et al.[45] showed that Doppler-derived aortic PWV was elevated in patients with T2DM and also in individuals with impaired glucose tolerance and newly diagnosed patients with T2DM compared to non-diabetic controls. The Horn Study[97] showed that central arterial stiffness (assessed via carotid to femoral transit time) was increased in patients with T2DM compared to

individuals with normal glucose metabolism. Similarly, aortic compliance has been shown to be lower in patients with T2DM compared to non-diabetic individuals[98, 99].

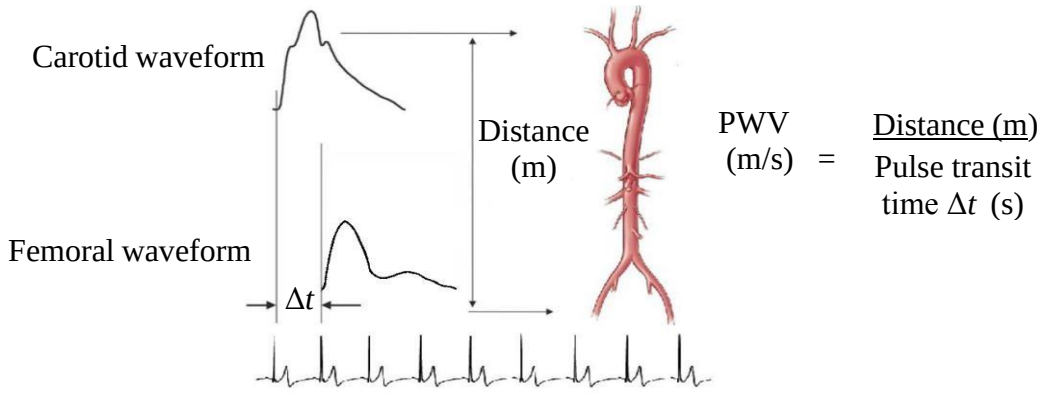


Figure 2.1.4. Estimation of aortic stiffness using PWV. Carotid-to-femoral PWV (expressed in meters per second [m/s]) is determined using the 'foot to foot' method. Using this method, the speed that the pressure waveform travels is determined as the ratio of the distance (m) from the common carotid artery to the femoral artery pressure sites and the time delay (Δt) between the foot of the pressure waveform at the two measurement sites.

Peripheral haemodynamics

Systemic vascular resistance

Studies have confirmed that systemic vascular resistance is either normal or reduced in obese individuals[100], however there is limited data available in patients with T2DM. Due to hyperinsulinaemia[66] and peripheral vasodilation mediated by nitric oxide release[101] systemic vascular resistance is likely to be reduced in patients with T2DM and is examined in *Chapter 3* of this thesis. A significant reduction in systemic vascular resistance in combination with increased arterial stiffness in patients with T2DM may give rise to increased pressure and/or flow being transmitted from the large vessels to the microcirculation where damage to the delicate microvessels may occur (discussed in more detail later in this chapter). However, this mechanism has never been examined in patients with T2DM before and thus was investigated in *Chapters 5* and *6* of this thesis.

Peripheral (brachial) BP

T2DM and elevated brachial BP (hypertension) are comorbid diseases, which independently predispose an individual to further cardiovascular complications. Although both diseases have independent aetiology, they both serve to exacerbate the other in terms of further cardiovascular

complications[102]. Indeed, hypertension accelerates the progression of microvascular and macrovascular complications in patients with T2DM[103]. Evidence suggests that maintaining brachial systolic BP levels of <140 mmHg and diastolic BP <90 mmHg markedly reduces the risk of cardiovascular disease related morbidity and mortality and the development of end- stage renal disease in patients with T2DM[104-106]. Furthermore, a reduction in systolic BP of 10 mmHg is associated with a 12% decrease in any complications related to T2DM and a 15% decrease in risk of mortality related to T2DM[107].

Peripheral pulse pressure

Patients with T2DM demonstrate increased peripheral pulse pressure compared to non-diabetic individuals[70, 108-110] which is likely to be a result of an increase in arterial stiffness[97]. Peripheral pulse pressure is associated with macro and microvascular complications in patients with T2DM[111], and is a strong predictor of mortality in individuals with impaired glucose tolerance[112] and coronary heart disease in patients with T2DM[110]. Importantly, patients with T2DM have a 27% increased risk of death related to cardiovascular disease per 10 mmHg increase in peripheral pulse pressure[97].

Central to peripheral systolic BP amplification

The central to peripheral (brachial) BP relationship, or BP amplification, is altered in individuals with cardiovascular risk factors including hypertension, hyperlipidaemia and T2DM[2, 4]. In patients with T2DM in particular, central to brachial systolic BP may be blunted compared to non-diabetic individuals[4]. The altered relationship is likely due to changes in arterial compliance (increased arterial stiffness) and autonomic function (increased heart rate), which would preferentially affect central BP compared to brachial BP[43]. However, the magnitude and variation in central to brachial systolic BP amplification is currently unknown in patients with T2DM. Clarifying this would be useful in guiding future treatment and management of hypertension in this population, given that the level of amplification can vary from 2- 33 mmHg in healthy individuals and in those with suspected coronary disease, and can result in a significant number of people being misdiagnosed in terms of risk related to hypertension[2, 4]. *Part II* of this *Review of Literature* is a systematic review and meta-analysis of the currently available literature on this topic. Central to brachial systolic BP amplification has been compared in patients with T2DM and non-diabetic individuals to determine the magnitude and variation in central to brachial systolic BP amplification.

Peripheral arterial stiffness

In patients with T2DM, brachial PWV (carotid-radial PWV; a marker of peripheral arterial stiffness) is higher than in non-diabetic individuals[3, 113]. Indeed patients with T2DM[114] and also obese[115] individuals have increased peripheral arterial diameters, which is likely to be associated with an increase in peripheral arterial stiffness and stretching of collagen fibers[116].

2.1.4 Relation of central haemodynamics to target organ damage and clinical outcomes in patients with type 2 diabetes mellitus

Accelerated target organ damage is a common feature of T2DM however, the reasons for this remain unclear. Only a few studies (summarised in table 2.1.3) have examined the relationship between central haemodynamics and markers of target organ damage or clinical outcomes, independently of conventional measures of brachial BP in patients with T2DM. Sharman et al.[77] demonstrated that central pulse pressure predicted left ventricular mass index in patients with T2DM independently of brachial BP and other known risk factors for left ventricular hypertrophy. The Strong Heart Study showed that central pulse pressure was more strongly related to carotid intima media thickness and plaque score than brachial pulse pressure in 3520 individuals (of which 46.5% had diabetes)[32]. These authors have also demonstrated that central pulse pressure is independently related to cardiovascular events (including myocardial infarction and stroke) and mortality in individuals free from cardiovascular disease (including patients with T2DM)[78]. In another study[83] central systolic BP and augmentation pressure but not brachial systolic BP, were independently related to carotid intima media thickness, with the authors speculating that this could be due to changes in arterial mineralisation. Other studies have demonstrated that an increase in arterial stiffness (determined either as heart to femoral PWV or carotid to femoral PWV) is independently related to retinopathy[117] and kidney dysfunction[118, 119], as well as an overall increase in cardiovascular disease risk[96, 120, 121] and cardiovascular and all-cause mortality[45]. Finally, studies in patients with type 1 diabetes have shown that central haemodynamic parameters may be related to adverse changes in the brain[122], however, this is yet to be confirmed in patients with T2DM. Taken together, these previous studies suggest that central haemodynamic parameters may be related to various markers of target organ damage and clinical outcomes in patients with T2DM independently of traditional measures of brachial BP however, the underlying pathophysiological mechanisms remain unknown. Therefore, *Chapter 4* and *5* of this thesis explore some of the potential factors contributing to brain structural defects in patients with T2DM compared to non-diabetic individuals, whilst *Chapter 6* examines factors associated with kidney dysfunction.

Table 2.1.3. Studies indicating the relationship between haemodynamic parameters and markers of target organ damage or clinical events in patients with type 2 diabetes mellitus, independently of brachial blood pressure.

Haemodynamic parameter	Evidence of target organ damage and clinical events
Central blood pressure	Increased carotid intima media thickness[83]
Central pulse pressure	Increased left ventricular mass[77]
	Myocardial infarction, coronary heart disease, congestive heart failure, stroke and sudden death [78]
Augmentation index	Increased carotid intima media thickness[123]
Augmentation pressure	Atherosclerosis[81]
	Increased carotid intima media thickness[83]
	Ischemic heart disease[96]
Heart to femoral pulse wave velocity	Reduced glomerular filtration rate[118, 119]
	Retinopathy[117]
	White matter lesions[124]
	Reduced glomerular filtration rate[125]
	Albuminuria[125-127]
Carotid to femoral pulse wave velocity	Cardiovascular disease risk[96, 128, 129]
	Cardiovascular disease mortality[45, 129]
	All cause mortality[45]

2.1.5 Possible mechanisms linking abnormal central haemodynamics and target organ damage in patients with type 2 diabetes mellitus

Pulsatility

When the left ventricle contracts a pressure wave is generated that travels towards the periphery through the arterial network. Under optimal conditions, the elasticity of the proximal ascending aorta plays an important role in minimising excessive rises in BP and left ventricle work[130] and acts to buffer the pulsatile fluctuations in BP to ensure a more steady flow of blood is delivered to the periphery and microcirculation. However, when the pressure buffering capacity of the aorta and central arteries is diminished (i.e. increased arterial stiffness) there is a greater proportion of the highly pulsatile stroke volume (pressure and/or flow) that is transmitted to the periphery following left ventricular ejection. Studies in non-diabetic individuals[131-133] have shown that the transmission of pulsatile stress from the large vessels to the periphery, may extend deep into the microvasculature and cause excessive cyclic shear stress and damage to the delicate capillary networks. The brain and kidneys in particular have a vascular system supplying blood with high flow, but low resistance and, therefore, these organs can potentially be exposed to damaging levels of pressure and/or flow pulsatility due to increased aortic stiffness and pulse pressure[132, 134, 135]. This phenomenon has not been studied in patients with T2DM, yet may serve as a possible explanation for accelerated organ damage. Importantly, patients with T2DM demonstrate increased arterial stiffness and when combined with systemic vasodilation may result in a highly pulsatile pressure waveform that is transmitted directly to the organs thus contributing to accelerated brain atrophy and renal dysfunction. Indeed, it is this mechanism that may link abnormal central haemodynamics with target organ damage in patients with T2DM and, therefore, has been investigated in *Chapters 5* and *6* of this thesis.

Arterial stiffness and wave reflection

Arterial stiffness also increases the speed of the forward traveling pressure wave generate by left ventricular ejection. Conventional theory would argue that at sites of impedance mismatch, such as major arterial bifurcations, some of the energy from the incident wave is reflected back towards the heart and thus, the measured arterial pressure is the sum of the forward traveling wave and the backward traveling reflected wave[43, 136]. In young individuals with compliant vessels, the reflected pressure wave arrives back at the heart during diastole, aiding coronary perfusion and has a minimal affect on central systolic BP and left ventricular afterload. However, in older or diseased individuals (such as T2DM) with increased arterial stiffness, the reflected pressure

wave returns earlier during systole, causing augmentation in the systolic peak and an increase in central systolic BP and left ventricular work. However, recently this explanation of waveform morphology has been challenged[137-139]. Indeed, a recent meta-analysis demonstrated that there is no significant shift in the timing of the reflected wave[140] that contributes to an increase in central BP (and augmentation pressure) which typically occurs with age, whilst others have suggested that the rise in central BP should not merely be described by changes in reflected wave timing[141].

The wave reflection theory further suggests that augmentation in central BP is significantly influenced by the magnitude of the reflected wave, which is dependent on the magnitude of the incident wave and arterial impedance properties[43]. However, this is based on a number of assumptions relating to the cardiovascular system, perhaps most importantly, the compliant properties of the arteries have been largely ignored[142, 143]. Failing to account for the compliant nature (i.e. the ability to expand and contract in response to a increase in volume), or pressure buffering role of the large elastic arteries may result in incorrect explanation of the physiology underlying the central pressure waveform[139]. Moreover, recent studies have suggested that reflected waves contribute minimally to a rise in central BP[82, 144] and that due to dispersion of the reflected waves along the aorta, the compliance of the aorta may indeed play a more prominent role in determining central BP than previously described[145]

Aortic reservoir-excess pressure

The aortic reservoir-excess pressure theory is an alternate physiological model that describes the shape of the central pressure waveform, whilst taking into account the compliant properties of the arterial system[8]. The reservoir-excess pressure paradigm proposes that the central pressure wave may be separated into a reservoir pressure, which is representative of the changes in proximal aortic volume (distension during systole to store blood and recoil during diastole to release blood) and; an excess pressure component which is analogous to left ventricular flow (figure 2.1.5)[139]. The reservoir pressure is representative of the minimum amount of work the left ventricle must do to expel blood into the aorta, and the excess pressure is, therefore, any excess work required above this minimum[146]. The reservoir-excess pressure model does not omit the existence of reflected waves in the arterial system, however, when considering the 'reservoir function', the influence of reflected waves on augmentation of central systolic BP is significantly reduced[82]. In a recent sub-study of the Anglo-Scandinavian study[147], excess pressure was shown to predict adverse cardiovascular events in patients with cardiovascular disease, independently of known

cardiovascular risk factors and, therefore, it is likely that this novel parameter is of prognostic importance in describing other end organ damage. For the first time, the associations between aortic reservoir characteristics and target organ damage in patients with T2DM have been examined in *Chapters 5* and *6* of this thesis. In *Chapter 5* the association between reservoir pressure and excess pressure and grey matter atrophy has been examined in patients with T2DM compared to non-diabetic individuals, whilst in *Chapter 6* the association between reservoir characteristics and renal function has been explored.

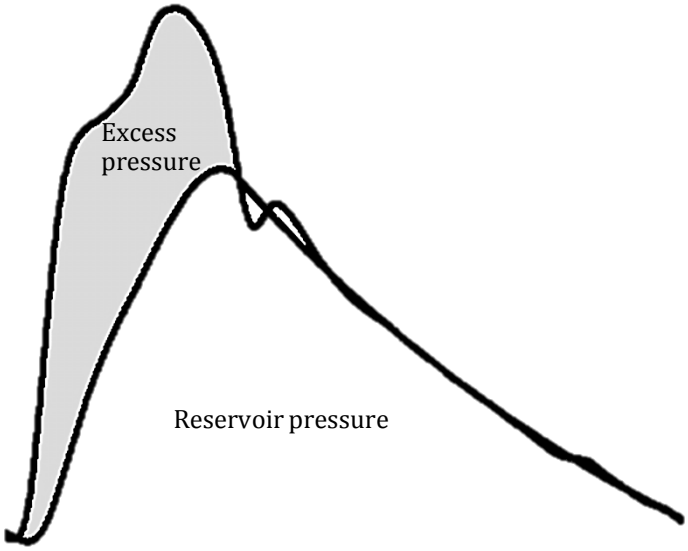


Figure 2.1.5. Example aortic pressure waveform separated into reservoir pressure and excess pressure. Total measured pressure is equal to the sum of reservoir pressure and excess pressure. Aortic reservoir pressure represents the cyclic increase in aortic volume (aortic distension that occurs during systole) and decrease in volume (aortic recoil that occurs during diastole). Excess pressure is representative of the excess work required by the left ventricle for ejection of stroke volume and is analogous to left ventricular flow[144].

2.1.6 Exercise – a method to identify abnormal haemodynamics in patients with type 2 diabetes mellitus?

Although resting BP indices are clinically important, haemodynamic responses to exercise may have stronger prognostic value in terms of cardiovascular risk[5], suggesting that pathophysiological insight may be gained from exercise haemodynamics beyond that of resting conditions. This is likely because individuals spend a proportion of their day doing some form of light to moderate physical activity[6] and, therefore, the BP response to physical activity is more akin to the chronic BP loading that occurs during normal daily activity[7]. In normotensive men with no prior history of coronary heart disease, an exaggerated systolic BP response to exercise (≥230 mmHg)

is associated with a 2.74 fold increased risk of acute myocardial infarction, independently of resting systolic BP and other cardiovascular risk factors[148]. Furthermore, an increase in exercise BP is associated with an increased relative risk of cardiovascular mortality, and the change in systolic BP from rest to exercise is associated with both cardiovascular and non-cardiovascular related mortality[149].

Most previous studies have examined the BP response to maximal intensity exercise, however, in a recent systematic review and meta-analysis Schultz et al.[5] showed that an exaggerated BP response to moderate intensity exercise was associated with a 36% increase in rates of cardiovascular events and mortality, independently of resting BP (figure 2.1.6). Weiss et al.[150] demonstrated that systolic BP measured during moderate intensity exercise at stage 2 of the Bruce protocol was more closely related to risk of cardiovascular mortality than systolic BP measured in response to maximal intensity exercise. Furthermore, independent of resting BP, light to moderate exercise haemodynamics have been shown to unveil BP abnormalities in individuals with increased cardiovascular risk[151] and also predict kidney function in elderly men[152].

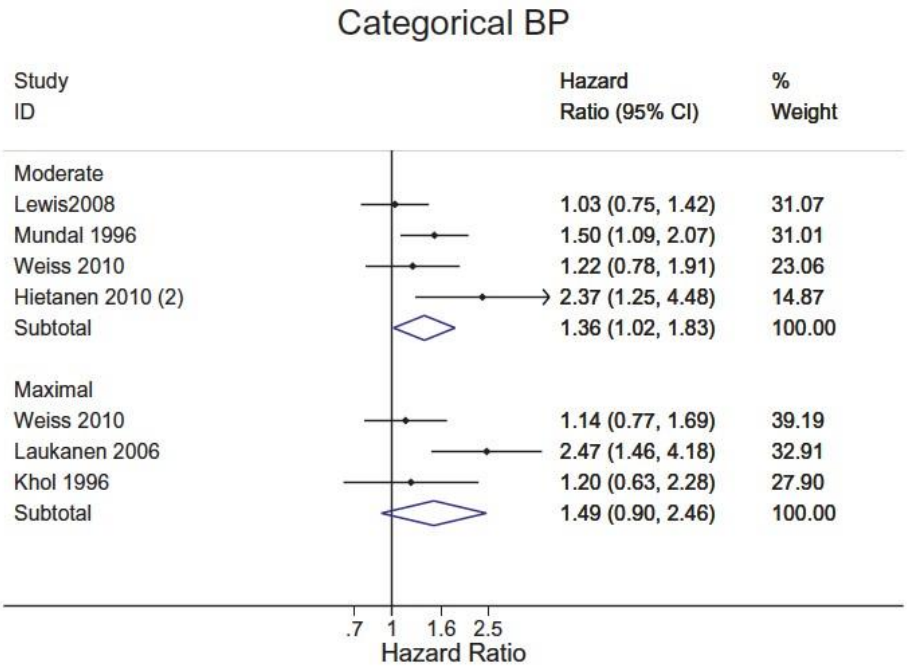


Figure 2.1.6. Pooled hazard ratios and 95% confidence intervals for a hypertensive response to moderate and maximal intensity exercise, adjusted for age, resting blood pressure (BP) and multiple cardiovascular risk factors. Moderate exercise p value=0.039, I^2=51.8%. Maximal exercise p value=0.12, I^2=65.0%[5].

Relative to non-diabetics, patients with T2DM have excessive increases in exercise brachial and central BP[28, 153]. Scott et al.[28] showed that the prevalence of a hypertensive response to exercise was significantly higher in patients with T2DM compared to non-diabetic individuals (51% vs 22%), and that this abnormal response was related to an increase in left ventricular relative wall thickness and also increased exercise central BP. This finding is likely to be clinically significant because a hypertensive response to exercise is related to future onset hypertension, cardiovascular morbidity and mortality in other populations[27, 154, 155]. Taken together, these data suggest that the modality of exercise may be a useful method to uncover haemodynamic abnormalities that would otherwise not be evident at rest. However, the association between exercise central haemodynamics and target organ damage has been seldom explored in patients with T2DM.

2.1.7 Summary and conclusion

Patients with T2DM are at an increased risk of target organ damage compared to their non-diabetic counterparts and while hypertension (measured conventionally by a cuff placed over the brachial artery of the upper arm) may explain some of the increased risk, it does not explain all of the variance in target organ damage. Although traditional measures of brachial BP taken in the clinic are useful for screening individuals at risk related to hypertension, substantial evidence now suggests that central BP (and related haemodynamics) may be more closely related to the chronic BP load experienced by the heart and other truncal organs and thus, may more accurately determine target organ damage risk. Importantly, the pathophysiological mechanisms, which contribute to the development of T2DM, are known to elicit vascular irregularities that predispose to abnormal central BP and haemodynamics, which may in turn contribute to accelerated target organ damage in this population. Moreover, the BP response to light to moderate intensity exercise has stronger prognostic value in terms of cardiovascular risk compared to corresponding resting BP. To date, few studies have examined the associations between central haemodynamics (either at rest or in response to light to moderate intensity exercise) and target organ damage in patients with T2DM. Understanding such associations may enable more targeted treatment and management strategies and help to reduce the risk of morbidity and mortality due to organ failure in this population.

Chapter 2 Part II. Central to brachial blood pressure amplification in type 2 diabetes mellitus: Systematic review and meta-analysis

Part II of *Chapter 2* was in the final stages of preparation for publication at the time of submission of this thesis.

Climie RED, Schultz MG, Otahal P, Fell JW, Srikanth V, Sharman JE. Central to brachial blood pressure amplification in type 2 diabetes mellitus: Systematic review and meta-analysis.

2.2.1 Abstract

Background. Brachial blood pressure (BP) may not reflect the pressure centrally (central BP) due to amplification in systolic BP (SBP). Patients with type 2 diabetes mellitus (T2DM) elicit vascular irregularities that may effect SBP amplification and other central BP indices (including pulse pressure [PP], augmentation pressure [AP] and augmentation index [AIx]). By systematic review and meta-analysis, this study aimed to determine the magnitude and variation of central to brachial SBP and PP amplification, AIx and AP in T2DM compared to non-diabetic controls.

Methods. Online databases were searched for published studies reporting central and brachial SBP in T2DM and non-diabetic controls. Random effects meta-analyses and meta-regression were used to analyse the studies.

Results. We identified 17 studies with a total of 2,711 T2DM and 10,460 non- diabetic controls. There was no significant difference in SBP amplification between groups (T2DM=10.8, non-diabetic=10.2mmHg;pooled estimate=0.6mmHg, 95%CI - 0.3,1.5, p=0.21), but large variation in both (T2DM range=2.0-16.6mmHg, non- diabetic range=1.0-16.1mmHg). In the meta-regression, the difference in glycated haemoglobin (HbA_1c) explained 50.9% of the variance in the pooled data (p=0.03) and duration of T2DM explained 15.9% (p=0.16); the difference in amplification between groups increasing by 0.3mmHg per year of T2DM. PP amplification was not significantly different between groups (p=0.16). AIx (p=0.010), AIx corrected for heart rate (p<0.001) and AP (p=0.001) were all significantly higher in T2DM.

Conclusions. There is no difference in SBP (or PP) amplification in T2DM compared to non-diabetic individuals but the difference varies with duration of T2DM. There is also large variation in SBP amplification. These data suggest that central SBP cannot be estimated from brachial SBP.

2.2.2 Introduction

High blood pressure (BP) or hypertension, is associated with adverse cardiovascular outcomes[22, 23]. In clinical practice, BP is typically measured at the brachial artery (brachial BP)[30]. However, due to amplification in systolic BP (SBP) as the pressure wave prorogates peripherally, brachial SBP may not accurately reflect the pressure at the heart and aorta (central SBP), and studies have demonstrated that central SBP and the corresponding load on the left ventricle may be elevated despite brachial SBP being within the normal range[4]. The left ventricle must overcome aortic pressures with each cardiac ejection to expel blood into the systemic circulation and thus, it is reasonable to expect that central SBP may be more closely related to the chronic load experienced by the heart and aorta, rather than other surrogate measures (such as brachial SBP)[34, 39]. Furthermore, central BP indices (including central pulse pressure [PP], augmentation pressure [AP; the difference between the second and first central systolic peaks] and augmentation index [AIx; AP expressed as a percentage of PP]) have been identified as predictors of cardiovascular events[156], morbidity and mortality[33], independently of brachial SBP. Together this suggests that measurement of central SBP and related indices may improve cardiovascular risk assessment.

The magnitude and variation of central to brachial SBP (and PP) amplification may be influenced by a number of demographic (including age and sex[35]) and physiological factors (including hyperlipidaemia, mean arterial pressure[35], arterial stiffness[4] and heart rate[36]) and has been shown to vary considerably in healthy individuals and patients with coronary heart disease (up to 33 mmHg)[2]. Patients with type 2 diabetes mellitus (T2DM) elicit vascular irregularities (including elevated cardiac output[63], and central[45, 97] and peripheral[46] arterial stiffening) compared to non-diabetic individuals, which may affect the magnitude and variation of central to brachial SBP (and PP) amplification, as well as AIx and AP. Indeed, we have previously observed substantial variability in central to brachial SBP amplification in patients with T2DM[2], however to our knowledge, this has never been thoroughly examined in comparison to non-diabetic controls by systematic review and meta-analysis. If significant amplification and variation exists in patients with T2DM compared to non- diabetic controls, this could mean that a measure of brachial SBP may not accurately reflect the true risk related to BP in this patient group. This could have therapeutic implications. Therefore, the aim of this study was to determine the magnitude and the range of variation of central to brachial SBP and PP amplification, and to determine the difference in AIx and AP in patients with T2DM compared to non-diabetic individuals.

2.2.3 Methods

Literature search and methods

The search methods used in this study followed the Preferred Reporting Items for Meta-analyses[157] and the Meta-analyses of Observational Studies in Epidemiology[158] reporting guidelines. Two independent reviewers (RC and MS) conducted a literature search of seven electronic databases (CINAHL, Cochrane, EMBASE, PubMed, Scopus, SPORTDiscus and Web of Science) including all studies reporting central to brachial SBP amplification in patients with T2DM for all years up to March 2015. The search strings included the following terms: ('type' AND ('2' OR 'two') AND 'diabetes' OR 'non-insulin dependent diabetes') AND ('blood pressure' OR 'brachial blood pressure' OR 'peripheral blood pressure' OR 'upper arm blood pressure' OR 'central blood pressure' OR 'aortic blood pressure' OR 'blood pressure amplification' OR 'pulse pressure' OR 'pulse pressure amplification' OR 'amplification' OR 'augmentation index' OR 'augmentation pressure'). Search filters for human studies and adults aged >18 years of age were included. Additionally, the reference list of any other relevant original and review articles were also searched.

Criteria for study inclusion

Studies were included in the systematic review if they met the following criteria; 1) full length publication in a peer reviewed journal; 2) a human study in adults >18 years of age; 3) reported central SBP and brachial SBP and diastolic BP using non- invasive or invasive techniques; 4) central and brachial SBP was measured at the same time period (either simultaneous or consecutive measurements) and; 5) a control (non-diabetic) group was included in the study. Studies were not included if data for central or brachial SBP for either patients with T2DM or the control group were not reported separately.

Outcome measures

The main outcome measure was central to brachial SBP amplification. Central to brachial PP amplification, AIx, (including AIx corrected for a heart rate of 75 beats per minute [bpm]) and AP were secondary outcome measures. SBP amplification was determined by the method specified by the study authors within each individual paper, or calculated as brachial SBP – central SBP. Similarly, PP amplification was determined by the method adopted by the individual paper or by brachial PP divided by central PP. If central PP was not reported, it was calculated as central SBP – central (or brachial where central was unavailable) diastolic BP. Where AIx was not

reported but central PP and AP were available, AIx was calculated using equation 1 below and the standard deviation was calculated using the Delta method[159]. AP could not be calculated if not reported in the individual studies due to insufficient availability of data.

Equation 1:
AIx = Augmentation pressure/central PP x 100

Data extraction

Two reviewers (RC and PO) extracted the data independently. All discrepancies were reviewed and resolved. For the systematic review the following data were extracted from each individual paper; the characteristics of the study population (including the age, proportion of male participants, body mass index [BMI], medications, disease status and duration of diabetes), central and brachial SBP and diastolic BP, central PP, peripheral PP, AIx, AP, heart rate, statistical methods (corrected, uncorrected analysis) and method of determining central and brachial SBP and diastolic BP. The study by Maple Brown et al.[160] was performed in two distinct populations (indigenous Australians and Australians with European ancestry) in which data was presented for both a diabetic and non-diabetic subgroup. Therefore, we decided *a priori* to treat these estimates as separate studies.

Statistical analysis

Random effects analyses were performed comparing the difference in central to brachial SBP and PP amplification, AIx and AP between patients with T2DM and non-diabetic individuals. Five meta-analyses were performed separately and studies could be included in more than one meta-analysis if the appropriate data was reported. Heterogeneity between studies was examined using meta-regression analyses to examine the effect of age, BMI, diabetes duration (in the diabetic group), heart rate, and use of antihypertensive medication on the difference in central to brachial SBP between individuals with and without T2DM.

The majority of the studies measured central SBP using radial applanation tonometry and only two[161, 162] used alternate methods. Sensitivity analyses were performed to assess whether the two studies that used different methods to determine central SBP caused any difference in effect size. A number of studies[70, 126, 162] reported variance as either interquartile range or 95% confidence intervals and, therefore, these were converted to standard deviations for the analysis. Two studies containing data from similar cohorts were included in separate analyses, one in the analysis of central to brachial SBP and PP amplification[163] and one in the analysis of AIx and AP[164]. All

data from each individual study was reported as uncorrected. Publication bias was assessed visually with funnel plots and with Eggers test for bias.

2.2.4 Results

Literature search and systematic review

A summary of the literature search procedure adopted in the current study is shown in figure 2.2.1. The original search of seven online databases revealed 45,086 original articles of which 45,021 were excluded (due to being duplications or based on review of title or abstract or both), leaving 65 potentially relevant articles that required full text reviews. Forty-seven of these were excluded, leaving 18 articles for the final systematic review (table 2.2.1) and 17 for the primary meta-analysis. The 17 studies included in the meta-analysis had a total of 2,711 patients with T2DM and 10,460 non-diabetic controls for meta-analysis.

Central to brachial SBP amplification

Central and brachial SBP were elevated in patients with T2DM (124, range 113-147 mmHg and 134, range 121-160 mmHg) compared to non-diabetic individuals (114, range 103-146 mmHg and 124, range 114-158 mmHg). The pooled estimate showed that there was minimal difference in central to brachial SBP amplification between patients with T2DM and non-diabetic controls (0.6 mmHg, 95%CI -0.3, 1.5, p=0.21; figure 2.2.2). The mean central to brachial SBP amplification was 10.8 mmHg and ranged from 2.0 to 16.6 mmHg in patients with T2DM and was 10.2 mmHg and ranged from 1.0 to 16.1 mmHg in non-diabetic individuals.

The difference in age between individuals with and without T2DM, did not explain the variance in the pooled data (R^2 = 0%) nor did the difference in sex (R^2 = 0%), BMI (R^2 = 0%), heart rate (R^2 = 0%) or use of antihypertensive medication (R^2 = 0%). However, glycaemic control (HbA_1c levels) explained 50.9% (p=0.03) and diabetes duration explained 15.9% (p=0.16) of the variance in the pooled data. As the duration of diabetes increased in patients with T2DM, the difference in central to brachial SBP amplification between the groups also increased (figure 2.2.3). The meta-regression suggests that amplification is lower in patients with T2DM relative to non-diabetic individuals at five years of disease duration (-0.7 mmHg), and increases with each additional year of having T2DM by 0.3 mmHg per year to be 1.1 mmHg higher for participants with an average T2DM duration of 11 years. Of note, in the study by Chirinos et al.[162] central SBP was estimated from the carotid artery rather than the aorta, however, removal of this study from the analysis made little difference to the overall pooled result (0.6 mmHg, 95%CI -0.4, 1.6, p=0.25).

Furthermore, the removal of the two studies[161, 162] that used alternate methods to determine central SBP other than radial tonometry, made no difference to the overall pooled result (0.6 mmHg, 95%CI -0.4, 1.6, p=0.28).

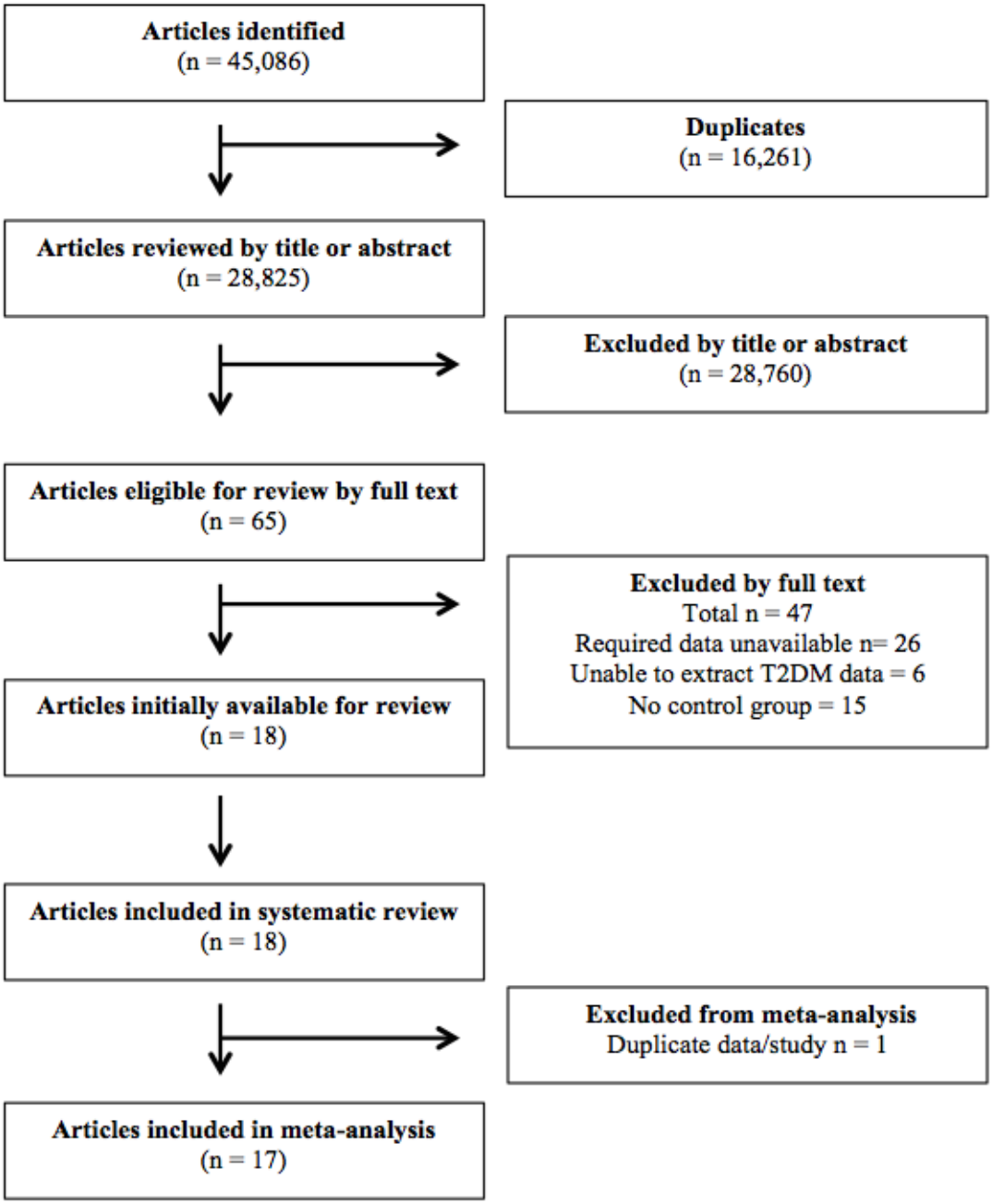


Figure 2.2.1. Summary of literature search and selection procedure for articles included in the systematic review and meta-analysis.

