CENTRAL BLOOD PRESSURE IN AN URBAN DEVELOPING COMMUNITY IN SOUTH AFRICA

Michelle Redelinghuys

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ABSTRACT

Contemporary notions of the adverse effects of blood pressure (BP) incorporate the increasingly recognised damaging effects of not only distending pressure (indexed by mean arterial pressure-MAP) but also pulse pressure (PP) (the difference between systolic and diastolic BP) on the cardiovascular system. Although the factors which determine brachial artery PP are similar to those affecting central (aortic) PP (PPc), some factors may affect central PP preferentially, and thus PP calculated from brachial artery BP measurement may not closely reflect the PP that accounts for cardiovascular damage. In order that therapeutic strategies are developed that modify PPc independent of distending pressures, there is considerable interest in the pathophysiological mechanisms that explain increases in PPc. In this regard, aortic PP is comprised of the forward or incident pressure component (P1), which is largely determined by stroke volume, aortic compliance or stiffness and aortic diameter; and the augmented pressure component (AP), which is determined by wave reflection. Whilst currently employed antihypertensive agents may modify AP independent of distending pressures, there is little evidence to indicate a similar effect on the structural aortic changes responsible for P1.

Although changes in AP as opposed to P1 largely account for age-related increases in PPc across the adult lifespan in normotensives, the relative contribution of AP and P1 to PPc in communities with a high prevalence of uncontrolled BP is unknown. In 1015 randomly recruited participants (range 16-88 years) from a community sample, 37.7% of whom had uncontrolled BP, I demonstrated that independent of MAP and other confounders, P1 contributes as much as AP to age-related increases in PPc and to variations in PPc across the adult lifespan.

As no previous studies have assessed the relationship between P1 and cardiovascular damage, in 503 randomly recruited participants from a community with a high prevalence of uncontrolled BP, the relative contribution of P1 and AP to increases in left ventricular mass index (LVMI) was subsequently evaluated. In this regard, independent of distending pressures, P1 was associated with LVMI, highlighting the need to understand the

potential mechanisms which contribute to P1. Could the pathophysiological mechanisms that determine hypertension account for the contribution of P1 to PPc? In this regard, I evaluated the potential role of three mechanisms.

First, in 635 randomly selected participants with 24-hour urine samples that met with pre-specified quality control criteria, I provide the first data to demonstrate that urinary sodium-to-potassium ratio (an index of Na^+ and K^+ intake) is independently associated with PPc, but not brachial PP independent of distending pressures, a relationship that could be accounted for by changes in both AP and P1, but not aortic pulse wave velocity. Second, I explored the possibility that low grade inflammation as indexed by circulating high-sensitivity C-reactive protein concentrations (hs-CRP) may contribute toward PPc and the component pressures. In this regard, although hs-CRP has been associated with changes in central haemodynamics in small study samples, in a large community sample of participants these findings could not be reproduced. However, in that study the community had a low prevalence of risk-related hs-CRP concentrations. In 836 randomly recruited participants from a population sample with a high prevalence of risk-related hs-CRP concentrations (~57%), although on univariate analysis I showed that hs-CRP was strongly associated with PPc and the component pressures, this relationship did not persist with adjustments for confounders. Last I evaluated the potential contribution of genetic factors toward PPc and the component pressures. Although three prior studies had demonstrated heritability of PPc, AP and P1, two studies failed to adjust for MAP and a third assessed the heritability in females only. In none of these studies was the contribution of aortic PWV to the heritability estimates of PPc, AP and P1 assessed. In 568 participants from 183 nuclear families, I showed that independent of MAP, multivariable adjusted PPc, AP, P1 and PWV aggregated in families and were inherited. However, adjustments for aortic PWV failed to modify the extent of intra-familial aggregation and heritability of PPc, AP, or P1.

In conclusion, in the present thesis I have advanced our understanding of the mechanisms responsible for increases in PPc. In this regard, I provide evidence to suggest that independent of distending pressures and stroke volume, P1 accounts for a significant

proportion of the age-related increases in PPc and the variability of PPc across the adult lifespan in communities with a high prevalence of uncontrolled hypertension; that P1 contributes substantially to the relationship between PPc and LVMI; and that PPc and both the AP and P1 component pressures are associated with a urinary index of salt intake as well as genetic factors, but not to an index of low-grade inflammation. These findings suggest that to achieve optimal cardiovascular risk reduction in hypertension, therapeutic strategies that target the aortic structural changes responsible for P1 are likely to be required across the adult lifespan, and that this therapy must in-part address the impact of salt intake and genetic factors, but not necessarily low-grade inflammation on PPc.

DECLARATION

I declare that this thesis is my own unaided work except as indicated in the acknowledgements. It is being submitted for the degree of Doctor of Philosophy in the Faculty of Health Science, University of the Witwatersrand, Johannesburg. The work contained in this thesis has not been submitted for any degree or examination in this University or any other university.

Michelle Redelinghuys ..... signed on the .....day of ..... 2011.

I certify that the studies contained in this thesis have been approved by the Committee for Research in Human Subjects of the University of the Witwatersrand, Johannesburg. The ethics approval number is M02-04-72 and renewed as M07-04-69.

Michelle Redelinghuys ..... signed on the .....day of ..... 2011.

Prof. Gavin Norton (supervisor 1)

Date: .....

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Prof. Angela Woodiwiss (supervisor 2)

Date: .....

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PUBLICATIONS AND PRESENTATIONS ARISING FROM THE THESIS

Publications

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Oral presentations

Redelinghuys M, Norton GR, Woodiwiss AJ, Singh S, Dessein P. The relative contribution of different adiposity indices to an inflammatory marker in the general population. 35th Meeting of the Physiology Society of Southern Africa (PSSA). Muldersdrift, Johannesburg, 2007.

Redelinghuys M, Norton GR, Woodiwiss AJ. Heritability and familial aggregation of indices of large artery function in a community with a high prevalence of excess adiposity. 37th Meeting of the Physiology Society of Southern Africa (PSSA). Stellenbosch, 2009.

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Poster Presentations

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'The most exciting phrase to hear in science, the one that heralds the most discoveries, is not "Eureka!" (I found it!) but "That's funny"…' ***Isaac Asimov***

STATEMENT OF CONTRIBUTION TO DATA COLLECTION AND ANALYSIS

I declare that I designed the studies described in this thesis, in consultation with my supervisors. I collected a major component of the clinical data with the assistance and supervision of the health professionals registered to practice in South Africa and Dr. Carlos Libhaber, a cardiologist and echocardiographer who analysed the echocardiographic data in the APOGH study. I performed all of the data analysis for this thesis and interpreted the data.

LIST OF ABBREVIATIONS

ACEI	angiotensin converting enzyme inhibitor
AI	augmentation index
AIc	central (aortic) augmentation index
AIx	central (aortic) augmentation index (chapter 2)
AP	augmentation pressure
ARB	angiotensin receptor antagonist
BMI	body mass index
BP	blood pressure
C	compliance of conduit artery (aorta)
CCB	calcium channel blocker
CHD	coronary heart disease
CI	confidence intervals
cm	centimetre
CRP	c-reactive protein
D	diameter of conduit artery (aorta) in water hammer equation
DBP	diastolic blood pressure
DM	diabetes mellitus
ECG	electrocardiogram
ERD	effective reflecting distance
h^2	heritability estimate
HbA_{1C}	glycated haemoglobin
Hs-CRP	high sensitivity CRP
ISH	isolated systolic hypertension
IVS	interventricular septal wall thickness
K^+	potassium ion
K+/creatinine	urinary potassium ion corrected for urine creatinine

kg	kilogram
kg/m²	kg per meter²
LV	left ventricle
LVED	LV end diastolic
LVEDD	LV end diastolic diameter
LVEDV	LV end diastolic volume
LVESD	LV end systolic diameter
LVESV	LV end systolic volume
LVH	LV hypertrophy
LVM	LV mass
LVMI	LVM indexed for height$^{1.7}$
m/sec	meters per second
MAP	mean arterial pressure
mg/l	milligrams per litre
mHz	megahertz
ml	millilitres
mls/beat	millilitres per beat
mm Hg	millimetres of mercury
mmol	millimole
mmol/day	millimoles per day
msec	milliseconds
mV	millivolts
n	number (sample size)
Na-K-2Cl	sodium-potassium-chloride co-transporter
Na^+	sodium ion
Na^+/creatinine	urinary sodium excretion corrected for urine creatinine
Na^+/K^+	sodium-to-potassium ratio
p	fluid density in conduit artery (aorta)

P1	central (aortic) forward component pressure
p value	probability value
PP	pulse pressure
PPc	central (aortic) PP
PWT	posterior wall thickness
PWV	pulse wave velocity
r	correlation coefficient
RAAS	renin-angiotensin-aldosterone system
RDA	recommended daily allowance
RWTT	reflected wave transit time
SAGE	statistical analysis for genetic epidemiology software
SAS	statistical analyses software
SBP	systolic blood pressure
SBPc	central SBP
SD	standard deviation
SEM	standard error of the mean
SP	Systolic blood pressure
SV	stroke volume
SV_t	stroke volume calculated using the Teichholz method
SV_z	stroke volume (z-derived)
WHO	World Health Organization

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PREFACE

There is presently an unprecedented increase in the prevalence of hypertension across continents. Hypertension contributes significantly to the burden of disease in economically developing countries such as South Africa, particularly in urban developing communities of African descent. Although there is strong evidence that brachial artery blood pressure (BP) measurements predict cardiovascular outcomes, there is also data to show that central (aortic) systolic BP and pulse pressure (PP) predict cardiovascular outcomes beyond brachial BP. Therefore there is considerable interest in developing therapeutic strategies that reduce central systolic BP and PP. In order to develop these strategies a comprehensive understanding of the mechanisms responsible for increases in central BP are required.

The present thesis was prompted by a need to address some outstanding issues regarding the mechanisms and impact of the pressure components of central BP, namely the forward (incident) (P1) and the augmented (AP) pressure components in an urban economically developing African community with a high prevalence of uncontrolled hypertension. This question was considered to be of importance as current evidence suggests that although presently available therapeutic agents and strategies may reduce AP, there is little evidence to indicate that antihypertensive therapy is able to reverse the structural aortic changes responsible for P1. Hence, if AP is the major pressure component that accounts for increases in central BP, then current therapeutic agents are likely to effectively reduce cardiovascular risk. However, if P1 also accounts for increases in central BP, then current therapeutic agents are unlikely to effectively reduce cardiovascular risk. In this regard, there is considerable uncertainty as to the relative role of AP and P1 as determinants of central BP. In the present thesis I therefore focussed my efforts on attempting to clarify the contribution of P1 and AP toward increases in central BP as well as target organ changes, and to identify some of the potential mechanisms that may explain increases in AP and P1.

The present thesis is written as a series of semi-independent chapters, each with its own introduction, methods, results and discussion section. The thesis begins with a review chapter which highlights the current understanding and controversies in the field and leads the reader through a series of arguments in support of conducting the studies described in the present thesis. Furthermore, the present thesis concludes with a summary chapter which consolidates the findings of each chapter and underscores the novelty of the findings by placing the studies in the context of our present understanding of the field. In support of the present thesis, the data presented in chapters 4 and 5 have been published in the journal *Hypertension* (Redelinghuys et al 2010) and the *American Journal of Hypertension* (Redelinghuys et al 2011) respectively. The data provided in the other chapters are currently in preparation for submission to international journals for review.

CHAPTER 1

INTRODUCTION

The Current Understanding and Controversies in Central (Aortic) Haemodynamics

1.1 Introduction

Hypertension currently affects 1 billion individuals globally and is becoming more prevalent in both economically developed and developing countries (World Health Organisation [WHO] 2003, Chobanian et al 2007). Estimates of the prevalence of hypertension range from 28% in North America to 44% in some European countries (Wolf-Maier et al 2003). In South Africa, an economically developing country, hypertension affects approximately 26% of the entire population (Steyn et al 2008) but in urbanized populations of African descent living in South Africa, the prevalence may lie between 40 and 50% (Malhotra et al 2008, Maseko et al 2010).

Globally, hypertension contributes to 62% of cerebrovascular disease including strokes, and to 49% of coronary heart disease (CHD) (WHO 2003). These percentage contributions are similar in the South African context, with 50% of strokes and 42% of CHD being attributed to hypertension (Norman et al 2007). In urban communities of African ancestry in South Africa, hypertension may contribute to up to a third of heart failure cases (Stewart et al 2008), hypertension is strongly associated with myocardial infarctions (Steyn et al 2005) and hypertension accounts for a significant proportion of strokes (Connor et al 2009). As these conditions are the leading causes of death in the elderly and the third most common cause of death in younger age groups in African populations (Tollman et al 2008) hypertension clearly accounts for a significant burden of disease in groups of African descent living in developing countries.

With respect to the management and control of hypertension, despite the evidence to indicate that hypertension contributes to a substantial proportion of cardiovascular events in any country, in the United States of America only ~34-35% of all hypertensives and only ~55% of treated hypertensives are controlled to target blood pressure (BP) levels (Hertz et al 2005, Cutler et al 2008). By comparison, the control of BP in some European countries may be far worse (Wolf-Maier et al 2004, Wang et al 2007). Moreover, in economically emerging countries such as South Africa where the majority of the population are of black African

ancestry, only ~14% of hypertensives at a national level (Steyn et al 2001) and ~33-44% of hypertensives in primary care settings (Dennison et al 2007, Steyn et al 2008) are controlled to target BP levels. Moreover, in urban, developing communities of African descent in South Africa, in which 46% may have hypertension, more than half with hypertension may not receive antihypertensive therapy (Maseko et al 2010) and only 36% receiving antihypertensive therapy (18% of hypertensives) achieve target BP values (Maseko et al 2010). Thus, globally and particularly in groups of African descent, hypertension is poorly controlled. Does the poor BP control in groups of African ancestry translate into excessive cardiovascular damage?

There is a greater prevalence of cardiovascular disease in groups of African as compared to European origins. Indeed, in the United States of America, the prevalence of strokes and major cardiovascular intermediate phenotypes for stroke, namely left ventricular hypertrophy and urinary albumin-to-creatinine ratios, are higher amongst groups of African as compared to European origins (Gillum 1999, Howard 2001, Sacco et al 2001, Lorber et al 2003, Murtaugh et al 2003, Skelton et al 2003, Hollar et al 2004, Jamerson 2004, McGruder et al 2004, Kizer et al 2004, Rodriguez et al 2004, Drazner et al 2005, Nunez et al 2005, Bryson et al 2006). As strokes, left ventricular hypertrophy and urinary albumin-to-creatinine ratios are strongly determined by BP, these differences could be explained by the lack of BP control in groups of African ancestry. In many of these studies however, the excessive cardiovascular damage in groups of African ancestry persisted after adjustments for BP measured at the brachial artery. The question therefore arises as to why excessive cardiovascular damage may exist in groups of African descent even with adjustments for brachial BP values? One potential mechanism relates to the possibility that BP measured at the brachial artery may not accurately predict the extent of cardiovascular damage in these groups.

Although there is no question as to the value of BP measurements performed at the brachial artery in cardiovascular risk prediction and indeed the definition of hypertension is based on brachial BP measurements (WHO 2003, Chobanian et al 2003, Williams et al

2004, Mancia et al 2007), brachial BP may not fully account for the deleterious effects of an increased BP. In this regard there is now emerging evidence to indicate that central (aortic) BP, which is mediated by different mechanisms from brachial artery BP, may cause cardiovascular damage beyond brachial artery BP. In the present thesis I have explored outstanding aspects of the pathogenesis and impact on cardiovascular damage of increases in central aortic BP. I have specifically performed these studies in a community sample of African descent, because, as previously mentioned, there is a high prevalence of uncontrolled BP and cardiovascular damage in these communities. Therefore, in the present chapter I will describe the current understanding of central aortic BP and highlight the outstanding evidence that led me to perform the studies described in the present thesis. However, I will first outline the evidence to show that brachial distending and pulsatile pressures predict cardiovascular outcomes, as it is this evidence that has gradually led up to the hypothesis that central (aortic) BP may be important in contributing toward cardiovascular damage beyond brachial artery pressures.

1.2 The role of brachial blood pressure and its relation to cardiovascular outcomes

Hypertension is defined as a brachial systolic BP greater than or equal to 140 mm Hg, and/or diastolic BP greater than or equal to 90 mm Hg (WHO 2003, Chobanian et al 2003, Williams et al 2004, Mancia et al 2007). Over the years there has been a gradual shift in recognition of the importance of both systolic and diastolic BP measured at the brachial artery in the pathogenesis of cardiovascular disease as opposed to a preferential role of either systolic or diastolic BP considered separately. More recently, this view has been expanded to incorporate recognition of the role of pulsatile or pulse pressure (PP) (systolic - diastolic BP) independent of distending pressures as measured at the brachial artery. The following sections review the evidence responsible for the shift in this paradigm.