Table 2.2.1. Studies included in the systematic review reporting central to brachial systolic blood pressure (SBP) amplification in patients with type 2 diabetes mellitus (T2DM) and non-diabetic controls.

No.	Study	Participants (n)	Age (years)	Male (%)	Duration of diabetes (years)	Body mass index (kg/m^2)	Smoking history (%)	Hypertensive medication (%)	Hyperlipidaemic medication (%)	Hyperglycaemic medication (%)
1	Afsar et al. 2014[161]	*146	61±11	18	9.8	28.2±5.7	29	36	18	NR
		238	51±16	21		30.5±5.6	39	41	21	NA
2	Agnoletti et al. 2013[165]	*126	63±10	56	11.0	28.4±3.9	69	58	53	47
		203	57±15	52		25.8±4.6	94	51	30	NA
3	Brooks et al. 2001[70]	*88	56±11	58	7.5	28.7±5.3	61	31	NR	67
		85	55±16	47		25.0±4.1	40	17	NR	NA
4	Chirinos et al. 2013[162]	*37	53±5	68	4.8	30.9±4.8	NR	46	NR	71
		2025	45±7	45		24.8±3.9	NR	9	NR	NA
5	Climie et al. 2013[63]	*53	61±8	51	NR	30.8±5.0	NR	57	57	NR
		53	58±6	51		25.4±3.5	NR	6	2	NA
6	Climie et al. 2014[164]	*37	63±9	47	6.0	30.5±4.8	NR	63	66	68
		37	52±8	51		25.9±3.3	NR	0	0	NA
7	Climie et al. 2015[163]	*39	63±9	49	6.0	30.5±4.8	8	64	67	72
		39	53±9	49		24.9±3.3	10	NR	NR	NA
8	Kolade et al. 2012[71]	*211	56±10	55	NR	31.8±6.1	NR	NR	NR	NR
		208	50±14	70		26.2±3.8	NR	NR	NR	NA
9	Maple Brown et al. 2005[167]	*43	47±11	42	>10	27.3±4.9	45	50	NR	NR
		54	46±9	35		29.5±5.7	57	13	NR	NA
10 a)	Maple Brown et al. 2007[160]	*38	54±8	45	5.0	30.8±5.0	8	47	NR	NR
		83	42±9	34		25.8±6.0	9	4	NR	NA
10 b)	Maple Brown et al. 2007[160]	*60	48±10	48	5.0	27.3±6.0	46	45	NR	NR
		102	42±12	38		24.6±5.0	56	8	NR	NA

11	McEniery et al. 2008[4]	*356 5648	65±14 45±21	64 51	NR	29.4±NR 25.2±NR	NR NR	NR NR	NR NR	NR NA
12	Recio-Rodriguez et al. 2012[166]	*100 92	59±11 55±12	65 53	NR	29.9±5.2 25.7±3.5	20 26	70 0	58 0	87 NA
13	Sacre et al. 2012[72]	*106 106	56±9 56±9	58 58	NR	32.0±6.0 27.0±4.0	NR NR	51 0	45 10	71 NA
14	Schultz et al. 2012[67]	*21 20	61±9 53±8	48 45	11.0	29.0±6.0 26.0±5.0	37 37	52 0	NR NR	74 NA
15	Scott et al. 2008[28]	*73 73	54±10 53±12	62 63	NR	31.5±5.9 26.2±3.8	NR NR	25 0	29 0	53 NA
16	Sharman et al.	*224 222	56±10 50±14	55 68	NR	31.8±6.1 26.0±3.7	NR NR	NR NR	NR NR	NR NA
17	Tamminen et al. 2002[168]	*16 19	54±2 51±2	68 75	7.0	29.1±1.1 28.9±0.9	30 20	NR NR	NR NR	NR NA
18	Wier et al. 2011[126]	*974 1170	NR NR	NR NR	NR	NR NR	NR NR	NR NR	NR NR	NR NA

Table 2.2.1 continued.

No.	Central SBP	Brachial SBP	Brachial DBP	Method to determine brachial SBP	Method to determine central SBP
1	*119±16 116±15	136±17 132±16	86±10 83±10	Automatic, oscillometry	Brachial BP cuff (Mobil-o-Graph arteriograph), calibrated via brachial SBP and DBP
2	*147±26 146±27	160±28 158±29	94±15 96±15	Automatic, oscillometry	Radial applanation tonometry (SphygmoCor), calibrated via brachial SBP and DBP
3	*129±18 114±16	139±18 124±16	82±8 76±10	NR	Radial applanation tonometry (PWV Medical Blood Pressure Analysis System), calibration method NR
4	*141±19 130±23	143±3 131±0	87±2 77±0	Automatic, oscillometry	Carotid applanation tonometry (SphygmoCor), calibrated via brachial MAP and DBP
5	*114±13 107±12	124±13 117±11	71±9 68±8	Automatic, oscillometry	Radial applanation tonometry (SphygmoCor), calibrated via brachial SBP and DBP
6	*114±11 103±10	124±12 114±9	68±8 65±6	Automatic, oscillometry	Radial applanation tonometry (SphygmoCor), calibrated via brachial SBP and DBP
7	*115±12 103±10	125±13 114±9	69±8 65±6	Automatic, oscillometry	Radial applanation tonometry (SphygmoCor), calibrated via brachial SBP and DBP
8	*125±16 114±15	136±17 125±15	82±9 75±10	Mercury sphygmomanometer	Radial applanation tonometry (SphygmoCor), calibrated via brachial SBP and DBP
9	*113±19 114±21	121±20 121±21	76±11 75±12	Automated sphygmomanometer	Radial applanation tonometry (SphygmoCor), calibration method NR
10 a)	*125±15 107±16	132±15 118±17	78±8 73±10	Automated sphygmomanometer	Radial applanation tonometry (SphygmoCor), calibration method NR
10 b)	*117±17 112±23	130±23 123±27	75±11 73±12	Automated sphygmomanometer	Radial applanation tonometry (SphygmoCor), calibration method NR

^11	*130±21 108±12	143±21 120±11	79±11 74±8	Automatic, oscillometry	Radial applanation tonometry (SphygmoCor), calibration method NR
12	*129±18 108±14	137±19 115±14	83±11 74±9	Automated sphygmomanometer	Radial applanation tonometry (SphygmoCor), calibration method NR
13	*122±13 114±12	135±14 125±13	79±9 75±8	Mercury sphygmomanometer	Radial applanation tonometry (SphygmoCor), calibrated via brachial SBP and DBP
14	*121±17 106±13	136±18 119±12	72±9 70±9	Automatic, oscillometry	Radial applanation tonometry (SphygmoCor), calibrated via brachial SBP and DBP
15	*116±10 113±11	127±11 124±12	77±7 76±8	Mercury sphygmomanometer	Radial applanation tonometry (SphygmoCor), calibrated via brachial SBP and DBP
^16	*125±17 113±14	136±18 124±13	82±9 74±9	Mercury sphygmomanometer	Radial applanation tonometry (SphygmoCor), calibrated via brachial SBP and DBP
17	*119±13 116±18	129±4 125±13	79±2 80±2	NR	Radial applanation tonometry (SphygmoCor), calibration method NR
18	*117±21 111±16	130±22 120±16	68±13 72±13	Aneroid sphygmomanometer	Radial applanation tonometry (SphygmoCor), calibration method NR

Data are mean ± standard deviation unless otherwise indicated. NR, not reported; NA, not applicable; DBP, diastolic BP; PWV, pulse wave velocity.

*represents data for patients with T2DM. ^SBP amplification reported in individual study, for all other studies SBP amplification was calculated as brachial SBP – central SBP.

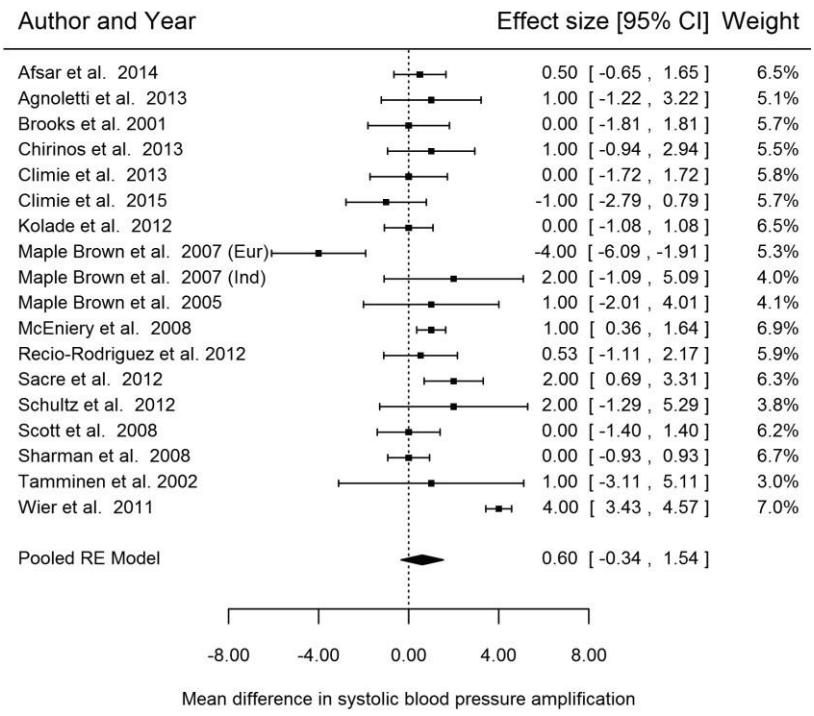


Figure 2.2.2. Pooled estimates and 95% confidence intervals for amplification in central to brachial systolic blood pressure (SBP) in patients with type 2 diabetes mellitus (T2DM) compared to non-diabetic individuals. I^2=88.0% p=0.21. The forest plot indicates that central to brachial systolic blood pressure was slightly, although not significantly, higher in patients with T2DM.

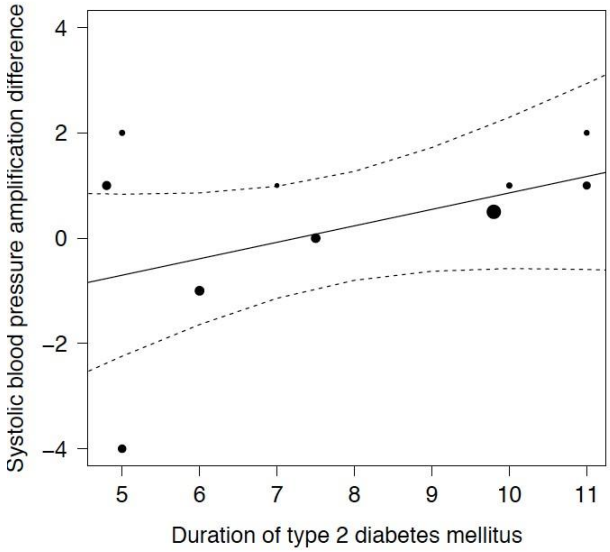


Figure 2.2.3. Association between the level of central to brachial systolic blood pressure amplification between patients with and without type 2 diabetes mellitus and the duration of diabetes. R^2=15.9%, p =0.16.

Central to brachial PP amplification

The total number of patients with T2DM included in the meta-analysis of PP was 2,622 and 10,368 non-diabetic controls. The pooled estimate showed that there was no difference in central to brachial PP amplification between patients with T2DM and non-diabetic controls (-0.03 mmHg, 95%CI -0.07, 0.01, p=0.16; figure 2.2.4 A). The mean PP amplification was 1.3 mmHg and ranged from 1.0 to 1.4 mmHg in patients with T2DM, and was 1.3 mmHg and ranged from 1.0 to 1.4 mmHg in non-diabetic controls.

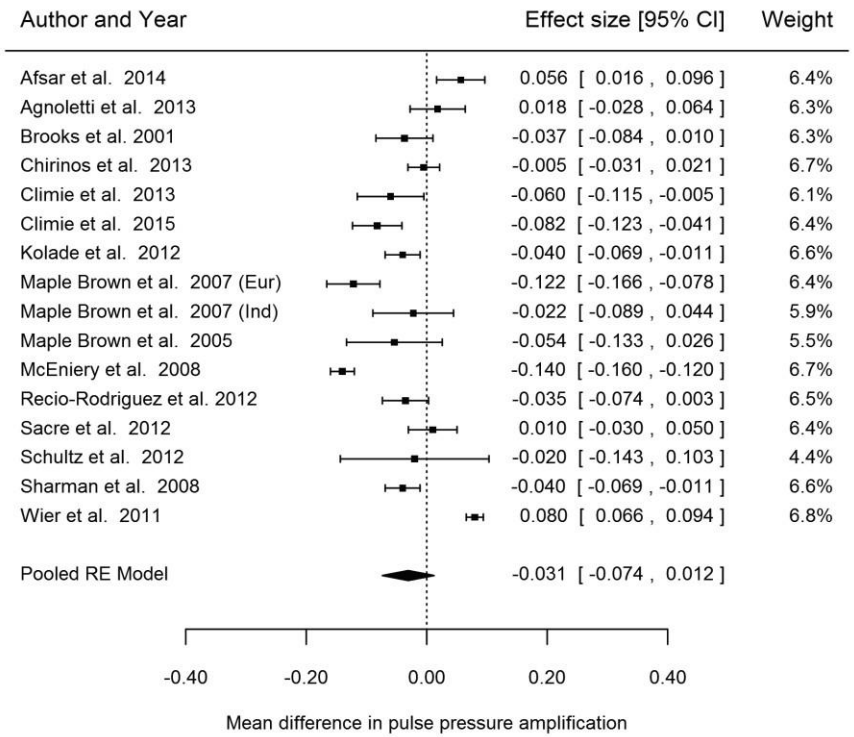
Augmentation index and augmentation pressure

Unadjusted AIx was calculated using equation 1 in two studies[70, 165]. However, insufficient data was provided to calculate AIx in six studies[28, 71, 126, 161, 162, 167] and AP in nine studies[28, 71, 72, 126, 161, 162, 165-167] and therefore, these studies were excluded from the respective meta-analyses. Data for AIx corrected for heart rate was only available in seven studies[28, 63, 67, 72, 126, 161, 164]. There was a total of 1,046 patients with T2DM and 6,504 non-diabetic controls included in the meta-analysis of unadjusted AIx; 712 patients with T2DM and 6,101 non-diabetic controls included in the meta-analysis of AP and; 1,410 patients with T2DM and 1,697 non-diabetic controls included in the meta-analysis of AIx corrected for heart rate. The pooled estimate showed that AIx was significantly elevated in patients with T2DM (3.1%, 95%CI 0.7, 5.4, p=0.010; figure 2.2.4 B) compared to non-diabetic controls, as was corrected AIx (4.3%, 95% CI 2.7, 6.0, p<0.001; figure 2.2.4 C). Additionally, AP was significantly greater in patients with T2DM compared to non-diabetic controls (3.2 mmHg, 95% CI 1.3, 5.1, p=0.001; figure 2.2.4 D).

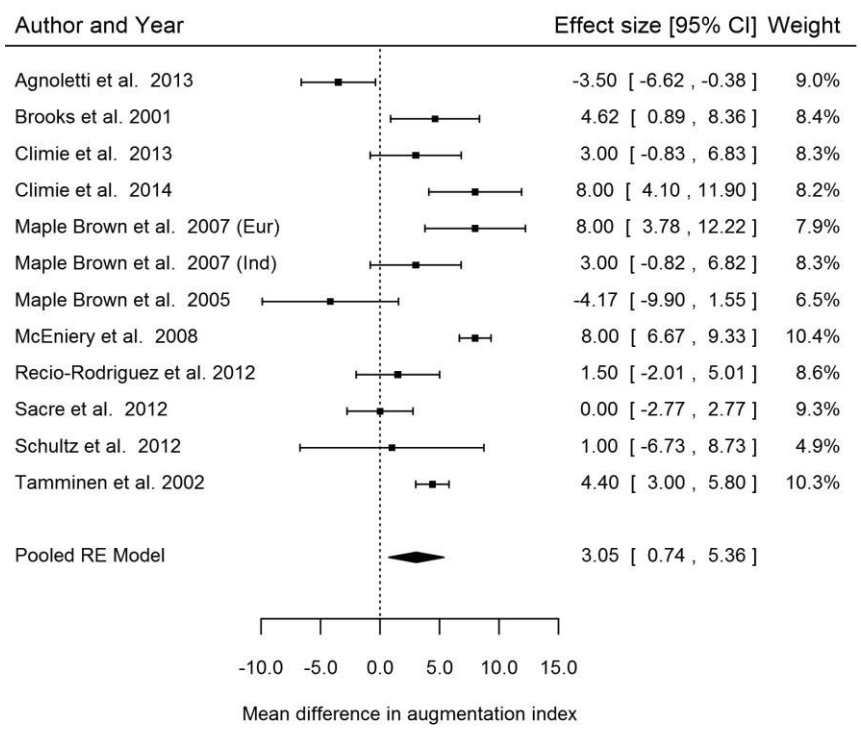
Publication bias

It is difficult to determine publication bias from a relatively small number of individual studies, however, funnel plots (figure 2.2.5) and Egger's test indicated that there was relatively little influence of any publication bias.

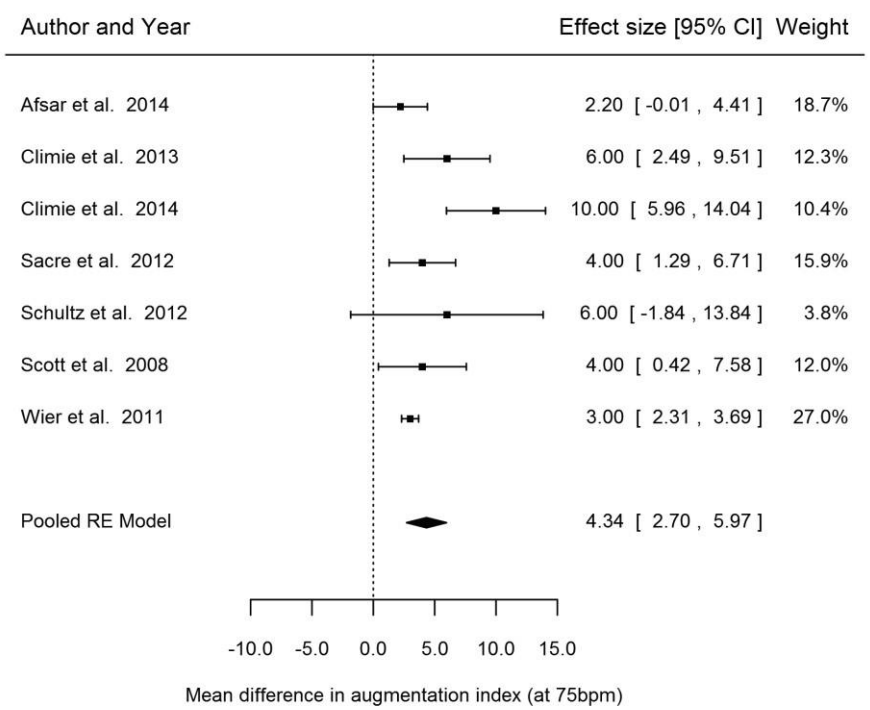
A


Author and Year
Effect size [95% CI]
Weight
Afsar et al. 2014 0.056 [0.016 , 0.096] 6.4%
Agnoletti et al. 2013 0.018 [-0.028 , 0.064] 6.3%
Brooks et al. 2001 -0.037 [-0.084 , 0.010] 6.3%
Chirinos et al. 2013 -0.005 [-0.031 , 0.021] 6.7%
Climie et al. 2013 -0.060 [-0.115 , -0.005] 6.1%
Climie et al. 2015 -0.082 [-0.123 , -0.041] 6.4%
Kolade et al. 2012 -0.040 [-0.069 , -0.011] 6.6%
Maple Brown et al. 2007 (Eur) -0.122 [-0.166 , -0.078] 6.4%
Maple Brown et al. 2007 (Ind) -0.022 [-0.089 , 0.044] 5.9%
Maple Brown et al. 2005 -0.054 [-0.133 , 0.026] 5.5%
McEniery et al. 2008 -0.140 [-0.160 , -0.120] 6.7%
Recio-Rodriguez et al. 2012 -0.035 [-0.074 , 0.003] 6.5%
Sacre et al. 2012 0.010 [-0.030 , 0.050] 6.4%
Schultz et al. 2012 -0.020 [-0.143 , 0.103] 4.4%
Sharman et al. 2008 -0.040 [-0.069 , -0.011] 6.6%
Wier et al. 2011 0.080 [0.066 , 0.094] 6.8%
Pooled RE Model -0.031 [-0.074 , 0.012]
-0.40
-0.20
0.00
0.20
0.40
Mean difference in pulse pressure amplification

B


Author and Year
Effect size [95% CI]
Weight
Agnoletti et al. 2013 -3.50 [-6.62 , -0.38] 9.0%
Brooks et al. 2001 4.62 [0.89 , 8.36] 8.4%
Climie et al. 2013 3.00 [-0.83 , 6.83] 8.3%
Climie et al. 2014 8.00 [4.10 , 11.90] 8.2%
Maple Brown et al. 2007 (Eur) 8.00 [3.78 , 12.22] 7.9%
Maple Brown et al. 2007 (Ind) 3.00 [-0.82 , 6.82] 8.3%
Maple Brown et al. 2005 -4.17 [-9.90 , 1.55] 6.5%
McEniery et al. 2008 8.00 [6.67 , 9.33] 10.4%
Recio-Rodriguez et al. 2012 1.50 [-2.01 , 5.01] 8.6%
Sacre et al. 2012 0.00 [-2.77 , 2.77] 9.3%
Schultz et al. 2012 1.00 [-6.73 , 8.73] 4.9%
Tamminen et al. 2002 4.40 [3.00 , 5.80] 10.3%
Pooled RE Model 3.05 [0.74 , 5.36]
-10.0
-5.0
0.0
5.0
10.0
15.0
Mean difference in augmentation index

C



D

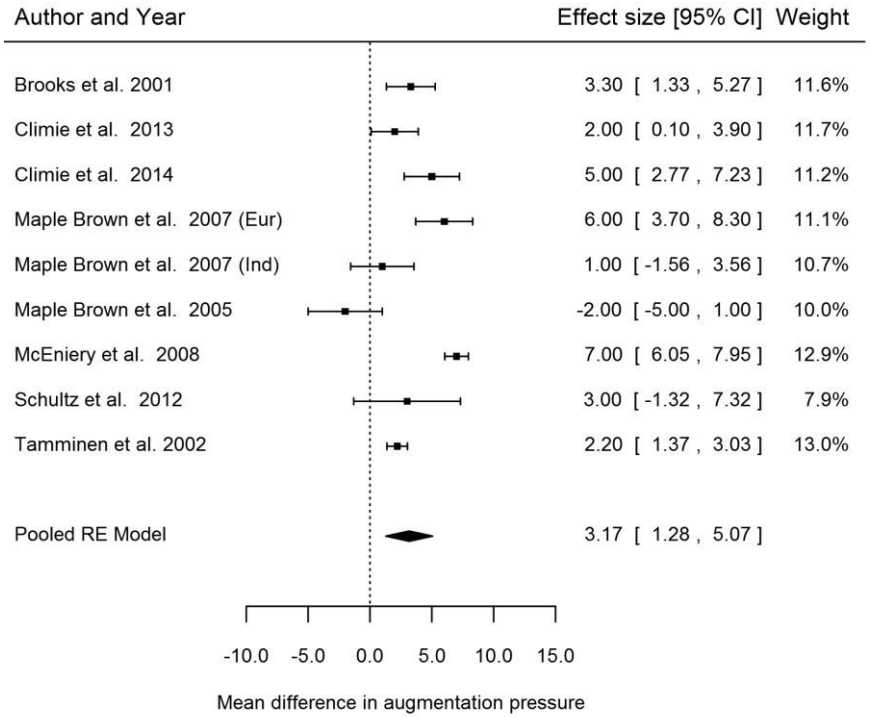


Figure 2.2.4. Pooled estimates and 95% confidence intervals for; (A) Amplification in central to brachial pulse pressure, I^2=96.1% p=0.16; (B) augmentation index, I^2=86.5% p=0.010; (C) augmentation index adjusted for a heart rate of 75 beats per minute (bpm), I^2=61.0% p<0.001; (D) augmentation pressure, I^2=90.6% p=0.001.

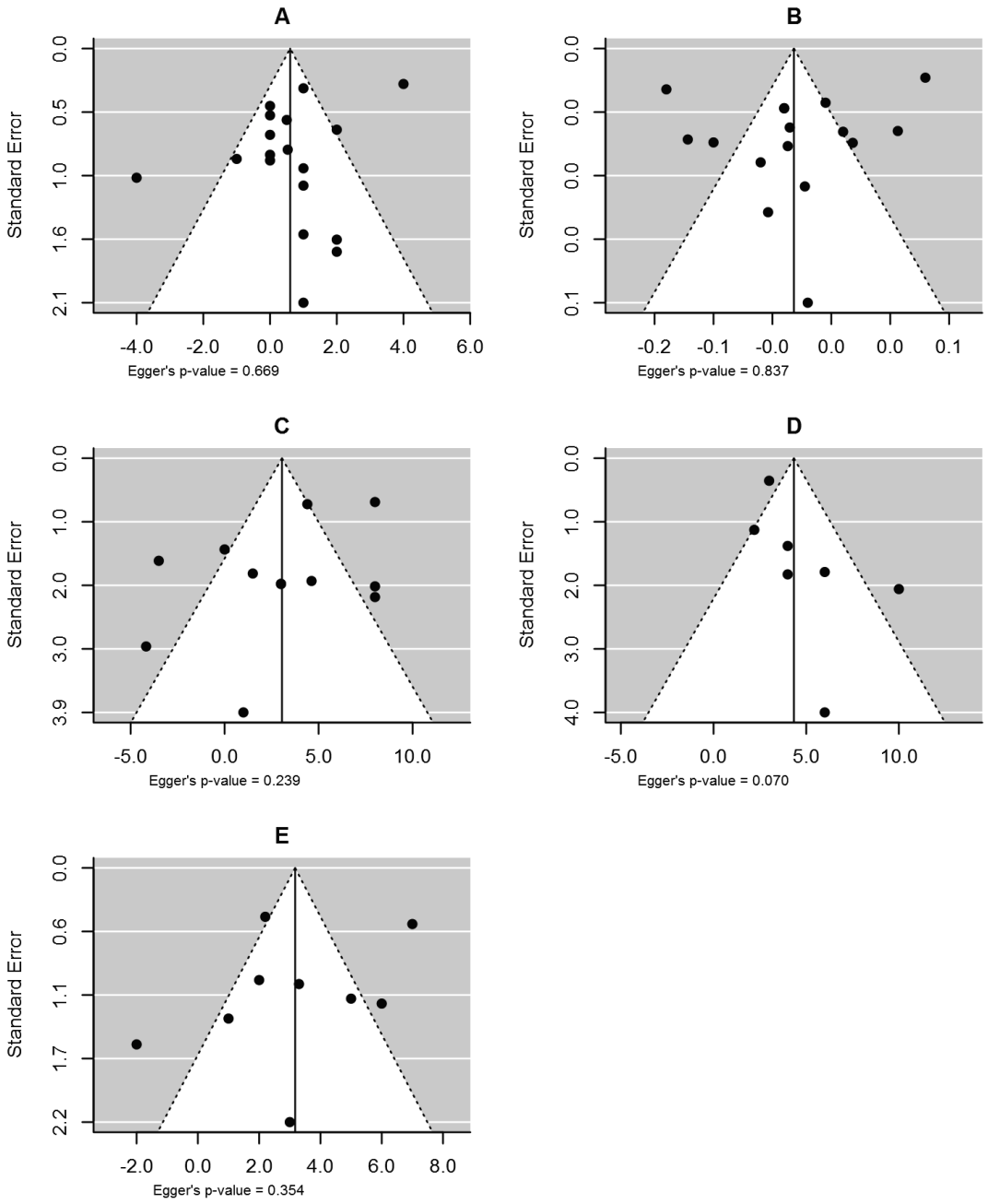


Figure 2.2.5. Funnel plots representing the publication bias for individual studies for each meta-analysis. (A) Central to brachial systolic blood pressure amplification; (B) central to brachial pulse pressure amplification; (C) augmentation index; (D) augmentation index corrected of heart rate of 75 beats per minute; (E) augmentation pressure. The results depict the relative absence of any publication bias.

2.2.5 Discussion

The salient finding of this study, comprising of data from 2,711 patients with T2DM and 10,460 non-diabetic individuals, was that there is no difference in central to brachial SBP or PP amplification in patients with T2DM compared to non-diabetic controls, despite differences in both central and brachial SBP, as well as central BP indices (AIx and AP). Furthermore, we have shown that large variation in SBP amplification exists and is similar for both individuals with and without T2DM. Additionally, the difference in the level of amplification between patients with T2DM and non-diabetic controls differs depending on the duration of diabetes in the diabetic group, increasing in magnitude with increasing disease progression. These novel findings highlight that central SBP cannot be estimated from a measure of brachial SBP and that assessment of risk related to BP should not be based on a measure of brachial BP alone.

Central to brachial SBP and PP amplification in patients with T2DM compared to non-diabetic controls

The level of central to brachial SBP amplification is predominantly influenced by factors affecting vessel stiffness and pressure wave travel[4] but studies have shown that amplification may also be influenced by a number of demographic[35] and physiological factors[35, 36]. Furthermore, the discrepancy between central and brachial SBP may be magnified by the administration of antihypertensive medication[40, 169]. In patients with T2DM, vascular abnormalities and cardiovascular risk factors that have a greater influence on central, rather than brachial SBP (including hypertension[170], hyperlipidaemia[79] and smoking[171]) may further effect the magnitude and variation in amplification by causing a rise in central SBP and a dampening of central to brachial SBP amplification. Indeed, in a large cohort of individuals from the Anglo-Cardiff Collaborative Trial, McEniery et al.[4] showed that diabetes (as well as cardiovascular disease) was associated with an increase in PP ratio (higher central relative to brachial BP), beyond other cardiovascular risk factors. This suggests that central to brachial SBP amplification should be lower in patients with T2DM compared to non-diabetic individuals. On the other hand, we have previously shown that there is no difference in central to brachial SBP amplification (brachial-aortic SBP difference) between patients with T2DM and healthy individuals[167].

In the current study, patients with T2DM had elevated central (and brachial) SBP, AIx and AP compared to non-diabetic controls, all of which are markers of increased cardiovascular risk[33, 156]. Despite this, there was no difference in central to brachial SBP or (PP) amplification between

patients with T2DM and non-diabetic controls. Importantly, we observed substantial variation in amplification in both groups, similar to previous findings[172]. This suggests that two people (either with or without T2DM) may have similar brachial SBP, but could have significantly different central SBP. Furthermore, the magnitude of SBP amplification varies depending on the degree of glycaemic control and throughout disease progression in patients with T2DM. Indeed, in newly diagnosed patients (duration of diabetes of 5 years) central to brachial SBP amplification is lower compared to non-diabetic controls, but rises to be 1.1 mmHg higher after 11 years duration of T2DM. Further, chronic and uncontrolled hyperglycaemia can lead to vascular dysfunction[56] and an eventually results in an increase in brachial SBP. Indeed, the difference in the degree of glycaemic control in patients with T2DM had a substantial influence on the heterogeneity observed in SBP amplification between studies. Therefore, in patients with T2DM, in whom central systolic stress is increased (i.e. elevated central SBP, AIx and AP), risk related to BP may not be captured by a conventional measure of brachial BP. These findings have relevance for the management of BP in patients with T2DM and decisions surrounding the administration of therapeutic agents, which may be misguided based on a measure of brachial BP alone.

A large proportion of the studies included in the meta-analysis (15 from a total of 17 studies) used radial applanation tonometry (mostly calibrated with brachial SBP and diastolic BP) to estimate central SBP. However, a recent meta-analysis and systematic review[173] demonstrated that there is significant error introduced by calibrating central SBP obtained via radial applanation tonometry with brachial SBP and diastolic BP measures. In particular using the SphygmoCor device, central SBP calibrated with non-invasive brachial SBP and diastolic BP was underestimated by 8 ± 11.6 mmHg. While this method advocates the use of brachial SBP and diastolic BP to calibrate the radial waveform, we have shown that by failing to account for brachial to radial SBP amplification, central SBP estimated via radial tonometry is underestimated in patients with T2DM[174] and may result in SBP amplification being overestimated. Additionally, brachial to radial SBP amplification is significantly dampened in patients with T2DM compared to non-diabetic controls (9 ± 8 vs 14 ± 7 mmHg, p=0.042)[174] and when the radial waveform is re-calibrated with radial (rather than brachial) SBP, the change in central SBP is lower in patients with T2DM (9 ± 6 vs 12 ± 6 mmHg). This suggests that patients with T2DM may have less underestimation in central SBP and thus, higher central to brachial SBP amplification compared to non-diabetic individuals. Further, underestimation of brachial BP itself by devices that utilise oscillimetric SBP and diastolic BP as calibration points may further contribute to underestimation in central SBP[175,

[176]. Thus calibrating the radial waveform with brachial mean and diastolic BP may be a reasonable alternative to brachial SBP and diastolic BP[177, 178] as thesevpressures remain almost entirely unaltered throughout the arterial tree[43]. Indeed, recent evidence suggests that the use of oscillometric mean arterial pressure for calibration generates a derived central SBP that is closer to the true (invasive) central SBP[75]. Furthermore, Laugesen et al.[179] demonstrated that by calibrating the radial waveform with mean arterial pressure and diastolic BP using a 40% form factor substantially improved the accuracy of estimating central SBP in patients with T2DM. Taken together, this suggests that the minimal difference in amplification observed between patients with T2DM and non-diabetic controls may be influenced by the non-invasive BP methods used to determine central SBP, potentially masking any difference in amplification observed between the groups. It should also be noted that the observed difference in SBP amplification between patients with T2DM and non-diabetic controls that varies with disease progression may also be influenced by the non-invasive methods used to estimate SBP amplification. The degree of influence is difficult to discern from the current study and is a limitation of using non-invasive methods to determine the level of amplification in patients with T2DM, which may be overcome with further invasive studies.

Limitations

There are a few limitations to our study. Firstly, the different devices used in the individual studies to estimate central BP non-invasively may influence the level of amplification observed in the current study. However, this is unlikely as all but two studies[161, 162] used radial applanation tonometry to measure central SBP and in the sensitivity analysis these studies did not affect the pooled estimate for central to brachial SBP amplification. Secondly, we relied on published combined data rather than acquiring individual patient data for each study and thus, it was not possible to correct for potential biases within the individual studies. Finally, only a small number of studies met our inclusion criteria and, therefore, publication bias was unable to be thoroughly assessed.

2.2.6 Conclusions

This is the first systematic review and meta-analysis to examine central to brachial SBP amplification in patients with T2DM compared to non-diabetic individuals. These findings are significant as they show that despite central and brachial SBP (as well as other markers of systolic stress) being elevated in patients with T2DM, there is no difference in the level of SBP

amplification compared to non-diabetic individuals. Importantly, we observed large variation in amplification in both populations, which suggests that risk related to central SBP may be overestimated in some individuals whilst underestimated in others based on a measure of brachial SBP. However, methodological errors exist when calibrating central SBP estimated non-invasively and may have confounded the level and variation in amplification observed in the current study. Therefore, further studies are required to confirm the true magnitude of central to brachial SBP amplification in patients with T2DM compared to non-diabetic controls.

2.2.7 Contribution of Chapter 2 Part II to thesis aims

The study presented in *Part II* of *Chapter 2* showed that despite patients with T2DM having elevated central and brachial SBP compared to non-diabetic individuals, there is minimal difference in central to brachial SBP amplification between the groups. The difference in amplification between individuals with and without T2DM varies depending on the length of disease (diabetes) duration. Importantly, this systematic review and meta-analysis has demonstrated that large variation in amplification occurs in patients with T2DM, which suggests that the risk related to elevated central systolic stress may not be captured based on a measure of brachial BP alone in this population. However, methodological errors in estimating central SBP non-invasively, especially via radial applanation tonometry, may limit the value of central BP as a clinically useful tool. Whilst this study is an important contribution to current knowledge surrounding the non-invasive measurement of central SBP in patients with T2DM, more work is warranted to resolve these methodological errors, given the potential supremacy of central, beyond brachial, BP for determining risk related to BP. In *Chapter 7* of this thesis, the effect of brachial to radial SBP amplification on central SBP estimated via radial applanation tonometry is, therefore, examined. Furthermore, substantial debate surrounds the issue as to whether AIx should be considered as a marker of arterial stiffness in patients with T2DM, as it is purported to be. This line of inquiry is further investigated in the next chapter.

Chapter 3. Augmentation index and arterial stiffness in patients with type 2 diabetes mellitus

This chapter has previously been published;

Climie RED, Nikolic SB, Otahal P, Keith LJ, Sharman JE. Augmentation index and arterial stiffness in patients with type 2 diabetes mellitus. *Artery Research*, September 2013; 7:194- 200.

3.1 Abstract

Background. Augmentation index (AIx) is a measure of left ventricular afterload that predicts mortality and is regarded as a marker of systemic arterial stiffness. Patients with type 2 diabetes mellitus (T2DM) have increased arterial stiffness, but not AIx, which suggests that mechanisms contributing to AIx in T2DM may differ from non-diabetic individuals and be unrelated to arterial stiffness. The aim of this study was to examine the cardiovascular and clinical determinates of AIx (including arterial stiffness) in patients with T2DM compared with controls.

Methods. Clinical characteristics and haemodynamic variables (including aortic and brachial pulse wave velocity [stiffness], cardiac output, systemic vascular resistance and heart rate) and AIx (by radial tonometry) were recorded in 53 T2DM (aged 61±8 years) and 53 matched controls (aged 58±6). Correlates of AIx unadjusted for heart rate were assessed by uni- and multi-variable analysis.

Results. Compared with controls, T2DM patients had significantly higher aortic stiffness (7.6±1.6vs6.7±1.9 m/s p=0.016), cardiac output, heart rate, brachial and central BP; lower brachial stiffness and systemic vascular resistance, but no significant difference in AIx (27±9vs24±11% p=0.184). AIx (adjusted or unadjusted) was not significantly related to aortic or brachial stiffness in either group (p>0.198 all). Independent predictors of AIx in T2DM patients were height and heart rate, whereas in controls, AIx was independently related to height.

Conclusions. Determinants of AIx in patients with T2DM differ from non-diabetic individuals. Moreover, AIx is not significantly related to regional large artery stiffness and should not be regarded as indicative of systemic arterial stiffness.

3.2 Introduction

Increased arterial stiffness is an independent predictor of cardiovascular events and total mortality in both healthy and diseased populations[180]. Augmentation index (AIx) is defined as the difference between the second and first systolic peaks on the central (aortic) pressure waveform expressed as a percentage of pulse pressure. AIx represents the pressure over time that the heart is exposed to during each contraction and is, therefore, a measure of left ventricular afterload[141]. AIx is inversely related to heart rate, and is purported to be a marker of systemic arterial stiffness. This is based on the notion that the magnitude and speed of arterial wave travel is increased in the presence of stiffened vasculature through increased wave reflection[43].

Several studies have shown that patients with type 2 diabetes mellitus (T2DM) have generalised vascular dysfunction and increased arterial stiffness compared to non-diabetic, age-matched people[3, 85, 86, 98, 120, 181]. Specifically, patients with T2DM have been shown to have increased aortic stiffness (assessed by aortic pulse wave velocity)[85, 86, 120, 181], higher carotid intima media thickness[3] and elevated cardio-ankle vascular stiffness index[182]; as well as decreased systemic arterial compliance[44, 181] and arterial distensibility[98]. Taken all together these data lead to the expectation that AIx should be significantly elevated in patients with T2DM. Indeed, this has been reported in some cross sectional case-control comparison studies[44, 70, 84]. On the other hand, several studies have shown that despite significant increases in arterial stiffness among people with T2DM, no significant differences in AIx were found when compared with healthy subjects, and this was observed with[87, 88] or without[85, 86] adjusting for heart rate. The above information brings into question the concept that AIx is indicative of systemic arterial stiffness and necessitates further investigations to determine reasons for the inconsistency in these findings. Thus, the aim of this study was to examine the cardiovascular and clinical determinates of AIx (including arterial stiffness) in patients with T2DM compared with controls. We hypothesised that arterial stiffness would be significantly elevated in patients with T2DM but would not be related to AIx and that the determinants of AIx would differ from healthy individuals.

3.3 Methods

Study participants

Exclusion criteria for participation in the study included; pregnancy or a clinical history of cardiovascular disease including cardiac arrhythmia. A total of 152 eligible participants responded to community advertisement and were examined between June 2010 and February 2011. The sample comprised 53 patients with T2DM (51% male), and each of these were matched with one non-diabetic control participant selected from the remaining 99 non- diabetic participants. Matching was made on the basis of the same sex and the nearest age (total n=106). Diabetes mellitus was determined by self-reported diagnosis by a physician. Hypertension was defined as: clinic brachial BP ≥140/90 mmHg; use of antihypertensive medications or self-reported diagnosis of hypertension by a physician. Participant characteristics are summarised in table 3.1.

Study protocol

Participants attended the research clinic for assessment on two occasions. At visit one, all standard anthropometric (including height, weight, waist and hip circumference) and BP variables were measured in a temperature controlled room (23°C ± 1°C). Prior to this visit, participants were asked to refrain from alcohol consumption and exercise on the day of testing and to avoid consuming heavy meals (i.e. were in a post-absorptive state), smoking and caffeine containing products in the three hours prior to testing. At visit two, fasting blood samples were taken and all participants were fitted with a 24-hour ambulatory BP monitor. All participants signed informed consent and the study was approved by the Tasmanian Health and Medical Human Research Ethics Committee.

Arterial stiffness

After the participant had been resting supine on a bed for 10 minutes, duplicate measures of brachial pulse wave velocity (PWV) were measured in the carotid-to-radial arterial segments using ECG-gated hand held applanation tonometry (SphygmoCor 8.1, AtCor Medical, Sydney, Australia). Aortic PWV was measured in duplicate from the carotid-to-femoral arterial segments using the same tonometry apparatus. Arterial length was estimated by subtracting the transcutaneous distance between the sternal notch and carotid sampling site from the distance between the sternal notch and the radial sampling site (for brachial PWV) and femoral site (for aortic PWV)[10].

Table 3.1. Participant characteristics for non-diabetic participants (n=53) and patients with type 2 diabetes mellitus (T2DM; n=53).

	Non-diabetic	T2DM	P value
Male, n (%)	27 (51)	27 (51)	1.00
Age (years)	58±6	61±8	0.082
Waist-hip (ratio)	0.92±0.10	0.96±0.16	0.196
Weight (kg)	75±14	88±16	<0.001
Height (cm)	171±10	169±10	0.159
Body mass index (kg/m^2)	25.4±3.5	30.8±5.0	<0.001
Ambulatory day-time systolic BP (mmHg)	136±13	141±15	0.105
Ambulatory day-time diastolic BP (mmHg)	83±8	80 ±9	0.092
Duration of diabetes, years (range)	-	6 (1-18)	-
Hypertensive medication, n (%)	3 (6.1)	30 (56.6)	<0.001
Oral hyperglycaemia medications (including metformin and sulfonylurea), n (%)	0 (0)	33 (62.3)	-
Insulin, n (%)	0 (0)	11 (20.7)	-
Statins, n (%)	1 (1.9)	30 (56.6)	<0.001
Glucose (mmol/L)	4.8±0.6	7.6±1.9	<0.001
Cholesterol (mmol/L)	5.7±1.0	4.6±1.1	<0.001
Triglycerides (mmol/L)	1.0±0.4	1.5±0.7	<0.001
High density lipoprotein (mmol/L)	1.7±0.5	1.4±0.4	<0.001
Low density lipoprotein (mmol/L)	3.5±1.0	2.5±0.8	<0.001
Glycated haemoglobin (%)	5.3±1.0	7.2±0.8	<0.001

Data expressed as mean ± standard deviation or %. p value is for between group analyses. BP, blood pressure.

Brachial and central blood pressure

After supine measures, participants were moved into a seated position with feet flat on the floor, back supported by the chair and with a pillow placed under the arm so that the BP cuff was at the same height as the heart. After 10 minutes of rest, duplicate brachial BP measurements were recorded by a validated automatic device (Omron HEM-907; OMRON Europe B.V. (OMCE), Hoofddorp, The Netherlands)[183] using an appropriately sized cuff in accordance with guidelines[184]. Central BP was measured in duplicate by radial applanation tonometry (SphygmoCor 8.1, AtCor Medical, Sydney, Australia) immediately following the brachial BP measurements. A validated[11]

generalised transfer function was applied to the measured radial artery pressure waveforms to allow for the reconstruction of the central (aortic) pressure waveform. Pulse pressure amplification was calculated as the ratio of brachial to central pulse pressure and heart rate was determined from the electrocardiogram recording during the radial waveform measurement by the device.

Augmentation index

AIx was determined from the radial (radial AIx) and aortic pressure wave (central AIx) and was calculated as the difference in pressure between the second and first systolic peaks (augmented pressure on the central waveform) expressed as a percent of pulse pressure. Because AIx is significantly influenced by heart rate[92], it was also adjusted to a heart rate of 75 beats per minute using SphygmoCor software.

Cardiothoracic bioimpedance

Cardiac output, stroke volume and systemic vascular resistance were measured throughout the assessment by cardiothoracic bioimpedence (Physio Flow; Manatec Biomedical; Macheren, France). This device has previously been validated against invasive measures[185] and has good reproducibility[186].

Blood biochemistry

Venous blood samples were taken from the antecubital fossa following an overnight fast in order to assess blood biochemistry (including glucose, insulin, total cholesterol, triglycerides and glycated haemoglobin [HbA_{1C}]) in all participants. Analytical biochemistry was performed using accredited hospital pathology laboratory methodologies**.**

Statistical analysis

All data were analysed using SPSS for windows software version 19.0 (IBM SPSS Statistics, New York, USA). Data are presented as mean ± standard deviation unless otherwise stated and $p<0.05$ was considered statistically significant. Data were assessed for normality and all variables were normally distributed. Independent t-tests assuming unequal variance were performed for continuous variables to compare characteristics between control participants and patients with T2DM and Chi Square tests were performed for dichotomous variables. Univariable associations between variables were assessed by Pearson's correlations. Analysis of covariance was additionally undertaken to assess between group differences in AIx (correcting for age, gender, height and heart

rate) and aortic PWV (correcting for age, gender and mean arterial pressure). Multivariable regression analyses for the predictors of AIx were performed separately in patients with T2DM and controls. Models examined variables that significantly correlated with AIx and variables of clinical relevance (including age, height, heart rate, body mass index [BMI], antihypertensive medication and statin use). These variables were added separately into the regression model.

3.4 Results

Participant characteristics

As shown in table 3.1, there was no significant difference between patients with T2DM and non-diabetic controls with respect to sex, age, waist to hip ratio, height or 24 hour ambulatory determined day-time BP. Patients with T2DM were significantly heavier, had higher BMI, were more likely to be taking medication for hypertension (including angiotensin receptor blockers, beta blockers and angiotensin converting enzyme inhibitors), hyperlipidemia (statins) and hyperglycaemia (including metformin, sulfonylurea or insulin), had lower total cholesterol and high and low density lipoprotein, and had poorer glycemic control compared to non-diabetic controls. The average duration of diabetes was 6 years and ranged from 1-18 years.

Arterial stiffness

Compared to non-diabetic controls, patients with T2DM had significantly increased aortic PWV and significantly lower brachial PWV ($p<0.05$ for both, table 3.2). Furthermore, after adjusting aortic PWV for age, gender and mean arterial pressure, aortic PWV remained significantly higher in patients with T2DM ($p<0.005$).

Augmentation index

There was no significant difference in AIx between groups (radial or central; table 3.2), however, when AIx was normalised to a heart rate of 75 beats per minute, patients with T2DM had significantly increased AIx compared to the non-diabetic controls. Furthermore, AIx remained significantly higher in patients with T2DM after adjusting further for age, gender, height and heart rate.

Table 3.2. Haemodynamic comparison between non-diabetic controls (n=53) and patients with type 2 diabetes mellitus (T2DM; n=53).

	Non-diabetic	T2DM	P value
Arterial stiffness			
Aortic pulse wave velocity (m/s)	6.7±1.9	7.6±1.6	0.016
^Aortic pulse wave velocity (m/s)	6.8±1.8	7.6±1.8	0.023
Brachial pulse wave velocity (m/s)	8.5±1.0	8.1±0.9	0.037
Haemodynamics			
Brachial systolic blood pressure (mmHg)	117±11	124±13	0.004
Brachial diastolic blood pressure (mmHg)	68±8	71±9	0.178
Mean arterial pressure (mmHg)	80±22	88±16	0.039
Brachial pulse pressure (mm Hg)	49±5	54±10	0.004
Radial augmentation index (%)	76±16	80±12	0.171
Central systolic blood pressure (mmHg)	107±12	114±13	0.004
Central diastolic blood pressure (mmHg)	69±8	72±9	0.147
Central pulse pressure (mmHg)	37±6	43±10	0.001
Heart rate (bpm)	57±7	64±9	<0.001
Stroke volume (ml)	78±13	85±14	0.006
Cardiac output (l/min)	4.49±0.72	5.54±1.15	<0.001
Systemic vascular resistance (d.s.cm^{-5})	1562±281	1326±249	<0.001
Augmentation pressure (mmHg)	10±5	12±5	0.032
Central augmentation index (%)	24±11	27±9	0.184
Central augmentation index (heart rate 75 bpm)	15±11	21±7	0.002
*Central augmentation index (%)	24±9	27±9	0.043
Pulse pressure amplification (ratio)	1.33±0.15	1.27±0.14	0.043

Data expressed as mean ± standard deviation. p value is for between group analyses. ^Aortic pulse wave velocity was adjusted for age, gender and mean arterial pressure. *Central augmentation index was adjusted for age, gender, height and heart rate.

Haemodynamics

Patients with T2DM had significantly increased brachial systolic BP, mean arterial pressure, pulse pressure, central systolic BP, central pulse pressure, heart rate, stroke volume, cardiac output and

augmentation pressure, but significantly lower systemic vascular resistance and pulse pressure amplification ($p<0.05$ for all, table 3.2) compared to non-diabetic controls. There was no difference in brachial or central diastolic BP between groups.

Univariable associations with augmentation index

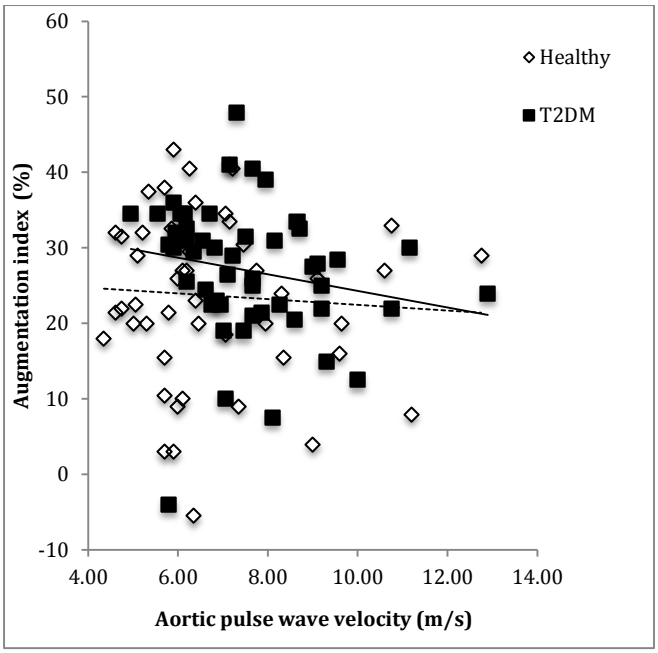
Table 3.3 summarises the univariable associations between AIx and different haemodynamic variables. AIx was not significantly correlated with either aortic PWV (figure 3.1a) or brachial PWV (figure 3.1b) in patients with T2DM or non-diabetic controls ($p>0.05$ for both, table 3.3). Moreover, after adjusting AIx for a heart rate of 75 beats per minute, there was still no significant association between AIx and aortic or brachial PWV in either patients with T2DM ($r=-0.091$, $p=0.527$ and $r=0.090$, $p=0.527$ respectively) or non-diabetic controls ($r=-0.023$, $p=0.872$ and $r=-0.015$, $p=0.311$ respectively). In patients with T2DM, AIx significantly correlated with age, height, HbA_{1C}, central systolic BP, heart rate, cardiac output and systemic vascular resistance ($p<0.05$ for all, table 3.3). In non-diabetic controls, AIx did not significantly correlate with age or heart rate ($p>0.05$), but was significantly correlated with height ($p=0.002$) and central systolic BP ($p<0.001$, table 3.3).

Table 3.3. Univariable associations of augmentation index in non-diabetic controls (n=53) and patients with type 2 diabetes mellitus (T2DM; n=53).

Independent variable	Non-diabetic		T2DM	
	r	P value	r	P value
Age (years)	0.11	0.427	0.47	<0.001
Height (cm)	-0.42	0.002	-0.46	<0.001
Body mass index (kg/m^2)	0.14	0.310	0.06	0.673
Glycated haemoglobin (%)	-0.24	0.193	0.33	0.042
Arterial stiffness				
Aortic pulse wave velocity (m/s)	-0.04	0.776	-0.19	0.198
Brachial pulse wave velocity (m/s)	-0.14	0.327	0.03	0.828
Haemodynamics				
Brachial systolic blood pressure (mmHg)	0.26	0.057	0.07	0.596
Brachial diastolic blood pressure (mmHg)	0.35	0.010	-0.18	0.211
Central systolic blood pressure (mmHg)	0.58	<0.001	0.33	0.015
Heart rate (bpm)	0.07	0.631	-0.63	<0.001
Stroke volume (ml)	-0.14	0.320	-0.23	0.101
Cardiac output (l/min)	-0.18	0.194	-0.60	<0.001
Systemic vascular resistance (d.s.cm^{-5})	0.23	0.104	0.54	<0.001

R refers to Pearson's correlation coefficient and p value is for the correlation between augmentation index and variables.

a)



b)

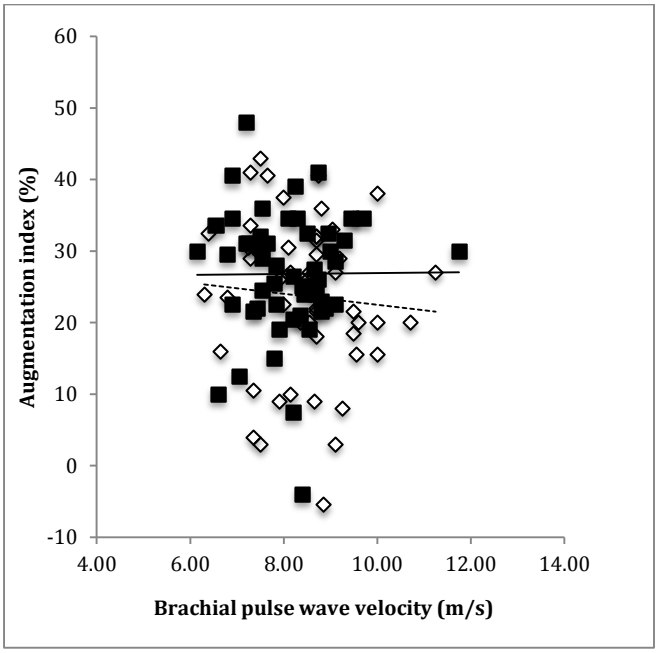


Figure 3.1. The univariable relationship between augmentation index and arterial stiffness (aortic pulse wave velocity (a) and brachial pulse wave velocity (b)) in non-diabetic participants (healthy; dashed line) (r=-0.03, p=0.863 and r=-0.13, p=0.373 respectively) and patients with type 2 diabetes mellitus (T2DM; solid line) (r=-0.19, p=0.198 and r=0.03, p=0.828).

Predictors of augmentation index

Separate regression models were performed for non-diabetic controls and patients with T2DM and are shown in table 3.4. The models included the covariates of age, height, BMI, HbA_{1C}, heart rate, cardiac output antihypertensive medication and statin use. In patients with T2DM, the strongest predictors of AIx (model adjusted R^2 =0.47, p=0.001) were height and heart rate, which accounted for 13% and 15% of the variance in AIx respectively. Further adjustment for insulin use did not affect the model (model adjusted R^2 =0.49, p=0.001). The strongest predictor of AIx in controls (model adjusted R^2 = 0.17, p=0.012) was height, which explained 20% of the variance in AIx. Only a few of the non-diabetic participants were being treated for hypertension (n=3) or hyperlipidemia (n=1), and the addition of these variables in the multivariable analysis did not affect the model.

Table 3.4. Multivariable regression for associations between augmentation index and cardiovascular and clinic characteristics in non-diabetic controls (n=53) and patients with type 2 diabetes mellitus (T2DM; n=53).

Independent variable	Unstandardised β (95% CI)	p value
Healthy		
Age (years)	-0.06 (-0.40, 0.53)	0.782
Height (cm)	-0.53 (-0.83, -0.23)	0.001
Body mass index (kg/m^2)	0.66 (-0.16, 1.48)	0.110
Heart rate (bpm)	-0.19 (-0.61, 0.22)	0.353
T2DM		
Age (years)	0.14 (-0.97, 0.37)	0.240
Height (cm)	-0.32 (-0.53, -0.10)	0.005
Body mass index (kg/m^2)	0.01 (-0.46, 0.47)	0.961
Hemoglobin A1c (%)	1.82 (-0.12, 3.78)	0.066
Heart rate (bpm)	-0.43 (-0.67, -0.13)	0.004
Cardiac output (l/min)	0.24 (-2.29, 2.77)	0.846
Antihypertensive medication	0.24 (-2.63, 6.05)	0.427
Statin use	0.90 (-3.13, 4.95)	0.649

Models were performed separately for non-diabetic controls and patients with T2DM. Data are unstandardised β coefficient and p value relates to the independent variable in the model.

3.5 Discussion

The main finding of this study was that despite patients with T2DM having significantly increased arterial stiffness (aortic PWV), there was no difference in AIx unadjusted for heart rate, compared to age and sex matched non-diabetic controls. Secondly, whether adjusted for heart rate or otherwise, AIx was not significantly related to aortic or brachial artery stiffness in non-diabetic controls or patients with T2DM. Furthermore, the determinants of AIx were different in patients with T2DM compared to non-diabetic individuals. In patients with T2DM, the independent predictors of AIx were height and heart rate whilst in non-diabetic controls only height was independently related to AIx. Our findings suggest that AIx is not a reliable marker of arterial stiffness in patients with T2DM or non-diabetic individuals and separate factors are likely to influence AIx between these populations.

Arterial stiffness in patients with T2DM

Our observation of increased aortic PWV in patients with T2DM is consistent with numerous studies showing that patients with T2DM have accelerated arterial stiffening compared to non-diabetic matched controls[3, 44, 85, 86, 120, 181]. The Strong Heart Study[187] also found that arterial stiffness assessed using the ratio of pulse pressure to stroke volume, was significantly increased in diabetic patients compared to normoglycaemic individuals[188] . AIx has been heralded as a surrogate marker of systemic arterial stiffness[189, 190] however studies examining the association between AIx and measures of vascular stiffness in patients with T2DM are not conclusive. Indeed, similar to our study, no difference in AIx has been observed between patients with T2DM and healthy participants[85-88]. On the other hand, others[70, 84, 97] have reported AIx to be significantly increased in patients with T2DM compared with non-diabetic individuals, but this was only after adjusting for heart rate or only evident in male study participants. A possible explanation for these discrepancies may be that AIx is influenced by a multitude of factors beyond arterial stiffness that result in altered waveform patterns in patients with T2DM and contribute to inconsistent findings in terms of the overall effect on AIx.

Determinants of AIx in patients with T2DM

In both healthy participants and patients with T2DM, AIx was significantly and inversely related to height. This supports previous findings and conventional theory suggests this is because people of shorter stature have reduced distance to arterial pressure reflecting sites and this influences the timing and magnitude of arterial wave travel, causing early return to the heart (during systole) and

resulting in an increase in AIx[43, 191]. In our study, patients with T2DM had significantly increased heart rate compared to healthy participants and, similar to previous studies[28, 44] heart rate was significantly related to AIx in patients with T2DM. An increase in heart rate shortens the ejection duration and corresponding lower AIx is purported to be due to reflected pressure waves being moved into the diastolic (rather than systole) phase[43], however, aortic reservoir function should also be considered when interpreting physiological mechanisms of AIx[82, 192]. In our findings, the effect of heart rate on AIx was greater than the effect of arterial stiffness (aortic PWV) because AIx was significantly increased in patients with T2DM after correcting for heart rate. Other studies have demonstrated the same effect whereby significantly increased AIx compared to healthy people was only seen after adjusting for heart rate[28, 92, 97]. On the other hand, Lacy et al.[88] found no difference in AIx between people with and without diabetes even after adjustment for heart rate. Insulin increases vasodilation of the large arteries and has a diminishing effect on AIx[193], which may explain the lack of difference in AIx between patients with and without T2DM. It should be noted that in the current study 20.7% of patients with T2DM were being administered insulin, however insulin use was not related to, or a determinant of, AIx. Further, in the current study, some patients with T2DM had poorly controlled blood glucose levels which can lead to vascular impairment via a number of pathways (including impaired vasodilatory processes and vascular smooth muscle cell dysfunction)[56]. HbA_1c was borderline significantly related to AIx in patients with T2DM suggesting that abnormalities in vascular function due to chronic hyperglycaemia may result in elevated AIx.

In our study, patients with T2DM had significantly increased cardiac output (due to a rise in both heart rate and stroke volume) and decreased systemic vascular resistance, compared to healthy participants. Elevated cardiac output has previously been observed in people with insulin resistance and patients with T2DM[67, 194]. Although not independent predictors of AIx, both cardiac output and systemic vascular resistance were significantly correlated with AIx in patients with T2DM, but not in healthy participants. The increase in left ventricular flow output together with the reduction in systemic vascular resistance could together be contributing to the relative reduction in AIx in diabetic individuals. The high flow output may be suggestive of increased dilation of the proximal aorta (among other possibilities) but would need to be assessed in future studies. Interestingly, patients with T2DM had significantly lower brachial PWV, which may also be suggestive of muscular peripheral artery dilation beyond that of controls.

Limitations

This is a relatively small case-control comparison study that cannot attribute causality and further studies are required in order to determine the exact mechanisms contributing to AIx in patients with T2DM. In our study, we determined arterial stiffness via PWV, however, the addition of other markers of arterial stiffness would strengthen the findings with respect to the relation between AIx and systemic arterial stiffness. Finally, more than half the patients with T2DM were taking medication for hypertension or hyperlipidaemia, and the vasoactive properties of these medications could have influenced the results. Future studies in drug naïve participants could overcome this problem.

3.6 Conclusions

The main finding of this study was that AIx (whether adjusted or unadjusted for heart rate) was not related to arterial stiffness in patients with T2DM and that the determinants of AIx in these patients were significantly different to that of non-diabetic participants. Our findings indicate that AIx should not be referred to as a surrogate maker of arterial stiffness and further work is needed in order to understand the disparate systemic haemodynamics that may explain the difference in AIx between people with and without T2DM.

3.7 Contribution of Chapter 3 to thesis aims

Prior to this study it was unclear whether AIx (a purported marker of arterial stiffness) was indeed related to arterial stiffness in patients with T2DM, as previous studies had shown conflicting results. Importantly, this current study has confirmed that AIx is not related to arterial stiffness in either individuals with or without T2DM and furthermore, has identified the different correlates of AIx within each group. Although the finding of no difference in unadjusted AIx between individuals with and without T2DM is somewhat contrary to the finding in *Part II* of *Chapter 2*, this may be explained by the relatively small sample size of the current study, as the magnitude of difference in AIx between the groups was similar in both studies. Nonetheless, this study further supports that patients with T2DM have abnormal central haemodynamics compared to non-diabetic individuals. However, it remains unclear how these alterations in central haemodynamics, in particular the high flow (elevated cardiac output), low resistance (low systematic vascular resistance) state is related to target organ damage and is further investigated in *Chapters 5* and *6*. Furthermore, it is also unclear how changes in central arterial function affect the accuracy of methods that estimate central BP non-invasively, such as radial applanation tonometry, which is r subsequently examined in *Chapter 7* of this thesis.

Chapter 4. Abdominal obesity and brain atrophy in type 2 diabetes mellitus

This chapter has previously been published;

Climie RED, Moran C, Callisaya M, Blizzard L, Sharman JE, Venn A, Phan TG, Beare R, Forbes J, Blackburn NB, Srikanth V. Abdominal obesity and brain atrophy in type 2 diabetes mellitus. *PloS One*, November 2015; 10: e0142589.

4.1 Abstract

Background. Type 2 diabetes mellitus (T2DM) is associated with gray matter atrophy. Adiposity and physical inactivity are risk factors for T2DM and brain atrophy. We studied whether the associations of T2DM with total gray matter volume (GMV) and hippocampal volume (HV) are dependent on obesity and physical activity.

Methods. In this cross-sectional study, we measured waist-hip ratio (WHR), body mass index (BMI), mean steps/day and brain volumes in a community dwelling cohort of people with and without T2DM. Using multivariable linear regression, we examined whether WHR, BMI and physical activity mediated or modified the association between T2DM, GMV and HV.

Results. There were 258 participants with (mean age 67±7 years) and 302 without (mean age 72±7 years) T2DM. Adjusting for age, sex and intracranial volume, T2DM was independently associated with lower total GMV ($p=0.001$) and HV ($p<0.001$), greater WHR ($p<0.001$) and BMI ($p<0.001$), and lower mean steps/day ($p=0.002$). After adjusting for covariates, the inclusion of BMI and mean steps/day did not significantly affect the T2DM-GMV association, but WHR attenuated it by 32% while remaining independently associated with lower GMV ($p<0.01$). The T2DM-HV association was minimally changed by the addition of BMI, steps/day or WHR in the model. No statistical interactions were observed between T2DM and measures of obesity and physical activity in explaining brain volumes.

Conclusions. Abdominal obesity or its downstream effects may partially mediate the adverse effect of T2DM on brain atrophy. This requires confirmation in longitudinal studies.

4.2 Introduction

People with type 2 Diabetes Mellitus (T2DM) are at high risk of developing cognitive impairment[195] and dementia[196]. We have recently shown that T2DM is associated with lower total gray matter volume (GMV) and that GMV loss may explain the association between T2DM and cognitive dysfunction[197]. However, the pathways leading to loss of GMV in T2DM are unknown.

Obesity and physical inactivity are commonly seen in people with T2DM, and have also been associated with brain atrophy[198-202] and dementia[203, 204]. The distribution of body fat may also play a role in explaining these associations. In particular, abdominal adiposity is linked to chronic inflammation and reduced insulin sensitivity[205], both potentially important factors in determining neuronal health[203, 204]. In support of this concept, a recent imaging study demonstrated that visceral fat accumulation was associated with reduced cortical thickness independent of BMI[206]. Low levels of physical activity[202] or cardiovascular fitness[207], which are determinants of low grade inflammation, vascular health and metabolic health[208] have also been associated with lower GMV.

The roles of obesity and physical activity in determining gray matter loss in people with T2DM have not been studied. Since these are modifiable risk factors, a better understanding of their relative contributions to brain health in T2DM will help guide interventions aimed at preserving cognition in people with T2DM who represent a high-risk group for developing dementia. We hypothesised that the association between T2DM and GMV will either be modified or mediated by measures of obesity or physical inactivity.

4.3 Methods

Study participants

The sample consisted of participants recruited into the Cognition and Diabetes in Older Tasmanians study, the recruitment details of which have previously been described[197]. Those with T2DM were selected from the National Diabetes Service Scheme (NDSS) register if aged >55 years and living in the Southern Tasmanian postcodes 7000-7199. The NDSS is managed by Diabetes Australia and provides information and support for individuals with diabetes who enroll voluntarily. The diagnosis of T2DM within NDSS is based on physician assessment using standard criteria including; fasting plasma glucose $\geq$7. 0 mmol/L, random plasma glucose $\geq$11.1 mmol/L, or 2 hour glucose $\geq$11.1mmol/L post oral glucose tolerance test. The population-based comparison group consisted of individuals who were aged $\geq$60 years without T2DM randomly selected from the same Southern Tasmanian postcodes (7000-7199) into the Tasmanian Study of Cognition and Gait[197].

The absence of T2DM in the comparison group was determined by the following; fasting plasma glucose <7.0mmol/L, random plasma glucose <11.1mmol/L and glycated haemoglobin (HbA_1c) ≤6.5% (48mmol/mol) in those individuals without a history of T2DM. All potential participants received invitation letters followed by telephone contact for enrolment into the study. Excluded were people living in a nursing home and those with any contraindication to magnetic resonance imaging (MRI). The Southern Tasmanian Health and Medical Human Research Ethics Committee and the Monash University Human Research Ethics Committee approved the study and all participants signed informed consent.

Measurements

Standardised questionnaires were administered to obtain demographic data, clinical information about the duration of T2DM, years of formal education, health and medical history including that of cardiovascular disease and risk factors, and medication use. The 15-item Geriatric Depression Scale (GDS)[209] was used to determine mood.

Obesity

Waist and hip circumference were measured in duplicate unless there was a difference of more than two centimeters between the first and second measurement, in which case a third measurement was taken and the average of all three measures was used in the analysis. Waist-hip ratio (WHR) was calculated as a measure of abdominal obesity dividing waist circumference (cm) by hip circumference (cm). Height (m) and weight (kg) were measured and body mass index (BMI) was calculated as weight divided by height squared.

Physical activity

Daily physical activity was measured using a Yamax pedometer. Participants were instructed to attach the pedometer to the waistband of trousers/skirt above their dominant leg and to wear the pedometer for 7 consecutive days, whilst going about normal daily activity. They were instructed to reset the pedometer at the start of every day and to record the number of steps displayed on the monitor in a pedometer diary at the end of each day. Mean steps/day were calculated by dividing the total number of steps on days where the participant wore the pedometer for ≥eight hours a day, by the number of days that the pedometer was worn. In a sub analysis (n=115) we determined that a cut off value for wear time of ≥eight hours a day would result in 95% of mean steps/day being captured.

Brain MRI

MRI brain scans were performed using a 1.5T General Electric Signa Excite T scanner with sequences as follows: High-resolution T1 weighted spoiled gradient echo (TR 35ms, TE 7ms, flip angle 35º, field of view 24 cm, voxel size 1 mm^3) comprising 120 contiguous slices; T2 weighted fast spin echo (TR 4300 ms; TE 120 ms; NEX 1; turbo factor 48; voxel size 0.90 x 0.90 x 3 mm); FLAIR (fluid attenuated inversion recovery) (TR=8802 ms, TE=130 ms, TI=2200ms, voxel size 0.50 x 0.50 x 3 mm); GRE (TR0.8ms, TE 0.015, flip angle 30º, voxel size 0.9 x 0.9 x 7 mm). All processing and segmentation steps were performed by investigators blinded to T2DM status. The scans were registered to a standard 152 brain Montreal Neurological Institute template in stereotaxic coordinate space. Gray and white matter were automatically segmented using methods in statistical parametric mapping software SPM5[210]. Hippocampi were manually segmented using standard methodology and landmarks with high test-retest reliability[211]. Total GMV and hippocampal volume (left, right and total HV) were calculated using standard in-house voxel counting algorithms.