1.2.1 The association of brachial systolic and diastolic blood pressure with cardiovascular outcomes.

The Epidemiological Pooling Project, which combined data from five observational studies conducted in middle-aged Caucasian men, demonstrated that diastolic BP makes a substantial contribution to cardiovascular risk (The Pooling Project Research Group, 1978). Furthermore, as noted in a meta-analysis of 14 studies, a reduction in diastolic BP through the use of diuretics and β-adrenergic receptor-blockers was associated with a 42% reduction in the incidence of strokes and a 14% lower risk of CHD in hypertensive patients (Collins et al 1990). Based on this evidence diastolic BP lowering was considered to be the primary target of antihypertensive therapy. However, the data included in the two meta-analyses were obtained from studies which had recruited comparatively young participants and were conducted over relatively short time periods (The Pooling Research Group 1978, Collins et al 1990). Thus the effect of age-related changes in brachial systolic and diastolic BP on cardiovascular risk was not taken into consideration.

In contrast to the view that diastolic BP is more important than systolic BP in mediating cardiovascular disease, as early as 1971 the Framingham Heart Study had demonstrated that in 5127 participants with a follow-up duration of 14 years, although both systolic and diastolic BP are related to coronary events, diastolic BP becomes less important with advancing age (Kannel et al 1971). Systolic BP was identified as a stronger predictor of cardiovascular risk than diastolic BP, particularly with increasing age (Kannel et al 1971). These results were extended to the risk of cerebrovascular disease in the same study group, in that diastolic BP added little to predicting the risk of strokes beyond systolic BP as assessed over a 20 year follow-up period (Kannel et al 1981). In a more recent prospective study of 12866 middle-aged men, elevated systolic BP was found to contribute to a greater risk of end-stage renal disease than diastolic BP (Klag et al 1996). In 2002, a landmark meta-analysis incorporating 61 prospective observational studies of more than a million participants, reported that in individuals between 40 and 69 years of age, for every 20 mm

Hg increase in systolic BP, the risk of cardiovascular disease is doubled (Lewington et al 2002). Thus epidemiological studies clearly demonstrate that systolic BP measured at the brachial artery is a strong predictor of cardiovascular risk and events.

Are there intervention studies to support the notion that systolic BP measured at the brachial artery is an important determinant of cardiovascular outcomes? In this regard there are also some landmark studies (SHEP Cooperative Research Group 1991, Staessen et al 1997, Staessen et al 2000, Wang et al 2000) conducted in patients with an increased systolic BP, whilst diastolic BP may be normal or even decreased. This condition is commonly known as isolated systolic hypertension and most frequently occurs in the elderly (see subsequent paragraph). In these studies, patients with isolated systolic hypertension received antihypertensive therapy despite having normal or even decreased diastolic BP values (SHEP Cooperative Research Group 1991, Staessen et al 1997, Staessen et al 2000, Wang et al 2000) and treatment resulted in a marked decrease in cardiovascular outcomes. These studies therefore provided direct evidence to support the notion that increases in systolic BP without increases in diastolic BP produce cardiovascular damage.

In addition to highlighting the importance of brachial systolic as compared to diastolic BP in predicting cardiovascular events, the results of the aforementioned studies (Kannel et al 1971, Kannel et al 1981, SHEP Cooperative Research Group 1991, Klag et al 1996, Staessen et al 1997, Staessen et al 2000, Wang et al 2000, Lewington et al 2002) also underscore the differential impact of aging on brachial diastolic and systolic BP. Indeed, in individuals older than 50-60 years of age, brachial diastolic BP begins to level off and in some instances may even decrease, although brachial systolic BP continues to increase with age (Franklin et al 1997). Thus with increasing age PP as determined from BP measurements at the brachial artery widens. Widening of PP is considered to be important in individuals with isolated systolic hypertension, whom characteristically have an increased PP. In this regard, the Framingham study identified not only a two-to-five fold increase in cardiovascular risk in isolated systolic hypertension compared to normotensive participants, but more importantly, in patients with isolated systolic hypertension with a diastolic BP lower

than 95 mm Hg, cardiovascular risk increased with increasing systolic BP values (Kannel et al 1980). In a more recent prospective study a combination of a decreased brachial diastolic BP and increased brachial systolic BP conferred a greater cardiovascular risk to middle-aged men as compared to an increase in systolic BP only, an increase in both systolic and diastolic BP, a decrease in diastolic BP only, or a decrease in both systolic and diastolic BP (Benetos et al 2000). Similarly, as noted in older hypertensives, at a given systolic BP cardiovascular risk is higher when diastolic BP is decreased (Blacher et al 2000). These results therefore suggest that a greater difference between systolic and diastolic BP, i.e. a greater brachial artery PP, may confer a larger cardiovascular risk than either an increased brachial systolic or diastolic BP alone. In other words, pulsatile pressures or PP may be more important than distending pressures (diastolic BP or mean arterial pressures-MAP) when considering the impact of BP on cardiovascular disease.

1.2.2 The association of brachial pulse pressure with cardiovascular outcomes, independent of distending pressures.

A number of studies have confirmed that elevated brachial PP independently predicts the incidence of cardiovascular and cerebrovascular events in hypertensives (Madhavan et al 1994, Chae et al 1999, Franklin et al 1999, Blacher et al 2000, Vaccarino et al 2001) and in normotensive participants (Benetos et al 1998, Franklin et al 1999). Indeed, normotensive men with an increased brachial PP may be at the same elevated cardiovascular risk as hypertensive men with a decreased brachial PP (Benetos et al 1998). Furthermore, brachial PP predicts cardiovascular risk beyond both systolic and diastolic BP (Chae et al 1999, Franklin et al 1999, Cockcroft et al 2005, Anderson et al 2009) and brachial PP is a predictor of cardiovascular outcomes independent of MAP (Benetos et al 1997, Benetos et al 1998, Millar et al 1999, Chae et al 1999, Lee et al 1999, Domanski et al 1999, Blacher et al 2000, Vaccarino et al 2000, Glynn et al 2000, Franklin et al 2001, Domanski et al 2001, Blacher et

al 2001). Considering the close relationship between PP and MAP (see later section for potential reasons), these findings are indeed striking.

1.3 Central aortic versus brachial artery blood pressure

The findings that PP is associated with cardiovascular outcomes beyond distending pressures provided the stimulus to hypothesise that central aortic PP or systolic BP may be more important than PP or systolic BP measured at the brachial artery. This line of thought was generated by the evidence that although distending pressures are constant from central to peripheral arteries, pulsatile pressures differ considerably in the aorta as compared to peripheral arteries. How does aortic and brachial artery BP differ and what is the evidence that these differences could translate into a distinct impact of aortic and brachial BP on cardiovascular outcomes?

1.3.1 Differences in pulse pressure in brachial and central arteries

Mean arterial pressure, an index of distending pressures, is calculated as 1/3PP + DBP, and assuming that pressure and flow remain constant throughout the cardiac cycle, MAP is the product of cardiac output and systemic (peripheral) vascular resistance. As MAP is largely determined by diastolic BP and diastolic BP is in-turn principally driven by arteriolar function which has similar effects on diastolic BP in the aorta as it does in large arteries found in the periphery, MAP remains relatively constant from the central arteries (aorta) to the periphery (Nichols et al 1998).

What generates pulsatile or dynamic pressures and are pulsatile pressures the same in the aorta as compared to peripheral arteries? In this regard, the heart pumps intermittently and with each ventricular ejection, an oscillating pressure wave (the forward or incident pressure wave) is generated. As a result flow is variable throughout the cardiac cycle and BP is subsequently pulsatile. The large conduit arteries, and indeed mostly the aorta, minimise

the pulsatility through a cushioning effect to provide a steady BP to the organs and periphery. In a healthy person, the cushioning effect of the aorta is achieved through compliant large arteries with a low characteristic impedance (see section 1.4 for an explanation) which are able to accommodate large blood flow rates and volumes without generating high pressures. However, dynamic or pulsatile pressures, as indexed by PP and systolic BP are not the same at the periphery (i.e. brachial artery) as compared to centrally (aorta). Indeed, brachial pressures can be considerably higher than central aortic pressures, a finding that has been termed “PP amplification” (Nichols et al 1998). The mechanisms of PP amplification are explained in section 1.4. This difference is particularly marked in the young, but with age-related changes in large vessels, this difference between central and brachial artery dynamic pressures gradually decreases (Nichols et al 1998). Therefore BP measurement at the brachial artery may not accurately reflect central pressures until much later in life. Taking into account that BP in the aorta is more likely to reflect what the heart and cerebral vasculature are exposed to, central BP may be a better predictor of cardiovascular events than brachial BP. Is there evidence to suggest that central aortic BP is more closely associated with cardiovascular risk than BP measured at the brachial artery?

1.3.2 Central (aortic) blood pressure is more closely related to cardiovascular outcomes than brachial pressure

Table 1.1 provides a summary of recent clinical studies which have shown central (aortic) systolic BP and/or PP to be independent predictors of cardiovascular risk beyond peripheral (brachial) PP or systolic BP (Safar et al 2002, Chirinos et al 2005, Williams et al 2006, Roman et al 2007, Jankowski et al 2008, Wang et al 2009, Roman et al 2009). With respect to the relative roles of central systolic BP and central PP as being more important in predicting cardiovascular outcomes there is still some uncertainty in this regard as PP is a strong determinant of systolic BP. In the Strong Heart Study conducted in an older American Indian population (mean age of 58 years), with a mean follow up of 4.8 years,

Table 1.1. Summary of recent clinical studies that have shown central systolic blood pressure (BP) or pulse pressure to predict cardiovascular events and mortality beyond brachial BP.

Patient group	Mean follow-up (years)	Sample size (n)	Outcome	Reference
End stage renal disease	4.3	180	Cardiovascular mortality All cause mortality	Safar et al 2002
Coronary artery disease	3.2	297	All cause mortality Composite end point of all major cardiovascular events	Chirinos et al 2005*
Treated hypertension	3.0	2073	Composite end point including all cardiovascular events, procedures and renal impairment	Williams et al 2006
American Indian population	4.8	3520	All cardiovascular events	Roman et al 2007
Elderly population sample	8.0	404	All cardiovascular events	Pini et al 2008
Coronary artery disease	4.5	1109	All cardiovascular events	Jankowski et al 2008*
Chinese population	10.8	1272	Cardiovascular mortality All-cause mortality	Wang et al 2009
American Indian population	5.6	2405	All cardiovascular events	Roman et al 2009

* Invasive methods of determining central aortic pressures were employed.

both peripheral and central PP were better predictors of cardiovascular outcomes than peripheral and central systolic BP, but central PP was the best overall independent predictor, demonstrating a 15% increase in risk of a cardiovascular event with every 10 mm Hg increase in central PP (Roman et al 2007). These results were confirmed in a subsequent study by the same group, where a central PP value greater than 50 mm Hg was associated with adverse cardiovascular outcomes (Roman et al 2009). In contrast, Pini et al (2008) although showing in an elderly European population that both central systolic BP and PP predict cardiovascular events independent of BP measured at the brachial artery, only central systolic BP predicted cardiovascular mortality. However, only age and sex were adjusted for in multivariable analysis (Pini et al 2008). Moreover, in another study both central systolic BP and PP were shown to predict cardiovascular mortality beyond BP measured at the brachial artery (Wang et al 2009). Irrespective of whether central systolic BP or PP are predictors of cardiovascular outcomes beyond brachial BP, the majority of studies have clearly demonstrated the better predictive power of central as compared to peripheral BP measurement. Is there any evidence to the contrary?

In contrast to the aforementioned studies demonstrating relationships between central aortic BP and cardiovascular outcomes beyond brachial BP measurements (Table 1.1), a study conducted in 484 elderly hypertensive women with a 4 year follow-up duration suggested that brachial BP is a better predictor of cardiovascular events than central BP (Dart et al 2006). However, all participants were treated with either a diuretic or angiotensin-converting enzyme-inhibitor (ACEI) over the duration of the study, but in multivariable analysis the use of antihypertensive therapy was not included as a potential confounder. In this regard, a subsequent study by the same group (Dart et al 2007) reported reductions in both central and brachial BP with antihypertensive treatments.

The evidence that aortic PP or systolic BP predicts cardiovascular outcomes beyond BP measured at the brachial artery obviously raises the question of whether all antihypertensive agents reduce central BP to the same extent as brachial BP. The following section describes the evidence for or against such effects.

1.3.3. Impact of anti-hypertensive drugs on aortic as compared to brachial artery BP

In prominent prospective studies, renin-angiotensin system inhibitors, such as angiotensin receptor blockers (ARBs) and ACEIs have been shown to reduce cardiovascular mortality and morbidity beyond alternative agents despite producing similar effects on brachial BP (Gerstein et al 2000, Lindholm et al 2002, Dahlof et al 2005). These data obviously beg the question of whether this occurrence reflects differential effects of antihypertensive agents on brachial and central BP. Indeed, there is evidence to suggest such an effect.

In a six-week study, eprosartan (an ARB) reduced central systolic BP and PP to a greater extent than atenolol (β-adrenergic receptor blocker) despite similar brachial artery BP lowering effects (Dhakam et al 2006). The Preterax in Regression of Arterial Stiffness in a Controlled Double-Blind (REASON) study similarly demonstrated that a combination of perindopril, an ACEI and indapamide, a diuretic agent, reduced central systolic BP and PP to a much larger degree than atenolol after one year of treatment (Asmar et al 2001, London et al 2004). Moreover, in the Conduit Artery Function Evaluation (CAFE) study central BP reduction occurred in the amlodipine (calcium channel blocker [CCB])-treated group but not in the atenolol-treated group (Williams et al 2006). Therefore, different drug classes do indeed have varying effects on central systolic BP and PP lowering, with the weight of the evidence suggesting that while ACEI, ARB, diuretics and calcium channel blockers may appropriately reduce central BP, β-adrenergic receptor blockers may not. These data therefore could translate into different cardiovascular outcomes when treating brachial BP to target values.

1.4 The forward and reflected pressure components of central pulse pressure.

To understand why aortic PP and systolic BP may better predict cardiovascular outcomes than brachial artery PP or systolic BP, it is important to recognise how pulsatile pressure is generated in the aorta as compared to peripheral arteries. As indicated in previous discussion, PP is in-part generated by ventricular ejection (producing a stroke volume) of blood into conduit arteries. In its simplest form, the Windkessel model describes the arterial system as parallel resistance and compliance components, where resistance is determined by systemic vascular resistance driven largely by small vessels (arterioles) and compliance by the capacity of the arterial system to accommodate increases in volume as the heart ejects blood, which is a function of large vessels. In this regard, compliance (C) can be approximated by stroke volume/pulse pressure (SV/PP). Using this overly-simplistic approach, PP=SV/C and hence PP is positively related to stroke volume and inversely related to compliance. However, the Windkessel model in its simplest form assumes that all pressure changes occur simultaneously, which is not the case. The simple Windkessel model does not account for the transmission characteristics of the vessel wall. How do transmission characteristics of the vessel wall influence PP?

The initial ejection wave produced by ventricular contraction generates a forward or incident pressure wave in vessel walls. With each forward wave produced by contraction of the heart, this waveform travels down large artery walls and reflects back off these walls as the vessel narrows (reflection points which may originate in muscular arteries in the major branches of the aorta). The reflected wave subsequently travels back toward the heart and meets the forward or incident wave generated by ventricular contraction. The reflected or backward pressure wave thus augments the forward pressure wave and hence this pressure is called augmentation pressure (AP). Thus the pulsatile pressure measured in conduit arteries is a summation of both the forward and the reflected pressure waves (Nichols et al 1998).