Blood biochemistry and genotyping

Following an overnight fast, venous blood samples were taken from the antecubital fossa. Analytical biochemistry of fasting plasma glucose, HbA_1c, insulin, lipid profile and C-reactive protein (CRP) were performed at the Royal Hobart Hospital, Tasmania, Australia using accredited laboratory techniques. We also measured serum levels of tumor necrosis factor alpha (TNFα) and interleukin 6 (IL6) using Multiplex Bead Arrays (Lincoplex, Linco Research Inc. Missouri, USA). Whole blood DNA extraction and apolipoprotein ε4 allele (*APOE*-ε4) SNP genotyping (rs429358 and rs7412) using Sequenom MassArray iPLEX technology was also performed as the presence of *APOE*-ε4 is known to increase the risk of Alzheimer's disease in patients with T2DM[212].

Other clinical measures

Mean systolic blood pressure was taken from three consecutive seated brachial blood pressure measurements from the right arm of each participant using an Omron M4 sphygmomanometer. Hypertension was defined as systolic blood pressure>140mmHg and/or diastolic blood pressure >90 mmHg and/or current use of anti-hypertension medication. Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated from fasting plasma glucose and insulin levels using the formula (Insulin x Glucose)/22.5[213]. Hyperlipidaemia was defined as total cholesterol $\geq$6 mmol/L and/or current use of statin. We also had measures of tissue advanced glycation endproduct (AGE) accumulation available in most participants using the skin autofluorescence technique[214].

Statistical analysis

Independent t tests were performed for continuous variables with normal distributions, Wilcoxon rank sum test for continuous measures with non-normal distributions, and Chi square tests for dichotomous variables while comparing characteristics between patients with and without T2DM. Firstly exploratory unadjusted correlations and regressions were conducted adjusting for age, sex and total intracranial volume to examine the association between T2DM and cortical volumes (GMV, HV), and associations of obesity and habitual physical activity (WHR, BMI, mean steps/day) with cortical volumes. Multivariable regression models were then used to examine whether the T2DM-brain volume relationships were confounded, modified or mediated by measures of obesity and physical activity. To study effect modification, we assessed for an interaction between T2DM and measures of obesity and physical activity in explaining brain volumes using a test of significance of the respective product terms (T2DM × WHR; T2DM × BMI, T2DM × mean steps/day), adjusting for age, sex, total intracranial volume, education, *APOE*-ε4 status (grouped as ε4 allele carriers or non-carriers), vascular risk factors (a summary variable coded for the presence of hypertension, and/or hyperlipidemia, and/or smoking, and/or history of stroke, and/or history of ischemic heart disease), years of formal education and GDS score. SAF was used as an additional covariate among participants in whom it was available. To examine potential mediation of the association between T2DM and brain volumes, we successively entered mean steps/day, BMI and WHR into multivariable regression models relating T2DM to the respective brain volume measure. Mediation was judged to be present, if the addition of the potential mediator (mean steps/day, BMI or WHR) attenuated the β coefficient for the association between T2DM and the brain volume measure by >30%, and the β coefficient and standard errors for the mediator remained relatively unchanged from its value without T2DM in the model. Finally, we explored the effects of potential mechanistic variables (HOMA-IR, HbA_1c, and inflammatory cytokines including CRP, TNFα and IL6) by adjusting for them in the final models. All statistical analyses were performed using STATA version 12 (StatCorp.College Station Tx.) and $p<0.05$ was considered statistically significant.

4.4 Results

The participant characteristics are summarised in table 4.1. Among a total of 560 participants, There were 258 with T2DM (mean age 67 ± 7 years) and 302 without T2DM (mean age 72 ± 7 years) with complete data on the primary exposure (obesity measures and mean steps/day) and outcome (brain MRI measures) variables. The median duration of T2DM was 6 years (interquartile range 3-11 years). In univariable comparisons against those without T2DM, people with T2DM had

significantly greater BMI, WHR, fasting blood glucose, HbA_1c, and triglyceride levels, were more likely to report a history of ischemic heart disease, stroke, hypertension, hyperlipidemia, and be on treatment for both (all $p<0.05$), but had similar mean steps/day.

T2DM, obesity, habitual physical activity and brain volumes

Associations of these variables with total GMV and HV are presented in table 4.2, adjusted for age, sex and total intracranial volume. T2DM was significantly associated with lower total GMV (β= –10.04, 95% CI –15.89 to –4.19, p=0.001), left HV (β= –0.39, 95% CI –0.47 to –0.32, p<0.001), right HV (β= –0.45, 95% CI –0.53 to –0.37, p<0.001) and total HV (β= –0.85, 95% CI –0.99 to –0.70, p<0.001). Greater WHR (p<0.001) and BMI (p=0.01), and fewer mean steps/day (p=0.02) were independently associated with lower total GMV. Greater WHR, greater BMI, and fewer mean steps/day were associated with lower left, right HV and total HV (all p<0.05).

4.1. Participant characteristics.

	T2DM (n=258) Mean (SD) or n (%)	**Non-T2DM (n=302) Mean (SD) or n (%)**	**P value**
Male sex	159 (62)	161 (53)	0.061
Age (years)	67 (7)	72 (7)	<0.001
Median duration of T2DM (years; IQR)	6 (3-11)	-	-
Ischemic heart disease	51 (20)	52 (17)	0.46
History of stroke	22 (8)	20 (6)	0.42
Smoked	140 (54)	155 (51)	0.49
^Hypertension	215 (83)	216 (72)	0.001
Systolic blood pressure (mmHg)	137 (19)	142 (22)	0.005
Diastolic blood pressure (mmHg)	77 (10)	80 (12)	<0.001
Hyperlipidaemia	173 (67)	143 (47)	<0.001
Blood pressure lowering medication	182 (70)	144 (48)	<0.001
Statin use	161 (62)	74 (25)	<0.001
Body mass index (kg/m^2)	30.0 (4.6)	27.2 (4.0)	<0.001
Overweight (BMI 25-30)	108 (42)	148 (49)	0.076
Obese (BMI>30)	115 (45)	59 (20)	<0.001
Waist-hip ratio	0.96 (0.08)	0.90 (0.08)	<0.001
Fasting blood glucose (mmol/l)	7.7 (2.2)	5.3 (0.55)	<0.001
Glycated haemoglobin (HbA_{1C}) (%)/ (mmol/mol)	7.1 (1.2)/ 54.1	5.6 (0.3)/ 37.7	<0.001
Total cholesterol (mmol/L)	4.4 (1.0)	5.3 (1.2)	<0.001
Triglycerides (mmol/L)	1.7 (0.8)	1.3 (0.6)	<0.001
HOMA-IR (IU)	2.18 (13.30)	5.93 (1.56)	<0.001
C-reactive protein (mg/dL)	3.31 (7.44)	3.70 (7.09)	0.53
Tumor necrosis factor alpha	1.15 (1.74)	2.82 (2.34)	<0.001
Interleukin 6	1.51 (2.17)	2.15 (3.48)	0.004
APOE-ε4 allele	70 (27)	72 (24)	0.53
Geriatric Depression Scale (GDS) score	2.2 (2.4)	1.7 (2.0)	0.02
Formal education (years)	12 (4)	11 (4)	0.051
Mean steps per day	6088 (3481)	6201 (3216)	0.67
MRI Cortical volumes			
Total grey matter volume (ml)	586.9 (60.2)	582.6 (61.1)	0.40
White matter volume (ml)	457.8 (58.6)	455.15 (55.5)	0.58
Total hippocampal volume (ml)	4.6 (0.8)	5.4 (0.9)	<0.001
Left hippocampal volume (ml)	2.2 (0.4)	2.6 (0.5)	<0.001
Right hippocampal volume (ml)	2.3 (0.4)	2.8 (0.5)	<0.001

T2DM – type 2 diabetes mellitus, SD – standard deviation, IQR – interquartile range, HOMA-IR – homeostatic model assessment of insulin resistance, MRI – magnetic resonance imaging. ^Hypertension - self-reported history of hypertension or mean systolic blood pressure >140 or mean diastolic blood pressure >90 mmHg. p value is for unadjusted comparisons. Wilcoxon rank sum tests for fasting glucose, HbA_{1C}, triglycerides, HOMA-IR, C-Reactive Protein, GDS score. Independent t-tests or chi-square tests for all other variables.

Table 4.2. Associations of type 2 diabetes mellitus (T2DM), waist-hip ratio (WHR), body mass index (BMI), mean steps/day and cortical volumes (n=560).

	T2DM	**P value**	**WHR**	**P value**	**BMI**	**P value**	**Mean steps/day**	**P value**
	β (95% CI)		**β (95% CI)**		**β (95% CI)**		**β (95% CI)**	
Total grey matter volume (ml)	-10.04 (-15.89, -4.19)	0.001	-107.77 (-146.81, -68.73)	<0.001	-0.82 (-1.47, 0.18)	0.01	0.001 (0.0001, 0.002)	0.02
Left hippocampal volume (ml)	-0.39 (-0.47, -0.32)	<0.001	-1.48 (-2.03, -0.93)	<0.001	-0.01 (-0.01, -0.001)	0.03	0.00002 (5.39^{-6}, 0.00003)	0.005
Right hippocampal volume (ml)	-0.45 (-0.53, -0.37)	<0.001	-1.18 (-1.77, -0.59)	<0.001	-0.009 (-0.02, -0.00002)	0.05	0.00002 (7.93^{-6}, 00003)	0.002
Total hippocampal volume (ml)	-0.85 (-0.99, -0.70)	<0.001	-2.70 (-3.76, -1.64)	<0.001	-0.02 (-0.04, -0.003)	0.02	0.00004 (0.00002, 0.00007	0.001

β is unstandardised coefficient. CI – confidence interval.

All regressions adjusted for age, sex and total intracranial volume.

Analysis of effect modification and mediation

There were 532 participants with complete data available for multivariable analysis excluding the variable SAF. Table 4.3 shows the change in the association between T2DM and total GMV (adjusted for age, sex, vascular risk, education, *APOE*-ε4 and GDS score) when each additional factor of interest (i.e. mean steps/day, BMI, WHR) is entered into the models. The addition of mean steps/day (Model 2) and BMI (Model 3) did not appreciably alter the association between T2DM and total GMV. The addition of WHR (Model 4) attenuated the association between T2DM and total GMV by 32% (compared with Model 3) rendering the T2DM-GMV relationship statistically non-significant, while WHR remained independently associated with total GMV ($p<0.001$), and the standard errors for T2DM and WHR remained unchanged. The association between T2DM and total HV (table 4.4) was unchanged by the addition of mean steps/day, BMI and WHR. Greater mean steps/day, but not BMI or WHR, was independently associated with greater total HV ($p<0.05$). The addition of HOMA-IR, HbA_1c, CRP, TNFα and IL6, and SAF (available only in 486 participants, data not shown) to the final models (Models 4) for both total GMV and HV did not change the observed associations. There were no significant interactions between T2DM and measures of obesity or physical activity in explaining cortical volumes ($p>0.05$ for all product terms).

Table 4.3. Effects of mean steps/day, body mass index (BMI) and waist-hip ratio (WHR) on the association between type 2 diabetes mellitus (T2DM) and total grey matter volume (n=532).

	Model 1	**Model 2**	**Model 3**	**Model 4**
	β (95%CI)	**β (95%CI)**	**β (95%CI)**	**β (95%CI)**
T2DM	-7.98 (-13.96, -2.01)*	-7.48 (-13.48, -1.45)*	-7.40 (-13.64, -1.16)*	-5.05 (-11.32, 1.22)
Mean steps per day		0.001 (-0.0002, 0.002)	0.001 (-0.0002, 0.002)	0.001 (-0.001, 0.02)
Body mass index			-0.14 (-0.85, 0.57)	0.24 (-0.50, 0.97)
Waist hip ratio				-72.26 (-117.55, -26.97)^

β – beta coefficient, CI – confidence interval, T2DM – type 2 diabetes mellitus, BMI – body mass index, WHR – waist-hip ratio.

All models adjusted for age, sex, years of education, total intracranial volume, vascular risk (hypertension and/or hyperlipidemia and/or smoking and/or history of stroke and/or history of ischemic heart disease), apolipoprotein ε4 allele and Geriatric Depression Scale score.

*p<0.05, ^p<0.01.

Model 1 – association between T2DM and total grey matter volume.

Model 2 – model 1 adjusted additionally for mean steps/day.

Model 3 – model 2 adjusted additionally for BMI.

Model 4 – model 3 adjusted additionally for WHR.

Table 4.4. Effects of mean steps/day, body mass index (BMI) and waist-hip ratio (WHR) on the association between type 2 diabetes mellitus (T2DM) and total hippocampal volume (n=532).

	Model 1	**Model 2**	**Model 3**	**Model 4**
	β (95%CI)	**β (95%CI)**	**β (95%CI)**	**β (95%CI)**
T2DM	-0.94 (-1.08, -0.79)^	-0.92 (-1.06, -0.77)^	-0.94 (-1.09, -0.78)^	-0.91 (-1.06, -0.75)^
Mean steps per day		0.00003 (8.67^{06}, 0.0001)*	0.00003 (0.00001, 0.0001)*	0.00003 (9.88^{06}, 0.0001)*
Body mass index			0.01 (-0.01, 0.03)	0.02 (-0.001, 0.04)
Waist hip ratio				-1.09 (-2.21, 0.03)

β – beta coefficient, CI – confidence interval, T2DM – type 2 diabetes mellitus, BMI – body mass index, WHR – waist-hip ratio.

All models adjusted for age, sex, years of education, total intracranial volume, vascular risk (hypertension and/or hyperlipidemia and/or smoking and/or history of stroke and/or history of ischemic heart disease), apolipoprotein ε4 allele and Geriatric Depression Scale score.

*p<0.05, ^p<0.01.

Model 1 – association between T2DM and total hippocampal volume.

Model 2 – model 1 adjusted additionally for mean steps/day.

Model 3 – model 2 adjusted additionally for BMI.

Model 4 – model 3 adjusted additionally for WHR.

4.5 Discussion

We found that the adverse association between T2DM and total GMV may be partially mediated by abdominal obesity. Moreover, WHR, but not BMI or mean steps/day, remained independently associated with total GMV. Mean steps/day did not affect the relationship between T2DM and total GMV. By contrast, neither WHR, BMI or mean steps/day appeared to affect the association between T2DM and total HV. However, across all individuals mean steps/day, but not WHR or BMI, remained independently associated with total HV.

Although previous studies have reported that obesity is associated with lower total brain or regional volumes in the general population[198, 199, 206, 215, 216], none, to our knowledge, have examined these relationships in people with T2DM. In our previous study[197], we were unable to demonstrate an independent association of T2DM with white matter volume. Therefore we did not explore white matter volume as an outcome, although others have demonstrated that obesity (overall and abdominal) is related to lower white matter volume in morbidly obese people[216]. We found that WHR, but not BMI, explains a large portion of the T2DM-GMV association suggesting that abdominal obesity and its related mechanistic factors may be important drivers of gray matter atrophy in T2DM. WHR was also independently associated with total GMV. An interpretation of this finding is that T2DM confounds the relationship between WHR and GMV, but the stability of standard errors in the models suggests this is less likely. T2DM is likely to represent a clinical state further downstream of abdominal obesity in the causal pathway to cortical atrophy. In support of this concept, abdominal adiposity often precedes the development of insulin resistance and T2DM[205]. The direction of causality between WHR and total GMV cannot be confirmed based on these cross-sectional analyses alone, because atrophy of brain regions that regulate dietary habits may theoretically explain the observed relationships[217]. However, a Mendelian randomisation analysis in the 3C-Dijon Study demonstrated that the association between WHR and lower total GMV in the general population[198] was likely to be causal. Our results are consistent with cross-sectional and longitudinal data from the general population showing that the associations between obesity and brain volumes are more pronounced for abdominal obesity rather than measures of global body mass such as BMI[198, 199, 218]. Abdominal fat differs in its metabolic activity compared with peripheral fat, is strongly linked to the production of pro-inflammatory cytokines and the generation of insulin resistance[205], and is more strongly predictive of cardiovascular disease than measures of global obesity (e.g. BMI)[219]. Although fewer mean steps/day were associated with T2DM as well as total gray matter and hippocampal atrophy, mean steps/day did not explain the

T2DM-GMV or T2DM-HV associations. Interventions involving moderate and vigorous aerobic[207, 220] or resistance training[221] interventions are known to preserve brain structure and function as well as improve glycemic control in older individuals[222]. It is possible that our measure of physical activity was not sufficiently sensitive to capture the exercise intensity and type necessary to influence T2DM related brain atrophy. However, similar to recent work[223], we showed that those individuals who engaged in more physical activity had lower WHR. Mean steps/day did remain independently related to total HV, in line with previous studies[224, 225], suggesting physical activity is important for maintaining total HV irrespective of diabetes status.

Chronic low grade inflammation, insulin resistance, advanced glycation endproducts (AGEs), hormonal effects and vascular disease may all be mechanisms that could explain the associations between T2DM, abdominal obesity and brain atrophy. The association of T2DM and WHR with GMV was independent of inflammatory cytokines in our study, however, peripheral inflammatory cytokine levels are poor measures of neuroinflammation which requires estimation with specialised neuroimaging[226]. Neuronal insulin resistance is associated with impaired amyloid clearance[227] and increased tau phosphorylation in the human brain[228] and in mouse models of T2DM[229]. However, adjustment in our final models (Model 4) for HOMA-IR did not alter the T2DM-GMV and T2DM-HV associations. Finally, the associations of T2DM, obesity measures and mean steps per day with GMV or hippocampal volume were independent of SAF, a measure of long-term tissue advanced glycation, although we were unable to adjust for measures of circulating AGEs. Abdominal obesity is also strongly associated with vascular mechanisms that may explain brain atrophy such as arterial stiffness[164, 230]. It is tempting to consider whether interventions targeting abdominal obesity or related factors may protect against brain atrophy in T2DM. Lifestyle interventions (such as increased physical activity and decreasing caloric intake) seem a reasonable option although they do not necessarily preferentially target abdominal adiposity[231] and may be difficult to maintain. Bariatric surgery in highly selected morbidly obese middle-aged individuals was shown to be associated with improved cognition in a small study (n=21), but the contribution of weight loss to this improvement was not explored in relation to other mechanistic variables[232]. There is renewed use of antidiabetic agents such as thiazolidinediones[233] and metformin[234] that have modest effects on abdominal obesity, as well as leptin analogs to determine whether use of these agents may ameliorate cognitive decline in individuals with T2DM[235]. Additionally, interventions that target adiposity-related mechanisms such as insulin signaling (e.g. analogs of glucagon-like peptide) deserve further study for preserving brain health in T2DM.

Strengths of this study include a large sample size, a robust definition of T2DM, quantitative measures of exposures (physical activity, BMI and WHR) and outcome (brain volumes) using validated and standardised techniques, adjustment for several potential confounders, and careful analysis for effect modification and mediation.

Limitations

The following are limitations of our study. Due to the cross sectional design, this study does not permit us to draw conclusions about causality. On the other hand, our findings are consistent with evidence linking abdominal obesity to cognitive decline[236, 237] and brain atrophy in non-diabetic populations[198, 199, 206, 215], and provide a good basis for the longitudinal study of abdominal obesity on brain atrophy in patients with T2DM. Secondly, as patients with T2DM were recruited based on their willingness to participate in research indicated on their NDSS membership, our sample might be over-represented by healthier individuals with T2DM. Nonetheless, we showed consistent and expected differences in anthropometric and biochemical measures between those with T2DM and the comparison group, (i.e. patients with T2DM had higher WHR, BMI, fasting blood glucose and HbA_1c). Although pedometers provide an objective measure of habitual physical activity and are simple and inexpensive[238], they do not provide information on sedentary behavior, non-ambulatory physical activity (i.e. swimming or resistance training), intensity or type of physical activity[239]. Finally, the pedometers were only worn for 7 days and, therefore, may not provide a good representation of long-term physical activity.

4.6 Conclusions

In summary, abdominal obesity appears to be an important factor in explaining the adverse impact of T2DM on total GMV and these results require confirmation in longitudinal studies. In people with T2DM, who represent a high-risk group for developing dementia and cognitive dysfunction, interventions targeting abdominal obesity or its related downstream factors may present promising avenues for reducing the risk of T2DM related total GMV atrophy.

4.7 Contribution of Chapter 4 to thesis aims

This chapter makes a significant contribution to understanding why patients with T2DM are more likely to demonstrate target organ damage, in particular brain structural abnormalities, compared to non-diabetic individuals. Although previously published studies have shown that abdominal obesity contributes to grey matter atrophy in healthy ageing populations whilst physical activity is

beneficial for maintaining brain structure, it was unknown whether these variables were related to brain structural abnormalities in patients with T2DM until now. For the first time, this study showed that abdominal obesity explains a large proportion of grey matter atrophy in patients with T2DM and importantly, this association was independent of a number of cardiovascular risk factors including resting brachial BP. A limitation of this study was that measures of central haemodynamics (either at rest or during exercise) were not available, and therefore, the association between these variables and brain structural abnormalities was not able to be determined. In the next chapter, measures of resting and exercise central haemodynamics and brain structure have been obtained in a cohort of individuals with and without T2DM and their relationships explored.

Chapter 5. Aortic reservoir characteristics and brain structure in people with type 2 diabetes mellitus; a cross sectional study

This chapter has previously been published;

Climie RED, Srikanth V, Beare R, Keith LJ, Fell J, Davies JE, Sharman JE. Aortic reservoir characteristics and brain structure in people with type 2 diabetes; a cross sectional study. *Cardiovascular Diabetiology*, October 2014;13.1:143.

5.1 Abstract

Background. Central haemodynamics help to maintain appropriate cerebral and other end-organ perfusion, and may be altered with ageing and type 2 diabetes mellitus (T2DM). We aimed to determine the associations between central haemodynamics and brain structure at rest and during exercise in people with and without T2DM.

Methods. In a sample of people with T2DM and non-diabetic controls, resting and exercise measures of aortic reservoir characteristics (including excess pressure integral [P_{excess}]) and other central haemodynamics (including augmentation index [AIx] and aortic pulse wave velocity [aPWV]) were recorded. Brain volumes (including grey matter volume [GMV] and white matter lesions [WML]) were derived from magnetic resonance imaging (MRI) scans. Multivariable linear regression was used to study the associations of haemodynamic variables with brain structure in the two groups adjusting for age, sex, daytime systolic BP (SBP) and heart rate.

Results. There were 37 T2DM (63±9years; 47% male) and 37 non-diabetic controls (52±8years; 51% male). In T2DM, resting aPWV was inversely associated with GMV (standardised β=-0.47, p=0.036). In non-diabetic controls, resting P_{excess} was inversely associated with GMV (β=-0.23, p=0.043) and AIx was associated with WML volume (β=0.52, p=0.021). There were no associations between exercise haemodynamics and brain volumes in either group.

Conclusions. Brain atrophy is associated with resting aortic stiffness in T2DM, and resting P_{excess} in non-diabetic controls. Central vascular mechanisms underlying structural brain changes may differ between non-diabetic controls and T2DM.

5.2 Introduction

Type 2 diabetes mellitus (T2DM) is an important vascular risk factor for cognitive impairment. It is associated with brain atrophy[197], infarcts and cerebrovascular lesions (white matter hyperintensity of presumed vascular origin [WML])[196], potentially leading to cognitive decline and greater risk for dementia. Age-related vascular factors such as hypertension and aortic stiffening are more prevalent in patients with T2DM[240] and may partly explain the associated structural brain abnormalities[241-243]. Aortic stiffening can limit buffering capacity of the large central arteries such that small changes in cardiac stroke volume can result in excessive rises in local pulsatile pressure[130]. These excess pressures may damage peripheral capillary networks[43], which is of relevance to the brain as a high flow organ with low resistance proximal large vessels and an extensive microcirculation. Consequent damage to the neurovascular unit may be a factor underlying the observed brain atrophy in T2DM.

Aortic reservoir function plays a role in the maintenance of normal central BP and may protect distal microcirculation by dampening excessive aortic pulsatile pressure, as well as reducing peripheral pressure transmission[139]. The aortic reservoir pressure paradigm proposes that the central (aortic) pressure wave may be separated into an aortic reservoir pressure component, representing proximal aortic volume; and an excess pressure (P_{excess}) component, representing excess left ventricular work required for stroke volume ejection, analogous to left ventricular flow (refer to figure 2.1.5)[8, 9]. Indeed, aortic reservoir pressure is related to aortic stiffness (aortic pulse wave velocity [aPWV]) and we have previously shown that reservoir pressure, not backward pressure (i.e. from peripheral wave reflections) is the largest contributory factor to an increase in augmented pressure[82]. Increased P_{excess} was recently shown to independently predict adverse cardiovascular events in patients with cardiovascular disease[147], possibly due to accelerated target organ damage, but this has never been examined.

Although resting BP indices are clinically important, hemodynamic responses to moderate exercise may have stronger prognostic value in terms of cardiovascular risk[5], suggesting that pathophysiological insight may be gained from exercise hemodynamics beyond that of resting conditions. This may be because individuals can spend a large proportion of their day ambulatory [6] (doing some form of light-moderate physical activity; standing, walking) and the BP response to this type of lower intensity exercise may, therefore, be a better representation of the chronic BP load. Indeed, we have shown that independent of resting BP, light-to-moderate exercise

hemodynamics can unveil BP abnormalities[151, 244] and also predict kidney function in older men[152]. We have also found that patients with T2DM have abnormal responses at higher exercise intensities[28, 72]. This study aimed to determine associations between central hemodynamics, including aortic reservoir characteristics, and brain structure in people with and without T2DM, during rest and light-moderate exercise.

5.3 Methods

Study sample

Eighty participants (T2DM n=40, non-diabetic controls n=40) were recruited from the community via local advertisements. Exclusion criteria were; pregnancy, arrhythmia, clinical history of cardiovascular disease (including coronary artery disease, myocardial infarction, heart failure or stroke), severe pulmonary disease and contraindication to brain magnetic resonance imaging (MRI). T2DM was determined by self-report of diagnosis by physician. All participants gave informed consent and the study was approved by the University of Tasmania Human Research Ethics Committee.

Study protocol

Participants attended the testing laboratory on two occasions and were scheduled for MRI assessment. At visit 1 participants were asked to avoid smoking, caffeine containing products and consuming heavy meals for a minimum of three hours prior to the testing, and were instructed to avoid heavy exercise and alcohol consumption within the 24 hours prior. Participants were not instructed to withhold BP medication. Anthropometric measures, questionnaires relating to BP, medical history and haemodynamic data were recorded. Following 10 minutes of semi-recumbent rest (torso at 45^{o}, arm supported at heart level), brachial BP was measured by a validated automatic device (Omron HEM-907; Hoofddorp, The Netherlands)[183], followed by central haemodynamic variables recorded by applanation tonometry (SphygmoCor, AtCor Medical, Sydney, Australia). All measures were repeated during moderate intensity exercise at 60% of age-predicted maximal heart rate. A validated[245]mercury free sphygmomanometer (UM-101, A&D Medical, Tokyo, Japan) and auscultation was used to measure exercise brachial BP. Using a 2-legged cycle ergometer positioned at the end of the bed, participants were asked to cycle at 50 revolutions per minute while the investigator increased the watts to 30. Data collection commenced after approximately 2-3 minutes once a steady state heart rate had been achieved and continued for approximately 20 minutes (with intermittent breaks to measure aortic stiffness as described below).

Further details of the exercise protocol can be found elsewhere[152]. Non-invasive impedance cardiography was continuously recorded throughout the protocol (PhysioFlow; Manatec Biomedical; Macheren, France). At visit 2, fasting venous bloods were taken and participants were fitted with a 24-hour ambulatory BP monitor (24ABPM; TM-2430, A&D Medical, Sydney, Australia). Hypertension was defined as clinic brachial BP ≥140/90 mmHg, self- reported diagnosis by a physician, or use of antihypertensive medications.

MRI analysis

Scans were performed on a 1.5T General Electric Signa Excite T scanner with the following sequences: High-resolution T1 weighted spoiled gradient echo (TR 35ms, TE 7ms, flip angle 35^{o}, field of view 24 cm, voxel size 1 mm^{3}) comprising 120 contiguous slices; T2 weighted fast spin echo (TR 4300 ms; TE 120 ms; NEX 1; turbo factor 48; voxel size 0.90 x 0.90 x 3 mm); FLAIR (TR=8802 ms, TE=130 ms, TI=2200ms, voxel size 0.50 x 0.50 x 3 mm). Scans were registered to a 152 brain Montreal Neurological Institute template in stereotaxic coordinate space. Brain tissue was classified as gray or white matter using statistical parametric mapping software SPM5. Hippocampi were manually segmented using standard landmarks with high test-retest reliability[246]. WML were segmented using a validated semi-automated method[247]. GMV, white matter, WML, and hippocampal volumes were calculated using standard voxel counting algorithms. MRI examiners were blinded to outcome variables and diabetes status.

Central haemodynamic measures

Aortic reservoir characteristics

Central (aortic) pressure waveforms were reconstructed as previously described[63]. Using custom MatLab software the averaged radial pressure waveforms were separated into reservoir pressure (representative of the cyclic changes in aortic volume that occur during systolic expansion to store blood, and diastolic recoil to allow for the discharge of blood from the proximal aorta) and excess pressure (excess work done by the left ventricle, see figure 2.1.5)[8, 9]. Reservoir pressure was calculated as previously described[147] and P_{excess} was determined by subtracting the reservoir pressure from the aortic pressure waveform[137].

Central BP and aortic stiffness

Central BP was measured in duplicate and augmentation index (AIx), augmentation pressure (AP), pulse pressure (PP) and PP amplification were calculated[63]. Duplicate right sided carotid-to-

femoral aPWV was measured as previously described[152]. The tonometry method was modified during exercise in order to obtain waveforms of sufficient quality. Once the participant reached a steady state heart rate, they were asked to increase the revolutions per minute in order to increase heart rate by approximately 10 beats per minute. Once the desired heart rate was reached, the investigator located the pulse site and told the participant to cease exercise. The participant's heart rate dropped to their steady state heart rate, during which time the investigator captured the waveform. The participant was then instructed to repeat the exercise until all data was collected.

Cardiothoracic bioimpedance

Measures of cardiac output, systemic vascular resistance, heart rate and stroke volume were recorded using a device with good reproducibility during rest and exercise[186]. Five minutes of continuous steady state monitoring was averaged and analysed offline.

Peripheral haemodynamic measures

Duplicate conventional brachial BP measures were averaged for analysis. 24ABPM was measured every 20 minutes during the daytime, and every 30 minutes during the nighttime.

Biochemistry

Fasting blood glucose, insulin, glycated haemoglobin (HbA_{1c}), and lipid profiles were obtained by accredited laboratory techniques (Royal Hobart Hospital pathology department). A resting urine sample was analysed for the presence of albumin by the Royal Hobart Hospital pathology department.

Statistical analysis

Data were analysed using SPSS for Windows software version 19.0 (IBM SPSS Statistics, New York, USA). Data were visually inspected for normality of distribution and were all normally distributed. All brain volume outcome measures were expressed as a ratio of total intracranial volume. To compare characteristics between patients with T2DM and non-diabetic controls, independent t-tests (continuous variables) and Chi square tests (dichotomous variables) were performed. Independent t-tests were used to compare unadjusted brain volumes between groups, followed by analysis of covariance (ANCOVA) adjusted for age and sex. To assess the relationships between resting and exercising central haemodynamic variables and brain volumes, Pearson's correlations and multivariable linear regression were performed. Z statistic scores were

determined to compare the regression slopes obtained from within-group correlations. Independent variables known (age and sex) or suspected (heart rate, ambulatory daytime systolic BP [SBP], body mass index [BMI] and total cholesterol) to contribute to variance in brain volumes were added separately into the regression model, and a $p<0.05$ was considered statistically significant. Based on previous reproducibility work[248], we calculated that a between- group difference of 10 mmHg in central SBP could be detected in 36 participants per group ($\alpha=0.05$ and $\beta=0.20$), therefore we recruited 40 participants for each group.

5.4 Results

Sample characteristics

One patient with T2DM and two non-diabetic controls withdrew consent for MRI due to claustrophobia. Brain volume data was unavailable for one patient with T2DM (due to a significant non-vascular abnormality on MRI) and technical difficulty rendered aortic reservoir data unavailable in two participants (one participant from each group), resulting in 37 participants in each group. Compared with non-diabetic controls, patients with T2DM were older, heavier, and had greater blood glucose and glycated haemoglobin (HbA_{1C}). None of the non-diabetic controls were on BP or cholesterol lowering medications (table 5.1).

Table 5.2 summarises the difference in brain volumes between groups whilst table 5.3 summarises the differences in resting and exercising central haemodynamics. There was no difference between the groups in any of the adjusted brain volumes. Those with T2DM had significantly greater values in most aortic reservoir characteristics and other haemodynamic variables at rest and during exercise. No between-group differences were observed for reservoir pressure integral and stroke volume during rest and exercise, and for peak reservoir pressure and cardiac output during exercise alone. Compared with non-diabetic controls, brachial SBP and PP were significantly higher in those with T2DM at rest and during exercise, whereas resting systemic vascular resistance was significantly lower. P_{excess} correlated with AP at rest in patients with T2DM ($r=0.49$, $p=0.001$) and in non-diabetic participants at rest and during exercise ($r=0.58$, $p<0.001$ and $r=0.34$, $p=0.032$ respectively). In patients with T2DM, there was a significantly greater change from rest to exercise in peak excess pressure, central SBP, central PP, aPWV, brachial SBP and brachial PP compared to non-diabetic participants ($p<0.05$ for all).

Table 5.1. Study participant characteristics.

	T2DM (n=37)	**Non-diabetic (n=37)**	**P value**
Male, n (%)	17 (47)	19 (51)	0.56
Age (years)	63±9	52±8	<0.001
Body mass index (kg/m^2)	30.5±4.8	25.9±3.3	<0.001
Waist-hip (ratio)	0.91±0.06	0.84±0.1	0.002
Current smoker, n (%)	3 (8)	3 (8)	0.97
Hyperlipidaemia, n (%)	25 (66)	10 (27)	0.001
Normotensive, n (%)	15 (39)	28 (76)	0.002
24 hour ambulatory systolic BP (mmHg)	134±13	130±11	0.21
24 hour ambulatory diastolic BP (mmHg)	75±8	79±6	0.016
Day-time ambulatory systolic BP (mmHg)	138±14	136±13	0.50
Night-time ambulatory systolic BP (mmHg)	119±12	113±11	0.016
Duration of diabetes (years)	6±6	-	-
Antihypertensive medications, n (%)	24 (63)	0 (0)	<0.001
Oral hypoglycaemic medications, n (%)	26 (68)	0 (0)	<0.001
Urinary albumin (mg/L)	9.00±11.19	7.85±7.59	0.60
Insulin, n (%)	5 (13)	0 (0)	0.016
Statin, n (%)	25 (66)	0 (0)	<0.001
Glucose (mmol/L)	7.5±1.8	4.7±0.4	<0.001
Glycated haemoglobin (%)	7.2±0.8	5.5±0.3	<0.001
Insulin (IU/mL)	10.2±8.6	2.4±4.7	<0.001
Total cholesterol (mmol/L)	4.4±1.0	5.4±1.0	<0.001
HDL cholesterol (mmol/L)	1.3±0.4	1.6±0.4	0.002
Triglycerides (mmol/L)	1.4±0.6	1.0±0.5	0.003

Data expressed as mean ± standard deviation or %. T2DM, type 2 diabetes mellitus; BP, blood pressure; HDL, high density lipoprotein. P is for between group analyses.

Table 5.2. Brain magnetic resonance imaging (MRI) volumes in patients with type 2 diabetes mellitus (T2DM) and non-diabetic controls.

MRI variable	T2DM Mean±SD (n=37)	Non-diabetic Mean±SD (n= 37)	Association of T2DM with MRI variable β coefficient (95% CI)	P for regression
Grey matter volume (ml)	567.36±77.81	607.81±63.01	0.014 (-17.23, 21.21)	0.84
White matter volume (ml)	583.92±76.03	604.84±80.53	-0.005 (-15.98, 14.48)	0.92
Left hippocampal volume (ml)	2.43±0.37	2.55±0.38	-0.019 (-0.17, 0.14)	0.86
Right hippocampal volume (ml)	2.51±0.36	2.56±0.39	0.046 (-0.14, 0.21)	0.70
White matter lesion volume (ml)	3.34±2.38	3.44±2.39	-0.148 (-1.93, 0.54)	0.26

Unadjusted MRI volumes are presented in the first two columns; β refers to standardised beta coefficient for the association between T2DM and MRI variables determined by ANCOVA and adjusted for age, sex and total intracranial volume. SD, standard deviation. P value is for relation of diabetes status with MRI variables.

Table 5.3. Differences in central and peripheral (brachial) haemodynamic variables between patients with type 2 diabetes mellitus (T2DM) and non-diabetic controls at rest, during exercise and the change from rest to exercise.

	Rest			Exercise			Change from rest to exercise		
	T2DM (n=37)	**Non-diabetic (n=37)**	**P value**	**T2DM (n=37)**	**Non-diabetic (n=37)**	**P value**	**T2DM (n=37)**	**Non-diabetic (n=37)**	**P value**
Peak reservoir pressure (mm Hg)	36±8	32±4	0.016	18±10	15±5	0.17	-19±10	-17±10	0.49
Reservoir pressure integral (Pa.s)	1872±520	1869±369	0.97	794±485	694±263	0.40	-1413±570	-1441±454	0.81
Peak excess pressure (mm Hg)	35±9	30±4	0.005	73±16	58±12	<0.001	37±19	27±11	0.013
Excess pressure integral (Pa.s)	630±197	493±98	<0.001	1644±437	1255±472	<0.001	970±468	776±470	0.079
Central systolic BP (mm Hg)	114±11	103±10	<0.001	132±14	114±13	<0.001	18±12	11±11	0.015
Central pulse pressure (mm Hg)	45±9	37±5	<0.001	52±12	39±7	<0.001	8±9	2±7	0.003
Pulse pressure amplification (ratio)	1.2±0.1	1.3±0.1	<0.001	1.5±0.1	1.6±0.1	0.007	0.26±0.11	0.23±0.09	0.37
Augmentation pressure (mm Hg)	13±4.8	8±5	<0.001	9±6	4±4	<0.001	-4±5	-4±3	0.78
Augmentation index (%)	29±6.8	21±10	<0.001	17±9	10±6	0.001	-12±7	-11±6	0.68
Augmentation index (at 75bpm)	23±6	13±11	<0.001	25±9	14±11	<0.001	0.8±9	1±7	0.73
*Adjusted augmentation index (%)	26±6.7	23±6.7	<0.001	14.6±8.0	11.9±7.9	<0.001	-11.6±6.7	-11.5±6.8	0.58
Aortic pulse wave velocity (m/s)	8.01±2.16	6.29±1.42	<0.001	9.73±2.10	7.02±1.43	<0.001	2.14±2.59	0.32±2.71	0.004
Heart rate (bpm)	64±8	58±8	0.001	92±12	86±12	0.043	27±9	28±9	0.58
Cardiac output (L/min)	5.24±0.90	4.50±0.73	<0.001	8.22±1.54	7.91±1.28	0.35	2.9±1.3	3.4±1.3	0.103
Stroke volume (mL)	82±11	78±15	0.26	90±13	93±14	0.36	7±12	14±11	0.017
Brachial systolic BP (mm Hg)	124±12	114±9	<0.001	155±17	134±14	<0.001	31±13	20±13	<0.001
Brachial diastolic BP (mm Hg)	68±8	65±6	0.064	77±9	73±9	0.097	8±6	9±8	0.93
Brachial pulse pressure (mm Hg)	55±10	49±5	0.002	78±15	60±10	<0.001	23±11	11±10	<0.001
Systemic vascular resistance ($d.s.cm^{-5}$)	1369±243	1503±268	0.027	1004.38±201	973±157	0.45	-354±206	-529±247	0.001

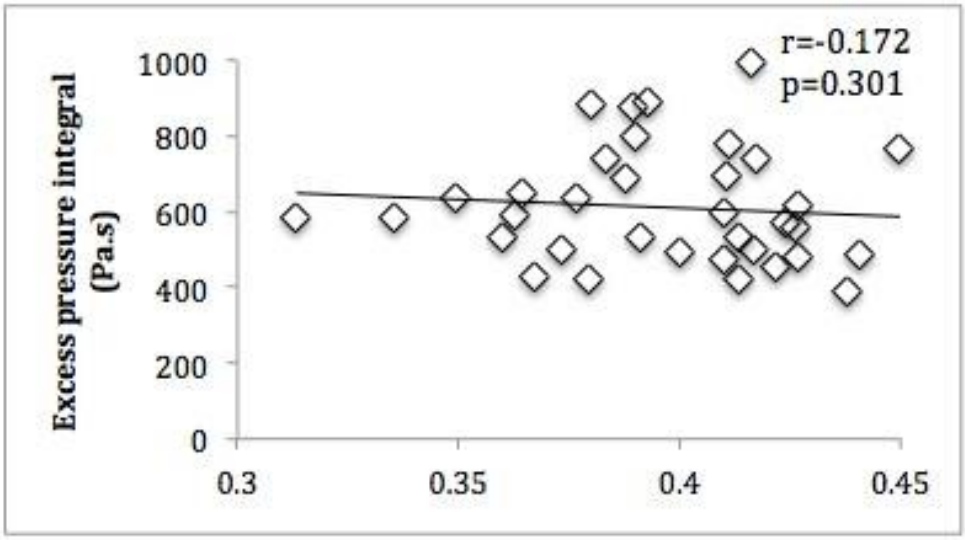
Data expressed as mean ± standard deviation. BP, blood pressure. P is for between group analyses. *Augmentation index adjusted for age, sex, heart rate and height.

Associations between central haemodynamic and brain MRI volumes

In patients with T2DM, resting aortic reservoir characteristics were not related to MRI volumes ($p>0.05$ for all). In non-diabetic controls, there was an inverse correlation between resting P_{excess} and GMV ($r=-0.41$, $p=0.011$), which remained after adjusting for age, sex, ambulatory daytime SBP and heart rate (table 5.4, figure 5.2). Further adjustment for BMI or urinary albumin did not attenuate the association ($\beta=-0.73^{-4}$, $p=0.028$, $\beta=-0.061^{-3}$, $p=0.045$ respectively) however, the addition of total cholesterol did ($\beta=-0.58^{-4}$, $p=0.060$). Adjusting for clinic SBP (in the place of ambulatory daytime SBP), did not affect the relationship between P_{excess} and GMV ($\beta=0.075^{-3}$, 95% CI -0.139^{-3} to -0.011^{-3}, $p=0.023$). There was a between-group difference in the strength of the association between resting P_{excess} and GMV in patients with T2DM compared to non-diabetic controls ($z=2.08$, $p=0.044$, figure 5.2).

In patients with T2DM, but not in non-diabetic participants, resting aPWV was inversely associated with GMV ($r=-0.45$, $p=0.005$) and remained associated after adjusting for age, sex, ambulatory daytime SBP, heart rate (table 5.4) and the use of antihypertensive medication. Additionally, adjusting for clinic SBP instead of ambulatory BP, did not affect the relationship between aPWV and GMV ($\beta=-0.009$, 95% CI -0.015 to -0.002, $p=0.009$). Further adjustment for BMI, urinary albumin or total cholesterol did not alter the association ($\beta=-0.007$, $p=0.036$, $\beta=-0.007$, $p=0.050$ and $\beta=-0.006$, $p=0.045$ respectively). There was no difference between non-diabetic participants and patients with T2DM in the strength of the association between aPWV and GMV ($z=1.76$, $p=0.088$).

T2DM



Healthy

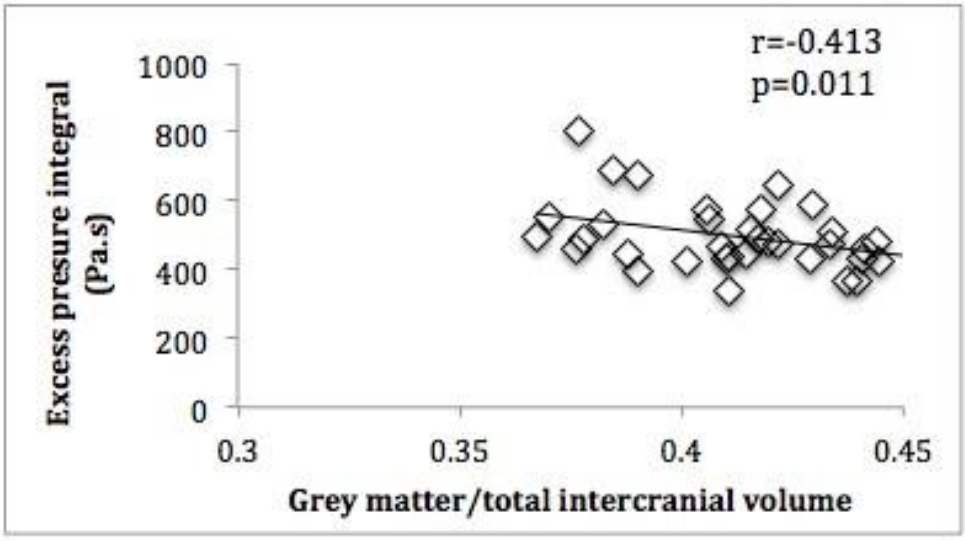


Figure 5.1. Univariate association (unadjusted) between grey matter volume and excess pressure integral in patients with type 2 diabetes mellitus (T2DM) and non- diabetic controls (healthy) at rest.

Table 5.4. Multivariable analysis of grey matter volume and resting haemodynamics in patients with type 2 diabetes mellitus (T2DM) and non-diabetic controls.

Brain MRI variable	Independent variable	β unstandardised (95% CI)	β standardised	P value	Model adjusted R^2
T2DM					
Gray matter/total intracranial volume	**Aortic pulse wave velocity**	**-0.007 (-0.014, -0.050^{-2})**	**-0.47**	**0.036**	0.16
	Age	-0.001 (-0.002, 0.001)	-0.15	0.44	
	Sex	0.006 (-0.019, -0.030)	0.088	0.64	
	24ABPM daytime systolic BP	2.94^{-5} (-0.001, 0.001)	0.013	0.94	
	Heart rate	0.001 (-0.001, 0.001)	0.18	0.28	
Healthy					
Gray matter/total intracranial volume	**Excess pressure integral**	**0.60^{-4} (-0.119^{-3}, -0.200^{-5})**	**-0.23**	**0.043**	0.68
	Age	-0.020 (-0.002, -0.001)	-0.49	<0.001	
	Sex	-0.028 (-0.039, -0.018)	-0.55	<0.001	
	24ABPM daytime systolic BP	4.30^{-5} (-0.390^{-3}, 0.477^{-3})	0.021	0.84	
	Heart rate	0.32^{-4} (-0.001, 0.001)	-0.010	0.93	
White matter lesion/total intracranial volume	**Augmentation index**	**5.91^{-5} (0.9^{-5}, 0.12^{-3})**	**0.52**	**0.021**	0.16
	Age	2.01^{-5} (0.29^{-4}, 0.7^{-4})	0.14	0.41	
	Sex	0.28^{-3} (-0.001, 0.001)	0.12	0.57	
	24ABPM daytime systolic BP	-0.10^{-4} (-0.39-^{4}, 0.22^{-4})	-0.093	0.58	
	Heart rate	9.17^{-6} (-0.41^{-4}, 0.59^{-4})	0.060	0.71	
	Central pulse pressure	**0.11^{-3} (0.28^{-3}, 0.19^{-3})**	**0.48**	**0.010**	0.19
	Age	2.79^{-5} (-0.18^{-4}, 0.74^{-4})	0.19	0.23	
	Sex	0.12^{-3} (-0.001, 0.001)	-0.045	0.79	
	24ABPM daytime systolic BP	0.17^{-4} (-0.49^{-4}, 0.14^{-4})	-0.093	0.58	
	Heart rate	1.40^{-5} (-0.35^{-4}, 0.63^{-4})	0.091	0.57	

R^2 refers analysis of variance adjusted R square and P value is for the independent variable. 24ABPM, 24 hour ambulatory blood pressure monitoring; BP, blood pressure. All models adjusted for age, sex, ambulatory daytime systolic BP and heart rate.

Aortic reservoir characteristics were not related to WML volume in either group ($p>0.05$ for all). In non-diabetic participants, resting AIx and central PP were the only haemodynamic variables associated with WML volume ($r=0.46$, $p=0.004$ and $r=0.47$, $p=0.003$ respectively) and remained related after adjusting for age, sex, ambulatory daytime SBP and heart rate (table 5.4). Alternatively adjusting for clinic SBP, in the place of ambulatory BP, did not attenuate the relationships between central PP and AIx with WML ($\beta = 0.122^{-3}$, 95% CI 0.013^{-3} to 0.230^{-3}, $p=0.029$ and $\beta = 8.354^{-5}$, 95% CI -0.018^{-3} to -0.149^{-3}, $p=0.014$ respectively). Further adjustment for BMI, urinary albumin or total cholesterol did not attenuate the association between AIx and WML volume ($\beta=5.40^{-5}$, $p=0.037$, $\beta=6.233^{-5}$, $p=0.020$ and $\beta=5.86^{-5}$, $p=0.025$) or central PP and WML volume ($\beta=9.83^{-5}$, $p=0.025$, $\beta=0.120^{-3}$, $p=0.007$ and $\beta=0.0001$, $p=0.006$ respectively). Neither exercise central haemodynamic variables nor peripheral haemodynamic variables were associated with MRI brain volumes in either group.

5.5 Discussion

To our knowledge, this is the first study to examine associations between aortic reservoir characteristics and brain structure. There are several new or noteworthy findings: 1) In non-diabetic individuals, P_{excess} (a novel marker of cardiovascular risk) was independently associated with GMV. 2) In patients with T2DM, aortic stiffness (a more traditional marker of cardiovascular risk and shown to be elevated in patients with T2DM) was independently associated with GMV. 3) Contrary to expectation, exercise haemodynamic variables were not stronger correlates of brain structural abnormalities than resting variables. Overall, these findings suggest that central haemodynamic mechanisms may play a role in leading to structural brain changes underlying cognitive impairment, but that these mechanisms may differ between non- diabetic individuals and patients with T2DM.

Unique to the brain is the continuous passive perfusion of high volume blood flow to the organ throughout systole and diastole[132]. High flow associated with low microvascular resistance could lead to brain vascular networks being sensitive to upstream changes in pressure and flow pulsatility[131, 249]. Maintenance of relatively low central BP (especially PP) could, therefore, be important in protecting the microcirculation from excess pressure and/or flow pulsatile energy which may lead to

microvascular remodeling, ischemia or structural brain changes[131]. This hypothesis appears to be consistent with data in our study showing an independent association of WML (a marker of small cerebral vessel disease) with raised central PP and AIx in healthy people. Moreover, we show that higher P_{excess} is related to lower GMV in this population. P_{excess} is representative of the excess left ventricular work required above the minimum to eject blood into the aorta and the P_{excess} waveform has been shown to correspond closely with the flow velocity waveform[8, 9, 137, 147]. Thus one interpretation of the association between high P_{excess} and low GMV is that greater pressure and/or flow transmission from the aorta to the cerebral circulation causes microvascular stress[242], unfavorable remodeling leading to ischemia[131] and neuronal loss.

Despite patients with T2DM being significantly older, of greater BMI and aortic stiffness compared to controls, there were no significant differences between the groups in any of the brain volume measures. This may be explained by the relatively small sample size or by the relatively younger age and shorter duration of T2DM than that of previous studies showing a significant reduction in brain volume compared to non-diabetic individuals[250, 251]. On the other hand WML volume has been shown to not differ between patients with T2DM and age and sex matched controls[124]. Interestingly, the relationship between high P_{excess} and low GMV was only evident in healthy individuals, whereas adverse structural brain changes were more highly related to aortic stiffness in patients with T2DM. These results may be influenced by the cross sectional design of the study, but it is also likely that alterations in central hemodynamic function associated with T2DM is an explanatory factor. Key differences in patients with T2DM compared with healthy individuals were increased aortic stiffness, higher cardiac output (mainly due to higher heart rate) and reduced systemic vascular resistance. Increased aortic stiffening has previously been described in these patients, and other study samples have observed similar high left ventricular flow output, reduced peripheral resistance and different central hemodynamic responses to postural stress[63, 67]. The association between aortic stiffness and brain structural defects has not been definitively established in patients with T2DM despite some studies showing evidence for[252], however also against[253], an association with cognitive impairment. Our findings agree with data from patients with type 1 diabetes mellitus[122] and the general community in which aortic stiffening was independently related to brain structural defects[131, 249].

We can only speculate as to the possible mechanistic differences between non-diabetic participants

and those with T2DM, which may contribute to brain atrophy. During systole, pressure rises due to increased aortic inflow relative to outflow[9]. A proportion of the pressure rise is dispersed via aortic reservoir function which is dependent on proximal aortic stiffness and peripheral resistance, both aiding in buffering BP fluctuations to allow steady blood flow to the periphery. Aortic reservoir pressure integral was not different in those with T2DM compared with healthy controls despite higher cardiac output and increased aortic stiffness in the former. This is similar to previous reports whereby patients with T2DM were shown to have reduced aortic elastic properties, however, there was no difference in aortic energy loss compared to non-diabetic controls[254]. This implies that the significant reduction in systemic vascular resistance in patients with T2DM may be a factor mitigating excessive increases in aortic reservoir pressure. Alternatively, or in conjunction, despite some studies showing smaller aortic root diameter in patients with T2DM[255], aortic diameter could have remodeled to be higher in patients with T2DM in the current study, thereby enabling relatively more inflow into the proximal aorta before a rise in pressure occurs. Others have suggested that alterations in aortic, rather than carotid arterial properties occur in patients with T2DM[162, 256]. Impedance mismatching between the aortic and carotid arteries have previously been associated with increased flow pulsatility in the carotid vasculature and may relate to cerebral microvascular remodeling and lower brain volumes[131]. Similarly, our data supports the probability that brain structural defects associated with aortic stiffness in patients with T2DM may be the product of excessive transmission of flow (rather than pressure) pulsatility to the cerebral circulation. Therapeutic methods (such as weight loss and reductions in insulin) that target aortic stiffness[257] may, therefore, be beneficial in patients with T2DM. Finally, and in opposition to our hypothesis, associations between exercise aortic reservoir characteristics and brain atrophy/WML were not enhanced compared to resting data, despite patients with T2DM having exaggerated hemodynamic responses indicative of central systolic stress (including increased central PP, AIx and aPWV) compared to healthy individuals. This was based on the expectation that moderate exercise (similar to ambulatory BP conditions) would be more representative of the chronic hemodynamic loading experienced during normal daily activity and, thus, would be more highly related to end organ disease. This appears to be relevant to cardiac structure[258] and kidney function[152] but the lack of relationship with brain morphology implies different pathophysiological pathways.

Limitations

The strengths of our study include comprehensive MRI measures and rigorous haemodynamic examination at rest and during moderate intensity exercise. Despite finding significant associations between central haemodynamic variables and GMV in both patients with T2DM and non-diabetic controls, we have performed multiple statistical tests in a relatively small study sample and, therefore, further studies in larger samples are required to confirm our results. We did not measure aortic root diameter and, therefore, our assumption of aortic dilation cannot be confirmed. Further, Study participants were not told to withhold BP medication as this may have resulted in some participants having abnormally high BP readings on the day of testing which is not reflective of their normal, controlled state. However, this does mean that haemodynmic data may have been influenced by antihypertensive medication in some patients with T2DM but not others. Finally, the cross sectional nature of the study limits inference regarding causality.

5.6 Conclusions

In summary, this is the first study to examine associations between aortic reservoir characteristics and brain structure. Our findings suggest that P_{excess} may be an important contributor to brain atrophy in healthily ageing individuals whereas in patients with T2DM, aortic stiffening may play a more prominent role. These findings suggest that there may be different vascular abnormalities contributing to brain dysfunction among diabetics compared with non-diabetics. However more work is required to determine the underlying central vascular mechanism/s.

5.7 Contribution of Chapter 5 to thesis aims

Chapter 5 represents the first investigation of central haemodynamics measured in response to light to moderate intensity exercise (similar to that of normal daily activity) in patients with T2DM. Importantly, this study showed that the central haemodynamic response to exercise is abnormal in patients with T2DM and that all central haemodynamic variables indicative of systolic stress were elevated compared to non-diabetic individuals. Although exercise central haemodynamics were not related to brain structural abnormalities, they may have important ramifications for other organ systems, such as the kidneys, the association between which is examined in the next chapter. Furthermore, this study was the first to examine the physiological and clinical relevance of the aortic reservoir characteristics in patients with T2DM and identified excess pressure as a novel

cardiovascular risk marker associated with brain atrophy, above and beyond traditional measures of brachial BP. This is important as it suggests that excess pressure may be a useful clinical marker for determining risk related to BP in future.

Chapter 6. Exercise excess pressure and exercise-induced albuminuria in patients with type 2 diabetes mellitus

This chapter has previously been published;

Climie RED, Srikanth V, Keith LJ, Davies JE, Sharman JE. Exercise excess pressure and exercise-induced albuminuria in patients with type 2 diabetes mellitus. *American Journal of Physiology – Heart and Circulatory Physiology*, May 2015; 308.9.

6.1 Abstract

Background. Exercise-induced albuminuria is common in patients with type 2 diabetes mellitus (T2DM) in response to maximal exercise, but the response to light-moderate exercise is unclear. Patients with T2DM have abnormal central haemodynamics and greater propensity for exercise hypertension. This study sought to determine the relationship between light-moderate exercise central haemodynamics (including aortic reservoir and excess pressure) and exercise-induced albuminuria.

Methods. Thirty-nine T2DM (63±9 years; 49% male) and 39 non-diabetic controls (53±9 years; 51% male) were examined at rest and during 20-minutes of light-moderate cycle exercise (30W; 50RPM). Albuminuria was assessed by albumin-creatinine ratio (ACR) at rest and 30 minutes post exercise. Haemodynamics recorded included brachial and central blood pressure (BP), aortic stiffness, augmentation pressure (AP), aortic reservoir pressure and excess pressure integral (P_{excess}).

Results. There was no difference in ACR between groups prior to exercise ($p>0.05$). Exercise induced a significant rise in ACR in T2DM but not controls (1.73±1.43 vs 0.53±1.0 mg/mol, $p=0.002$). All central haemodynamic variables were significantly higher during exercise in T2DM (i.e. P_{excess}, systolic BP and AP; $p<0.01$ all). In T2DM (but not controls), exercise P_{excess} was associated with post exercise ACR ($r=0.51$, $p=0.002$), and this relationship was independent of age, sex, body mass index, heart rate, aortic stiffness, antihypertensive medication and ambulatory daytime systolic BP ($\beta=0.003$, $p=0.003$).

Conclusions. Light-moderate exercise induced a significant rise in ACR in T2DM and this was independently associated with P_{excess}, a potential marker of vascular dysfunction. These novel findings suggest that P_{excess} could be important for appropriate renal function in T2DM.

6.2 Introduction

Type 2 diabetes mellitus (T2DM) is associated with maximal exercise-induced albuminuria which may be an early marker of diabetic nephropathy[259]. Vascular risk factors such as hypertension and aortic stiffening are associated with renal function and albuminuria under resting conditions in patients with T2DM[119, 260, 261], however, these risk factors only partly explain the variance in renal function. Patients with T2DM are more likely to have increased aortic stiffening, which can limit the buffering capacity of large central arteries and is hypothesised to expose the glomerular capillaries to damage from excess pulsatile stress. However, to our knowledge this has never been examined. The aortic reservoir-excess pressure concept suggests that the measured arterial pressure is the sum of the volume-related reservoir pressure (representing the cyclic changes in aortic volume that occur during systole to store blood, and during diastole to allow for the discharge of blood from the proximal aorta) and excess pressure, a potential marker of vascular dysfunction[8, 9, 139, 147]. Excess pressure integral (P_{excess}) was recently shown to predict cardiovascular events and mortality above and beyond common cardiovascular risk factors[147], with the authors suggesting that higher P_{excess} may reflect endothelial and circulatory dysfunction. This opens the possibility that P_{excess} could have an independent mediatory role on cardiovascular related end organ damage.

Moderate intensity exercise blood pressure (BP) measured at a fixed intensity has been shown to have stronger prognostic value than resting BP or maximal exercise BP in terms of cardiovascular risk[5]. This is likely because the BP responses to physical activity at moderate intensity are more akin to the chronic BP loading that occurs during normal daily activity[7]. Relative to non-diabetics, patients with T2DM have excessive increases in exercise brachial and central BP[28, 153] and it has recently been shown (in patients undergoing coronary angiography) that the dominant driver of an increase in central BP during light-moderate exercise is indeed P_{excess}[144]. Most studies examining the association between exercise-induced albuminuria have been at maximal exercise[262-264] and only one has shown that albuminuria may be induced by light-moderate treadmill exercise (2.9 to 4.3 average MET consumed during the exercise) in patients with T2DM[265]. However, the relationship between light-moderate exercise central hemodynamics and exercise-induced albuminuria in patients with T2DM is yet to be elucidated. This current study aimed to determine the association between exercise central hemodynamics (including reservoir pressure and P_{excess}; measured under a fixed resistance in order to mimic a standard light-moderate exercise intensity of normal daily activity) and exercise-induced

albuminuria in patients with T2DM. We hypothesised that compared with non-diabetics, exercise-induced albuminuria would be more pronounced in patients with T2DM and that this response would be independently related to exercise P_{excess}.

6.3 Methods

Study participants and protocol

Eighty consecutive participants from the local community were recruited via advertisements. The sample included a group of patients who were otherwise healthy but diagnosed with T2DM (n=40) and a group of non-diabetic control participants (n=40). Exclusion criteria included; pregnancy, arrhythmia or a clinical history of cardiovascular disease (including coronary artery disease, myocardial infarction, heart failure or stroke) or severe pulmonary disease. Aortic reservoir data was not available in one patient with T2DM and one healthy participant due to technical difficulties, leaving 39 participants for the final analysis in each group. T2DM was determined by self-report of previous diagnosis by a physician. Hypertension was defined as clinic brachial BP ≥140/90 mmHg, self-reported diagnosis by a physician or use of antihypertensive medications.

Participants attended the Menzies Research Institute Tasmania on two occasions. Prior to attendance, participants were asked to abstain from smoking, caffeine containing products and consuming heavy meals (i.e. were in a post-absorptive state) for a minimum of three hours. Participants were also asked to avoid heavy exercise and alcohol consumption 24 hours prior to testing. At visit 1, standard questionnaires relating to BP, medical history and physical activity were completed. The amount of moderate, vigorous and total physical activity MET minutes per a week the participants engaged in was determined as per the international physical activity questionnaire recommendations[266]. Following, anthropometric measures (including height, weight, waist and hip circumference) were obtained and resting and exercise haemodynamic data were recorded. A baseline sample of urine was provided by each participant (prior to exercise) and at 30 minutes post exercise, based on the data of Poortman et al.[267] who demonstrated that significant exercise-induced albuminuria can be detectable at this time point. Brachial and central BP and large artery stiffness measures were taken sequentially at rest and during exercise (semi-recumbent) on a bicycle ergometer at a light-moderate intensity in a temperature controlled room (23°C ± 1°C). At visit 2 (within 10 days of visit 1), a blood sample was taken following an overnight fast and participants were fitted with a 24 hour ambulatory BP

monitoring (24 ABPM) device (TM-2430, A&D Medical, Sydney, Australia). All participants provided informed consent and ethical approval was obtained from the University of Tasmania Human Research Ethics Committee.

Renal function measures

Urine samples were analysed for the presence of albumin and creatinine by the Royal Hobart Hospital pathology department using standard laboratory techniques as previously reported[152]. Serum creatinine was measured by IDMS-aligned technique to allow for the estimation of the glomerular filtration rate (eGFR) by the CKD-Epi eGFR equation. Venous blood samples were taken from the antecubital fossa and analysed for plasma glucose, glycated haemoglobin (HbA_{1c}), insulin, total cholesterol, high-density lipoprotein and triglycerides using accredited laboratory techniques.

Central haemodynamics

Central blood pressure

Following 10 minutes of semi-recumbent supine rest (torso at 45º, head and arm supported) central BP was synthesised using radial applanation tonometry and a validated (both at rest and during exercise)[11] and reproducible[248] generalised transfer function (SphygmoCor 8.1, AtCor Medical, Sydney, Australia). Augmentation pressure (AP) was calculated from the central pressure waveform as the difference in pressure between second and first systolic.

Aortic reservoir and excess pressure

The ensemble-averaged radial pressure waveforms were separated into reservoir and excess pressure using custom MatLab software as previously described[147].. All of the reservoir pressures and excess pressures are presented with diastolic pressure subtracted.

Aortic stiffness

Aortic pulse wave velocity (aPWV) was determined using electrocardiogram-gated hand-held applanation tonometry (SphygmoCor 8.1) in the right carotid-to-femoral arterial segments as previously described[10]. The average of duplicate measures of aPWV captured during rest and exercise was used in the analysis.

Non-invasive haemodynamic monitoring

Cardiac output, stroke volume and systemic vascular resistance were measured using cardiothoracic bioimpedance (Physio Flow; Manatec Biomedical; Macheren, France), which has been validated[185] and shown to have good reproducibility at rest and during exercise[186]. The average of five minutes of continuous steady state monitoring at rest and during exercise was analysed offline.

Brachial blood pressure

Brachial BP was recorded as the average of duplicate measures taken prior to central BP measurements and by a validated automatic device (Omron HEM-907 Hoofddorp, The Netherlands)[183] using an appropriately sized cuff as per recommendations. Exercise brachial BP was measured using a validated mercury free sphygmomanometer and auscultation technique (UM-101, A&D Medical, Tokyo, Japan) to minimise potential error due to movement artifact.

Exercise protocol

Following resting measurements, the participant remained in the semi-recumbent position and a bicycle ergometer (Rehab Trainer 881, MONARK Exercise AB, Vansbro, Sweden) was attached to the end of the bed. Exercise was commenced with two-legged cycling. Participants were instructed to gradually increase cadence until plateauing at 50 revolutions per minute. At the same time, resistance was progressively increased to 30 watts and participants maintained this exercise until a steady state heart rate was achieved after approximately 2-5 minutes. This exercise intensity equated to an average of approximately 55% of age predicted maximum heart rate for all participants. In order to record all waveforms of sufficient quality during exercise, the tonometry method was modified as follows; once the participant reached a steady state heart rate, they were asked to increase the revolutions per minute in order to increase heart rate by approximately 10 beats per minute. Once the desired heart rate was reached, the investigator located the carotid or radial arterial pulse and the participant was instructed to stop pedaling while the waveform was captured, during this time the participant's heart rate had returned to a rate similar to the steady state. During femoral tonometry, once the desired heart rate of 10 beats per minute above steady state had been reached, the participant was told to stop exercise and remove their right leg from the cycle ergometer and lay it horizontally on the bed whereupon the investigator recorded the arterial pulse waveform (from the femoral pulse site).

Statistical analysis

Continuous variables were analysed using independent t-tests and a repeated measures analysis of variance (ANOVA), and categorical variables were analysed by Chi-square test for independence. To assess the relationships between variables, Pearson's correlations and multivariable linear regression analyses were performed. Independent variables known (including age, sex and body mass index [BMI]) or suspected (24 ABPM daytime systolic BP, antihypertensive medication, blood glucose, HbA_1c, aPWV and heart rate) to contribute to the variance in post exercise ACR were added separately into the regression models, which were performed separately for patients with T2DM and non-diabetic participants. The accuracy of P_{excess} to predict an increase in ACR following exercise was evaluated with the use of receiver operator characteristics. Z statistic scores were calculated to compare the regression slopes obtained from within-group correlations. We also tested for an interaction between T2DM and P_{excess} by assessing the coefficient of the product term in the multivariable analysis. All data were analysed using SPSS for Windows software version 19.0 (IBM SPSS Statistics, New York, USA) and $p<0.05$ was considered statistically significant. We recruited 40 participants for each group based on previous reproducibility work[248] whereby we determined that a between-group difference of 10 mmHg in central SBP could be detected in 36 participants per group ($\alpha=0.05$ and $\beta=0.20$).

6.4 Results

Study participant characteristics

The study participant baseline characteristics are shown in table 6.1. Compared with non- diabetic controls, patients with T2DM were older and had higher BMI. There was no difference between the groups in 24 ABPM systolic BP, but 24 ABPM diastolic BP was significantly lower in patients with T2DM. Patients with T2DM were more likely to have hypertension and hyperlipidaemia and had significantly higher blood glucose, but significantly lower total cholesterol and high-density lipoprotein cholesterol. Over half of the patients with T2DM were receiving medication for hypertension (mean number of medications 2±2), hyperlipidaemia and hyperglycaemia.

Renal function and albuminuria

Table 6.2 summarises the difference in renal function measures between the patients with T2DM and non-diabetics prior to and post exercise. Albumin measured prior to exercise was undetectable in 16 patients with T2DM and 11 non-diabetic participants and in 18 patients with T2DM and 29 non-diabetics following exercise. Where albumin was undetectable in study participants (indicating normal renal function in relation to albuminuria) a '0' value was allocated for the analysis. Prior to exercise, patients with T2DM had significantly lower eGFR (CKD-Epi eGFR) compared to non-diabetic participants, but there was no difference between the groups in all other variables ($p>0.05$ for all). Following exercise, patients with T2DM had significantly increased urinary albumin and ACR compared to non- diabetic participants, and the difference in ACR measured prior to and post exercise was significantly higher in patients with T2DM. The increase in ACR was due to a slight but non- significant decrease from rest to exercise in urinary albumin in patients with T2DM (2.11±2.08) compared to a slightly greater decrease in non-diabetic controls (5.53±3.37, $p=0.32$ for between group difference in change). At the same time there was a decrease in urinary creatinine in both patients with T2DM and non-diabetic participants (2.14±0.14, 4.29±2.78 respectively, $p=0.06$ for between group difference in change). These findings were similar whether the data was analysed using a t test or a repeated measures ANOVA.

Table 6.1. Study participant baseline characteristics.