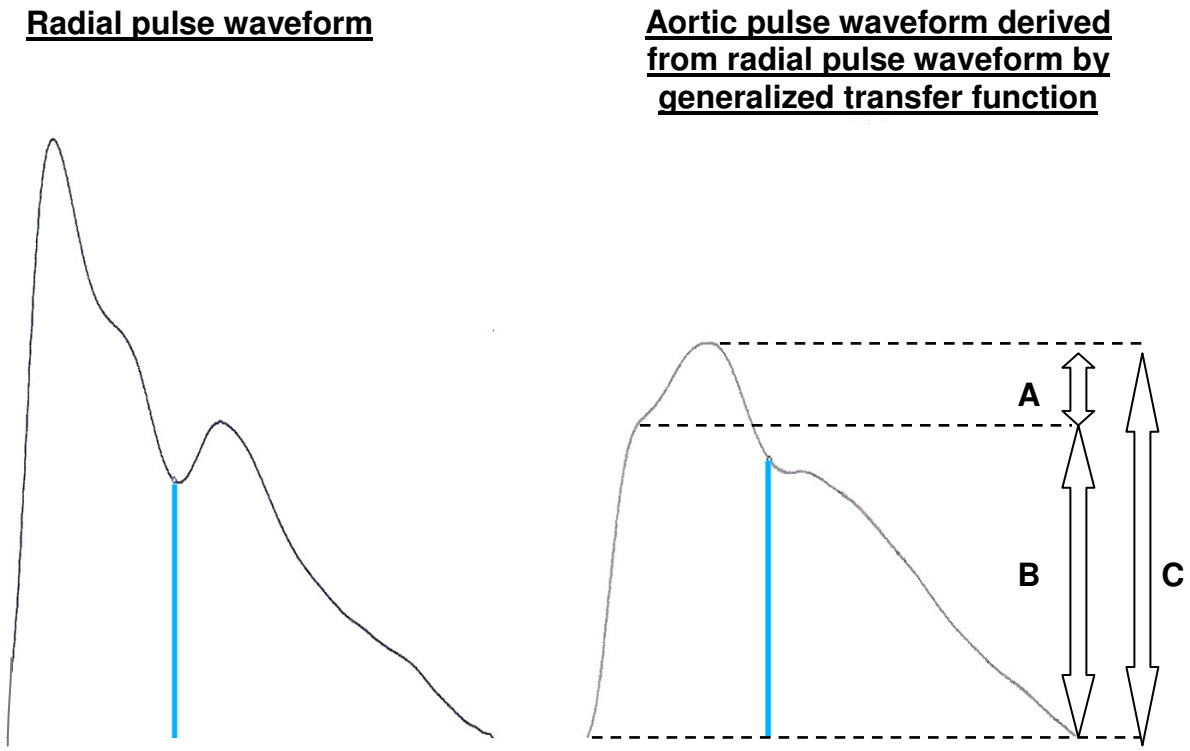
In the young the reflected pressure wave reaches the heart during diastole, increasing central (aortic) diastolic pressure and hence both improving coronary blood flow (which depends on perfusion pressures during diastole) and decreasing central PP. However, in adulthood the reflected pressure wave coincides with the forward wave generated during systole, thus increasing central (aortic) systolic pressures and central PP. In contrast, because of the distance between the brachial artery and distal reflection points and the characteristics of large arteries in the periphery, the chances of the reflected wave coinciding with the forward pressure wave in the brachial artery during systole, even in the elderly, is small. Thus during adulthood, the augmented pressure wave in the aorta increases central PP with aging, whilst the augmented pressure wave in peripheral arteries, including the brachial artery, increases diastolic BP and hence may continue to decrease brachial PP with aging.

The differences that exist between brachial and central aortic PP (see section 1.3.1) may be explained by variations in the impact of the forward and reflected pressure wave at different arterial sites. In this regard, aortic PP is determined by reflection sites off aortic branches, whereas brachial BP is determined by reflection sites in the forearm and these sites produce differential effects on wave reflection (Dart and Kingwell 2001). Moreover, the relationship between brachial PP and height (Asmar et al 1997) and the number of studies demonstrating a relationship between height and pressure augmentation (London et al 1995, Cameron et al 1998, Smulyan et al 1998) suggests that pressure amplification is closely associated with the length of the conduit arteries along which wave transmission occurs, which differ considerably in the aorta as compared to brachial arteries. Furthermore, the speed of forward wave travel is non-linear across vessels of varying sizes (Jones et al 1992), which, consequently, is likely to influence the timing with which the forward wave meets the reflected wave in the aorta as compared to peripheral arteries.

1.4.1 The measurement of aortic pulsatile pressures and the forward and reflected pressure components.

Central BP should ideally be measured invasively with a catheter paced in the carotid artery or proximal aorta, but invasive measurements are often impractical for large clinical and epidemiological studies. Therefore non-invasive techniques, namely applanation tonometry coupled with the use of a validated population-based generalized transfer function, are employed to non-invasively estimate central BP. Applanation tonometry is performed at the site of the radial or carotid artery. When the tonometer is placed over the artery, the artery is partially flattened and a pressure waveform generated in the arterial wall may be measured by a pressure transducer. By use of a generalized transfer function (in the case of radial tonometry) and calibration of the arterial waveform by brachial artery BP measurements, central aortic waveforms may be derived (Figure 1.1).

Accurate measurement of carotid waveforms is more likely to reflect central pressure waveforms than measurement of radial artery waveforms coupled with the use of validated population-based transfer functions to estimate central waveforms. However, radial tonometry has several advantages in comparison to carotid tonometry. The close proximity of the radial artery to bone is advantageous as pressure waveforms are easier to measure when the radial artery is partially flattened against the underlying bone (Papaioannou et al 2009). Furthermore, in obesity, adipose tissue in the neck can lead to a cushioning effect on the carotid pressure waveform (Papaioannou et al 2009). Moreover, calibration with brachial cuff pressures is better suited to radial measurements than carotid measurement because of pressure amplification between central and peripheral arterial sites (Papaioannou et al 2009). Therefore it is not surprising that carotid applanation tonometry has provided more variable results as compared to radial tonometry (O' Rourke and Nichols 2005). In this regard, Chen and colleagues were the first to validate the use of radial applanation tonometry to determine central BP (Chen et al 1997). However, more recent studies have demonstrated accuracy in



A: Augmentation pressure (AP)

B: Forward pressure component (P1)

C: Central (aortic) pulse pressure

Figure 1.1. The derivation of the aortic pulse waveform and component pressures (right panel) from the radial artery pulse waveform (left panel) obtained by applanation tonometry.

central BP measurement using radial tonometry in individuals at rest (Sharman et al 2006; Smuylan et al 2003, Hope et al 2004) and during exercise (Sharman et al 2006).

Importantly, estimation of the central (aortic) BP waveform not only allows for measurements of central aortic systolic and diastolic BP and PP, but also for the component pressures of central PP. Indeed, from the central pulse pressure waveform, both the forward (incident) and the augmented pressure component can be identified (Figure 1.1). The magnitude of the forward pressure component is determined as the difference between the inflection point at the end of the first systolic shoulder (i.e. pressure 1 or P1 as it reflects the pressure measured at the end of the first systolic shoulder) and diastolic BP (Figure 1.1). The augmented pressure (AP) is determined as the difference between central systolic BP and the pressure at the inflection point at the end of the first systolic shoulder (Figure 1.1). Although the use of a transfer function accurately estimates central aortic pressures, the reconstructed waveform does nevertheless underestimate the central AP because of inaccuracies in reconstructing waveform details and hence identifying the end of the first systolic shoulder (Chen et al 1997).

1.4.2 The potential determinants of the forward and reflected pressure components of aortic pulsatile pressures

The amplitude of the pressure that is generated by the forward (incident) pressure component (P1) is thought to depend on a number of factors including stroke volume, the elastic properties of the proximal aorta, and the diameter of the aorta. If stroke volume increases, the pressure generated by high flows will augment pressures during ventricular ejection and hence P1 will increase. Both the diameter and the elastic properties of the aorta influence the aortic characteristic impedance to blood flow (Nichols et al 1998, O' Rourke and Pauca 2004), where aortic characteristic impedance is the pressure generated by a given flow waveform in the proximal aorta during early systole, before return of the reflected wave. In this regard an increased aortic stiffness may enhance P1 by increasing the aortic

impedance to blood flow. Alternatively, an augmented aortic compliance (which is the inverse of stiffness) may decrease P1 by attenuating the aortic characteristic impedance to blood flow. As aortic characteristic impedance is also defined by the water hammer equation where impedance=4xaortic pulse wave velocity (PWV)x$p/\pi D^2$, and where p is the density of fluid (in this case blood) and D is diameter of the conduit (in this case the aorta), which although should theoretically only be applied to systems where wave reflection does not occur, it stands to reason that smaller aortic dimensions are associated with a greater aortic characteristic impedance, thus enhancing the magnitude of the forward pressure. Although there is considerable controversy in this regard (O'Rourke and Nichols 2005,Vasan 2008), consistent with inverse relationships noted between PP and aortic diameter in a number of prior studies, in patients with isolated systolic hypertension, an increased central PP is attributed in-part to a decreased aortic diameter (Mitchell et al 2008), an effect that may be mediated through increases in P1. However, aortic root diameter does not predict the development of hypertension or BP progression (Ingelsson et al 2008).

The magnitude of AP is thought to be determined by the amplitude and the timing of the reflective wave (Nichols et al 1998, O' Rourke and Pauca 2004) and the amplitude of P1 (law of conservation of energy). The amplitude of wave reflections may depend on the vascular tone of muscular arteries. The timing of wave reflections may depend on a number of factors. Earlier wave reflections are likely to reach the proximal aorta during systole, which may occur if the speed of wave travel (aortic pulse wave velocity) is increased or if the point of wave reflections moves closer to the heart by modifying vascular tone in branch points from the aorta and causing phase shifts at reflection sites. This occurrence is thought to explain why in contrast to what is noted in the young, where AP tends to be a negative value because the reflective wave is sufficiently late that diastolic pressures are augmented, in older subjects the reflected wave is early and hence AP is a positive value (Murgo et al 1980). Augmentation pressures may also increase without changing the speed or magnitude of wave reflections, but by extending the time taken for the generation of P1, such as may

occur in a bradycardia. In this instance, there is an increased chance that the reflected wave will coincide with the forward wave whilst the forward wave is in systole.

It is also important to note that AP is indirectly influenced by P1 height (law of conservation of energy) (Namasivayam et al 2009) and thus cannot be interpreted solely as an index of wave reflection. While both flow and pressure parameters are required to accurately separate the forward and reflected pressure waves, this approach is difficult with non-invasive measurements subject to errors created by turbulent flow in the ascending aorta.

Lastly, it is also important to note that possibly the strongest determinant of aortic pulsatile pressures, the component P1 and AP pressures and the determinants of these components is steady-state pressures. In this regard steady-state pressures determine distending pressures in large vessels. As the relationship between pressure and volume in large vessels is non-linear (exponential), with pressure changes being greater for a given volume at a higher distending pressure, large artery stiffness is increased at higher steady-state pressures. Thus, at higher steady-state or distending pressures PP and P1 will increase. In addition at higher steady-state or distending pressures the increased arterial stiffness will also increase the speed of wave reflection and consequently AP. Thus, a limitation of many studies conducted to-date when assessing factors associated with PP and the component pressures or the impact of PP and the component pressures on cardiovascular damage is that the investigators have not adjusted for distending pressures such as MAP. Where these adjustments have not been included in the analysis of studies assessing these questions, this will be highlighted in subsequent discussion as a limitation that needs to be addressed in further studies.

1.4.3 Augmentation index as an index of wave reflection

The ratio of AP to central PP, expressed as a percentage (central augmentation index or AIc) is a combined measure of both the timing and magnitude of wave reflection (Swillens

and Segers 2008). The advantage of using AIc as an index of wave reflections is that it does not depend on absolute pressures and hence is not subject to inaccuracies in brachial BP measurements employed to calibrate the aortic waveform. However, because P1 increases in individuals over 60 years of age, thus increasing PP but not necessarily AP, AIc becomes less sensitive as an index of wave reflection in individuals over the age of 60 years (McEniery et al 2005) and hence may not always indicate the timing and magnitude of wave reflection.

1.4.4 The contribution of the forward and reflected pressure components to cardiovascular damage.

As indicated in section 1.3.2, there is good evidence to indicate that central (aortic) PP contributes to the development of cardiovascular damage and disease independent of BP measured at the brachial artery (Table 1.1) As P1 and AP constitute the pressure components of central PP, the obvious question that arises is whether both AP or P1 contribute equally to this effect or whether one contributes more than the other to cardiovascular damage. The importance of this question relates to whether therapeutic strategies should target one, more or less or equally as the other. The answer to this question may appear intuitive as reducing either of these pressure components will reduce central BP. However, as shall be described in later sections, the pathophysiological mechanisms responsible for increases in central BP may not be that simple and as shall be discussed in the next section, the ability of currently available antihypertensive therapy to modify the structural aortic changes responsible for P1 appear to be limited at best.

The majority of studies assessing the impact of the pressure components of central PP on cardiovascular damage or outcomes have focused on the possible role of the reflective wave with AIc largely being employed as a measure of wave reflection (Table 1.2). In this regard, AIc predicts cardiovascular outcomes in normotensives (Saba et al 1993), hypertensives (Williams et al 2006, Hashimoto et al 2007) and patients with existing or

suspected cardiovascular damage (London et al 2001, Nurnberger et al 2002, Hayashi et al 2002, Ueda et al 2004, Weber et al 2004, Weber et al 2005, Chirinos et al 2005).

One cross-sectional (Weber et al 2004) and three prospective (Chirinos et al 2005, Williams et al 2006, Hashimoto et al 2007) studies have also explored the contribution of AP (as opposed to just AIc) to cardiovascular damage (Table 1.2). In this regard, AP has been shown to predict adverse cardiovascular events including fatal strokes, acute myocardial infarctions, unstable angina and death, in elderly predominantly Caucasian male patients with coronary heart disease after a mean follow-up of 3.2±1.2 years (Chirinos et al 2005). Moreover, the Conduit Artery Function Evaluation (CAFE) study noted AP to be an independent predictor of cardiovascular outcomes in 2073 Caucasian elderly treated hypertensives (Williams et al 2006). Furthermore Hashimoto and colleagues demonstrated that a reduction in AP through antihypertensive therapy is associated with a decrease in left ventricular mass, in 46 Japanese middle-aged individuals after a year of therapy (Hashimoto et al 2007).

With regard to the forward pressure component, the CAFÉ study noted no independent association between P1 and cardiovascular events (Williams et al 2006). However, as this was an intervention study, one has to consider the possibility that current antihypertensive therapy cannot reduce P1 independent of distending pressures (see subsequent section) and hence P1 is unlikely to predict cardiovascular damage under these circumstances. Moreover, there are no reports assessing the relative role of P1 versus AP in contributing toward indexes of cardiovascular damage or cardiovascular outcomes in study samples where P1 has been demonstrated to contribute substantially toward aortic PP or systolic BP. As shall be discussed in subsequent sections (1.5.1), in the present thesis I compared the relative contribution of P1 and AP toward central PP in a community with a high prevalence of uncontrolled BP. As shall be described in chapter 2, in contrast to a number of studies, I was able to show a marked contribution of P1 to age-related increases in aortic PP and to the variability of aortic PP across the adult lifespan in the community studied. Thus, this finding provided an ideal opportunity for me to assess the contribution of

Table 1.2. Summary of recent clinical studies that have shown central augmentation index or augmentation pressure to contribute to cardiovascular damage.

Patient group (n)	Study design	Parameter	Outcome	Reference
Normotensive (67)	Cross-sectional	AIc	Left ventricular mass	Saba et al 1993
			Carotid wall thickness	
End stage renal disease (180)	Prospective	AIc	All cause mortality	London et al 2001
			Cardiovascular mortality	
Healthy and cardiovascular disease (216)	Cross-sectional	AIc	Cardiovascular risk scores	Nurnberger et al 2002
Suspected coronary artery disease (190)	Cross-sectional	AIc	Coronary artery disease	Hayashi et al 2002
Suspected coronary artery disease (465)	Cross-sectional	AIc, AP	Coronary artery disease	Weber et al 2004
Coronary angioplasty (103)	Prospective	AIc	Restenosis	Ueda et al 2004
Coronary angioplasty (262)	Prospective	AIc	All cardiovascular events	Weber et al 2005
Coronary artery disease (297)	Prospective	AIc, AP	All cause mortality	
			All cardiovascular events	Chirinos et al 2005
Hypertensives (2073)	Prospective	AIc, AP	All cardiovascular events	Williams et al 2006
Hypertensives (46)	Prospective	AIc, AP	Left ventricular mass	
			Reduction (with treatment)	Hashimoto et al 2007

AIc: Central augmentation index, AP: augmented pressure

P1 toward the variability of left ventricular mass in the community studied. The results of this study are described in chapter 3 of the present thesis and provide substantial evidence to indicate that P1 does indeed contribute toward cardiovascular damage.