	T2DM (n=39)	Non-diabetic (n=39)	P value
Male, n (%)	19 (49)	19 (49)	0.75
Age (years)	63±9	53±9	<0.001
Body mass index (kg/m^2)	30.5±4.8	24.9±3.3	<0.001
Waist-hip (ratio)	0.9±0.1	0.8±0.1	<0.001
24 hour ambulatory systolic blood pressure (mmHg)	135±13	130±12	0.12
24 hour ambulatory diastolic blood pressure (mmHg)	75±8	79±6	0.02
Day-time ambulatory systolic blood pressure (mmHg)	140±15	137±13	0.32
Night-time ambulatory systolic blood pressure (mmHg)	121±13	113±11	0.002
Hyperlipidaemia (%)	26 (67)	10 (26)	0.001
Normotensive, n (%)	15 (38)	30 (77)	<0.001
Current smoker, n (%)	3 (8)	4 (10)	0.67
Time since diagnosis of T2DM (years)	6±6	-	-
Blood biochemistry			
Glucose (mmol/L)	7.5±1.8	4.7±0.5	<0.001
Glucose ≥ 7.0 mmol/L, n (%)	13 (33)	0 (0)	<0.001
Glycated haemoglobin (%)	7.2±0.8	5.5±0.6	<0.001
Insulin (IU/mL)	10.2±8.7	2.5±4.6	<0.001
Total cholesterol (mmol/L)	4.4 ±1.0	5.4±1.03	<0.001
High density lipoprotein cholesterol (mmol/L)	1.3±0.4	1.7±0.4	<0.001
Triglycerides (mmol/L)	1.5±0.7	1.02±0.5	0.001
Medications			
Antihypertensive medications, n (%)	25 (64)	0 (0)	<0.001
Angiotensin-converting-enzyme inhibitor, n (%)	8 (21)	0 (0)	0.003
Angiotensin receptor blocker, n (%)	16 (41)	0 (0)	<0.001
Beta-blocker, n (%)	3 (8)	0 (0)	0.07
Calcium antagonist, n (%)	9 (23)	0 (0)	0.001
Diuretic, n (%)	6 (15)	0 (0)	0.01
Statin, n (%)	26 (67)	0 (0)	<0.001
Diabetic medication, n (%)	28 (72)	0 (0)	<0.001
Oral hypoglycemic medications, n (%)	27 (69)	0 (0)	<0.001
Insulin, n (%)	5 (13)	0 (0)	<0.001
Physical activity			
Moderate activity (MET minutes/week)	718±1109	610±800	0.62
Vigorous activity (MET minutes/week)	903±1391	1001±1427	0.76
Total (MET minutes/week)	2534±2532	2677±2288	0.79

Data expressed as mean ± standard deviation or %. T2DM, type 2 diabetes mellitus. P value is for between group analyses.

Table 6.2. Differences in renal function measures between patients with type 2 diabetes mellitus (T2DM) and non-diabetic participants prior to and post exercise.

	T2DM (n=39)	Non-diabetic (n=39)	P value
Prior to exercise			
Plasma urea (mmol/L)	6±2	6±1	0.13
Plasma creatinine (μmol/L)	76±18	75 ±13	0.87
*Urine albumin (mg/L)	8.54±10.97	7.92±7.67	0.78
Urine creatinine (mmol/L)	8.11±4.02	9.24±6.07	0.34
Albumin creatinine ratio	0.87±1.09	0.90±1.30	0.95
CKD-Epi estimated glomerular filtration rate	94±11	100±8	0.01
Post exercise			
*Urine albumin (mg/L)	6.43±8.89	2.39±4.30	0.014
Urine creatinine (mmol/L)	5.97±4.16	4.95±3.29	0.23
Albumin creatinine ratio	1.73±1.43	0.53±1.0	0.002
Albumin creatinine ratio (post exercise minus prior to exercise)	0.15±1.04	-0.49±1.16	0.014

Data expressed as mean ± standard deviation. P value is for between group analyses. *Albumin measured prior to exercise was undetectable in 16 patients with T2DM and in 11 non-diabetic participants and in 18 patients with T2DM and in 29 non-diabetic participants following exercise.

Resting haemodynamics

Central haemodynamics including systolic BP, pulse pressure and AP were all significantly elevated in patients with T2DM compared to non-diabetics (table 6.3). Peak reservoir pressure, peak excess pressure and P_{excess} were all significantly higher in patients with T2DM compared to non-diabetic participants. aPWV, heart rate and cardiac output were all significantly elevated in patients with T2DM, however, systemic vascular resistance was significantly lower compared to non-diabetic participants ($p<0.05$ for all, table 6.3). Brachial systolic BP, diastolic BP and pulse pressure were all significantly higher in patients with T2DM ($p<0.05$ for all).

Exercise haemodynamics

During exercise, central systolic BP, pulse pressure and AP were all significantly higher in patients with T2DM. Peak excess pressure and P_{excess} were both significantly elevated in patients with T2DM, as were aPWV and heart rate ($p<0.05$ for all, table 6.3). Additionally, patients with T2DM had significantly higher exercising brachial systolic BP and pulse pressure ($p<0.05$ for all).

Association between resting haemodynamics and albuminuria (ACR)

At rest in patients with T2DM, aPWV was significantly associated with resting ACR ($r=0.39$, $p=0.019$). After adjusting for covariates (age, sex, BMI, 24 ABPM daytime systolic BP) the relationship between aPWV and resting ACR no longer remained (table 6.4). There were no significant associations between brachial or central haemodynamics in non-diabetics at rest and resting ACR ($p>0.05$ all**)**.

Table 6.3. Haemodynamic differences between patients with type 2 diabetes mellitus (T2DM) and non-diabetic participants at rest and during a bout of light-moderate intensity exercise.

	Rest			Exercise		
	T2DM (n=39)	**Non-diabetic (n=39)**	**P value**	**T2DM (n=39)**	**Non-diabetic (n=39)**	**P value**
Central systolic blood pressure (mmHg)	115±12	103±10	<0.001	132±14	114±12	<0.001
Central pulse pressure (mmHg)	45±9	37±5	<0.001	53±11	39±7	<0.001
Augmentation pressure (mmHg)	13±5	8±5	<0.001	9±6	4±4	<0.001
Peak reservoir pressure (mm Hg)	35±8	32±4	0.011	18±10	14±5	0.14
Reservoir pressure integral (Pa/s)	1897±536	1888±370	0.93	793±484	676±267	0.32
Peak excess pressure (mmHg)	35±9	30±4	0.003	74±16	58±12	<0.001
Excess pressure integral (Pa/s)	636±197	492±96	<0.001	1671±465	1272±467	<0.001
Aortic pulse wave velocity (m/s)	8.0±2.1	6.3±1.4	<0.001	9.7±2.1	7.1±1.4	<0.001
Heart rate (bpm)	64±8	58±8	0.001	92±12	86±11	0.050
Cardiac output (L/min)	5.4±1.01	4.5±0.7	<0.001	8.3±1.5	7.9±1.3	0.23
Stroke volume (mL)	83±113	79±14	0.13	91±13	92±14	0.62
Systemic vascular resistance ($d/s/cm^{-5}$)	1361±243	1504±260	0.015	1003±200	980±156	0.57
Brachial systolic blood pressure (mmHg)	125±13	114±9	<0.001	155±17	135±14	<0.001
Brachial diastolic blood pressure (mmHg)	69±8	65±6	0.028	77±9	74±8	0.094
Brachial pulse pressure (mmHg)	56±11	49±5	<0.001	78±15	61±10	<0.001

Data expressed as mean ± standard deviation. P value is for between group analyses.

Table 6.4. Multivariable analysis of the relationships between albumin creatinine ratio measured prior to and post exercise and haemodynamic variables in patients with type 2 diabetes mellitus (T2DM) and non-diabetic participants.

		T2DM (n=39)		Non-diabetic (n=39)	
	Independent variable	**β (95% CI)**	**P value**	**β (95% CI)**	**P value**
Prior to exercise					
Albumin creatinine ratio	Aortic pulse wave velocity (m/s)	0.081 (-0.079, 0.241)	0.069	-0.080 (-0.454, 0.293)	0.93
Post exercise					
Albumin to creatinine ratio	Excess pressure integral (Pa/s)	0.003 (0.001, 0.004)	0.001	0.000 (-0.004, 0.003)	0.60
	Peak excess pressure (mm Hg)	0.030 (-0.020, 0.080)	0.22	-0.039 (-0.147, 0.070)	0.47
	Central systolic blood pressure (mm Hg)	0.023 (-0.028, 0.074)	0.27	-0.003 (-0.115, 0.110)	0.63
	Cardiac output (L/min)	0.262 (-0.158, 0.682)	0.204	-0.044 (-0.799, 0.711)	0.62
	Brachial systolic blood pressure (mm Hg)	0.026 (-0.014, 0.065)	0.19	-0.022 (-0.118, 0.074)	0.56
	Brachial pulse pressure (mm Hg)	0.027 (-0.024, 0.078)	0.24	-0.065 (-0.193, 0.063)	0.34
Albumin to creatinine ratio (post exercise minus prior to exercise)	Excess pressure integral (Pa/s)	0.001 (0.000, 0.002)	0.034	004^{-3} (-0.001, 0.001)	0.99
	Cardiac output (L/min)	0.262 (0.003, 0.521)	0.27	0.068 (-0.27, -0.0408)	0.99

β refers to unstandardised beta coefficient for the independent variable; CI, confidence interval. P value relates to the independent variable in the model. All models adjusted for age, sex, body mass index and ambulatory daytime systolic blood pressure.

Association between exercise haemodynamics and exercise-induced albuminuria (ACR)

Following exercise, in patients with T2DM but not non-diabetic controls, exercising central systolic BP (r=0.33, p=0.043), P_{excess} (r=0.51, p=0.002), peak excess pressure (r=0.38, p=0.022), stroke volume (r=0.40, p=0.014), cardiac output (r=0.45, p=0.005), brachial systolic BP (r=0.36, p=0.026) and pulse pressure (r=0.34, p=0.035), but not aPWV (r=0.12, p=0.45), were significantly associated with post exercise ACR. Additionally, P_{excess} and cardiac output in patients with T2DM were significantly associated with the difference between ACR measured prior to and post exercise (r=0.44, p=0.008 and r=0.39, p=0.026 respectively). After adjusting for the same covariates as at rest, the only independent predictor of post exercise ACR in patients with T2DM was P_{excess} (table 6.4). After further adjusting for the use of antihypertensive medication, blood glucose or HbA_{1c} level, the association between P_{excess} and post exercise ACR in patients with T2DM remained unchanged (β=0.003, 95%CI 0.001 to 0.004, p=0.001). Adjusting for aPWV or heart rate did not attenuate the association between P_{excess} and post exercise ACR in patients with T2DM (β=0.003, 95%CI 0.001 to 0.004, p=0.003 and β=0.003, 95%CI 0.001 to 0.004, p=0.001 respectively) and P_{excess} remained independently associated with post exercise ACR in patients with T2DM after adjusting for exercise systolic BP and also the change from rest to exercise in systolic BP (β=0.002, 95%CI 0.001 to 0.004, p=0.002 and β=0.003, 95%CI 0.001 to 0.004, p=0.002 respectively). Furthermore, P_{excess} remained an independent predictor of the change in ACR from prior to post exercise. There were no significant associations between post exercise ACR and exercise brachial or central haemodynamics in non-diabetics. Individuals who had a high P_{excess} response during exercise (defined as ≥1439 Pa/s which was the median P_{excess}) were more likely to have T2DM (n=27 vs n=12, p<0.001), were of older age (60±8 vs 55±10 years, p=0.007), had greater BMI (29.2±5.5 vs 26.2±4.0 kg/m^2, p=0.008), higher blood glucose (6.6±1.9 vs 5.4±1.7 mmol/L, p=0.008) and HbA_{1c} levels (6.6±1.0 vs 6.0±1.0%, p=0.012) compared to individuals who had an exercise response below the median. A P_{excess} value of 1227 Pa/s predicted an increase in ACR from rest to exercise with 80% sensitivity and 60% specificity (AUC = 0.677; p=0.019). Z statistic scores were calculated to compare the correlation coefficients of haemodynamic variables and ACR between patients with T2DM and non-diabetics. There was a significant difference in the strength of the relationship between exercise P_{excess} and post exercise ACR in patients with T2DM compared with non-diabetics (Z statistic=2.85, p=0.007; figure 6.1). There was no significant interaction between the groups and P_{excess} in predicting ACR (p>0.05 for product term).

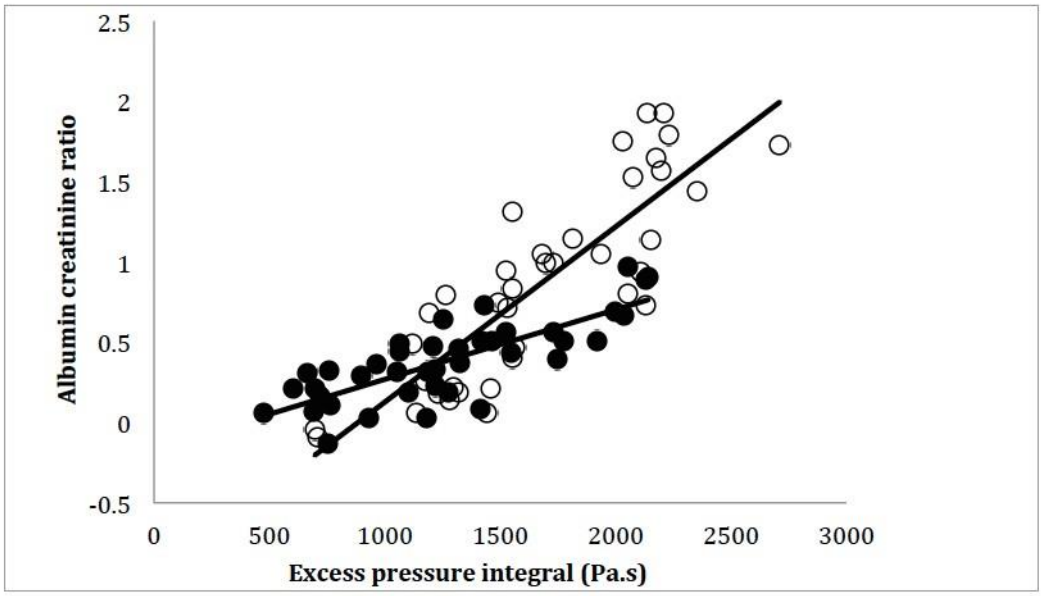


Figure 6.1. Association between albumin to creatinine ratio measured post exercise and excess pressure integral (P_{excess}) during exercise in patients with type 2 diabetes mellitus (open circles; r=0.510, p=0.002, n=39) and non-diabetic participants (solid circles; r=0.220, p=0.18, n=39). The correlation in patients with T2DM was stronger than for non-diabetic participants (Z statistic=2.85, p=0.007).

6.5 Discussion

In this study we have shown that 1) a bout of light-moderate intensity exercise induced a significant rise in ACR only in patients with T2DM; 2) central (not brachial) haemodynamics, specifically P_{excess}, was independently associated with exercise-induced albuminuria in patients with T2DM and, importantly, this association remained after correction for other variables known to be associated with end organ damage including age, BMI and 24 ABPM; 3) the association between P_{excess} and exercise-induced albuminuria was only evident under light-moderate intensity exercise, not resting conditions. These novel findings suggest that P_{excess}, a new marker representing vascular dysfunction, may be important for appropriate renal function in patients with T2DM, especially under the haemodynamic load induced by low level exercise similar to normal daily activities.

Altered central hemodynamics, flow wave patterns and albuminuria

Several investigations of subjects studied under resting conditions have reported an association between increased aortic stiffness and albuminuria, independent from brachial BP[268-270].

Observations such as this have led to the hypothesis that stiffening of the large central vasculature enhances transmission of pulsatile pressure[43] and/or flow energy to the peripheral microvasculature resulting in end organ injury. Indeed, data from Hashimoto and Ito[271] suggest that increased aortic stiffness may disturb femoral blood flow patterns, firstly by decreasing the normal diastolic flow reversal thought to be needed for appropriate circulation to the truncal organs, but also by reducing forward flow to the lower extremities. These investigators also demonstrate that femoral flow wave abnormalities are related to adverse renal artery hemodynamics, which in turn explained higher levels of urinary albumin excretion, even after correction for well known risk factors[133]. In the current study, patients with T2DM had significantly higher aPWV (stiffness) and central pulse pressure, both at rest and during exercise, but neither of these factors were related to ACR. This disparity is probably due to different study designs and patient populations.

In the current study, we found that P_{excess} (specifically measured under the stress induced by exercise) was the only significant predictor of exercise-induced albuminuria in patients with T2DM. Although speculative, a stiffened aorta (as observed in our patients with T2DM) may result in an increase in left ventricular work and a subsequent elevation in P_{excess}, ultimately leading to greater transmission of pulsatile stress towards the periphery, which may disrupt renal hemodynamics and induce a rise in albumin excretion. Indeed, when the normal 'reservoir' function of the aorta is less than optimal (i.e. due a reduction in vessel compliance) there must be an increase to left ventricular work and excess pressure in order to overcome the resistance caused by the stiffened aorta. However, reservoir pressure is influenced not only by aortic compliance, but also by resistance from the peripheral circulation, which probably has greater impact on 'discharge' of the reservoir during diastole[139]. Combined with systemic vasodilation (demonstrated in this study by a reduction in systemic vascular resistance in both non-diabetic participants and patients with T2DM during exercise), the increased excess pressure associated with light-moderate exercise may be transmitted with higher energy from the large vessels to the microcirculation. As P_{excess} is also analogous to flow output into the aorta[8], our data appears to conform with the 'flow hypothesis' which suggests that increased flow pulsation may extend into the renal microvasculature and cause excessive cyclic shear stress and eventual glomerular dysfunction[134, 272].

Hashimoto et al.[133] showed that the renal resistive index is inversely associated with renal diastolic flow (and femoral reverse flow) and resulted in reduced renal flow throughout diastole. This may align with the findings of the current study, whereby in patients with T2DM who not only have reduced 'reservoir' function but also higher heart rates (both at rest and during exercise and thus

shortened cardiac cycle time; predominantly affecting the diastolic phase), there will be less aortic recoil and discharge of blood from the proximal aorta to the distal vasculature, and thus normal blood flow throughout diastole will be reduced[8]. Our findings support the notion that a reduction in reservoir pressure from rest to exercise in both groups could give rise to a large majority of the arterial pressure wave, which is attributable to P_{excess} as shown previously[8, 144] and a resultant increase in pulsatile pressure and/or flow throughout systole. Taken together, our findings imply that abnormalities in the aortic reservoir and excess pressure components of the pressure wave and the relative increase in systolic flow and decrease in diastolic flow may play a role in impaired renal flow hemodynamics and end organ damage.

Exercise central hemodynamics and albuminuria. It is worth noting that central hemodynamics (P_{excess}) measured during light-moderate intensity exercise, but not at rest, were related to ACR, and this was independent of BP. To our knowledge, only one study has reported the association between exercise hemodynamics to exercise-induced albuminuria in patients with T2DM, showing that maximal exercise systolic BP was associated with exercise-induced albumin excretion[264]. The rise in noradrenalin that occurs during exercise may partially explain the increase in permeability of the glomerular membrane and increased urinary albumin excretion, a mechanism that may be reversed by sympathetic nerve inhibition[267]. Based on our findings under light-moderate exercise conditions, it is possible that patients with T2DM with an elevated central BP (and P_{excess}) response may be exposed to pronounced stress-induced hemodynamic changes during normal daily activity that allow for the transmission of excessive pressure to the microcirculation and ensuing susceptibility towards renal dysfunction. Having said this, the cross sectional design of this study limits inference regarding causality.

Limitations

Only one urine sample was taken at 30 minutes post exercise. The rationale for choosing this time point was based on previous literature showing that there is a significant increase in urine albumin excretion occurring 30 minutes following exercise[267], but the lack of multiple urine measures (considered *a priori* to be less feasible than one discrete sample) could have led to the peak ACR response being missed in some individuals. Multiple urine samples would also have provided more precise information on the integrated (area under the curve) exposure of haemodynamic renal damage from exercise. In a number of participants we were unable to detect a measureable level of urinary albumin following exercise (indicating that these participants had normal renal function in response to light-moderate intensity exercise) and therefore, this reduced

the sample size of participants with detectable albumin values and could have led to a type 2 error. That said, despite the small sample size we were still able to detect significant changes in renal function in response to exercise, which provides sound rationale for examining the underlying mechanisms in larger cohort studies. A further limitation is that all participants, irrespective of their age or disease status, exercised at the same intensity. The reason for using a set resistance protocol was to achieve a fixed light-moderate intensity exercise that approximated the intensity regularly achieved during daily activity. This approach is more generalisable to clinical exercise stress testing which is performed at fixed intensities. Finally, due to the cross sectional design we are unable to determine the degree to which chronic exposure to conventional risk factors may explain the abnormal kidney function response to exercise in patients with T2DM.

6.6 Conclusions

This is the first study to examine the association between exercise central haemodynamics and exercise-induced albuminuria in patients with T2DM. Our findings show that a bout of light-moderate exercise, similar to that of normal daily activity, induced albuminuria in patients with T2DM. Current guidelines for assessing urinary albumin in patients with T2DM suggest avoiding heavy exercise within the 24 hours prior to assessment[273]. However, our findings suggest that urinary albumin should be measured well clear of light to moderate physical activity as well, in patients with T2DM. Alternatively, our findings show that the modality of exercise may reveal renal abnormalities in patients with T2DM that are not evident at rest, however, further longitudinal studies are required to confirm this. Additionally, P_{excess}, a marker of possible vascular dysfunction, may be important for appropriate renal function in this population. Given the increased risk of albuminuria and renal dysfunction in patients with T2DM, more work is required to determine the exact underlying vascular mechanism contributing to such abnormalities and the implications of light to moderate exercise prior to a spot urine test in this population.

6.7 Contribution of Chapter 6 to thesis aims

The results from the study presented in *Chapter 6* showed, for the first time, that light to moderate intensity exercise can induce albuminuria in patients with T2DM. This is important, as until now, previous studies have only measured albuminuria in response to maximal intensity exercise. These findings suggest that exercise at a similar intensity to that of normal daily activity, can induce this abnormal renal state in patients with T2DM. Therefore, light to moderate intensity exercise may be a useful tool to unmask renal abnormalities in patients with T2DM. *Chapter 6* also

demonstrated that exercise central haemodynamics, in particular excess pressure, were related to exercise-induced albuminuria in patients with T2DM, independently of resting brachial BP. This is inline with the findings from *Chapter 5* and provides further support for excess pressure as a potential marker of increased cardiovascular risk. Furthermore, the findings from this study suggest that central haemodynamics measured in response to light to moderate intensity exercise may provide pathological insights above and beyond resting clinic measures of brachial BP.

The findings from the studies presented in *Chapters 3, 5* and *6* highlight that patients with T2DM have abnormal central haemodynamics compared to their non-diabetic counterparts, which may influence the accuracy of clinical methods including the estimation of central BP non-invasively. Therefore, in *Chapter 7*, the effect of these haemodynamic abnormalities on the accuracy of central BP determined via radial applanation tonometry (the most widely utilised non-invasive method during the candidature) is examined.

Chapter 7. Brachial-to-radial systolic blood pressure amplification in patients with type 2 diabetes mellitus

This chapter has previously been published;

Climie RED, Picone DS, Keske MA, Sharman, JE. Brachial-to-radial systolic blood pressure amplification in patients with type 2 diabetes mellitus. *Journal of Human Hypertension*, October 2015; 10.1038/jhh.2015.101

Chapter 7 formed part of a larger study for which 40 healthy younger participants and 40 older participants (20 patients with type 2 diabetes mellitus and 20 non-diabetic, healthy controls) were recruited. The findings from the study in the healthy participants are presented in *Appendix I*. In this larger study, the effect of light to moderate intensity exercise on brachial to radial systolic blood pressure amplification in patients with type 2 diabetes mellitus and non-diabetic controls was also examined. This data was not included in the final submitted paper (*Chapter 7*) but is presented in *Appendix II*.

7.1 Abstract

Background. Brachial-to-radial-systolic blood pressure amplification (Bra-Rad-SBP_{Amp}) can affect central SBP estimated by radial tonometry. Patients with type 2 diabetes mellitus (T2DM) have vascular irregularities that may alter Bra-Rad-SBP_{Amp}. By comparing T2DM to non-diabetic controls, we aimed to determine the 1) magnitude of Bra-Rad-SBP_{Amp}; 2) hemodynamic factors related to Bra-Rad-SBP_{Amp}; and 3) effect of Bra-Rad-SBP_{Amp} on estimated central SBP.

Methods. Twenty T2DM (64±8 years) and 20 non-diabetic controls (60±8 years; 50% male both) underwent simultaneous cuff deflation and two-dimensional ultrasound imaging of the brachial and radial arteries. The 1st Korotkoff sound (denoting SBP) was identified from the first inflection point of Doppler flow during cuff deflation. Bra-Rad-SBP_{Amp} was calculated by radial minus brachial SBP. Upper limb and systemic hemodynamics were recorded by tonometry and ultrasound.

Results. Radial SBP was higher than brachial SBP for T2DM (136±19vs127±17mmHg; $p<0.001$) and non-diabetic controls (135±12vs121±11mmHg; $p<0.001$), but Bra-Rad-SBP_{Amp} was significantly lower in T2DM (9±8vs14±7mmHg, $p=0.042$). The product of brachial mean flow velocity*brachial diameter was inversely and independently correlated with Bra-Rad-SBP_{Amp} in T2DM (β=-0.033 95% CI-0.063 to -0.004, $p=0.030$). When radial waveforms were calibrated using radial, compared with brachial SBP, central SBP was significantly higher in both groups (T2DM; 116±13vs125±15mmHg and controls; 112±10vs124±11mmHg, $p<0.001$ both) and there was a significant increase in the number of participants classified with 'central hypertension' (SBP≥130 mmHg; $p=0.004$).

Conclusions. Compared with non-diabetic controls, Bra-Rad-SBP_{Amp} is significantly lower in T2DM. Regardless of disease status, radial SBP is higher than brachial SBP and this results in underestimation of central SBP using brachial-BP-calibrated radial tonometry.

7.2 Introduction

Central blood pressure (BP) indices are predictive of cardiovascular events and all-cause mortality above and beyond brachial BP[33, 274]. Accumulating evidence suggests that central BP could be useful in routine clinical management of hypertension[275-277]. The most accurate measurement of central BP is obtained invasively, however, this is not suitable for routine use. The most common non-invasive method to estimate central BP to date has been radial applanation tonometry[10]. Using this method, the radial pressure waveform is calibrated with brachial systolic BP (SBP) and diastolic BP, and a generalised transfer function applied to synthesise the central (ascending aortic) waveform[10, 11]. This method relies on the assumption of minor differences in SBP from the brachial to the radial artery[278]. However, we recently found major brachial-to-radial SBP amplification (Bra-Rad-SBP_{Amp}) in healthy older people, with wide inter-individual variation (range from 3 to 27 mmHg)[279]. Other studies confirm that significant Bra-Rad-SBP_{Amp} is likely to be a common finding[178, 280-283]. Importantly, Bra-Rad-SBP_{Amp} contributes to underestimation of central SBP using radial applanation tonometry[173, 178, 279], and this could result in misclassification of individual risk based on central hypertension thresholds[42].

Inter-individual differences in Bra-Rad-SBP_{Amp} may be influenced by disease related changes in arterial structure and function. Patients with type 2 diabetes mellitus (T2DM) have cardiovascular irregularities including increased cardiac output (predominantly due to increased heart rate but also stroke volume)[63], increased central and peripheral[46] large artery stiffness, reduced systemic vascular resistance[63], adverse structural remodeling of the peripheral arterioles[49, 67] and impaired nitric oxide mediated endothelial function[284]. Abnormalities such as these could impact on the magnitude of Bra-Rad-SBP_{Amp} which could in turn affect the accuracy of central BP estimated using brachial BP-calibrated radial tonometry, but whether this amplification is different in patients with T2DM compared to non-diabetic controls is unknown. The aims of this study were to determine the: 1) magnitude of Bra-Rad-SBP_{Amp}; 2) hemodynamic factors related to Bra-Rad-SBP_{Amp} and; 3) effect of Bra-Rad-SBP_{Amp} on estimated central SBP in patients with T2DM compared to healthy age-matched non-diabetic controls.

7.3 Methods

Study participants.

Twenty patients with T2DM and 20 non-diabetic controls were consecutively recruited from the community via advertisements. Exclusion criteria included pregnancy, arrhythmia (due to affecting the quality of the waveforms captured), clinical history of cardiovascular disease (including coronary artery disease, myocardial infarction, heart failure or stroke), or severe pulmonary disease. The presence of T2DM was determined by self-report of previous diagnosis by a physician. Hypertension was defined as clinic brachial BP ≥140/90 mmHg or self-reported diagnosis by a physician, or use of antihypertensive medications. All participants signed informed consent and the study was approved by the University of Tasmania Human Research Ethics Committee.

Study protocol.

The study protocol has previously been described elsewhere[279]. Briefly, each participant attended the testing laboratory on one occasion. Participants were asked to refrain from vigorous exercise for the 24 hours prior to their visit; avoid alcohol consumption on the day; and to fast and refrain from caffeine and cigarettes three hours prior to their appointment. Participants on medications maintained their normal treatment schedule on the study day. Standard anthropometric measurements (including height, weight, waist and hip circumference) were recorded. All hemodynamic data were collected with the participant in a semi-recumbent position (with the upper section of the bed elevated so that the head and torso were at a 45 degree angle) and the arm supported at the level of the heart. At the completion of the study, participants completed a standard questionnaire relating to BP and medical history and were fitted with a validated[285] oscillometric 24 hour ambulatory BP monitor (TM-2430, A&D Medical, Sydney, Australia) which measured brachial BP every 20 minutes during the day and 30 minutes during the night.

Bra-Rad-SBP$_{Amp}$.

After 10 minutes of rest, six measures of brachial and radial SBP (three at each site) were measured sequentially (approximately 45 seconds apart), in random order, using the same arm for brachial and radial SBP measurements. Appropriately sized cuffs were placed on the upper arm (~7cm above the antecubital fossa) and forearm (~7cm above the anatomical snuff box) of the participant to measure brachial and radial SBP respectively. SBP was identified during BP cuff deflation from the first inflection point of Doppler flow (Figure 1) and the audible Doppler signal (denoting SBP) [286]. When the first Doppler flow inflection (and audible signal) during cuff deflation was observed, SBP was recorded as the value displayed on the–sphygmomanometer; a validated mercury-free

device (UM-101, A&D Medical, Tokyo, Japan)[287]. We could not identify diastolic BP using the Doppler flow signal and accurate auscultation was not possible at the radial artery. We assumed brachial diastolic BP was equal to radial diastolic BP, based on previous data that shows diastolic BP remains consistent throughout the arterial system (within 1-3 mmHg)[74]. This resulted in the magnitude of Bra-Rad-SBP_{Amp} being equivalent to pulse pressure amplification; therefore, we only reported the former. The BP operator was blinded to the ultrasound measurement site by a partition screen that blocked the view of the participant's arm and the arterial image on the ultrasound screen, but permitted sight of the Doppler flow signal and the sphygmomanometer to allow SBP to be determined. Bra-Rad-SBP_{Amp} was calculated as radial minus brachial SBP. We tested the validity of this method of determining SBP from the Doppler flow by comparing brachial SBP obtained by auscultation with brachial SBP obtained from the Doppler flow in all participants. There was strong agreement between measures (intraclass correlations [ICC] r=0.963, p<0.001 and mean difference = -0.10 ± 3.38 mmHg, p=0.85). The reproducibility of the brachial and radial SBP obtained from the Doppler flow was tested in a subset of 10 participants who completed an additional assessment within 5 ± 2 days of their initial assessment. The between-visit ICC were r=0.944 for brachial SBP and r=0.937 for radial SBP (p<0.001 both) and the mean differences between visits were 1 ± 5 mmHg, p=0.45 and -1 ± 6 mmHg, p=0.72 for brachial and radial SBP respectively.

Arterial diameter and blood flow velocity

Brachial and radial arterial imaging was performed using a two dimensional ultrasound (Philips iU22, Philips Healthcare, Bothell, WA, USA) with a linear-array transducer with a transmission frequency of 12-5 MHz and arterial diameters were analysed offline, using QLAB software (figure 7.1). Brachial and radial artery mean blood flow velocities were recorded by Doppler ultrasound with the same Philips device and the average of 10 heart beats was used for analysis. The difference between brachial and radial mean flow velocity was determined by brachial minus radial flow velocity. Exploratory variables such as brachial mean flow velocity x brachial diameter were derived based on sound physiological rationale (including patients with T2DM having increased flow output and reduced vascular resistance compared to their non-diabetic counterparts[63]), which may explain any observed differences in Bra-Rad-SBPAmp between the groups."

Brachial artery blood flow and radial artery blood flow (in ml/min) were calculated using equation 1 and 2 respectively;

Equation 1:

Brachial artery flow

$$= \Pi \; x \; brachial \; artery \; radius^2 \; x \; brachial \; time \; averaged \; mean \; velocity \; x \; 60$$

Equation 2:

Radial artery flow

$$= \Pi \; x \; radial \; artery \; radius^2 \; x \; radial \; time \; averaged \; mean \; velocity \; x \; 60$$

Where, Π = pi; radius expressed as cm; and velocity expressed as cm/s.

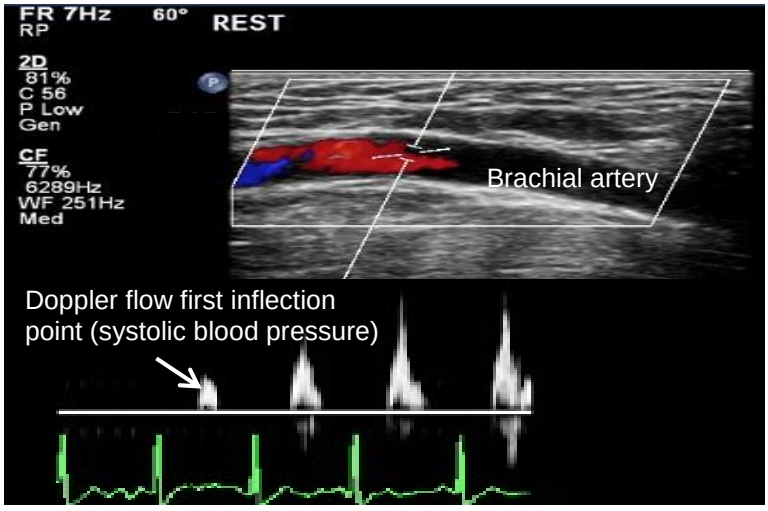


Figure 7.1. Measurement of systolic blood pressure (SBP) using brachial artery Doppler ultrasound and sphygmomanometry. As per normal SBP measurement, the brachial cuff was inflated to occlude flow at suprasystolic pressure and then steadily deflated at ~ 2 mmHg/sec. The point at which the first Doppler flow inflection (which also corresponded to the first audible Doppler signal) returned during cuff deflation (as indicated by the left arrow) was defined as SBP. Upon observing the first Doppler flow inflection, the BP operator immediately recorded SBP from the sphygmomanometer (as indicated by the right arrows). The same method was used to determine SBP at the radial artery by inflating a cuff placed at the forearm.

Arterial stiffness

Brachial pulse wave velocity (PWV; carotid-radial) was measured as previously described[10]. The average upper limb distensibility was calculated using equation 3 below and is a measure of passive expansion and contraction of the arterial wall relative to changes in pressure[288]. The average upper limb distensibility was determined as the average of brachial and radial distensibility;

Equation 3:

Average upper limb distensibility

$$\frac{[= Brachial\ (Ds - Dd)/Dd\ /\ PP)]\ + [radial\ (Ds - Dd)/Dd\ /\ PP)]}{2} \times\ 10\ 000$$

Where, Ds = End systolic diameter (cm); Dd = End diastolic diameter (cm); PP = brachial and radial pulse pressure (mmHg) respectively. Multiplied by 10 000 for better display.