1.4.5. Impact of anti-hypertensive drugs on the forward and augmented pressure components of central BP.

As indicated in section 1.3.3, not all antihypertensive agents are able to reduce central PP and systolic BP to a similar extent. Can this be attributed to differential effects of some classes of agents on AP or P1? In this regard, after 10 weeks of treatment of patients with untreated isolated systolic hypertension, despite atenolol (β-adrenergic receptor blocker), perindopril (ACEI), lercanidipine (CCB) and bendrofluazide (thiazide diuretic) producing similar changes in brachial artery BP, AP was reduced only by perindopril and lercanidipine (Mackenzie et al 2009). Although P1 was decreased by all agents, these effects are most likely to be attributed to reductions in distending pressures (Mackenzie et al 2009). Indeed, in that study (Mackenzie et al 2009) changes in neither P1 nor AP were adjusted for decreases in distending pressures. In contrast to these findings, in the Conduit Artery Function Evaluation (CAFÉ) study, after 4 years of therapy, P1 was significantly lower in the atenolol-treated group compared to the amlodipine-treated group. However, the reduced P1 value in the atenolol-treated group may be attributed to either a P1-lowering effect produced by atenolol, or an increase in P1 in the amlodipine-treated group, an effect that may be accounted for by a decrease in peripheral resistance and a consequent increase in stroke volume. Furthermore, although AP was decreased in the amlodipine treated group (Williams et al 2006), the change in AP from baseline in the two treatment groups was not reported on, and thus the greater benefit of amlodipine as compared to atenolol, could have been attributed to adverse effects on AP in the atenolol-treated group mediated through heart rate reduction (Williams et al 2006). Therefore there is limited evidence regarding the effects of differing antihypertensive drug classes on the forward and augmented pressure components

of central PP. Nevertheless, taken together, the current consensus is that there is little evidence to support a beneficial effect of current agents on the aortic structural changes that determine P1 independent of MAP, whilst the current weight of evidence is that many of the presently available antihypertensive agents may modify AP (Zieman et al 2005, Laurent et al 2007). What is the evidence to suggest that antihypertensive agents may modify the pathophysiological determinants of P1 independent of distending pressures?

As reviewed by Dart and Kingwell (2001) a number of studies have demonstrated that ACEIs, CCBs and some β-adrenergic receptor blockers can decrease aortic stiffness as indexed by carotid-femoral or brachial-radial pulse wave velocity (PWV) through effects that cannot be attributed entirely to an impact on distending pressures. However, in all of these studies the beneficial effects were noted over acute or relatively short-term periods, thus raising the question as to whether the benefits were indeed mediated through structural aortic changes, which after all take years to produce. As shall be highlighted in subsequent discussion, whether PWV is a close proxy for aortic stiffness or compliance changes produced by long-term damage to large vessels is nevertheless controversial.

If the pathogenesis of increases in aortic PP and systolic BP is principally through increases in AP, then it is unlikely that novel agents that target P1 will be required. An important question is therefore whether increases in central PP and systolic BP are principally through increases in AP or do increases in P1 contribute substantially toward increases in central PP and systolic BP? The following section will address this issue.

1.5 Potential determinants of the forward and augmented pressure components, independent of distending pressures

A number of factors have been identified as important determinants of aortic PP and systolic BP. Do these factors modify the aortic structural changes that account for P1 or do they influence mainly AP? The following sections will highlight our current understanding of the factors that influence aortic BP, review the potential mechanisms involved and

underscore the outstanding evidence that prompted me to perform the studies described in the present thesis.

1.5.1 Age as an important determinant of increases in the forward and reflected pressure components.

Aging is one of the primary determinants of increases in aortic systolic BP and PP. In this regard, it is well established that with increasing age, the elastin fibres in the media of large arteries, in particular the aorta, disintegrate and are replaced by collagen fibres. Collagen is at least 500 times stiffer than elastin and the amount of collagen in the aorta doubles from the age of 20 to 70 years (Nichols et al 2005). Thus, with the loss of elastin fibres and the replacement with collagen at increasing ages, the aorta becomes stiffer and less compliant. The increased aortic stiffness could produce two possible effects, either of which could account for age-related increases in aortic PP and systolic BP. First, a loss of aortic compliance and increased arterial stiffness could increase characteristic aortic impedance and thus P1. Second, an increased aortic stiffness could augment the speed of wave reflection producing an enhanced AP. Aging is also associated with a decrease in aortic dimensions, and as pointed out in section 1.4.2, a smaller aortic diameter increases aortic impedance and hence could enhance P1. Indeed, in elderly hypertensive men and women recruited as part of the 2nd Australian National Blood Pressure Study (ANBP2) aortic diameter was noted to be reduced (Dart et al 2008). Moreover, patients with isolated systolic hypertension have an increased central PP which has in-part been attributed to a decreased aortic diameter (Mitchell et al 2008). However, aortic root diameter does not predict the development of hypertension or BP progression (Ingelsson et al 2008). Despite these age-related structural changes in large vessels, consideration of the pressure components that account for aging effects on aortic systolic PP independent of distending pressures still generates considerable debate. What is the evidence for the role of AP and P1 or their determinants as mediators of age-related increases in aortic PP?

Whereas AP contributes toward aortic PP and systolic BP across the adult lifespan (Segers et al 2007, Cecelja et al 2009, Namasivayam et al 2009), P1 has been shown to contribute only modestly toward age-related increases in aortic PP and this effect only occurs after the age of 60 years (Mitchell et al 2004, Namasivayam et al 2009, Cecelja et al 2009) Furthermore, age-related increases in AP across the lifespan cannot be attributed to increases in aortic stiffness as indexed by aortic pulse wave velocity (PWV), but rather to the relative diameter of muscular arteries (Cecelja et al 2009), diameters that can easily be modified by alterations in vascular tone and hence antihypertensive agents.

Nevertheless, studies showing that AP accounts for most of the age-related increases in central PP and systolic BP across the adult lifespan (Mitchell et al 2004, Namasivayam et al 2009, Cecelja et al 2009) and studies that have demonstrated that the age-related increases in AP are not attributed to aortic PWV (Cecelja et al 2009), have been conducted in largely normotensive study samples. In contrast, P1 (Mitchell et al 2003) and the determinants of P1 (aortic stiffness and diameter) (Mitchell et al 2008) have been shown to account for increases in aortic PP in older persons with systolic hypertension in case-control study designs. Nevertheless, there are presently no studies that have assessed the role of P1 as a determinant of aortic PP across the adult lifespan in communities or populations with a high prevalence of uncontrolled hypertension. The lack of studies in this regard is particularly important because as previously pointed out in section 1.4.5, the current consensus is that there is little evidence to support a beneficial effect of available agents on the aortic structural changes that determine P1 independent of MAP, whilst the weight of evidence is that many of the existing antihypertensive agents may modify AP (Zieman et al 2005, Laurent et al 2007). Moreover, as shall be discussed in section 1.5.5, the aorta is a target organ of the ill effects of uncontrolled BP or hypertension, and indeed hypertension is associated with increases in aortic stiffness (or decreases in aortic compliance) which could increase P1.

As there is considerable uncertainty as to the role of P1 as a determinant of aortic PP in uncontrolled BP across the adult age range as opposed to in the elderly with isolated

systolic hypertension, in chapter 2 of the present thesis I assessed whether the presence of uncontrolled BP influences the relative contribution of P1 and AP or their determinants to age-related increases in aortic PP across the adult lifespan in 1015 randomly selected participants of a community sample with a high prevalence of uncontrolled BP.

1.5.2 Increases in aortic pulse wave velocity as a determinant of the forward and reflected pressure components.

As discussed in previous sections, an increased arterial stiffness may enhance the speed of wave reflection (as indexed by the speed of wave travel down the aorta assessed using carotid-femoral [aortic] pulse wave velocity [PWV]) and consequently increase AP. Furthermore, according to the water hammer equation and assuming the absence of wave reflection, aortic impedance =4xaortic pulse wave velocity (PWV)x$\rho/\pi D^2$ (see section 1.4.2). As aortic impedance is an important determinant of P1, PWV could also contribute toward P1. Many studies have evaluated the role of aortic pulse wave velocity (PWV) as a contributor of aortic PP or systolic BP. Although advancing age promotes aortic PWV (Benetos et al 2002, Mitchell et al 2004, McEniery et al 2005) a number of studies have failed to show that age-related changes in aortic PWV parallel age-related increases in central BP, AP or AIc (Mitchell et al 2004, McEniery et al 2005, Cecelja et al 2009). Indeed, whilst with age central BP and AP follow a gradual linear increase across the adult age-range, aortic PWV increases to only a minor extent in the young-to-middle aged with a subsequent marked increase in the elderly (McEniery et al 2005).That is, age-related increases in aortic PWV approximate an exponential function. Furthermore, age-related changes in aortic impedance are similarly not paralleled by age-related changes in aortic PWV (Segers et al 2007). In addition, in isolated systolic hypertension increases in aortic PWV do not account for increases in characteristic impedance, but characteristic impedance does account for increases in central BP independent of distending pressures (Mitchell et al 2003). Therefore

the role of aortic PWV as a determinant of aortic BP independent of distending pressures has been questioned.

Although aortic PWV may not parallel age-related increases in P1, AP or central PP, or contribute toward increases in central PP associated with isolated systolic hypertension, aortic PWV may contribute toward the impact of alternative factors on central PP and the component pressures. Indeed, 6.6% of the variation in central PP may be attributed to the effect of aortic PWV on P1 (Cecelja et al 2009) and aortic PWV contributes to central PP independent of central augmentation index in men but not in women (Farasat et al 2008) a finding that suggests an effect on P1. Although this may not be mediated by an impact on central PP or the component pressures, aortic PWV is also a predictor of cardiovascular risk and outcomes independent of distending pressures or brachial artery BP (Table 1.3). If the ability of aortic PWV to predict cardiovascular outcomes beyond brachial BP or distending pressures (Table 1.3) is not mediated through alterations in central BP, then one has to consider the possibility that aortic PWV is a target organ change produced by hypertension, diabetes mellitus, smoking, obesity or alternative risk factors and hence acts as a surrogate rather than a mediator of long-term cardiovascular damage.

1.5.3 The effective reflecting distance and reflected wave transit time as measures of the contribution of wave reflection to the augmented pressure component

The effective reflecting distance (ERD) is the distance from the heart to the site of peripheral wave reflection. By reducing the ERD, the time taken for wave reflection to occur will be decreased, and hence the chances that the reflective wave coincides with the forward wave during systole instead of diastole will increase. This will subsequently increase AP and hence central PP and systolic BP. The reflective wave transit time (RWTT) is a measure of the time it takes for the reflected pressure wave to travel from the site of wave reflection back to the heart. Decreasing the RWTT may result in an earlier return of the reflected wave to the

Table 1.3. Summary of recent clinical studies that have shown aortic pulse wave velocity to predict cardiovascular events and mortality.

Patient group	Mean follow-up (years)	Sample size (n)	Outcome	Reference
Essential hypertension	12.0	710	Cardiovascular mortality	Blacher et al 1999
Essential hypertension	9.3	1980	All cause mortality	Laurent et al 2001
			Cardiovascular mortality	
Elderly participants	2.5	141	Cardiovascular mortality	Meaume et al 2001
End stage renal disease	4.3	180	All cause mortality	London et al 2001
			Cardiovascular mortality	
Type 2 diabetes mellitus	10.7	397	All cause mortality	Cruickshank et al 2002
			Cardiovascular mortality	
Essential hypertension	5.7	1045	Incidence of coronary event	Boutouyrie et al 2002
End stage renal disease	6.5	242	All cause mortality	Blacher et al 2003
			Cardiovascular mortality	
Essential hypertension	7.9	1715	Incidence of fatal stroke	Laurent et al 2003
Healthy population	9.4	1678	Cardiovascular mortality	Hansen et al 2006
Healthy population	4.1	101	Coronary heart disease and stroke	Mattace-Raso et al 2006
Elderly participants	7.8	2232	Major cardiovascular events	Mitchell et al 2010

Recently published studies which independently relate aortic PWV to clinical outcomes, after multivariable adjustment for potential confounders.

heart, increasing the chances that the reflected wave coincides with the forward wave during systole instead of diastole thus enhancing AP and hence central aortic PP and systolic BP.

In the Framingham study, ERD has been demonstrated to increase with advancing age, whereas the RWTT decreases with age (Mitchell et al 2004). However, whether these parameters can account for age-related increases in central PP through alterations in AP is unknown. In this regard, there are no studies that have assessed whether adjustments for either of these parameters modify the contribution of AP to age-related increases in central PP and thus further investigation is required. However, the aims of the present thesis did not address this issue.

1.5.4 Impact of stroke volume on the forward pressure component.

As previously indicated (section 1.4.2) stroke volume may contribute to increases in central PP through an enhanced P1. In this regard, to my knowledge few studies have evaluated relationships between stroke volume and central PP or the component pressures. Stroke volume has been demonstrated to account for central PP in young individuals with isolated systolic hypertension (McEniery et al 2005) and in a normotensive population sample of middle-aged participants (Segers et al 2007). However, in a study conducted in a normotensive sample (Segers et al 2007) over a narrow age-range (35-55 years), consistent with previous studies conducted over a wide age range (17-76 years) (Alfie et al 1999), stroke volume did not increase with age and hence stroke volume could not account for age-related increases in PP (Segers et al 2007). However, whether stroke volume contributes toward age-related increases in central BP in samples where P1 accounts for a significant proportion of age-related increases in central BP has not been evaluated. As in the present thesis I could show that P1 and AP contributed equally to age-related increases in central PP across the lifespan in a community with a high prevalence of uncontrolled BP, the present thesis allowed me to test this hypothesis. Therefore, in chapter 2 of the present thesis I also assessed whether stroke volume accounts for age-related increases in P1 and central PP.

1.5.5 *Contribution of hypertension, diabetes mellitus and dyslipidaemia toward the pressure components of central pulsatile pressures.*

As indicated in an aforementioned section (1.5.1) P1 (Mitchell et al 2003) and the determinants of P1 (aortic stiffness and diameter) (Mitchell et al 2008) have been shown to account for increases in aortic PP in older persons with systolic hypertension (isolated systolic hypertension) in case-control studies. These changes have largely been attributed to aging effects. However, as previously highlighted in section 1.5.1, although aging is associated with marked increases in AP and AP makes a strong contribution to age-related increases in aortic PP across the lifespan, P1 only makes a modest contribution to age-related increases in aortic PP and only after 60 years of age (Namasivayam et al 2009, Cecelja et al 2009). However, these studies (Namasivayam et al 2009, Cecelja et al 2009) were conducted in largely normotensive participants. Presently there is no data to show the relative contribution of P1 and AP to central PP across the adult lifespan in community or population samples with a high prevalence of uncontrolled hypertension. It is nevertheless well-accepted that hypertension causes large artery stiffening (Schiffrin et al 2004, Blacher and Safar 2005, Laurent and Boutouyrie 2007, Laurent et al 2007) and hence it is possible that hypertension could enhance age-related increases in P1 through decreases in aortic compliance, increases in AP through an increased wave reflection, or increases in both of the pressure components across the adult lifespan independent of distending pressures. However, this phenomenon is only likely to occur in communities with poorly controlled hypertension. To test this hypothesis, in the present thesis in chapter 2 I assessed the relative contribution of P1 and AP or their determinants to age-related increases in aortic PP across the adult lifespan in 1015 randomly selected participants of a community sample with a high prevalence of uncontrolled hypertension.

Other than age and hypertension, alternative cardiovascular risk factors such as diabetes mellitus and an impaired glucose control, tobacco smoking, or dyslipidaemia have

also been associated with changes in large artery function. In type II diabetes mellitus a reduced distensibility of large arteries may occur (Lehmann and Sonsken 1992). Moreover, an increased arterial stiffness has been noted to occur in response to an impaired blood glucose control (Cruickshank et al 2002, Henry et al 2003, Schram et al 2004, Cameron and Cruickshank 2007), changes which may be accounted for by an increased glycosylation and hence stiffening of vascular wall collagen, or through the development of atheroma. However, the role of diabetes mellitus as a determinant of the aortic forward and augmented pressure component and hence central PP has not been fully evaluated and hence further work in this regard is still required. Although this was not an aim of the present thesis, it is nevertheless important to note that because of these findings, and the fact that in the community studied in the present dissertation a high proportion of participants had type II diabetes mellitus or a high prevalence of an HbA_{1C}>6.1%, I ensured that all relationships were adjusted for these factors.

There is indeed also evidence for a role of hypercholesterolaemia in contributing toward AP as indexed by augmentation index and hence central PP (Wilkinson et al 2002). The exact mechanisms of this effect are nevertheless obscure and unlike other determinants of aortic PP may not relate to large artery stiffness or compliance. In this regard, the slope of age-related increases in arterial stiffness is decreased rather than increased in patients with marked hypercholesterolaemia (Dart et al 1991) and in familial hypercholesterolaemia large arteries are more rather than less compliant (Lehmann et al 1992). Moreover, although one study has demonstrated that older patients with familial hypercholesterolaemia have reduced large artery compliance (Pitsavos et al 1998) an alternative study showed no change (Toikka et al 1999). As the community studied in the present thesis is recognised as having remarkably low median concentrations for cholesterol and on exploratory analysis I therefore could not reproduce the results of Wilkinson et al (2002), I did not pursue the role of hypercholesterolaemia as a potential determinant of central PP and the pressure components of aortic PP. Moreover, because of the lack of relationship between cholesterol concentrations and any of the measures of aortic function on exploratory analysis, in the

present thesis I also did not adjust for plasma cholesterol concentrations when assessing the impact of alternative effects.

1.5.6 Smoking as a potential determinant of aortic haemodynamics

Several studies suggest that tobacco smoking may play a role in contributing to central haemodynamic measurements (Wilkinson et al 2002, Mahmud and Feely 2003, Rehill et al 2006, McEniery et al 2008, Minami et al 2009, Woodiwiss et al 2011). Indeed, tobacco smoking has been associated with an increased aortic stiffness (Jatoi et al 2007), potentially mediated through changes in large artery wall thickness and loss of elastin fibres (Liang et al 2001). With regard to central systolic BP and PP, two studies demonstrated an increase in central systolic BP in smokers compared to non-smokers (Mahmud and Feely 2003, Minami et al 2009). In contrast, Woodiwiss et al (2011) could not demonstrate an independent relationship between central systolic BP or PP and smoking. However, the average number of cigarettes smoked per day in that study (Woodiwiss et al 2011) was considerably lower compared to prior studies (Mahmud and Feely et al 2003, Minami et al 2009). Indeed, the independent contribution of smoking to central BP appears to be dependent on the severity of smoking status i.e. number of cigarettes smoked daily (Minami et al 2009). Moreover, evidence suggests that smoking accounts for an increased AP, as indexed by AIc (Wilkinson et al 2002, Rehill et al 2005, Mahmud and Feely 2003, Jatoi et al 2007, Minami et al 2009, McEniery et al 2010) and thus central PP, but whether smoking contributes to P1 is unknown. Therefore, the role of tobacco smoking as a determinant of central PP and the component pressures requires further investigation. However, the contribution of tobacco smoking to central PP was not an aim of the present thesis, but because of these previous findings, I ensured that all independent relationships assessed in this thesis were adjusted for tobacco smoking as a potential confounder.

1.5.7 Role of sodium intake as a potential determinant of aortic pulsatile pressures and the component pressures.

Until the time of the work described in chapter 4 of the present thesis, there has been little evidence to support a role for salt (sodium [Na^+]) intake as a potential determinant of aortic PP and the component pressures independent of distending pressures. As part of the present thesis I assessed the hypothesis that the impact of salt intake on BP may occur preferentially in central as opposed to brachial arteries, whether these effects were independent of distending pressures and I identified whether these effects can be explained by changes in P1, AP or both pressure components of aortic PP. These data have been published in the journal *Hypertension* (Redelinghuys et al 2010). What is the evidence that led me to hypothesise that the relationship between salt intake and BP may occur preferentially in central as opposed to brachial arteries?

Observational and intervention studies have documented the adverse effects of Na^+ intake on brachial BP. The INTERSALT study and Scottish Heart study were the first observational studies conducted in large study samples (n=7354-10079) to show a relationship between urinary indices of Na^+ intake and brachial systolic and diastolic BP in the general population (Intersalt Cooperative Research Group 1988, Smith et al 1988). In this regard, the INTERSALT study showed 24-hour urinary Na^+ intake to be related to brachial systolic BP and both the INTERSALT and the Scottish Heart Study showed urinary Na^+/(potassium [K^+]) to be related to brachial BP (Intersalt Cooperative Research Group 1988, Smith et al 1988). More direct evidence for an important role of salt intake in modifying BP come from intervention studies. Intervention studies have reported that brachial BP is decreased with a reduced Na^+ intake in both normotensive (He et al 2000) and hypertensive (Benetos et al 1992, Sacks et al 2001, Seals et al 2001, He at al 2005, Gates et al 2004, He et al 2009) individuals. What is the evidence to suggest that salt intake can influence pulsatile pressures independent of distending pressures?

Although an impact of excessive Na^+ intake on increases in brachial PP has been demonstrated (du Cailar et al 2004, Buyck et al 2009, Bankir et al 2007, Haijar et al 2001, He et al 2009) in only one study was distending pressure, as indexed by MAP, accounted for (du Cailar et al 2004) and this analysis was sex and age-specific (du Cailar et al 2004). Thus, in the majority of studies the relationship between Na^+ intake and brachial PP (Haijar et al 2001, Bankir et al 2007, Buyck et al 2009, He et al 2009) may be attributed to the well-recognised effect of Na^+ intake on distending pressures. Importantly, in none of these studies (Haijar et al 2001, Bankir et al 2007, Buyck et al 2009, He et al 2009) was the relationship between Na^+ intake and central PP or its component pressures assessed.

Two studies have assessed the relationships between Na^+ intake and central aortic or carotid as opposed to brachial PP (Gates et al 2004, Starmans-Kool et al 2010), one of which (Starmans-Kool et al 2010) was published after I published the data described in Chapter 4 (Redelinghuys et al 2010), and both of which failed to adjust for distending pressures (Gates et al 2004). Moreover, these studies were conducted in small study samples (n=10-12) (Gates et al 2004, Starmans-Kool et al 2010) and hence may not reflect effects across a wide age range or alternative characteristics of the populations sampled. Furthermore, these studies failed to assess whether the relationships between Na^+ intake and central BP independent of distending pressure was more pronounced than the relationship with brachial PP (Gates et al 2004, Starmans-Kool et al 2010). Thus, whether relationships between salt intake and central BP are because salt intake modifies the properties of the aorta that cannot be detected from brachial BP measurements and is independent of distending pressure is uncertain. Moreover, as P1 is largely determined by aortic structural changes which occur in the elderly and which are likely to take many years to develop, no conclusions can be drawn on the impact of changes in Na^+ intake over a 4 week (Gates et al 2004) or 6 week (Starmans-Kool et al 2010) study period on P1. Thus, further studies in large population samples, with adjustments for distending pressures; which assess whether the effect of salt intake on aortic PP is more robust than that produced on

brachial BP; and with a study design that accounts for a potential long-term effect of salt intake on aortic PP and the pressure components, in particular P1, are still required.

The chances that a relationship exists between salt intake and aortic PP and the component pressures beyond distending pressures and brachial BP is most likely in communities of African ancestry, which have been reported to have a high prevalence of salt-sensitive hypertension (Sowers et al 1988, Morris et al 1999, Sacks et al 2001, Wright et al 2003, Jurgens et al 2008). In this regard, salt-sensitive hypertensive patients have diminished large artery distensibility compared to salt-resistant hypertensive patients with the same cardiac output, plasma volume, brachial BP and MAP (Draaijer et al 1993). Therefore in salt-sensitive communities salt intake is likely to enhance arterial stiffening independent of the volume changes which are usually associated with a high Na^+ intake (Draajier et al 1993) and through effects that cannot be detected by brachial BP measurements or through effects on distending pressures. This notion is also in-keeping with the association between an increased Na^+ intake and increases in indices of arterial stiffening in a number of studies (Avolio et al 1985, Avolio et al 1986, Draaijer et al 1993, Seals et al 2001, Gates et al 2004, He et al 2009). Whether these associations between salt intake and indices of arterial stiffening can be attributed to aortic structural changes that affect P1 is highly questionable. Indeed, modifying Na^+ intake for relatively short periods is able to change indices of arterial stiffness (Seals et al 2001, Gates et al 2004, He et al 2009). Importantly, in a multi-ethnic study conducted in mild hypertensives, a modest reduction in Na^+ intake reduced brachial BP in the groups of European, African, and Asian origin (He et al 2009). However, aortic PWV, employed as an index of large artery stiffness, was diminished only in the group of African ancestry (He et al 2009). Thus, there is significant evidence to suggest that salt-sensitivity may modify indices of large artery stiffness, particularly in groups of African ancestry. However, whether this translates into increases in either P1 or AP and consequently increases in aortic BP beyond distending pressures and produce a more robust effect on aortic as compared to brachial PP has not been determined.

The contribution of salt intake to aortic PP is also particularly important in salt-sensitive populations, as salt-sensitivity is a predictor of cardiovascular mortality independent of hypertension status (Weinberger et al 2001). Whether this may be accounted for by salt effects on aortic PP and the pressure components beyond that produced by effects on brachial BP or distending pressures has yet to be assessed. However, evidence is first required to show that in salt-sensitive populations, salt intake is associated with aortic PP and the component pressures beyond distending pressures and that the association is more robust with aortic PP as compared to brachial artery PP.

To address the questions of whether salt intake is associated with aortic PP beyond distending pressures, whether this effect is more marked for aortic as compared to brachial PP, whether this effect involves P1 or AP, and whether this effect involves an impact on aortic PWV, an index of aortic stiffness, or other determinants of aortic PP, in chapter 4 of the present thesis I therefore assessed the relationship between urinary indices of salt intake and brachial PP, aortic PP, the pressure components of aortic PP and the determinants of aortic PP in a large, randomly selected community sample of African ancestry. As previously indicated these data have been published in-part in the journal *Hypertension* (Redelinghuys et al 2010).

1.5.8 Relationship between C-reactive protein and central PP or the pressure components

There are a number of reasons to support a hypothesis that low-grade inflammation produced by pro-inflammatory substances either released from adipocytes (Maachi et al 2004), stimulated by tobacco smoking (Mendenhall et al 1996, Tracy et al 1997), produced by genetic factors that control the expression or activity of pro-inflammatory substances (Pankow et al 2001 Worns et al 2006) or produced because of the presence of low-grade systemic infections associated with a poor dental hygiene or other chronic infective states, may contribute toward aortic BP independent of distending pressures and that these increases in aortic BP may not be reflected in BP measured at the brachial artery. One

potential mechanism is through the development of atherosclerotic plaque which is associated with structural alterations in large vessels thus potentially changing aortic compliance and diameter and hence characteristic impedance. In this regard, atheroma formation involves an increased expression of adhesion molecules on the endothelial surface of the arterial wall and the production of chemo-attractant chemokines (Pasceri et al 2000, Pasceri et al 2001). These changes lead to the migration of leucocytes and macrophages into the subendothelium. Macrophages engulf oxidized low density lipoproteins in the subendothelium to form foam cells, the nidus of the atherosclerotic plaque. This process is thought to be mediated by the inflammatory molecule C-reactive protein (CRP) (Zwaka et al 2001). C-reactive protein may also mediate structural changes in the arterial wall through an increased production of metallo-proteinases which degrade components of the interstitium and through a loss of elastane fibres (Yasmin et al 2005) thus also modifying aortic structure and potentially aortic compliance and diameter and hence characteristic impedance. What is the evidence to suggest that CRP or low-grade inflammation may potentially contribute to the pathogenesis of increases in central BP?

A causal relationship between acute inflammatory states induced by vaccination and reversible increases in aortic stiffness, as indexed by aortic PWV, has been demonstrated (Vlachopoulos et al 2005). However, the fact that inflammation mediated by vaccinations produces increases in aortic PWV that is acute and reversible (Vlachopoulos et al 2005) suggests that it is not mediated through structural changes in the aorta. Nevertheless, several studies have shown relationships between the inflammatory marker CRP (high sensitivity CRP [hs-CRP]) and central haemodynamic variables in participants largely free of inflammatory or cardiovascular disease (Kampus et al 2004, Yasmin et al 2004, Duprez et al 2005, Nagano et al 2005, Kullo et al 2005, Saijo et al 2005, Andoh et al 2006). Nevertheless, in contrast to these studies conducted in small study samples (n=78-427) (Kampus et al 2004, Yasmin et al 2004, Duprez et al 2005, Kullo et al 2005, Andoh et al 2006), in a large (n=2409) community-based study, Schnabel et al (2008) were unable to show independent relationships between hs-CRP concentrations and central PP, P1 or aortic pulse wave

velocity (PWV). However, the inclusion of interleukin-6, an inflammatory marker which shows marked co-linearity with hs-CRP, as a confounder in the regression models, may have eliminated the independent relation between hs-CRP and central haemodynamic variables (Schnabel et al 2008). Furthermore, in that study (Schnabel et al 2008) the median values for hs-CRP concentrations were well below the threshold for a high cardiovascular risk (Pearson et al 2003). The role of hs-CRP as a potential determinant of central aortic haemodynamics in communities with a significant prevalence of high risk hs-CRP concentrations therefore requires investigation.

In chapter 5 of the present thesis I assessed whether hs-CRP concentrations are associated with increases in central BP, its pressure components, and potential determinants, namely aortic PWV, reflected wave transit time (RWTT) and effective reflecting distance (ERD), independent of steady state pressure and other confounders in a large community-based sample with a high prevalence of risk-related hs-CRP concentrations. As shall be discussed in chapter 5 of the present thesis, in the community studied the high prevalence of risk-related hs-CRP concentrations is driven by the high prevalence of obesity. Indeed, obesity has previously been demonstrated to be strongly associated with increased hs-CRP concentrations (Ford et al 1999, Visser et al 1999, Festa et al 2001). This relationship exists presumably because adipocytes release pro-inflammatory cytokines, namely tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6), both of which are able to stimulate the production of hs-CRP in the liver (Maachi et al 2004).

1.5.9 Genetic factors and the forward and augmented pressure components of aortic pulse pressure

There is considerable evidence to suggest that genetic factors contribute to the variability of brachial systolic and diastolic BP (Snieder et al 2000, Adeyemo et al 2002, Camp et al 2003, Fava et al 2004, Bayoumi et al 2007, Bochud et al 2005, van Rijn et al 2007). As a consequence, in order to identify novel therapeutic targets for managing brachial

BP, a number of genome-wide association and linkage studies have been performed or are currently underway. Are the genetic determinants of distending pressures likely to be the same as those that determine pulsatile pressure?

As highlighted in the present chapter, the haemodynamic determinants of PP are considerably different from those of distending pressures. Thus, if PP is indeed inherited, the genetic determinants of PP may be distinct from those that determine distending pressures (Camp et al 2003). In the past decade there have also been a number of studies that have reported on heritability estimates of brachial PP in different populations, with estimates ranging from 13 to 53% (Snieder et al 2000, Adeyemo et al 2002, Snieder et al 2003, Fava et al 2004, Bochud et al 2005, Bielinski et al 2005, Van Rijn et al 2007, Seidlerova et al 2008). Thus, for brachial pressures at least, genetic factors are important determinants of the pulsatile component of BP. However, as highlighted throughout the present chapter, aortic PP, which may be more important in mediating the damaging effects of pulsatile pressures than brachial PP, are determined by a variety of factors that are unique to changes in large arteries. Thus, if aortic PP is indeed inherited, the genetic factors that account for aortic PP may be different from those that account for brachial PP. Nevertheless, a number of relatively recent studies have demonstrated that central PP, the AP and P1 pressure components of aortic PP and indices of aortic stiffness, including aortic PWV are indeed inherited (Mitchell et al 2005, Sayed-Tabatabaei et al 2005, Pilia et al 2006, Levy et al 2007, Seidlerova et al 2008, Cecelja et al 2009). Is there outstanding evidence that still requires further studies being conducted to assess the overall genetic contribution to the AP and P1 pressure components of aortic PP and indices of aortic stiffness, including aortic PWV?

Of the studies that have assessed the heritability of central PP, the AP and P1 pressure components of aortic PP and indices of aortic stiffness (Mitchell et al 2005, Sayed-Tabatabaei et al 2005, Pilia et al 2006, Levy et al 2007, Seidlerova et al 2008, Cecelja et al 2009) three studies have reported on the heritability of the P1 and AP pressure components of central PP (Mitchell et al 2005, Levy et al 2007, Cecelja et al 2009). However, in two of these studies (Mitchell et al 2005, Levy et al 2007) heritability estimates were not adjusted for

an index of distending pressures. Thus, the heritability of either P1 or AP in these studies (Mitchell et al 2005, Levy et al 2007) could be attributed to the well-known role of genetic effects on distending pressures (MAP). Moreover, in the remaining study (Cecelja et al 2009) the heritability estimates of AP and P1, although adjusted for MAP, were determined in one sex only and hence these effects could be sex-specific. Furthermore, in none of the studies evaluating the heritability of central PP and the component pressures (Mitchell et al 2005, Levy et al 2007, Cecelja et al 2009) was the contribution of aortic PWV to the heritability of central PP and the pressure components formally evaluated. In this regard, heritability estimates for central PP, AP and P1 can be determined before and after adjustments for aortic PWV. Consequently the haemodynamic mechanisms responsible for the impact of genetic factors on central PP are uncertain. Without knowledge of these haemodynamic mechanisms, identifying the genetic determinants that may lead to novel drug development targeting central PP will remain limited. Consequently, in chapter 6 of the present thesis in nuclear families of African ancestry I evaluated whether independent of distending pressures, significant intra-familial aggregation and heritability of central PP, P1, AP, aortic PWV and alternative haemodynamic determinants of central PP, occurs. Moreover, I assessed whether aortic PWV can in-part mediate the effect of genetic factors related to central PP, P1 and AP.