Cardiothoracic bioimpedance

Non-invasive cardiothoracic bioimpedance (PhysioFlow, PF-05, Manatec Biomedical, Paris, France) was performed continuously throughout the study to monitor additional cardiovascular parameters including heart rate, stroke volume, cardiac output and systemic vascular resistance. This device has been previously validated[185] and has good reproducibility[186].

Central BP

Following the SBP measurements, duplicate central BP was estimated via radial applanation tonometry (SphygmoCor 8.1, AtCor Medical Pty Ltd, Sydney, Australia). Augmentation index (AIx) was calculated as the difference in pressure between the first and second systolic peaks (augmented pressure), expressed as a percentage of pulse pressure and was adjusted for a heart rate of 75 beats per minute. To quantify the effect of Bra-Rad-SBP_{Amp} on central SBP estimation, radial waveforms were calibrated firstly using brachial SBP and diastolic BP and secondly using radial SBP and brachial diastolic BP. Brachial, rather than radial diastolic BP was used to calibrate the radial waveforms on the assumption that diastolic BP varies little from central (i.e. aorta, carotid) to peripheral (brachial, radial) large arterial beds[74], and also because radial artery diastolic BP was unable to be detected accurately using auscultation and Doppler flow. A central SBP cutoff value of ≥130 mmHg was used to delineate 'central hypertension'[42].

Blood biochemistry

Following a three hour fast a venous blood sample was drawn from the antecubital fossa. Sample analysis was performed for blood glucose and lipid profiles using accredited laboratory techniques.

Statistical analysis

Data were analysed using SPSS for Windows software version 20.0 (IBM SPSS Statistics, New York, USA). Data were visually inspected for normality of distribution and were all normally distributed. Differences between patients with T2DM and non-diabetic controls were assessed using

independent T-tests for continuous variables and Chi square test for categorical variables. Pearson correlation analysis was used to determine associations between variables. Multiple regression analysis was performed in patients with T2DM and non-diabetic controls separately adjusting for potential confounders (including age, sex, clinic SBP, antihypertensive use, body mass index [BMI] and heart rate) to determine the independent predictors of Bra-Rad-$SBP_{Amp.}$ $P< 0.05$ was considered statistically significant. Based on previous reproducibility work[279], we calculated that a between-group difference of 5 mmHg in Bra-Rad-SBP_{Amp} could be detected in 16 participants per group (α=0.05 and β=0.20), therefore we recruited 20 participants for each group.

7.4 Results

Participant characteristics

The participant characteristics are displayed in table 7.1. The groups were well matched for age and sex. Patients with T2DM had significantly greater body mass index and waist-to-hip ratio. There was no difference between the groups in overall 24 hour ambulatory SBP or diastolic BP. None of the controls were taking medication for the treatment of hypertension, however, 70% of the patients with T2DM were taking antihypertensive medication, 20% were taking oral hypoglycaemic and 40% were taking statins. Patients with T2DM had significantly higher blood glucose but significantly lower total cholesterol and low-density lipoprotein cholesterol.

7.1. Participant characteristics of patients with type 2 diabetes mellitus (T2DM) and non-diabetic controls.

	T2DM (n=20)	**Non-diabetic (n=20)**	**P value**
Male, n (%)	10 (50)	10 (50)	1.0
Age (years)	63±8	60±7	0.21
Body mass index (kg/m^2)	30.7±6.1	25.6±3.3	0.002
Waist-hip ratio	0.92±0.09	0.86±0.10	0.036
24 hour ambulatory systolic BP (mmHg)	127±13	129±11	0.74
24 hour ambulatory diastolic BP (mmHg)	73±7	77±9	0.10
Antihypertensives, n (%)	14 (70)	0 (0)	<0.001
Oral hypoglycaemics, n (%)	4 (20)	0 (0)	0.03
Statins, n (%)	8 (40)	0 (0)	0.001
Glucose (mmol/L)	7.6±2.4	5.7±0.6	0.007
Total cholesterol (mmol/L)	4.5±1.1	5.7±1.0	0.002
LDL cholesterol (mmol/L)	2.2±0.7	3.5±0.9	<0.001
HDL cholesterol (mmol/L)	1.3±0.6	1.6±0.5	0.095
Triglycerides (mmol/L)	2.3±1.8	1.5±0.8	0.10

Data are mean ± standard deviation. BP, blood pressure; LDL, low-density lipoprotein; HDL, high density lipoprotein.

Bra-Rad-SBP$_{Amp}$

As shown in table 7.2, patients with T2DM had significantly lower Bra-Rad-SBP$_{Amp}$ compared to non-diabetic controls. Brachial SBP was 6 mmHg higher in patients with T2DM than non-diabetic controls, but this was non-significant. Radial SBP was significantly higher than brachial SBP for patients with T2DM and non-diabetic controls.

T**able 7.2.** Brachial to radial systolic blood pressure (BP) amplification (Bra-Rad- SBP_{Amp}) and effect on central BP estimation in patients with type 2 diabetes mellitus (T2DM) and non-diabetic controls.

	T2DM (n=20)	**Non-diabetic (n=20)**	**P value**
Bra-Rad-SBP_{Amp} (mmHg)	9±8	14±8	0.042
Brachial systolic BP (mmHg)	127±17	121±11	0.14
Brachial diastolic BP (mmHg)	68±7	72±7	0.12
Radial systolic BP (mmHg)	136±19	135±12[#]	0.9
*Central systolic BP (mmHg)	116±13	112±10	0.28
**Central systolic BP (mmHg)	125±15^	124±11^	0.80

Data are mean ± standard deviation. *Central systolic BP calibrated with brachial systolic and diastolic BP. **Central systolic BP calibrated with radial systolic and brachial diastolic BP. [#] P value =0.001 for radial vs brachial systolic BP. ^P value <0.001 for the difference in central systolic BP calibrated using radial compared to brachial systolic BP.

Differences in hemodynamic and arterial properties between groups and associations with Bra-Rad-SBP_{Amp}

Hemodynamic and arterial differences between patients with T2DM and non-diabetic controls are shown in table 7.3. AIx adjusted for heart rate of 75 beats per minute was significantly higher in patients with T2DM and upper limb distensibility was significantly lower in patients with T2DM compared to non-diabetic controls.

None of the measured hemodynamic variables shown in table 7.3 were significantly correlated with Bra-Rad-SBP_{Amp} in non-diabetic controls. However, in patients with T2DM, brachial mean and peak flow velocities significantly and inversely correlated with Bra-Rad-SBP_{Amp} (r=-0.628, p=0.003 and r=-0.563, p=0.010 respectively). The product of brachial mean flow velocity and brachial diameter was significantly and inversely correlated with Bra-Rad-SBP_{Amp} (r=-0.598, p=0.007; figure 7.2) and this relationship remained significant after adjusting for age, sex and clinic SBP (β=-0.033 95% CI -0.063 to -0.004, p=0.030). The relationship between brachial mean flow velocity*brachial diameter and Bra-Rad SBP_{Amp} remained significant after further adjustment for BMI (β=-0.033 95% CI -0.064 to -0.001, p=0.043) and heart rate (β=-0.035 95% CI -0.065 to -0.006, p=0.024). A similar but non-significant relationship existed between brachial blood flow and

Bra-Rad SBPAmp (r=-0.405, p=0.077). No other hemodynamic variables shown in table 3 significantly correlated with Bra-Rad-SBP_{Amp} in patients with T2DM.

Table 7. 3. Haemodynamic and arterial differences between patients with type 2 diabetes mellitus (T2DM) and non-diabetic controls.

	T2DM (n=20)	**Non-diabetic (n=20)**	**P value**
Augmentation index (%)	29±9	26±7	0.24
Augmentation index at heart rate of 75bpm (%)	24±7	18±8	0.017
Brachial artery diameter (mm)	3.96±0.92	3.59±0.60	0.14
Radial artery diameter (mm)	1.81±0.37	1.81±0.40	0.96
Brachial mean flow velocity (cm/s)	7.98±4.26	6.76±4.33	0.37
Brachial peak flow velocity (cm/s)	14.30±7.41	11.91±7.57	0.32
Brachial mean flow velocity x brachial diameter (cm/s/mm)	304±132	243±166	0.21
Radial mean flow velocity (cm/s)	6.70±3.91	5.87±4.98	0.56
Radial peak flow velocity (cm/s)	12.85±7.74	11.90±10.51	0.75
Difference between brachial and radial mean flow velocity (cm/s)	1.28±3.45	0.88±2.23	0.67
Brachial blood flow (ml/min)	54±29	42±32	0.22
Radial blood flow (ml/min)	10±7	11±13	0.8
Brachial pulse wave velocity (m/s)	8.13±1.09	7.99±1.55	0.73
Upper limb arterial distensibility (%/mmHg)	6.89±3.50	9.83±4.20	0.031
Heart rate (bpm)	67±10	60±10	0.051
Stroke volume (ml)	75±22	74±13	0.93
Cardiac output (l/min)	5.03±1.88	4.39±0.73	0.19
Systemic vascular resistance (dyne/s/cm^{-5})	1455±414	1676±269	0.071

Data are mean ± standard deviation.

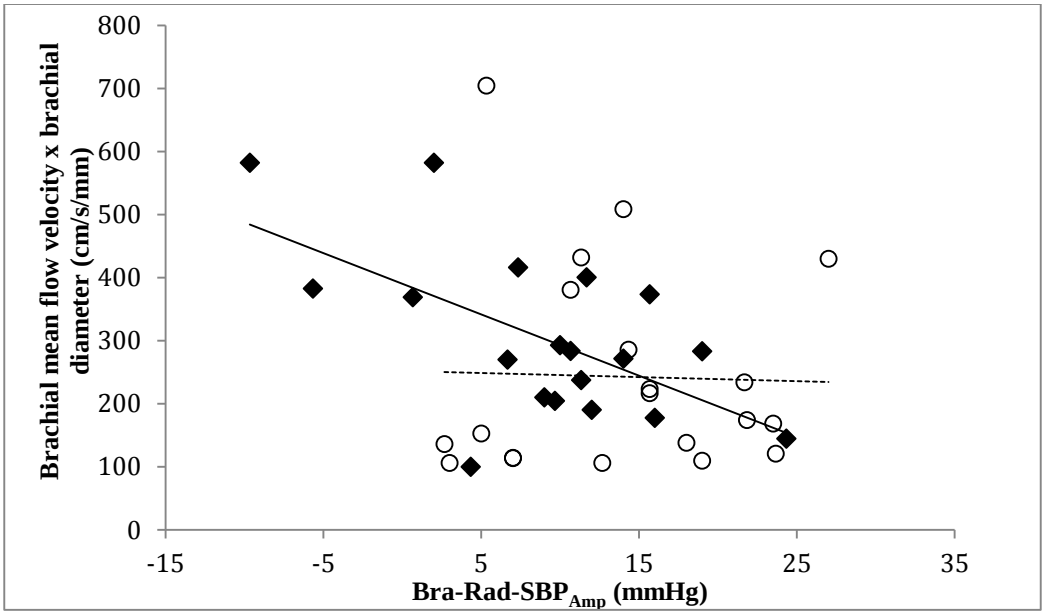


Figure 7.2. Univariate association between the product of brachial mean flow velocity and brachial diameter and brachial to radial systolic blood pressure amplification (Bra-Rad-SBP_{Amp}) in patients with type 2 diabetes mellitus (black diamonds and solid trend line; r=-0.598, p=0.007) and non-diabetic controls (open circles and dashed trend line; r=-0.028, p=0.905).

The effect of Bra-Rad-SBP_{Amp} on central BP estimation

There was no difference between the groups in central BP estimated using brachial SBP and diastolic BP to calibrate radial pressure waveforms. However, central SBP was significantly higher in both non-diabetic controls and patients with T2DM when radial pressure waveforms were calibrated using radial SBP and brachial diastolic BP (change in central SBP of 12±6 mmHg for non-diabetic controls and 9±6 mmHg for patients with T2DM, $p<0.001$ for both; table 7.2). One control participant (5%) and three patients with T2DM (15%) had central SBP $\geq$130 mmHg (indicative of high central blood pressure[42]) when brachial SBP and diastolic BP were used for calibration. However, when brachial SBP was replaced with radial SBP to calibrate the radial pressure waveforms, there was a significant (p=0.004) increase in the number of participants (6 non-diabetic controls [30%] and 12 patients with T2DM [60%]) who had high central SBP (i.e. $\geq$130 mmHg.

7.5 Discussion

This is the first study to directly, non-invasively measure Bra-Rad-SBP_{Amp} in patients with T2DM. The novel findings were: 1) Bra-Rad-SBP_{Amp} was significantly lower in patients with T2DM compared to age and sex matched non-diabetic controls; 2) the product of brachial mean flow velocity and diameter was inversely and independently related to Bra-Rad-SBP_{Amp} in patients with T2DM; and 3) central SBP was significantly higher in both patients with T2DM and non-diabetic controls when radial pressure waveforms were calibrated using radial, compared with brachial SBP. These findings suggest that compared with non-diabetic controls, patients with T2DM have abnormal upper limb hemodynamics that result in lower Bra-Rad-SBP_{Amp}, but regardless of disease status, Bra-Rad-SBP_{Amp} may lead to underestimation of central SBP by radial tonometry and brachial BP calibration of radial waveforms.

Bra-Rad-SBP_{Amp} and abnormal upper limb hemodynamics in patients with T2DM

In an optimally functioning system, the structure of the arterial tree encourages SBP amplification and widening of the pressure pulse, as the pressure wave generated by left ventricular contraction is transmitted from large central elastic arteries to relatively muscular and tapered peripheral large arteries. Only a few studies[178, 281-283, 289] have attempted to assess the magnitude of Bra-Rad-SBP_{Amp}. In another investigation, using the same methods as this current study, we found that Bra-Rad-SBP_{Amp} averaged 8±7 mmHg in healthy young adults, but this was significantly higher in healthy older adults (14±7 mmHg)[279]. Although it is generally accepted that SBP amplification decreases with age from the aorta to brachial artery[43], until now it was unclear as to the amount of SBP amplification that may occur down the forearm. Interestingly, in this previous study[279] we observed that in fact central to radial SBP amplification did decrease with age, but only when central SBP was estimated using radial SBP to calibrate the radial waveform. Furthermore, the magnitude of this SBP amplification was similar to several other well-conducted studies[74, 282, 290] that measured central and radial SBP using simultaneous invasive measurements. Verbeke et al.[178] showed in a cohort of healthy subjects that there was significant Bra-Rad-SBP_{Amp} (e.g. 6±5 mmHg), and a recent invasive study in individuals undergoing percutaneous coronary intervention (15% of which had diabetes) found that SBP was 12±8 mmHg higher in the radial compared to the brachial artery [289]. To our knowledge, no studies have examined Bra-Rad-SBP_{Amp} specifically in patients with T2DM compared with non-diabetic controls. We expected that cardiovascular abnormalities in patients with T2DM would give rise to an increase in Bra-Rad-SBP_{Amp}. In particular, the expected increase in cardiac output[63], together with increased aortic[44] and brachial[46] artery stiffness associated with T2DM were hypothesised to result in an increased amplitude of SBP from brachial

to radial arteries. On the contrary, compared to healthy age and sex matched non-diabetic controls, Bra-Rad-SBP_{Amp} was significantly reduced in patients with T2DM.

We believe the explanation for reduced Bra-Rad-SBP_{Amp} observed in patients with T2DM is due to a relatively higher flow velocity, but a lower pressure gradient from the brachial to radial arteries, despite having significantly reduced upper limb distensibility (as previously reported[46]). This conclusion is based on the independent association between higher brachial flow velocity*brachial diameter and reduced Bra-Rad-SBP_{Amp} in patients with T2DM. In these patients there were also trends toward increased cardiac output (owing to higher heart rate) and lower systemic vascular resistance; altogether suggesting a hyperdynamic system of flow into and out of the upper limb vasculature. The higher inflow does not produce a pressure rise because even though brachial SBP was significantly higher in patients with T2DM compared to controls, there were no differences between groups in radial SBP. In keeping with our findings, we and others have previously reported that patients with T2DM or obese individuals have increased diameter of the brachial and radial arteries as well as increased cardiac output[67] but decreased systemic vascular resistance[63].

Effect of Bra-Rad-SBP_{Amp} on estimated central BP

Estimated central SBP was significantly higher in both groups after accounting for Bra-Rad-SBP_{Amp} and calibrating radial pressure waveforms with radial SBP. We have previously shown this in healthy individuals[279] and is not unexpected given the higher radial SBP calibration point. Interestingly, a recent meta-analysis[173] showed that central SBP estimated by radial tonometry calibrated with brachial BP, underestimated invasive central SBP by -8.2±11.6 mmHg. Our results are similar to that paper, whereby calibration of radial tonometry with brachial SBP resulted in the underestimation of central SBP (compared to central SBP estimated via radial SBP calibration of radial tonometry) in patients with T2DM (9±6 mmHg) and non-diabetic controls (12±6 mmHg). Due to technical difficulties in accurately measuring radial BP, previous studies have attempted to at least partially account for Bra-Rad-SBP_{Amp} by calibrating radial waveforms with brachial mean arterial pressure and diastolic BP, which seems to be a reasonable alternative because both mean and diastolic BP are thought to be relatively constant throughout the arterial tree compared with SBP[43, 178, 283]. However, if mean arterial pressure is calculated from either 1) brachial tonometry and integration of the brachial waveform[178, 283] or 2) using 1/3 or 40% form factor equations[291], these calibration methods can be subject to error due to dependence on brachial SBP. Indeed, recent evidence suggests that calibration of radial waveforms with oscillometric mean arterial pressure (which is less dependent on brachial SBP) and diastolic BP may substantially improve precision of

waveform calibration and subsequent central SBP estimation[75].

Cut-off values denoting high central SBP have recently been proposed[42]. Accordingly, we stratified participants based on these values (<130 or ≥130 mmHg)[42] to determine the possible clinical implications of central SBP underestimation by neglecting to account for Bra-Rad-SBP_{Amp}. Importantly, there was a significant increase in the number of participants categorised as having increased risk associated with 'central hypertension' (central SBP≥130 mmHg) when radial (as opposed to brachial) SBP was used to calibrate radial pressure waveforms (e.g. from 15% to 60% in T2DM and from 5% to 30% in controls; p=0.004). In clinical decision making, central BP values may be more beneficial than traditional cuff based estimates for excluding a diagnosis of hypertension[292] and our findings suggest that consideration of Bra-Rad-SBP_{Amp} could help to refine management decisions through more accurate diagnosis of central hypertension.

Limitations

Despite our Doppler methodology to assess SBP being valid in comparison with brachial SBP auscultation, we were unable to compare radial SBP because accurate auscultation was not possible at the radial artery. Having said this, our results are similar to previous invasive studies of Bra-Rad-SBP_{Amp}[280, 289]. Nevertheless, simultaneous measurement of invasive brachial SBP and radial SBP would have been an optimal study design. However, this approach would not have been possible in healthy participants. Additionally, anatomical differences between the brachial and radial arteries may have differentially affected the pressure required to compress the radial artery compared with the brachial artery. However, as the radial cuff was placed approximately seven centimetres from the proximal end of the anatomical snuff box (to allow for the placement of the ultrasound transducer), the cuff was inflated over the muscular area comprising the brachioradialis and flexor carpi radialis muscles, which could have compressed the radial artery similar to that of the biceps brachii compressing onto the brachial artery in the upper arm during cuff inflation. Furthermore, there is no reason to suspect differences between diabetics and non-diabetics in the pressure required to compress the radial artery, but in any case the above speculation can only be confirmed with invasive measures. Finally, we cannot rule out the effect of a reactive rise in either brachial or radial SBP from the measurement process itself[293] in our data. However, we consider this an unlikely given the consistency of our findings with invasive studies of Bra-Rad-SBP_{Amp}[280, 289].

7.6 Conclusions

In summary, Bra-Rad-SBP_{Amp} is significantly reduced in patients with T2DM compared to healthy age and sex matched non-diabetic controls. In patients with T2DM, vascular irregularities (in particular the product of brachial mean flow velocity and diameter) influence the magnitude of Bra-Rad-SBP_{Amp}. Furthermore, central BP is significantly underestimated when determined non-invasively by radial applanation tonometry calibrated with brachial, rather than radial SBP. These findings are of clinical importance if central BP is going to be used to guide hypertension management.

7.7 Contribution of Chapter 7 to thesis aims

The findings from *Chapter 7* further highlight that patients with T2DM elicit central haemodynamic abnormalities compared to their non-diabetic counterparts. For the first time, this study has shown that there is significant amplification in SBP from the brachial to radial artery in patients with T2DM and that due to Bra-Rad-$SBP_{Amp,}$ central SBP is systematically underestimated using radial applanation tonometry. Given the potential value of central BP beyond measures of brachial BP to identify individuals at increased BP risk, these findings (in combination with those presented in *Part II* of *Chapter 2*) have relevance to how central BP is measured in future. However, further work is required to refine the methods that estimate central BP (and haemodynamics) non-invasively so that there is little influence of Bra-Rad-SBP_{Amp}, prior to central BP being established as a clinically useful tool.

Chapter 8. Conclusions and future directions

Through a number of original studies, this research program has confirmed that patients with type 2 diabetes mellitus (T2DM) have abnormal central blood pressure (BP) and related haemodynamics (*Chapters 2, 3* and *7*) and that these patients are more likely to suffer damage to target organs compared to their non-diabetic counterparts (*Chapters 4, 5 and 6*). For the first time, this thesis has shown that there is substantial variation in the level of central to brachial systolic BP amplification in patients with T2DM (*Chapter 2*); that patients with T2DM have abnormal central haemodynamics in response to light to moderate intensity exercise (similar to the intensity of normal daily activity) and finally; that central haemodynamics (measured both at rest and during exercise) provide pathological insights relating to target organ damage, above and beyond conventional measures of clinic BP taken from the brachial artery (brachial BP) (*Chapters 5 and 6*). Taken together, this research thesis provides novel information and represents a significant advancement in understanding the haemodynamic differences between individuals with and without T2DM, the physiology and clinical relevance of exercise central haemodynamics and their relation to target organ damage.

For the first time, the systematic review and meta-analysis presented in *Part II* of *Chapter 2* shows that despite patients with T2DM having elevated central and brachial systolic BP and other central BP indices, there is no difference in the level of central to brachial systolic BP amplification compared to non-diabetic individuals. However, large variation in amplification was observed (in both individuals with and without T2DM) and thus, this data suggests that the true risk related to BP (i.e. the chronic loading on the heart and central organs) may be inadequately assessed via a measure of brachial BP. These findings have relevance for the management of BP in patients with T2DM (as well as non-diabetic individuals) and the design of future clinical trials. However, due to the complexity of methodological errors inherent in a non-invasive central BP measurement, the level of amplification between patients with T2DM and non-diabetic individuals needs to be confirmed invasively. Future case-control studies that measure the magnitude of central (aortic) to brachial systolic BP amplification via invasive catheterisation in patients with T2DM compared to non-diabetic individuals are required. More broadly, if central BP is going to be measured in clinical practice, further large-scale prospective studies that include measures of hard endpoints (such as cardiovascular events and/or mortality) are required to determine cut-off values of central systolic BP that denote increased cardiovascular risk in patients with T2DM.

The study presented in *Chapter 3* was the first to specifically determine the relationship between arterial stiffness and augmentation index (AIx) in individuals with and without T2DM. This is an important comparison as AIx has been suggested to be a surrogate marker of arterial stiffness and increased cardiovascular risk. The findings from this study clarify that AIx is not related to, and should not be used as a surrogate marker of arterial stiffness in patients with T2DM in future. Following on from this work, a longitudinal study that examines whether the changes in arterial stiffness are related to the changes in AIx over time may be useful to definitively determine the relationship between AIx and arterial stiffness in patients with T2DM. However, it remains unknown what factors are contributing to AIx in patients with T2DM compared to non-diabetic individuals. Thus, given that AIx is an independent predictor of increased cardiovascular risk, further large-scale studies that involve a comprehensive cardiovascular assessment (including measures of both left ventricular and vascular function) are required to determine the exact underlying pathophysiology of AIx in this population. Furthermore, clinical trials that examine whether interventions such as exercise are beneficial in reducing AIx (and thus cardiovascular risk) in patients with T2DM are warranted.

Chapter 4 makes an important contribution to understanding why patients with T2DM have abnormal brain structure compared to non-diabetic individuals. Although measures of central haemodynamics were not available, the findings show that abdominal obesity was associated with grey matter atrophy, independently of resting brachial BP and other cardiovascular risk factors. Thus, future interventions that target abdominal obesity may prove to be advantageous in preserving the integrity of brain structure in patients with T2DM. Further to this, the exact mechanistic pathway linking abdominal obesity and grey matter atrophy in patients with T2DM remains to be elucidated and, therefore, further studies should aim to investigate other mechanisms that may explain this association including neuroinflammation and insulin signaling pathways (which have been suggested as possible causative factors) as well as the role of exercise central haemodynamics.

Despite physical activity being previously shown to be beneficial for maintaining brain structure in non-diabetic populations, this was not the case in the study presented in *Chapter 4*, possibly due to the relatively low intensity of physical activity adopted (mean step count), compared to that in previous studies. Additional work is required to determine the beneficial effect of a more vigorous exercise regime on maintaining brain structure in patients with T2DM. Indeed, following on from this study, a pilot randomised control trial is currently underway (Cognition and

Diabetes in Older Tasmanians– a randomised control trial of exercise [CDOT-X]) that aims to determine the effects of regular aerobic exercise on brain structure in patients with T2DM. In this trial, 50 patients with T2DM have been randomised to either undergo 6-months of aerobic exercise training (experimental group) or flexibility training (control group) in order to determine whether regular aerobic exercise is beneficial in preserving brain volumes in this population. Furthermore, central haemodynamics are being measured in response to exercise (during an exercise stress test) and also following the intervention. This will enable the beneficial effects of exercise on improving vascular function, and the subsequent role in maintaining brain structure in patients with T2DM to be examined. The findings from this intervention study may help to guide exercise recommendations in patients with T2DM in future.

Chapter 5 constitutes the first investigation of central haemodynamics measured in response to light to moderate intensity exercise in patients with T2DM. This study showed that exercise central haemodynamics are abnormal in patients with T2DM compared to non-diabetic controls and although these variables were not related to brain structure, they may explain why patients with T2DM have accelerated decline in other organ systems such as the kidneys (as shown in *Chapter 6*) and also the eyes. Although it appears that abnormalities in pressure and/or flow pulsatility may be a likely factor linking central haemodynamics and target organ damage in patients with T2DM, the exact underlying mechanism remains to be elucidated. Mechanistic studies that examine whether the pulsatility in the large vessels is indeed reflected in the microcirculation using methods such as Laser Doppler Flow techniques are warranted. Furthermore, this study (and also the study presented in *Chapter* 6) was limited by the cross sectional design. Therefore, further longitudinal studies are required that include comprehensive measures of haemodynamic function and aim to examine the changes in central haemodynamics in patients with T2DM and their relation with target organs over time. To this end, a longitudinal study was commenced in 2014 and aims to determine the association between the 3-year change in central haemodynamics (measured at rest and in response to exercise) and target organ damage in the same study population of that in *Chapters 5* and *6*. The results from this study will likely allow for more causative conclusions to be drawn and will help to define the clinical relevance of resting and exercising central haemodynamics in patients with T2DM.

The study presented in *Chapter 5* was also the first to examine the physiological and clinical relevance of the aortic reservoir characteristics in patients with T2DM and identified excess pressure as a novel cardiovascular risk marker associated with grey matter atrophy (in non-

diabetic controls). This is important as excess pressure was related to brain structural abnormalities independently of brachial BP and the current "gold standard" of BP measures, 24-hour ambulatory BP, suggesting that excess pressure may be a useful clinical marker for determining risk related to BP in future. The development of efficiently and reliably non-invasive methods to measure aortic reservoir and excess pressure may facilitate a large-scale prospective study to definitely determine the clinical significance of abnormal reservoir and excess pressures, and may enable more widespread use of aortic reservoir characteristics in clinical practice.

The results presented in *Chapter 6* show that light to moderate intensity exercise can induce albuminuria in patients with T2DM. This may suggest that the chronic stress brought on by normal daily activity (i.e. light to moderate intensity exercise) may contribute to accelerated renal damage in patients with T2DM. Alternatively, or in conjunction, exercise may be beneficial for unmasking renal abnormalities in high- risk populations and for identifying individuals at risk of exercise-induced albuminuria, and may represent a useful tool to reveal cardiovascular abnormalities in future that are not otherwise evident at rest. This novel finding has relevance for how albuminuria is measured in clinical practice as currently guidelines suggest that strenuous exercise should be avoided prior to a measurement of albuminuria. However, these results suggest that the contribution of light to moderate intensity exercise should also be considered. Further large-scale prospective studies that determine the association between exercise-induced albuminuria (beyond a resting measure) and cardiovascular outcomes would be useful in deciphering whether exercise should be avoided or encouraged prior to a measurement of albuminuria in high-risk individuals. Furthermore, given that exercise is beneficial for maintaining vascular function, further randomised control trials that examine the benefit of an exercise intervention on improving vascular function and reducing the risk associated with exercise-induced albuminuria are warranted.

The study presented in *Chapter 6* also showed that exercise central haemodynamics, in particular excess pressure, were related to exercise-induced albuminuria in patients with T2DM, independently of resting brachial BP. This may suggest that firstly; excess pressure may be a useful clinical marker to identify individuals at elevated cardiovascular risk in future and secondly; that haemodynamics measured in response to stress induced by light to moderate intensity exercise may provide pathological insights above and beyond corresponding resting measures. Pertaining to this, methods that measure central BP and haemodynamics whilst ambulatory (such as 24- hour ambulatory central BP devices, which are becoming increasingly

commercially available) need to be refined and validated. This will give way for the association between ambulatory central haemodynamics (beyond measures of resting brachial BP) and target organ damage to be defined. Further, a prospective study that examines the association between exercise central haemodynamics and measures of hard endpoints in patients with T2DM may enable the true clinical relevance of ambulatory central haemodynamics to be determined. Once achieved, this may lead to the measurement of ambulatory (i.e. light to moderate intensity exercise) central haemodynamics being incorporated into routine clinical practice.

The results from *Chapter 7* show that central systolic BP is underestimated using radial applanation tonometry, when the radial pressure waveforms are calibrated with brachial BP. Underestimation of central BP using this method only became apparent through the studies presented in *Chapter 7* and *Appendix I* and is an issue that is inherent in any non-invasive measure of BP, whether it be at the brachial artery or estimated central BP. Although this study was confined to a relatively small sample group and further verification is required in larger study populations, these findings, (as well as those presented in *Appendix I* and *II*) highlight the necessity for refinement of methods that estimate central BP non-invasively, so that there is minimal dependence on brachial to radial systolic BP amplification. This is crucial if central BP is going to be implemented into routine clinical practice as currently such issues surrounding the non-invasive measurement of central BP limit its effectiveness as a tool to identify individuals at elevated risk related to BP. At the commencement of the body of research contained in this thesis, radial applanation tonometry was the gold standard method for determining central haemodynamics non-invasively, and it only became apparent early in the research that there may be issues surrounding the amplification in systolic BP when estimating central BP using this method. Thus, the studies presented in Chapter 7 and Appendix I were conducted concurrently with the studies presented in Chapters 3, 5 and 6, to further investigate this issue. Following on from this work, the findings presented in *Chapter 7* and *Appendix I* are currently being used to inform an international task force which aims to determine the most appropriate method to validate devices that measure central BP non-invasively. However, further large-scale studies are still required to determine the most robust calibration method, which may be device specific, in a range of study populations by comparison with the true (invasive) central BP. This would represent a significant advancement for measuring central BP non-invasively and aid in paving the way for central BP as a clinically useful tool in future.

Appendix I. Additional publication – Brachial to radial systolic blood pressure amplification: implications of age and estimated central blood pressure from radial tonometry

Appendix I represents an additional manuscript that was published during the candidature. Whilst this study does not form part of the primary thesis it is closely related to the aim of this thesis and provided a lot of the background information for the study presented in *Chapter 7*.

Appendix I has previously been published;

Climie RED, Picone DS, Ahuja KD, Keske MA, Sharman JE. Brachial-to-radial systolic blood pressure amplification: implications of age and estimated central blood pressure from radial tonometry. *Journal of Hypertension*, April 2015; 33.9:1876-1833.

AI.1 Abstract

Background. The reference standard for non-invasive estimation of central blood pressure (BP) is radial tonometry calibrated using brachial systolic and diastolic BP (SBP, DBP). Brachial to radial SBP amplification (B-R-SBPAmp) may introduce error into central BP estimation, but the magnitude of such amplification is uncertain. This study aimed to determine 1) the magnitude, and effect of aging on B-R- SBPAmp; 2) the effect of B-R-SBPAmp on radial tonometry-estimated central SBP, and 3) correlates of B-R-SBPAmp.

Methods. Forty young (28±5 years) and 20 older (60±8 years) healthy participants underwent brachial and radial artery ultrasound to identify SBP from the first Doppler flow inflection during BP cuff deflation (first Korotkoff sound). Impedance cardiography, ultrasound, tonometry and anthropometric data were collected to explore B-R-SBPAmp correlates.

Results. Radial SBP was significantly higher than brachial SBP in younger (118 ± 12 mmHg versus 110 ± 10 mmHg; $p<0.001$) and older (135 ± 12 mmHg versus 121±11 mmHg; $p<0.001$) participants. The magnitude of B-R-SBPAmp (radial minus brachial SBP) was higher in older, compared to younger participants (14 ± 7 mmHg versus 8 ± 7 mmHg; $p=0.002$), independent of sex and heart rate. Estimated central SBP was higher in both age groups when radial waveforms were recalibrated using radial (versus brachial) SBP ($p<0.001$). The central SBP change relative to B-R-SBPAmp was associated with augmentation index ($r=0.739$, $p<0.001$), independent of age, sex and heart rate. Age, male sex and high-density lipoprotein each positively related to B-R-SBPAmp in multiple regression analysis ($p<0.05$).

Conclusions. Major B-R-SBPAmp occurs in healthy people and is higher with increasing age. Furthermore, B-R-SBPAmp contributes to underestimation of radial tonometry-derived central SBP.

AI.2 Introduction

Central blood pressure (BP) indices predict cardiovascular disease and mortality independent of brachial BP[33]. Although methods to assess central BP are not currently used in routine clinical practice (due to several reasons including the need for specialist equipment, technical expertise and costs), accumulating evidence suggests that central BP estimation could aid in the assessment of risk related to hypertension[294, 295]. Recently, reference values for central BP have been proposed[42, 296]. The current reference standard for non-invasive central BP estimation is radial applanation tonometry whereby a central (ascending aortic) waveform (and BP) is estimated by applying a generalised transfer function to the radial pressure waveform[10, 11, 297]. The radial pressure waveform is usually calibrated with brachial systolic BP (SBP) and diastolic BP (DBP) on the assumption of negligible difference in these BP values from the brachial to radial artery[278]. It is generally accepted that SBP is amplified from the aorta to the brachial artery, but mean arterial pressure (MAP) and DBP vary little (from approximately 1 to 3 mmHg)[74] between these sites. The magnitude of aorta to brachial SBP amplification decreases with increasing age and vascular disease[2, 298]. However, there is dispute as to the level of SBP amplification that may occur from the brachial to radial arteries (B-R-SBPAmp)[299]. The presence of significant B-R-SBPAmp may compromise the accuracy of radial pressure waveform calibration and consequently estimated central SBP, with a tendency towards underestimation[173, 178].