1.6 Summary of problem statements

The background regarding the outstanding issues addressed in the present thesis has been thoroughly described in the previous sections of this chapter. However, I will briefly summarise the problem statements addressed by studies performed in the present thesis. As mentioned, the present thesis was designed to address some outstanding issues regarding the mechanisms and impact of the component pressures of central BP, namely P1 and AP. Presently, the contribution of P1 and AP, and their determinants, to increases in central BP in communities or populations with a high prevalence of uncontrolled hypertension is unknown.

Moreover, there is uncertainty as to whether the pressure components of central PP, namely P1 and AP, contribute independently to cardiovascular damage.

In addition, the impact of salt intake on central PP and its pressure components, independent of distending pressures is unknown. Furthermore, whether the presence of low-grade inflammation, as indexed by hs-CRP, contributes towards central PP and the component pressures, is unclear. Last, whether genetic factors influence central PP, P1 and AP, independent of distending pressures and through alterations in aortic PWV is similarly uncertain. Thus the aims of the present thesis can be summarised as follows:

1.7 Aims

a) To evaluate the relative contribution of aortic P1 and AP pressure components to increases in PPc, independent of steady state blood pressure across the adult lifespan in a community sample with poor blood pressure control. These data are presented in chapter 2.

b) To determine the independent relationship between P1 or AP and left ventricular mass index in a community sample with a high prevalence of uncontrolled blood pressure. These data are presented in chapter 3.

c) To clarify whether indices of salt intake are associated with central (aortic) PP, its pressure components (P1 and AP) and its determinants independent of distending pressure. These data are presented in chapter 4 and published in the journal *Hypertension* (Redelinghuys et al 2010).

d) To evaluate whether increases in hs-CRP concentrations can independently account for age-related increases in central PP, AP and P1, or the determinants of central PP in a community sample of African ancestry, with a high incidence of risk-related hs-

CRP concentrations. These data are presented in chapter 5 and have been published in the *American Journal of Hypertension* (Redelinghuys et al 2011).

e) To identify the relative contribution of aortic PWV to the intra-familial aggregation and heritability of central PP, P1 and AP, independent of distending pressure. These data are presented in chapter 6.

CHAPTER 2

Relative Roles of Aortic Augmentation and Forward Pressures Across the Adult Lifespan in a Community of African Descent with a High Prevalence of Uncontrolled Hypertension.

Abstract

Background. Current antihypertensive therapy reduces the augmented pressure (AP) component but not the forward component (P1) of central aortic pulse pressure (PPc), independent of distending pressures. Although in largely healthy, normotensive population samples, AP may play a more important role in determining age-related increases in PPc than P1, whether similar effects are noted in communities with a high prevalence of uncontrolled hypertension is uncertain.

Aim. In the present study I aimed to determine the relative contribution of P1 and AP to increases in PPc in a community sample with poor blood pressure (BP) control.

Methods and results. Applanation tonometry and a population-based transfer function were employed to determine aortic BP in 1015 randomly recruited participants (range=16-88years) from a community sample of black African descent, 37.7% of whom had uncontrolled hypertension. Stroke volume was determined using echocardiography. Across the adult lifespan and independent of distending pressures (mean arterial pressures), for every 1 SD increase in age (18.4 years), PPc increased by 6.2±3.0mm Hg, AP by 3.6±1.7mm Hg and P1 by 2.6±2.1mm Hg ($p<0.001$ for all). The age-related increase in PPc and P1 was not associated with similar age-related increases in stroke volume. In addition, with appropriate adjustments, the relationship between P1 and PPc (partial $r=0.90$, $p<0.0001$) was equally as strong as the relationship between AP and PPc (partial $r=0.87$, $p<0.0001$).

Conclusions. In a community of African ancestry with a high prevalence of uncontrolled BP, P1 contributed as much as AP to age-related increases in PPc and to variations in PPc. These data support the use of antihypertensive therapy that targets both P1 and AP to achieve optimal decreases in PPc in communities with a high prevalence of hypertension.

2.1 Introduction

As highlighted in chapter 1 of the present thesis, the adverse actions of blood pressure (BP) are currently viewed in terms of distending effects, indexed by mean arterial pressure (MAP), and dynamic or pulsatile effects, indexed by pulse pressure (PP). In various clinical populations, aortic or central PP (PPc) or indexes of augmentation of PPc (augmentation index [AIc], augmentation pressures [AP] and the backward pressure wave) are more closely associated with cardiovascular outcomes than PP or MAP measured at the brachial artery (London et al. 2001, Safar et al 2002, Chirinos et al 2005, Weber et al 2005, Williams et al 2006, Roman et al 2007, Pini et al 2008, Wang et al 2009, Wang et al 2010, Vlachopoulos et al 2010). Developing therapeutic strategies that target the mechanisms responsible for increases in PPc without influencing MAP has therefore attracted considerable attention.

The component pressures of PPc include AP, which numerically contributes little to PPc, and a forward or incident pressure component (P1), which is largely determined by stroke volume and aortic structural changes and numerically contributes substantially more to PPc than does AP. Considering the relatively small numerical contribution of AP to PPc, the ability of AP, and AIc to predict cardiovascular outcomes beyond brachial pressures (London et al 2001, Chirinos et al 2005, Weber et al 2005, Wang et al 2010, Vlachopoulos et al 2010) may appear surprising. However, some studies have demonstrated that AP contributes substantially more to age-related increases in PPc and variations in PPc than P1 (McEniery et al 2005, Cecelja at l 2009, Namasivayam et al 2009), findings which are nevertheless, not supported by other studies (Mitchell et al 2004, Mitchell et al 2010). However, all of these prior studies (McEniery et al 2005, Cecelja at l 2009, Namasivayam et al 2009, Mitchell et al 2004, Mitchell et al 2010) have been performed in samples with largely well-controlled BP values, whereas the relationship between AP or AIc and cardiovascular outcomes beyond brachial BP has been noted in unselected or specific clinical populations (London et al 2001, Chirinos et al 2005, Weber et al 2005, Wang et al 2010, Vlachopoulos et

al 2010). As described many years ago (O' Rourke, 1970) and subsequently further confirmed (Mitchell et al 2003, McEniery et al 2005, Mitchell et al 2008) in hypertension in the young and the elderly, P1 and the aortic functional determinants of P1 are considerably modified. Therefore, it is possible that the relative contribution of P1 and AP to PPc in communities with a high prevalence of uncontrolled BP is modified. In the present study I therefore evaluated the relative contribution of P1 and AP to age-related increases in PPc across the adult lifespan and to variations in PPc in a randomly selected community sample with a high prevalence of uncontrolled hypertension (Norton et al 2008, Woodiwiss et al 2009, Redelinghuys et al 2010).

2.2 Methods

2.2.1 Study participants.

The present study was approved by the Committee for Research on Human Subjects of the University of the Witwatersrand (approval number: M02-04-72 and renewed as M07-04-69) and was conducted according to the principles outlined in the Helsinki declaration. Participants gave informed, written consent. The present study design has previously been described (Maseko et al 2006, Norton et al 2008, Woodiwiss et al 2009). Nuclear families of black African descent (Nguni and Sotho chiefdoms) with siblings older than 16 years were randomly recruited from the South West Township (SOWETO) of Johannesburg, an urban developing community in South Africa, using the population census figures of 2001. Of the 1029 participants enrolled in the study, 1015 had central haemodynamic measurements and in a sub-study, 503 had stroke volume measurements obtained from echocardiography.

2.2.2 Clinical, demographic and anthropometric assessments.

A standardized questionnaire was administered to obtain demographic and clinical data as previously described (Norton et al 2008, Woodiwiss et al 2009). Included in the questionnaire were specific requests for date of birth, sex, previous medical history, the presence of hypertension, diabetes mellitus and kidney disease, prior and current drug

therapy (analgesic use included), smoking status (including the number of cigarettes smoked in the past and at the present time), daily alcohol consumption (beer, traditional beer or other forms of alcohol and the daily quantity), and family history of hypertension and cardiovascular events. For females, menstrual history, history of pregnancies and oral contraceptive use was evaluated.

In order to avoid translational errors, the questionnaire was not translated into an African language, but trained study assistants familiar with all languages spoken in this township and who either previously lived in Soweto or currently reside in Soweto assisted with the completion of each questionnaire. Only same sex assistants were used to assist each family member with the completion of the questionnaire. Support was only provided when requested. The majority of participants were reasonably proficient in English. At an initial home visit, the questionnaire was explained to the participants. The questionnaire was then completed at an office visit. Medications, alcohol consumption and tobacco use reported on in the questionnaire were subsequently verified during the second home visit or through telephonic contact with families. A pilot study was conducted in 20 participants to ensure that data obtained in the questionnaires were reproducible when obtained with the assistance of two separate study assistants.

Height and weight were measured with participants standing, wearing indoor clothing and no shoes. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Participants were identified as being overweight if their body mass index (BMI) was ≥25 kg/m^2 and obese if their BMI was ≥30 kg/m^2.

2.2.3 Conventional blood pressure

A trained nurse-technician measured conventional (brachial) blood pressure (BP) using a standard mercury sphygmomanometer. After participants had rested in a seated position for five minutes brachial BP was measured five consecutive times, 30 to 60 seconds apart. The cuff was deflated at approximately 2 mm Hg per second and Korotkov phases I and V were employed to identify systolic and diastolic BP respectively. Care was taken to avoid auscultatory gaps. Standard cuffs were used with an inflatable bladder with a length of

22 cm and a width of 12 cm except when arm circumference exceeded 31 cm, when larger cuffs with a 31 x 15 cm bladder were employed. The five readings were averaged to obtain a single systolic and diastolic BP reading. Hypertension was defined as a mean BP≥140/90 mm Hg (Chobanian et al 2003, Williams et al 2004, Mancia et al 2007), or the use of antihypertensive medication. In the present study quality control of BP measurements was assessed as previously described (Majane et al 2007). Only 2.27% of visits had fewer than the planned BP recordings. The frequency of identical consecutive recordings was 0.30% for systolic BP and 1.50% for diastolic BP. The occurrence of BP values recorded as an odd number was 0.02%. Of the 9953 systolic and diastolic BP readings, 29.89% ended on a zero (expected =20%).

2.2.4 Laboratory (blood) tests.

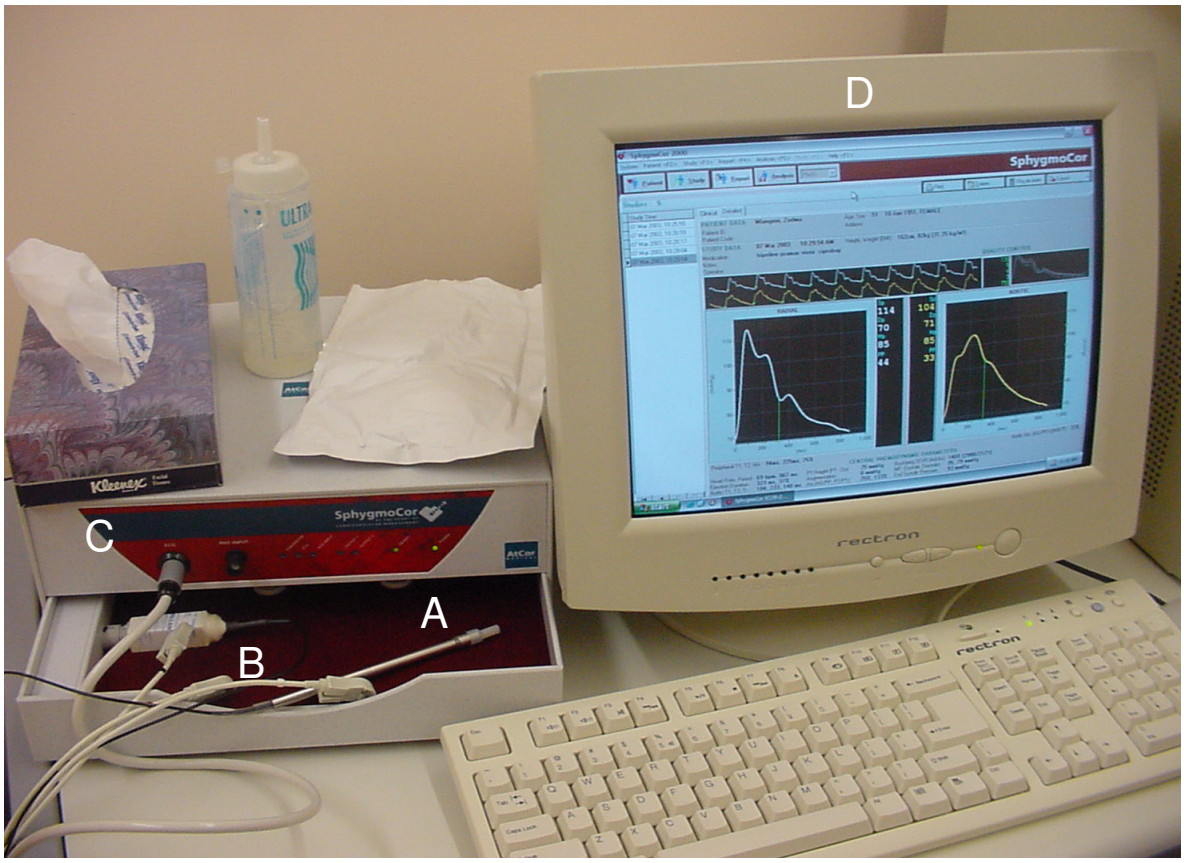
Standard laboratory blood tests of renal function, liver function, blood glucose, haematological parameters, and percentage glycated haemoglobin (HbA_{1C}) were performed. Blood samples were obtained on the day of the clinic visit and the tests included a full blood count and differential count, plasma urea, creatinine and electrolyte concentrations, alanine transaminase, aspartate transaminase, gamma gluteryl transaminase, alkaline phosphatase, albumin, total protein, total bilirubin, conjugated and unconjugated bilirubin, urate, total cholesterol, high density lipoprotein cholesterol, triglycerides, blood glucose, and a follicle stimulating hormone concentration (in females only to confirm menopausal status) (Bayer, Leverkusen, Germany). Diabetes mellitus (DM) or inappropriate blood glucose control was defined as the use of insulin or oral hypoglycaemic agents or an HbA_{1C} value greater than 6.1% (Bennett et al 2007).

2.2.5 Pulse wave analysis

Central BP, PP, P1, AP, aortic pulse wave velocity (PWV) and augmentation index (AIc) were estimated using techniques previously described (Shiburi et al 2006). To determine central pressures and the pressure components, after participants had rested for 15 minutes in the supine position, arterial waveforms at the radial (dominant arm) pulse were recorded by applanation tonometry during an 8-second period using a high-fidelity SPC-301

micromanometer (Millar Instrument, Inc., Houston, Texas) interfaced with a computer employing SphygmoCor, version 6.21 software (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia) (Figure 2.1). Recordings where the systolic or diastolic variability of consecutive waveforms exceeded 5% or the amplitude of the pulse wave signal was less than 80 mV were discarded. The pulse wave was calibrated by manual measurement (auscultation) of BP taken immediately before the recordings. From a validated inbuilt transfer function an aortic waveform was generated from which central systolic, diastolic and mean arterial BP were derived (Figure 2.2). The magnitude of the forward pressure component (P1) was determined as the difference between the inflection point at the end of the first systolic shoulder and central diastolic BP (i.e. the height of the first systolic shoulder). The magnitude of the augmented pressure wave (AP) was determined as the difference between central systolic BP and the inflection point at the end of the first systolic shoulder. Central PP (PPc) was calculated as the difference between central systolic BP and central diastolic BP and mean arterial pressure (MAP) was calculated as [central diastolic BP + 1/3(central PP)]. Although applanation tonometry at the carotid artery is the most accurate non-invasive assessment of the the forward and augmented pressures, carotid tonometry cannot be reliably applied in obesity (Laurent et al 2006). Considering the high prevalence of obesity in the study participants (≈43%) I therefore assessed the pressure components of PPc using radial tonometry. Central augmentation index was determined as the augmented pressure wave/pulse pressure, expressed as a percentage. The reflected wave transit time (RWTT) was determined from the beginning of the incident wave to the end of the first systolic shoulder (i.e., duration of the first systolic shoulder) (Mitchell et al 2004, Cecelja et al 2009).

Aortic PWV was measured from sequential waveform measurements at carotid and femoral sites as previously described (Shiburi et al 2006) (Figure 2.3). The distance which the pulse wave travels was determined as the difference between the distance from the femoral sampling site to the suprasternal notch, and the distance from the carotid sampling site to the suprasternal notch.



A Applanation tonometer.

B Electrocardiograph electrodes.

C SphygmoCor device.

D Image of radial artery and aortic pressure waves recorded from a participant (see Figure 2.2 for further details).

Figure 2.1 SphygmoCor device coupled to an applanation tonometer used to determine central (aortic) haemodynamics and aortic pulse wave velocity.

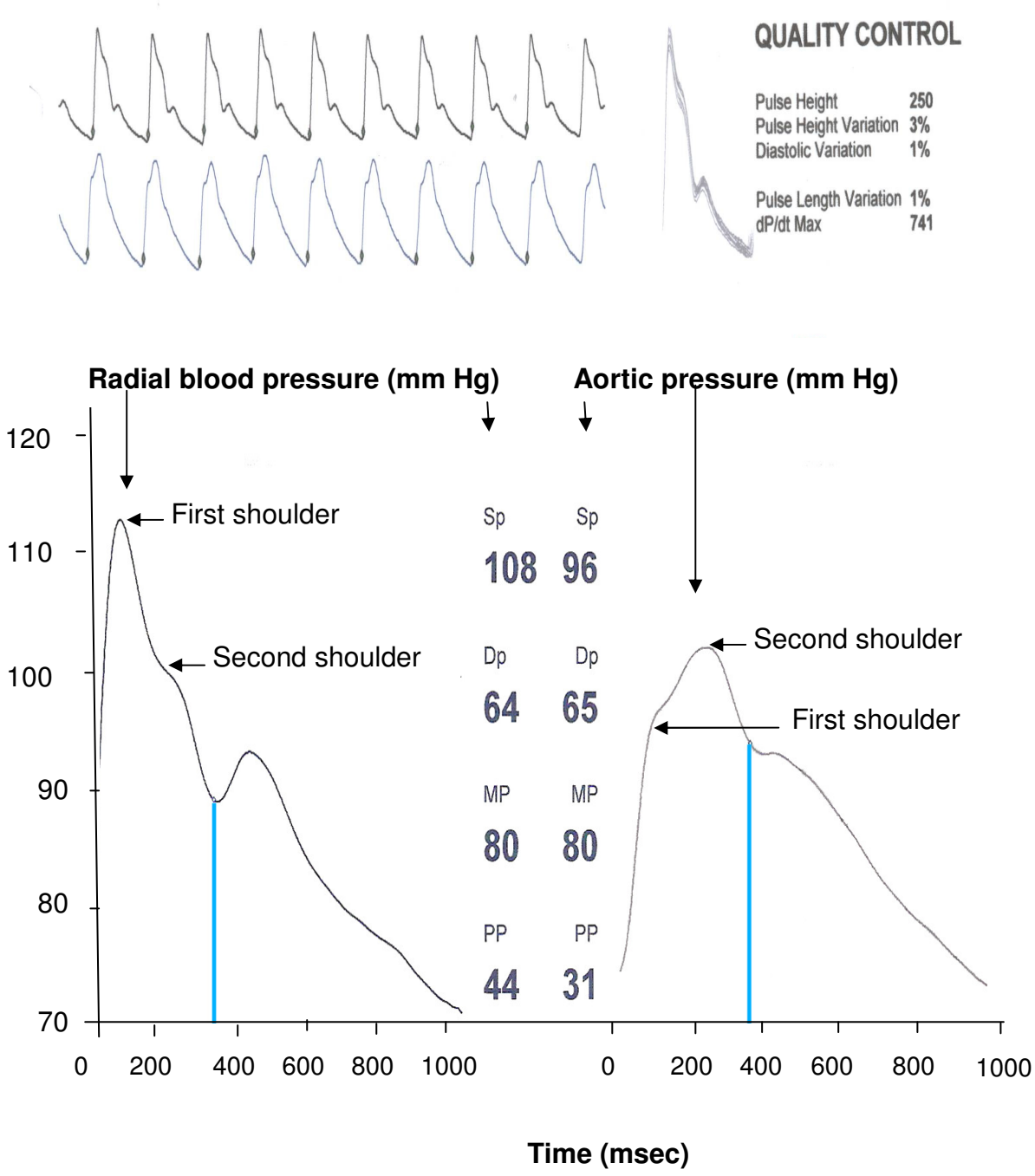


Figure 2.2. Examples of a pulse wave recording obtained to determine central haemodynamics. The figure shows the radial artery pulse wave obtained from applanation tonometry (lower left panel) and the aortic pulse wave derived from a population-based transfer function built into the software (lower right panel). The first and second systolic shoulders are identified. See text for a further description. Quality control assessments are shown in the top panel. Sp, systolic blood pressure (BP); Dp, diastolic BP; MP, mean arterial pressure; PP, pulse pressure.

Pulse wave transit time i.e. the time it takes the pulse wave to travel from the carotid to the femoral site, was determined as the difference between the times taken to generate the femoral and carotid pulse waveforms. To assess the differences in time of the generation of the femoral and carotid pulse waveforms, a single lead electrocardiogram was performed concurrently with pulse waveform sampling. Aortic PWV was calculated as distance (meters) divided by transit time (seconds). Aortic PWV could not be measured in 133 participants who were too obese to obtain reliable femoral pulse waves.

2.2.6 Stroke volume.

Two-dimensional guided M-mode echocardiography was performed with a Hewlett Packard-5500 (Palo Alto) recorder coupled to a 2.5 MHz transducer to determine short axis internal dimension measurements. The transducer was placed perpendicular to the wall of the chest, or pointed slightly laterally and inferiorly at the end of the long axis, in accordance with the guidelines recommended by the American Society of Echocardiography (Sahn et al 1978). All measurements were recorded on videotape and analysed off-line by one experienced investigator (Dr Carlos Libhaber) who was unaware of the clinical data of the participants. Left ventricular end systolic diameter (LVESD) and left ventricular end diastolic diameter (LVEDD) were measured when both the right and left septal surfaces could be visualised (Sahn et al 1978) (Figure 2.4). Left ventricular end diastolic and systolic volumes were determined using the Teichholz (Teichholz et al 1976) and the Z-derived (de Simone et al 1996) methods. Using the Teichholz method left ventricular end systolic volume (LVESV) is calculated using the equation $LVESV= [7.0/ (2.4 + LVESD)] \times (LVESD)^3$ and left ventricular end diastolic volume (LVEDV) using the equation: $LVEDV= [7.0/(2.4 + LVEDD)] \times (LVEDD)^3$ (Teichholz et al 1976). For the Z-derived method the validated equations $LVESV = 3.72(LVESD)^2$ and $LVEDV = 4.5(LVEDD)^2$ were used to determine LVESV and LVEDV (De Simone et al 1996). Stroke volume was determined as the difference between end diastolic and systolic volumes as derived from the two methods. . Stroke volume was indexed to body surface area when showing age-related changes or when adjusting for P1-PPc relations.

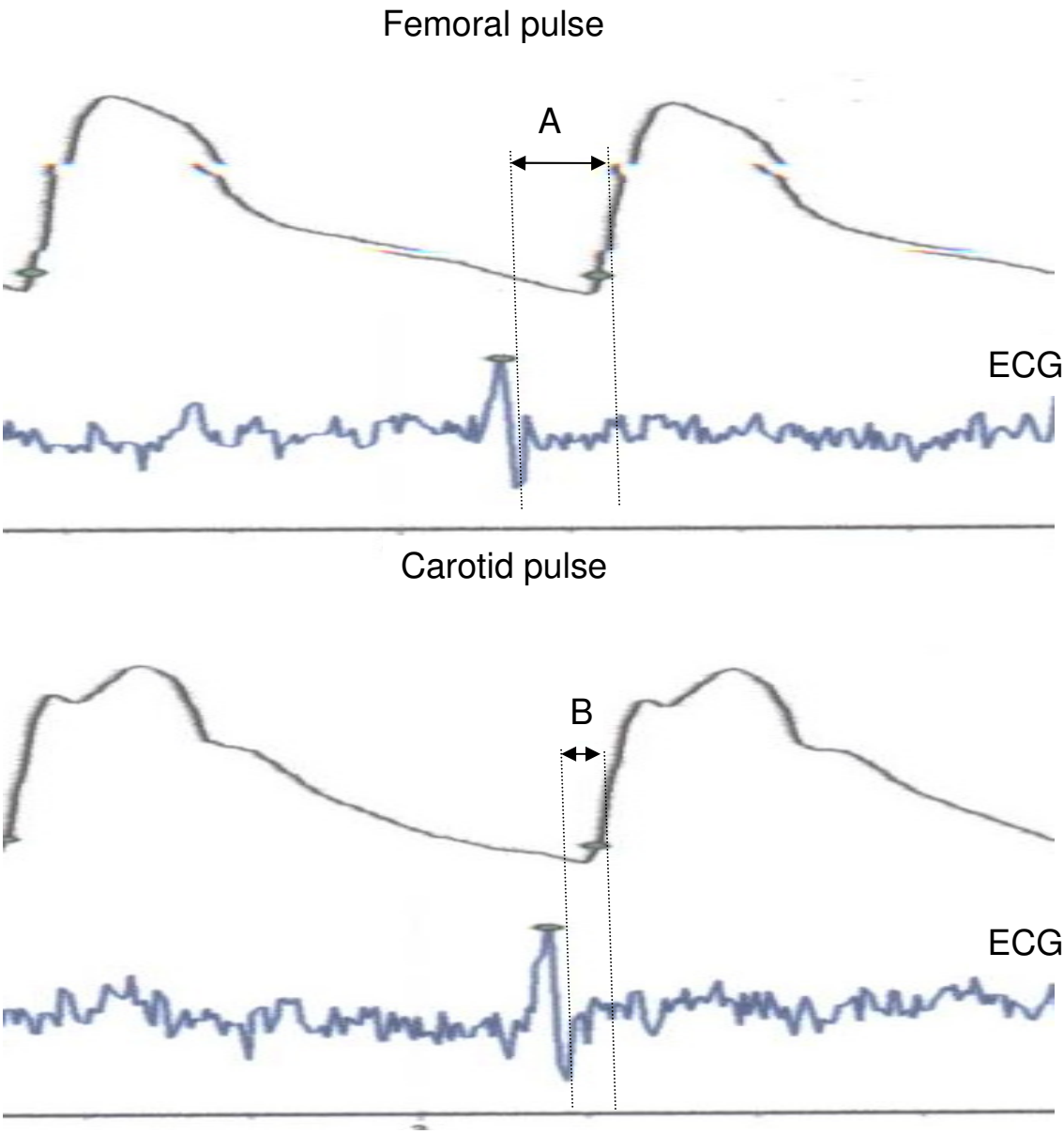


Figure 2.3. Examples of femoral and carotid artery pulse waves obtained using applanation tonometry from the same participants. Together with simultaneous electrocardiographic (ECG) recordings aortic pulse wave velocity (PWV) is calculated. The arrows indicate the time between electrical events and the arterial pressure changes in the carotid and femoral arteries used to calculate PWV. See text for a further description.

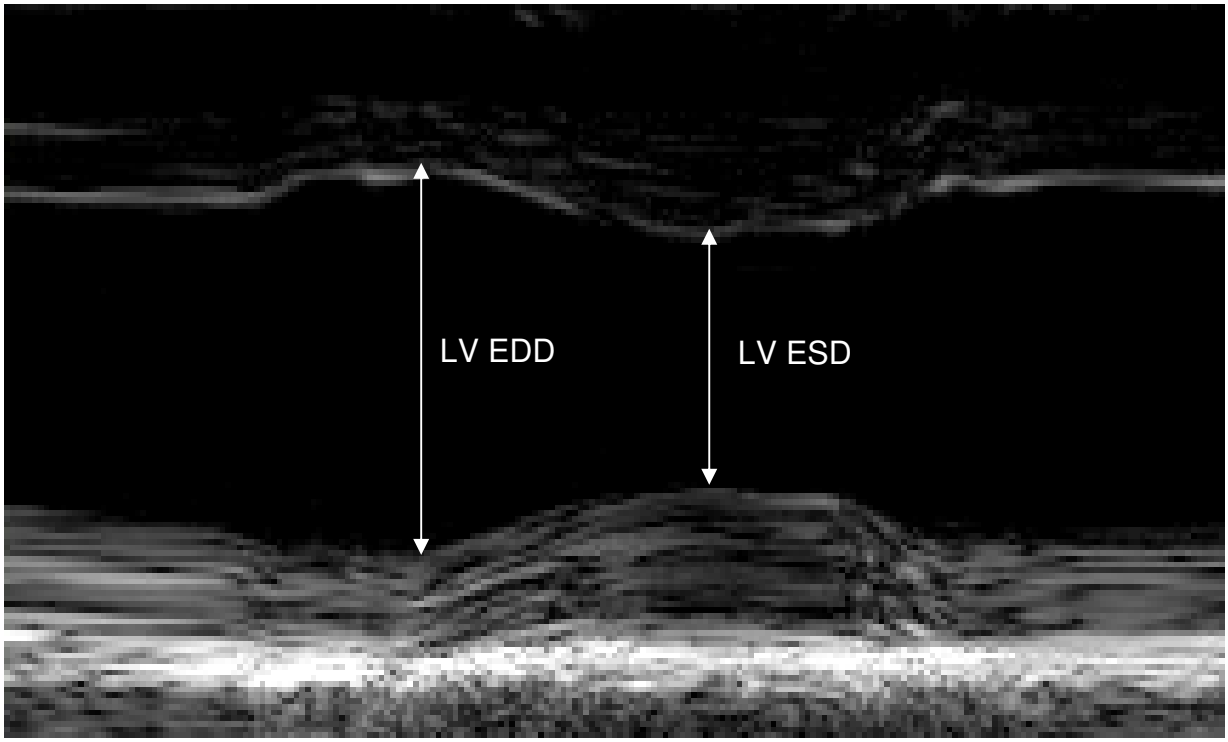


Figure 2.4. Example of M-Mode echocardiographic image of the left ventricle obtained to assess left ventricular end systolic (LV ESD) and end diastolic (LV EDD) internal diameters. These values were used for the calculation of stroke volume (see text for further details).

2.2.7. Data analysis

For database management and statistical analysis, SAS software, version 9.1 (SAS Institute Inc., Cary, NC) was employed. Regression analysis was performed with appropriate adjustments. When analysis was conducted in the whole group, additional adjustments were made for the treatment for hypertension. For the derivation of probability values, further adjustments were made for non-independence of family members using the mixed procedure as outlined in the SAS package. Sensitivity analysis was conducted in untreated participants.

2.3 Results

2.3.1 Characteristics of the participants.

Table 2.1 gives the demographic and clinical characteristics of all participants and of normotensives. More women than men participated. A high proportion of participants were overweight or obese, or had either DM or an HbA_{1C}>6.1%. Of the 1015 participants, 2.8% of participants had a history of cardiovascular disease. Importantly 37.7% of participants had uncontrolled hypertension.

2.3.2 Age-related associations with AP, P1 and PPc

Across the adult lifespan unadjusted AP increased linearly (Figure 2.5, upper panel Table 2.2), whilst unadjusted PPc and P1 increased exponentially (Figure 2.5, upper panel Table 2.2), with the first age at which P1 was noted to significantly increase being 50-60 years of age. With adjustments for distending pressures (MAP), sex and treatment for hypertension; age-related increases in PPc, AP and P1 were also noted across the adult lifespan (Figure 2.5, lower panel), following a linear relationship for AP and exponential relationships for PPc and P1 (Table 2.2). Again adjusted increases in AP occurred at an earlier age (30-40 years) than P1 (60-70 years) (Figure 2.5, lower panel). Although P1 began to increase later in life than AP, the extent to which it increased resulted in a similar quantitative effect as AP across the adult lifespan. Indeed, a one standard deviation (SD) increase in age resulted in a similar increase in P1 as compared to AP (Figure 2.6).

Table 2.1. Demographic, clinical, anthropometric and haemodynamic characteristics of all study participants and normotensives.

	All n=1015	Normotensives n=546
Sex (% female)	65.8	66.5
Age (years)	43.9±18.4	33.7±14.4
Body mass index (kg/m^2)	29.5±8.1	27.0±7.4
% overweight/obese	23/43	22/30
Regular tobacco (% subjects)	14.6	15.2
Regular alcohol (% subjects)	22.2	22.0
% with DM or HbA1c>6.1%	22.9	9.7
% women postmenopausal	44.9	19.6
% hypertensive	46.2	0
Pulse rate (beats/min)	64.6±11.5	63.7±11.4
Blood pressures (mm Hg)		
Conventional SBP/DBP	130±23/84±13	116±11/77±8
Conventional pulse pressure	45±16	38±9
Central SBP/DBP	121±23*/85±13	107±12/78±8
Mean arterial pressure	101±16	91±9
Central pulse pressure	36±15	29±8
Forward wave pressure (P1)	25±9	22±5
Augmented pressure (AP)	11±8	7±5
Other hemodynamic factors		
Central AIc (%)	142±25	134±24
Pulse pressure amplification	9.1±4.5	9.3±4.4
Aortic PWV (m/sec)	6.77±2.72*(n=882)	5.53±1.54 (n=487)
RWTT (msec)	105±13	106±14
SV (Teichholz) (mls/beat)	68±19(n=503)	66±19(n=265)
SV (Z-method) (mls/beat)	66±16(n=503)	65±16(n=265)

Data expressed as mean ± SD unless otherwise stated. DM, diabetes mellitus; HbA_{1C}, glycosylated haemoglobin; BP, blood pressure; SBP, systolic BP; DBP, diastolic BP; AI, central augmentation index; PWV, pulse wave velocity; RWTT, reflective wave transit time; SV, stroke volume.