Invasive catheterisation studies support the possibility of significant B-R-SBPAmp, even to levels $\geq$20 mmHg [280-282, 289, 300]. Two non-invasive studies of B-R-SBPAmp (assessed using oscillometric BP and applanation tonometry) have shown SBP to be greater in the radial artery compared to the brachial artery[178, 283]. Indeed, in apparently healthy cohorts an average (±SD) B-R-SBPAmp of 6 ± 5 mmHg (n=44)[178] and 7 mmHg (variance not provided) (n=1873)[283] has been reported. Additionally, it has been observed[301] in one study[302] that the derived aorta-radial transfer function was of a higher modulus than the derived aorta-brachial transfer function, indicating the presence of B-R-SBPAmp. However, a limitation of these studies was they were performed in either: 1) a small participant age range[281, 282]; 2) participants with significant cardiovascular comorbidities who were undergoing cardiac catheterisation[280, 289, 300] or; 3) used non-invasive methods to calculate B-R-SBPAmp[178, 283]. A summary of studies relating to the level of B-R-SBPAmp is presented in supplementary table 1. To our knowledge, no study has directly measured B-R-SBPAmp in healthy people of a wide age range. The aims of this study were to determine the magnitude of B-R-SBPAmp and the effect of aging on B-R-SBPAmp in healthy participants. Further we sought to determine the effect of B-R-SBPAmp on estimated central SBP using radial tonometry, as well as

to explore hemodynamic, arterial and anthropometric correlates of B-R-SBPAmp. Non-invasive Doppler ultrasound was used to directly measure brachial and radial SBP.

AI.3 Methods

Study participants

Forty healthy younger (19-40 years, 50% male) and 20 healthy older participants (49-75 years, 50% male) were consecutively recruited from the community via advertisements. Exclusion criteria included: a clinical history of cardiovascular disease, type 2 diabetes mellitus, high BP (defined as clinic BP ≥140/90 mmHg or self-reported diagnosis of hypertension by a physician, or use of antihypertensive medications), current smoking or pregnancy. Each participant provided informed written consent and the study was approved by the University of Tasmania Human Research Ethics Committee.

Study protocol overview

Each participant attended the research clinic on one occasion and the study was performed in a temperature-controlled room (24 ± 1°C). Participants were asked to refrain from vigorous exercise in the previous 24 hours, alcohol consumption on the day of the study, and to fast (including refraining from caffeine consumption) for three hours prior to the study. Participants were in a semi-recumbent position for the study, with the right arm outstretched and supported by a pillow on a bench top that was adjusted so that the heart, brachial and radial arteries were at the same level. After 10 minutes rest, ultrasound images of the structural and functional characteristics of the brachial and radial arteries were recorded. Following this, brachial and radial SBP measurements were recorded (for calculation of B-R- SBPAmp) in random order using Doppler ultrasound. Following this applanation tonometry was performed in duplicate to determine both central BP (from radial waveforms) and brachial pulse wave velocity (from carotid and radial waveforms). Central (ascending aortic) BP was determined by applying a validated generalised transfer function to the radial waveform (SphygmoCor 8.1, AtCor Medical Pty Ltd, Sydney, Australia)[11]. Augmentation index was calculated as the pressure augmentation above the systolic shoulder expressed as a percentage of pulse pressure (PP). MAP was calculated in three ways: using the SphygmoCor software via integration of the radial waveform calibrated with (a) brachial SBP and DBP or (b) radial SBP and brachial DBP or (c) calculated from brachial DBP + 0.4 x PP as proposed by Bos et al[303].

B-R-SBPAmp

Brachial and radial SBP were measured using Doppler ultrasound (Philips iU22, linear array 12-5 MHz transducer, Philips Healthcare, Bothell, WA, USA) with simultaneous sphygmomanometry via a validated mercury-free device (UM-101, A&D Medical, Tokyo, Japan)[287] and appropriately sized cuffs for both sites. To enable ultrasound transducer placement, the brachial and radial cuffs were placed approximately seven centimetres (the length of the ultrasound transducer head) proximal to the centre of the antecubital fossa and the proximal end of the anatomical snuff box respectively.

Six brachial and radial SBP measurements (three at each region) were taken in a random order by a BP operator who was blinded to the measurement site. Blinding was achieved by using a partition screen to block the BP operator's view of the participants arm and also to prevent sight of the arterial image on the ultrasound screen, whilst still allowing view of the Doppler flow signal and sphygmomanometer to record SBP. At each site, SBP was defined as the first inflection of the Doppler flow signal (during cuff deflation at approximately 2 mmHg/s), representing the first Korotkoff sound as previously described[304]. B-R-SBPAmp was calculated as radial minus brachial SBP.

The validity of this method was tested by comparing the brachial SBP determined only by Doppler ultrasound with brachial SBP determined only by auscultation in all 60 participants. SBP was acquired simultaneously by separate operators, each blinded to the others SBP reading. There was strong concordance between measures (intraclass correlation [ICC] r = 0.964, p<0.001; mean difference = 0.38 ± 3.15 mmHg; p=0.35). The reproducibility of brachial and radial SBP measures was tested in 10 participants who underwent an additional assessment with 5 ± 2 days between visits. Between-visit ICC were r=0.944 and r=0.937 (p<0.001 both) for brachial SBP and radial SBP respectively and the mean differences between visits were 1 ± 5 mmHg, p=0.45 and -1 ± 6 mmHg, p=0.72 for brachial and radial SBP respectively. The between-visit ICC and mean difference for B-R-SBPAmp were r=0.687; p=0.050 and -2 ± 6 mmHg, p=0.31.

Effect of B-R-SBPAmp on estimated central SBP

To quantify the effect of B-R-SBPAmp on central SBP estimation, radial waveforms were calibrated using brachial SBP and DBP and also using radial SBP and brachial DBP (radial DBP not measurable). Waveforms were also calibrated by MAP (brachial DBP + 0.4 x PP) and DBP. Equation 1 (a modified version of that used by Papaioannou et al[305]) was used to determine the

change in estimated central SBP (due to radial waveform calibration using radial SBP versus brachial SBP) expressed as a percentage of B-R-SBPAmp (Δ).

Equation 1:

$$\Delta = \frac{(central\ SBP\ derived\ from\ radial\ SBP\ calibration - central\ SBP\ derived\ from\ brachial\ SBP\ calibration)}{B-R-SBPAmp} \times 100$$

Haemodynamic, arterial and anthropometric correlates of B-R-SBPAmp

Impedance cardiography

A validated[185] non-invasive cardiothoracic bioimpedance device (PhysioFlow, PF-05, Manatec Biomedical, Paris, France) with good reproducibility[186] was used to assess haemodynamic parameters including heart rate, cardiac output, stroke volume and contractility index. Heart rate recorded during the same time period as SBP measurements was used in the analysis.

Ultrasound imaging and analysis

All ultrasound images were recorded using the Philips machine previously mentioned. Brachial and radial artery diameters were analysed offline using QLAB software (Philips Healthcare, Bothell, WA, USA). The average of three diameter measurements at end diastole were used for the analysis. Brachial and radial artery mean blood flow velocities were determined by Doppler ultrasound using a three lead electrocardiogram and were averaged over 10 cardiac cycles.

Anthropometric variables

Standard anthropometry was measured including height, weight, waist and hip circumference. In addition, brachial and radial arm circumferences were recorded from the distal end of each cuff. The distance between imaging sites was measured between the distal ends of brachial to radial cuffs.

Blood biochemistry

A venous blood sample was drawn from the antecubital fossa and analytical biochemistry was performed to derive glucose and lipid values using standard accredited laboratory techniques.

Statistical analysis

Data were analysed using SPSS for windows software version 20.0 (IBM SPSS Statistics, NY, USA). Data were visually inspected for normality of distribution and brachial and radial blood velocity data were logarithmically transformed to give a normal distribution. Differences between and within groups were assessed using two- tailed independent and paired t-tests respectively, as well as analysis of covariance (ANCOVA) adjusting for sex and heart rate. Associations between variables were determined using Pearson correlations and linear multiple regression analysis was performed to determine predictors of B-R-SBPAmp, adjusting for factors with known or suspected association with B-R-SBPAmp (as detailed in Results). Part correlation coefficients were used to assess the contribution of each independent variable to the overall variance in B-R-SBPAmp. Multicollinearity was assessed using variance inflation factors and Q-Q plots were used to determine normal distribution of the model. Analyses were not adjusted for MAP or SBP based on statistical singularity and multicollinearity potentially leading to unstable regression models. In the models examining independent predictors of B-R-SBPAmp, it was deemed inappropriate to adjust for brachial or radial SBP because both variables are included in the calculation of B-R-SBPAmp. Data are presented as mean ± standard deviation unless otherwise specified and $p<0.05$ was considered statistically significant.

AI.4 Results

Patient characteristics

Table AI.1 summarises the clinical characteristics of the study population. Body mass index, arm circumference (at brachial and radial sites), blood glucose, total cholesterol and low-density lipoprotein were all significantly higher in the older participants compared to younger participants ($p<0.05$ for all).

Table AI.1. Participant characteristics.

	Younger (n=40)	Older (n=20)	P value
Male, n (%)	20 (50)	10 (50)	1.00
Age (years)	28±5	60±8	< 0.001
Body mass index (kg/m^2)	23.7±3.1	25.6±3.3	0.038
Brachial arm circumference (cm)	25.0±2.1	26.7±2.0	0.004
Radial arm circumference (cm)	16.2±1.0	17.3±1.5	0.006
Distance between brachial and radial imaging sites (cm)	26.6±3.2	26.8±3.1	0.79
Glucose (mmol/L)	5.3±0.9	5.7±0.6	0.042
Triglycerides (mmol/L)	1.2±0.7	1.5±0.8	0.15
Total cholesterol (mmol/L)	4.8±0.8	5.8±1.0	0.001
Low-density lipoprotein (mmol/L)	2.6±0.8	3.5±0.9	0.001
High-density lipoprotein (mmol/L)	1.7±0.5	1.6±0.5	0.68

Data expressed as mean ± standard deviation or n (%). P value is for between group differences.

B-R-SBPAmp and effect of aging

Radial SBP was significantly higher than brachial SBP in the younger and older groups ($p<0.001$ for both groups). Radial and brachial SBP were significantly higher in older compared with younger participants (table AI.2). B-R-SBPAmp was significantly higher in the older group and remained significant after adjustment for sex and heart rate. The range of B-R-SBPAmp was -5 to 20 mmHg and 3 to 27 mmHg in the younger and older age groups, respectively.

Table AI.2. Blood pressure variables measured in both younger and older participants.

	Younger (n=40)	Older (n=20)	P value
Radial systolic BP (mmHg)*	118±12	135±12	<0.001
Brachial systolic BP (mmHg)	110±10	121±11	0.001
Brachial diastolic BP (mmHg)	67±6	72±7	0.012
B-R-SBPAmp (mmHg)**	8±7	14±7	0.004
MAP (a) (mmHg)	80±8	90±8	<0.001
MAP (b) (mmHg)	82±8	95±8	<0.001
MAP (c) (mmHg)	84±7	92±8	0.002
Brachial pulse pressure (mmHg)	43±9	49±8	0.011
Radial pulse pressure (mmHg)	51±11	63±12	<0.001

Data is mean ± standard deviation. P value represents between group analyses. BP, blood pressure; B-R-SBPAmp, brachial-to-radial systolic blood pressure amplification; MAP, mean arterial pressure. *P value of the difference between radial and brachial SBP was <0.001 for both groups. **Between group differences in B-R- SBPAmp remained significant after adjustment for sex and heart rate (p=0.002). MAP was calculated using three methods: SphygmoCor software via integration of the radial pressure waveform calibrated with either (a) brachial SBP and diastolic BP (DBP) or (b) radial SBP and brachial DBP or (c) calculated from brachial DBP + 0.4 x pulse pressure.

Effect of B-R-SBPAmp on estimated central SBP

Estimated central SBP was significantly increased when radial waveforms were calibrated with radial SBP and brachial DBP compared with brachial SBP and brachial DBP in both younger (100 ± 10 versus 95 ± 9) and older participants (124 ± 11 mmHg versus 112 ± 11 mmHg; p<0.001 for both groups). Central SBP calibrated with radial SBP was significantly greater than central SBP calibrated with brachial SBP, and this difference was significantly greater in older compared to younger participants (12 ± 6 mmHg versus 5 ± 4 mmHg, p<0.001). The difference remained significant after adjustment for sex and heart rate (difference between younger and older β = 6.23, 95% confidence interval [95%CI] 3.55 to 8.90, p<0.001). Central SBP calibrated with MAP (brachial DBP + 0.4 x PP) was also significantly higher than central SBP calibrated with brachial SBP and DBP, however, the difference was significantly lower in older compared to younger participants (5 ± 5 mmHg versus 11 ± 8 mmHg, p=0.001). The difference remained significant after adjusting for sex and heart rate (difference between younger and older β= -5.73, 95%CI -9.26 to -2.20, p=0.002). Figure AI.1 depicts the relationship between age and upper limb

SBP amplification (either central-to-brachial or central-to-radial). In each example radial tonometry waveforms were calibrated with either brachial or radial SBP and brachial DBP. As per expectation, with increasing age there was a decrease in central-to- brachial SBP amplification when radial waveforms were calibrated with brachial or radial SBP (figure AI.1 A, B). When using brachial SBP for calibration, there was no significant relationship between age and central-to-radial SBP amplification (figure AI.1 C). However, when radial waveforms were calibrated with radial SBP there was a decrease in central-to-radial SBP amplification with increasing age (18 ± 6 mmHg versus 12 ± 3 mmHg, $p<0.001$, younger versus older participants; figure AI.1 D). Augmentation index significantly correlated with the change in central SBP (due to radial SBP versus brachial SBP radial waveform calibration) relative to the magnitude of B-R-SBPAmp (figure AI.2) and remained significant after adjustment for age, sex and heart rate ($\beta=0.445$, 95%CI 0.25 to 0.65, $p<0.001$).

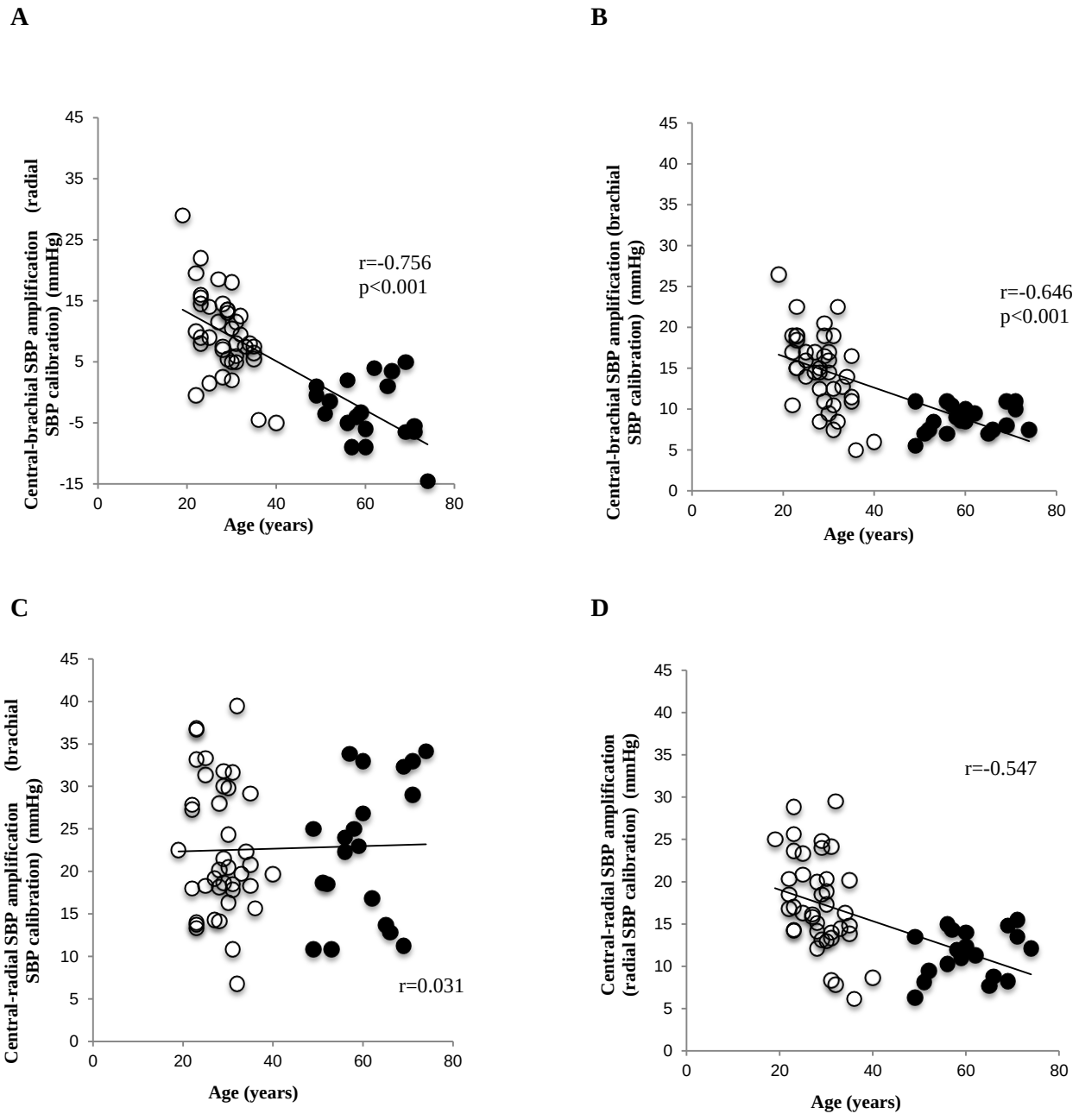


Figure AI.1. Associations between central to peripheral (brachial and radial) systolic blood pressure (SBP) amplification and age, and the effect of different methods to calibrate radial tonometry waveforms. A, B; central-to-brachial SBP amplification was significantly and negatively associated with age when using brachial or radial SBP for calibration. C; central-to-radial SBP amplification was not associated with age when using brachial SBP for calibration. D; central-to-radial SBP amplification was significantly and negatively associated with age when radial tonometry was calibrated with radial SBP.

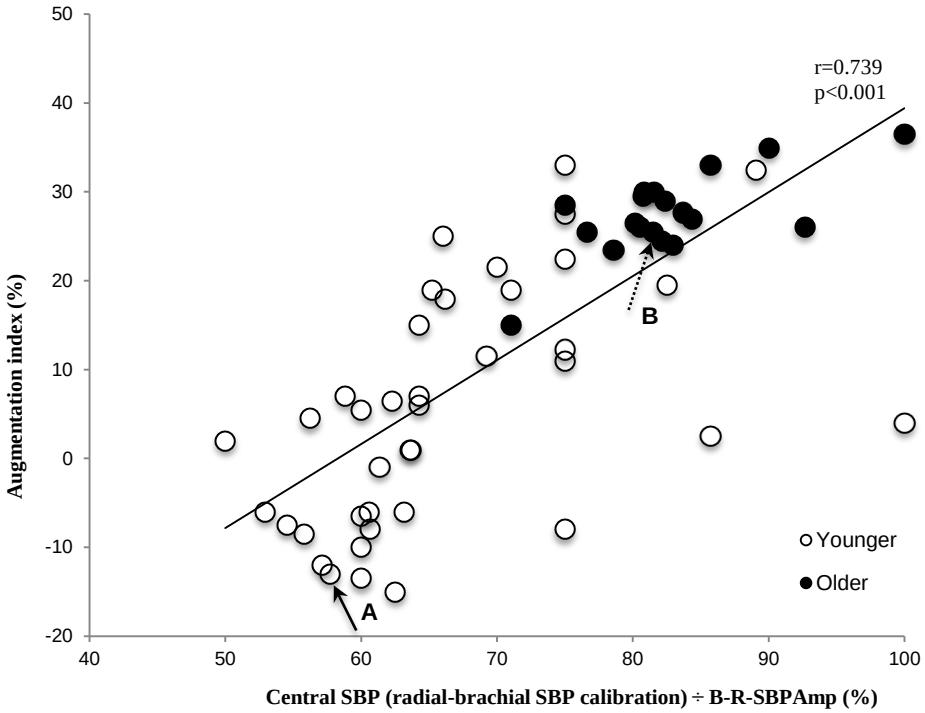


Figure AI.2. Association between augmentation index and the change in central systolic blood pressure (SBP) relative to the magnitude of brachial to radial SBP amplification (B-R-SBPAmp). The solid line represents the trend for the entire cohort. The solid and dashed arrows refer to two participants with 14 mmHg B-R-SBPAmp. Participant A had -12% augmentation index and their central SBP increased by 57% of B-R-SBPAmp when radial SBP was used for radial waveform calibration. Participant B had a higher augmentation index (24%) and their central SBP increased by 86% of B-R-SBPAmp

Haemodynamic and arterial variables

A comparison of the haemodynamic and arterial variables between the younger and older participants is shown in table AI.3. Systemic vascular resistance, augmentation index, mean radial blood flow velocity and the quotient of radial peak blood flow velocity and radial diameter were significantly higher in older participants. Contractility index was significantly higher in younger participants and there was a trend towards higher stroke volume and brachial blood flow velocity compared with the older participants. There was no significant difference between the groups in any of the other haemodynamic or arterial variables ($p>0.05$).

Table AI.3. Comparison of haemodynamic and arterial variables between younger and older participants.

	Younger (n=40)	Older (n=20)	P value
Heart rate (beats/min)	61±11	60±10	0.86
Cardiac output (L/min)	4.74±0.99	4.39±0.73	0.13
Stroke volume (mL)	79±14	74±13	0.25
Contractility index (AU)	173±62	85±40	<0.001
Systemic vascular resistance ($d/s/cm^{-5}m^2$)	1457±304	1676±269	0.008
Central augmentation index (%)	5±13	27±5	<0.001
Mean brachial blood flow velocity (cm/s)	0.63±0.23	0.76±0.25	0.07
Mean radial blood flow velocity (cm/s)	0.39±0.38	0.61±0.38	0.044
Brachial diameter (cm)	3.46±0.73	3.59±0.60	0.47
Radial diameter (cm)	1.67±0.33	1.81±0.40	0.19
Radial peak blood flow velocity / radial diameter (s^{-1})	-0.54±0.37	-0.34±0.36	0.046

Data expressed as mean ± standard deviation. P value represents between group analyses. AU, arbitrary units. Logarithmically transformed mean brachial and radial blood flow velocity data is presented.

Correlates of B-R-SBPAmp

In all 60 participants, age was significantly correlated with B-R-SBPAmp (r=0.449, p<0.001). Radial, but not brachial SBP was also significantly correlated with B-R- SBPAmp (r=0.585, p<0.001 versus r=0.087, p=0.51). Univariate correlations of B-R- SBPAmp with all variables listed in the methods were assessed, however, no significant associations were found (p>0.05 for all). To predict contributors to the variance in B-R-SBPAmp, a multivariable model was developed from variables with a univariate correlation of p<0.10 with B-R-SBPAmp. Radial SBP was not included in the multivariable analysis because it was used in the calculation of B-R-SBPAmp. Variables initially included in the model were glucose (r=0.22, p=0.09), high-density lipoprotein (HDL; r=0.24, p=0.06), contractility index (r=-0.25, p=0.06) and the quotient of radial peak flow velocity and radial artery diameter (r=0.28, p=0.028). Age, sex and heart rate were also included because of known or suspected association with B-R-SBPAmp. Variables were included in the final multivariable model if 1) they significantly predicted B-R-SBPAmp (p<0.05) or 2) upon removal of the variable there was a change in β coefficient >10%. The final model consisted of age, sex, heart rate and HDL. This model explained 32% of the variance in B-R-SBPAmp (table

AI.4).

Table AI.4. Independent predictors of brachial to radial systolic blood pressure (BP) amplification in the full study cohort (n=60).

	Unstandardised β coefficient (95% confidence intervals)	**P value**	**Part correlation coefficient**
Age (years)	0.21 (0.11, 0.31)	<0.001	0.46
Sex (0=female, 1=male)	4.36 (0.68, 8.04)	0.021	0.26
Heart rate (beats/min)	-0.11 (-0.27, 0.06)	0.194	-0.14
High density lipoprotein (mmol/L)	5.51 (1.89, 9.13)	0.004	0.33

Data are unstandardised β coefficient and 95% confidence intervals. Dependent variable is brachial to radial systolic blood pressure amplification. Adjusted R^2 = 0.32; p<0.001. Part correlation coefficient quantifies the unique contribution of each independent variable to the R^2 of the model.

AI.5 Discussion

This study used a direct, non-invasive method, for the first time to our knowledge, to measure brachial and radial SBP in healthy individuals. The main findings were: 1) radial SBP was significantly higher than brachial SBP, thus resulting in major B-R- SBPAmp; 2) the magnitude of B-R-SBPAmp was significantly greater in older compared with younger people; 3) owing to B-R-SBPAmp, the estimation of central SBP using radial tonometry and calibration with brachial SBP and DBP resulted in significant underestimation of central SBP and; 4) the magnitude of B-R-SBPAmp was not predicted from local or systemic haemodynamic, arterial or anthropometric characteristics.

SBP amplification from the aorta to the brachial artery is an established principle and while SBP amplification beyond the brachial artery is physiologically plausible[43], it has been argued that this is likely to be minimal[278]. In keeping with our findings, several studies have found major B-R-SBPAmp (e.g. ≥20 mmHg)[178, 280-283, 289, 300]. One invasive study[289] reported B-R-SBPAmp of 12.4 ± 8.2 mmHg (mean ± SD), whilst another[300] conducted in patients prior to cardiopulmonary bypass showed radial SBP was on average 7 mmHg higher than brachial SBP.

Estimated central SBP was significantly higher when accounting for B-R-SBPAmp with radial waveform calibration. Similarly, Verbeke et al.[178] showed that estimated central SBP increased by an average 4 mmHg when radial waveforms were calibrated with radial SBP compared with brachial SBP (B-R-SBPAmp = 6 mmHg average) and the ratio of B-R-SBPAmp:central SBP underestimation was comparative to that in our study (1.45 versus 1.42 respectively). A recent meta-analysis[173] reported -8.2 ± 11.6 mmHg disparity between estimated central SBP (determined by radial tonometry calibrated with non-invasive brachial SBP and DBP) and invasive catheter central SBP. Multiple factors may be contributing to this central SBP underestimation, including B-R-SBPAmp and underestimation of cuff brachial SBP[306, 307]. One method advocated to take into account B-R-SBPAmp is the calibration of radial waveforms using MAP calculated by brachial DBP + 0.4 x PP[32, 33]. When we used this method to calibrate radial waveforms (versus brachial SBP and DBP calibration), central SBP increased in both groups, but the increase was significantly higher among younger participants. On the other hand, when radial waveforms were calibrated with radial SBP and brachial DBP the estimated central SBP increased significantly more in older participants when compared to the brachial SBP and DBP calibration method. These disparate results may be due in part to the equation to derive MAP from brachial DBP + 0.4 x PP. This may not reflect the true MAP of the radial pressure waveform due to a relatively narrower and more peaked systolic phase compared to the brachial[308] artery, which differs between younger and older people[283].

Underestimation of cuff brachial SBP may also result in systematic underestimation of central SBP in the newer brachial cuff waveform devices that utilise oscillometric SBP and DBP as calibration points. However, this could be overcome by calibration with oscillometric MAP and DBP[75]. The value of this calibration method has been debated[309] due to potential for estimated central SBP to be higher than brachial SBP[75, 310], which would be non-physiological. However, this apparent reverse SBP amplification is likely due to the aforementioned underestimation of true brachial SBP[306, 307] combined with more accurate estimation of the true (higher) central SBP when using oscillometric MAP and DBP calibration of radial waveforms[51, 75]. Thus, the reverse SBP amplification is an artefact of recording methods rather than representing true underlying physiology. Importantly, this oscillometric MAP and DBP calibration method has been shown to improve the relationship of estimated central SBP with end organ damage (i.e. left-ventricular mass index)[310], suggesting this is a more clinically relevant method.

This study highlights the problem that different radial waveform calibration methods may cause

differences in the apparently true levels of central-to-brachial and -radial SBP amplification. Population data on central-to-brachial SBP amplification shows that this decreases with aging[296], although most of these data were non-invasive and central SBP was estimated on the assumption that brachial SBP was roughly equal to radial SBP. Our data supports two previous postulations: 1) the assumption of minimal B-R-SBPAmp contributes to underestimation of central SBP[178] and; 2) this assumption may inflate the true level of central-to-brachial SBP amplification[283]. Importantly, central-to-radial SBP amplification decreased with aging only when radial SBP was used to calibrate radial waveforms and the magnitude of amplification is comparable to several invasive catheter studies that simultaneously measured ascending aortic and radial SBP[74, 281, 282, 290, 311].

The mechanism underlying the change in central SBP by recalibration with radial SBP is related to waveform morphology as exemplified in figure 2. Despite individuals having similar B-R-SBPAmp, the magnitude of central SBP underestimation may be considerably different, with greater underestimation of central SBP associated with higher augmentation index. This is probably because augmentation index is not pressure dependent and, therefore, does not change when waveforms are recalibrated. However, if the SBP calibration value is increased there will be a relatively greater increase in central SBP (i.e. greater underestimation using brachial versus radial SBP calibration) when augmentation index is high. On the other hand, when augmentation is negative or close to zero, the change in the magnitude of estimated central SBP will be minimal as this will mainly be contingent on the first systolic peak rather than augmented pressure. This observation has clinical relevance because augmentation index increases with age (until approximately 50 years of age, at which point tends to plateau)[35] as well as disease processes related to arterial stiffening such as hypertension[43]. Therefore, greater underestimation of central SBP is likely to be more prevalent in these higher-risk patient populations, which may impact on treatment decisions if hypertension management is being guided by central BP values.

To assess the correlates of B-R-SBPAmp, a number of hemodynamic and anthropometric variables were measured. We found that radial SBP, not brachial SBP, was associated with B-R-SBPAmp, which suggests that B-R-SBPAmp cannot be predicted from traditional upper arm BP measurement, and that B-R-SBPAmp may be more dependent on vascular properties distal to the brachial artery. We expected that variables such as heart rate, flow resistance, flow input and arterial tapering may have correlated with B-R-SBPAmp[43], however, this was not the case. Increasing age, male sex and higher HDL values were all independent predictors of increased B-R-SBPAmp. The association between male sex and increased B-R-SBPAmp has been shown previously[178] and is consistent with

the case for central-to-brachial SBP amplification[2, 35]. The mechanisms of the association between B-R-SBPAmp and HDL are unclear, however, arterial compliance appears to be modified in familial hypercholesterolemia[91], and this may influence arterial pressure transmission.

Limitations

The gold standard method to determine B-R-SBPAmp would be simultaneous invasive pressure recording at the brachial and radial arteries, but due to ethical reasons this was not possible in healthy people. In any case, the Doppler ultrasound method has been shown to provide an accurate and direct measurement of SBP[178] and our methodology had excellent agreement with sphygmomanometric brachial SBP and good reproducibility. Anatomical differences between the brachial and radial arteries may have affected the pressure required for cuff occlusion of these arteries, and thus affected the calculation of brachial and radial SBP. However, the strong concordance of our non-invasive results with well conducted invasive studies[75, , 282, 305, 306] suggests that this is unlikely to be a major confounder, although this can only be confirmed with invasive measurements. Finally, we used cardiothoracic bioimpedance to record cardiovascular parameters. Although, this method has been validated compared with invasive techniques[187] and has acceptable reproducibility[188], the accuracy of this non-invasive tool would be inferior to the invasive reference standard.

AI.6 Conclusions

Interest in the usefulness of central BP as a clinical tool emphasises the importance of ensuring the accuracy of central BP measurement devices. Our study shows that B-R- SBPAmp results in significant underestimation of central SBP from the radial pressure waveforms calibrated using brachial SBP and DBP. Emerging data suggests that a more appropriate calibration method is with oscillometric MAP and DBP whether waveforms are derived by radial tonometry or upper arm cuff technology[76]. Given the large range of B-R-SBPAmp values between participants, central BP estimated from upper arm cuffs that have been validated by comparison with invasive central BP measurements, may provide a more accurate non-invasive estimation of central BP than radial tonometry.

Appendix II. Additional data on brachial to radial systolic blood pressure amplification in response to exercise

Appendix II represents additional information and data analysis that was completed for the study presented in *Chapter 7*. This data, relating to the effect of light to moderate intensity exercise on brachial to radial systolic blood pressure amplification, was not included in the final manuscript submitted for publication.

AII.1 Background

Despite resting blood pressure (BP) being clinically important, the BP response to moderate intensity exercise may have stronger prognostic value in terms of cardiovascular risk[5]. This is because individuals spend a relatively large proportion of their day doing some form of light to moderate intensity exercise[6] and, therefore, the BP response to light to moderate exercise is more akin to the chronic BP loading that occurs during normal daily activity[7]. Measuring central BP in response to light to moderate exercise may, therefore, provide pathophysiological insights beyond that of resting measures. However, radial tonometry is currently the only validated[11] method to estimate central systolic BP (SBP) during exercise and brachial to radial systolic BP amplification (Bra-Rad-SBP_{Amp}) may indeed influence the accuracy of this technique. Therefore, this study also aimed to determine the influence of light- moderate exercise on Bra-Rad-SBP_{Amp} and the affect of Bra-Rad-SBP_{Amp} on exercise central SBP in patients with and without type 2 diabetes mellitus (T2DM).

AII.2 Methods

Exercise protocol

Exercise was performed via two legged semi-recumbent cycling using a portable ergometer, mounted on the end of the hospital bed. The resistance was fixed at 40 watts and participants were asked to pedal at 50 revolutions per minute. Once the participant reached as steady state heart rate, all haemodynamic data measured at rest was collected again during exercise.

AII.3 Results

Table AII.1 details the level of Bra-Rad-SBP_{Amp} as well as exercise brachial and central BP in patients with T2DM and non-diabetic controls. Exercise brachial SBP was greater in patients with T2DM, although not significantly, compared to non- diabetic controls, however, there was no difference in exercise radial SBP between the groups. The difference between exercise brachial and radial SBP was borderline significant in patients with T2DM ($p=0.076$) and significant in non-diabetic controls ($p<0.001$). Importantly, similar to at rest, exercise Bra-Rad-SBP_{Amp} was significantly blunted in patients with T2DM compared to non-diabetic controls. Estimated exercise central SBP calibrated with radial SBP was higher than when calibrated using brachial SBP in both patients with T2DM ($p=0.090$) and non-diabetic controls ($p<0.001$) however, there was no difference in exercise central SBP (calibrated by either brachial or radial SBP) between the groups ($p>0.05$ for both).

Table AII.1 Brachial to radial systolic blood pressure (BP) amplification (Bra-Rad- SBP_{Amp}) and effect on central BP estimation during exercise in patients with type 2 diabetes mellitus (T2DM) and non-diabetic controls.

	T2DM (n=20)	**Non-diabetic (n=20)**	**P value**
Bra-Rad-SBP_{Amp} (mmHg)	5±12	16±12	0.006
Brachial systolic BP (mmHg)	154±24	146±17	0.16
Radial systolic BP (mmHg)	159±25	162±25	0.97
Brachial diastolic BP (mmHg)	70±7	72±8	0.36
*Central systolic BP (mmHg)	128±15	121±13	0.13
**Central systolic BP (mmHg)	132±18	132±12	0.74

Data are mean ± standard deviation. T2DM, type 2 diabetes mellitus; BP, blood pressure; Bra-Rad-SBP_{Amp}, brachial to radial systolic BP amplification; *Central systolic BP calibrated with brachial systolic and diastolic BP; **Central systolic BP calibrated with radial systolic and brachial diastolic BP.

AII.4 Conclusions

Radial SBP is higher than brachial SBP under light to moderate exercise conditions in both patients with and without T2DM. However, in patients with T2DM and also non- diabetic controls, Bra-Rad-SBP_{Amp} is of similar magnitude during exercise to that at rest. Therefore, although there is significant underestimation in central SBP using radial tonometry during exercise, it is not augmented compared to resting data.

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