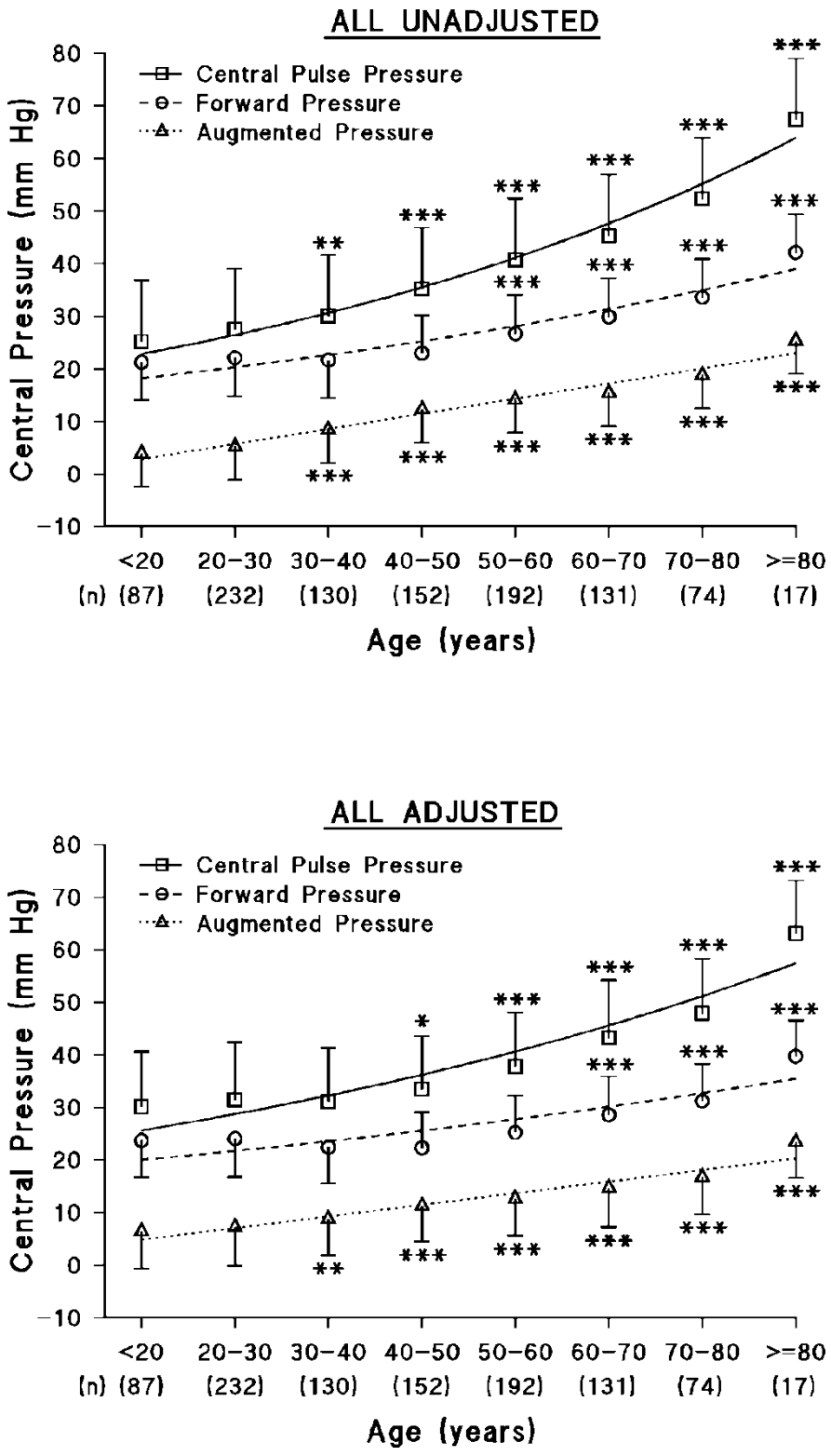


Figure 2.5. Changes in central (aortic) pulse pressure (PPc), and the forward (P1) and the augmented (AP) pressure components across the adult lifespan. Unadjusted (upper panel) and mean arterial pressure, treatment for hypertension and sex-adjusted (lower panel) data are shown. Probability values are further adjusted for the non-independence of family members. *p<0.05; **p<0.005; ***p<0.0001 vs reference sample<20 years of age.

Table 2.2. Regression equations for the relationships between age and central (aortic) pressures.

	All participants (n=1015)			Normotensives only (n=546)		
	Regression equation	r^2	p value	Regression equation	r^2	p value
Age versus			Unadjusted relationships			
PPc:	$PPc = -0.016age + 0.006age^2 + 24.399$	0.384	<0.0001	$PPc = 0.177age + 0.0005age^2 + 22.427$	0.156	<0.0001
P1:	$P1 = -0.285age + 0.006age^2 + 25.260$	0.268	<0.0001	$P1 = -0.215age + 0.003age^2 + 25.067$	0.020*	=0.004
AP:	$AP = 0.273age - 1.123$	0.388	<0.0001	$AP = 0.199age + 0.414$	0.299	<0.0001
Age versus			Adjusted for mean arterial pressure and sex (and treatment for hypertension in all)			
PPc:	$PPc = -0.412age + 0.008age^2 - 7.561$	0.548	<0.0001	$PPc = 0.035age + 0.0016age^2 + 3.788$	0.224	<0.0001
P1:	$P1 = -0.456age + 0.006age^2 + 12.167$	0.379	<0.0001	$P1 = -0.233age + 0.003age^2 + 21.400$	0.046*	<0.0001
AP:	$AP = 0.197age – 22.476$	0.555	<0.0001	$AP = 0.162age – 17.060$	0.394	<0.0001

PPc, Central (aortic) pulse pressure; P1, forward pressure component; AP, augmentation pressures. Probability values are further adjusted for the non-independence of family members. *p<0.001 as compared to r^2 for age-AP relationship in normotensive sample.

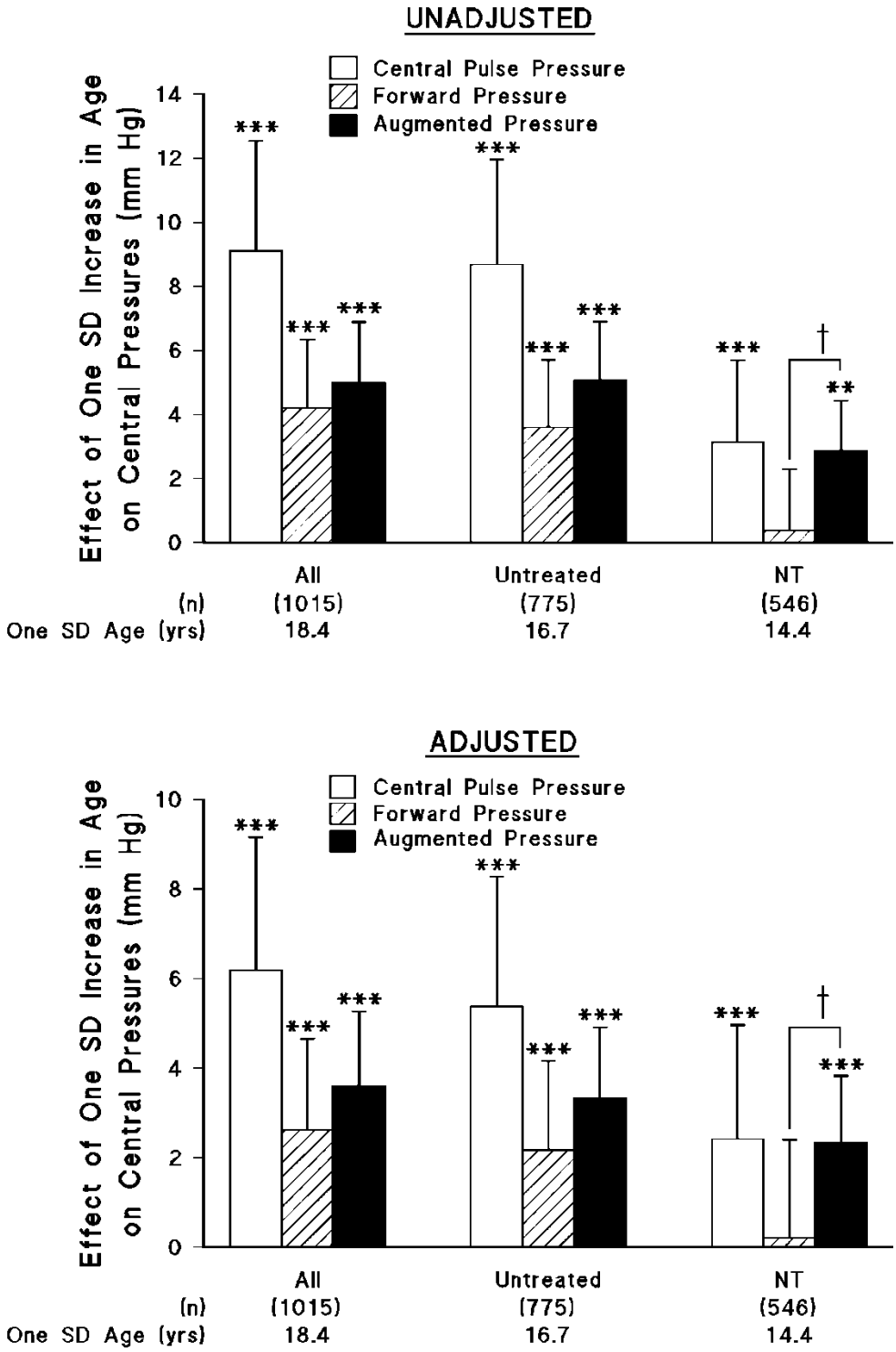


Figure 2.6. The quantitative impact of one standard deviation (SD) increase in age on the increase in central (aortic) pulse pressure (PPc), and the forward (P1) and the augmented (AP) pressure components across the adult lifespan. Both unadjusted and mean arterial pressure and sex-adjusted data are shown. Data for all participants, untreated participants and for normotensives (NT) only are shown. **p<0.005; ***p<0.0001 shows significant effects; †p<0.05.

In untreated participants, a one SD increase in age also resulted in a similar increase in P1 as compared to AP (Figure 2.6).

As increments in P1 may increase AP and hence result in an overestimation of the impact of age on AP, I also compared the impact of one SD increases in AP before the age at which P1 begins to increase, to a one SD increase in P1 across the adult lifespan. In these analyses again a one standard deviation (SD) increase in age resulted in a similar increase in P1 as compared to AP (Figure 2.7).

In normotensive participants only, with or without adjustments for distending pressures and sex; age-related increases in PPc and AP were noted across most of the adult lifespan (Figure 2.8, and Table 2.2) whilst P1 only began to increase at 70-80 years (Figure 2.8). In contrast to the whole group, in normotensives the age-P1 relationship was considerably smaller than the age-AP relationship (Table 2.2). Thus, in normotensives across the adult lifespan a one SD increase in age resulted in only a fraction of the increase in P1 as compared to AP (Figure 2.6).

2.3.3 Age-related associations with stroke volume

No differences in stroke volume were noted across the adult lifespan (Figure 2.9). No independent relationships between stroke volume and PPc (partial r=0.04 p=0.44), or P1 (partial r=0.07, p=0.23) were noted. A similar lack of age-related associations with stroke volume was noted in untreated participants (data not shown).

2.3.4 Age-related associations with AIc

Unadjusted and mean arterial pressure, treatment for hypertension and sex-adjusted AIc increased sharply from adolescence to middle age, reached a plateau at 40-50 years of age and did not continue to increase thereafter (Figure 2.10). The relationships best fitted a power function and similar relationships were noted in untreated participants (data not shown).

2.3.5 Independent contribution of P1 and AP to variations in PPc.

With adjustments for sex, both AP and P1 were equally as closely related to PPc (Figure 2.11). With additional adjustments for MAP, the relationship between P1 and PPc

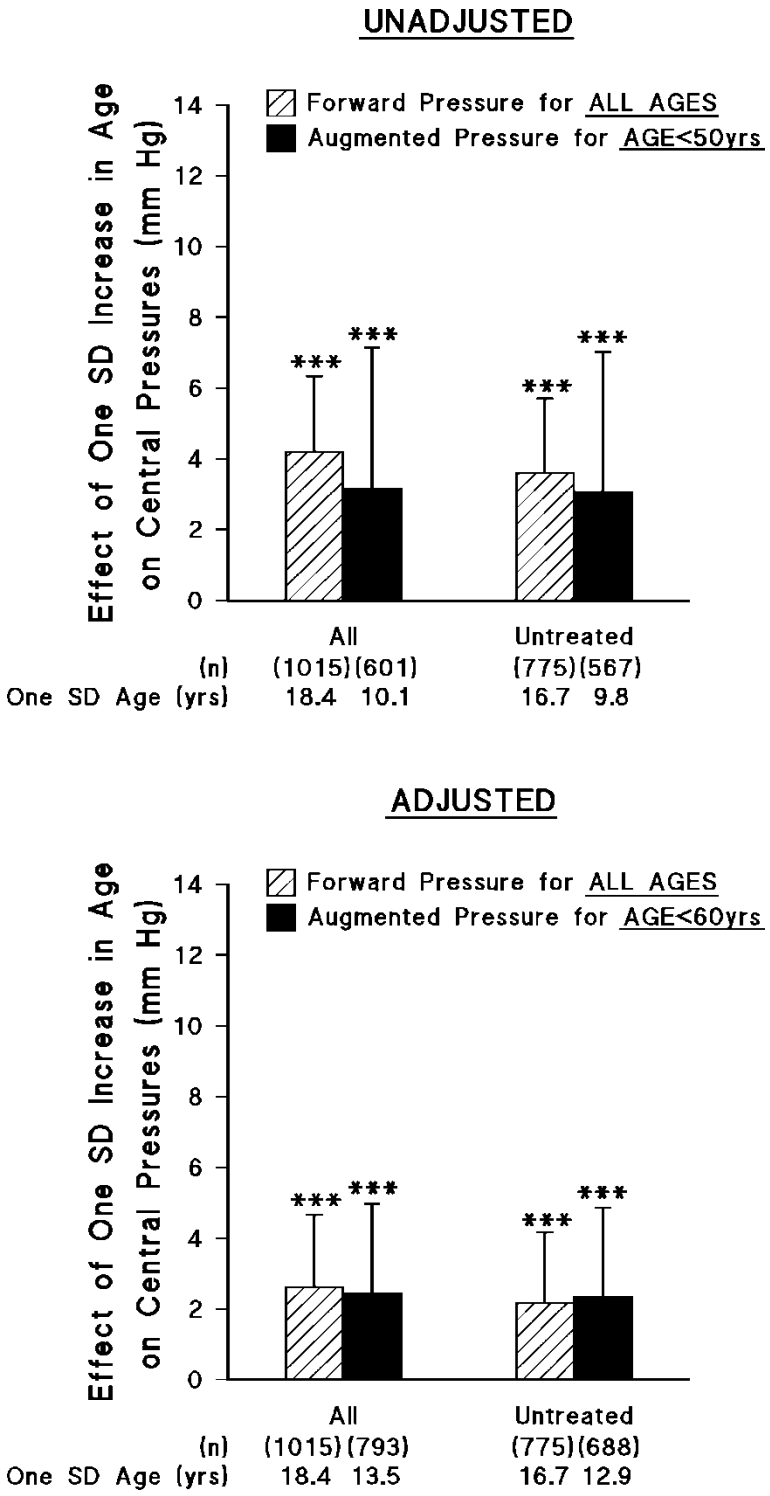


Figure 2.7. The quantitative impact of one standard deviation (SD) increase in age on the increase in the forward (P1) pressure wave across the adult lifespan and in the augmented (AP) pressure wave in all adults or <60 (adjusted) years of age. Both unadjusted and mean arterial pressure and sex-adjusted data are shown. Data for all participants and for untreated participants are shown. **p<0.005; ***p<0.0001 shows significant effects.

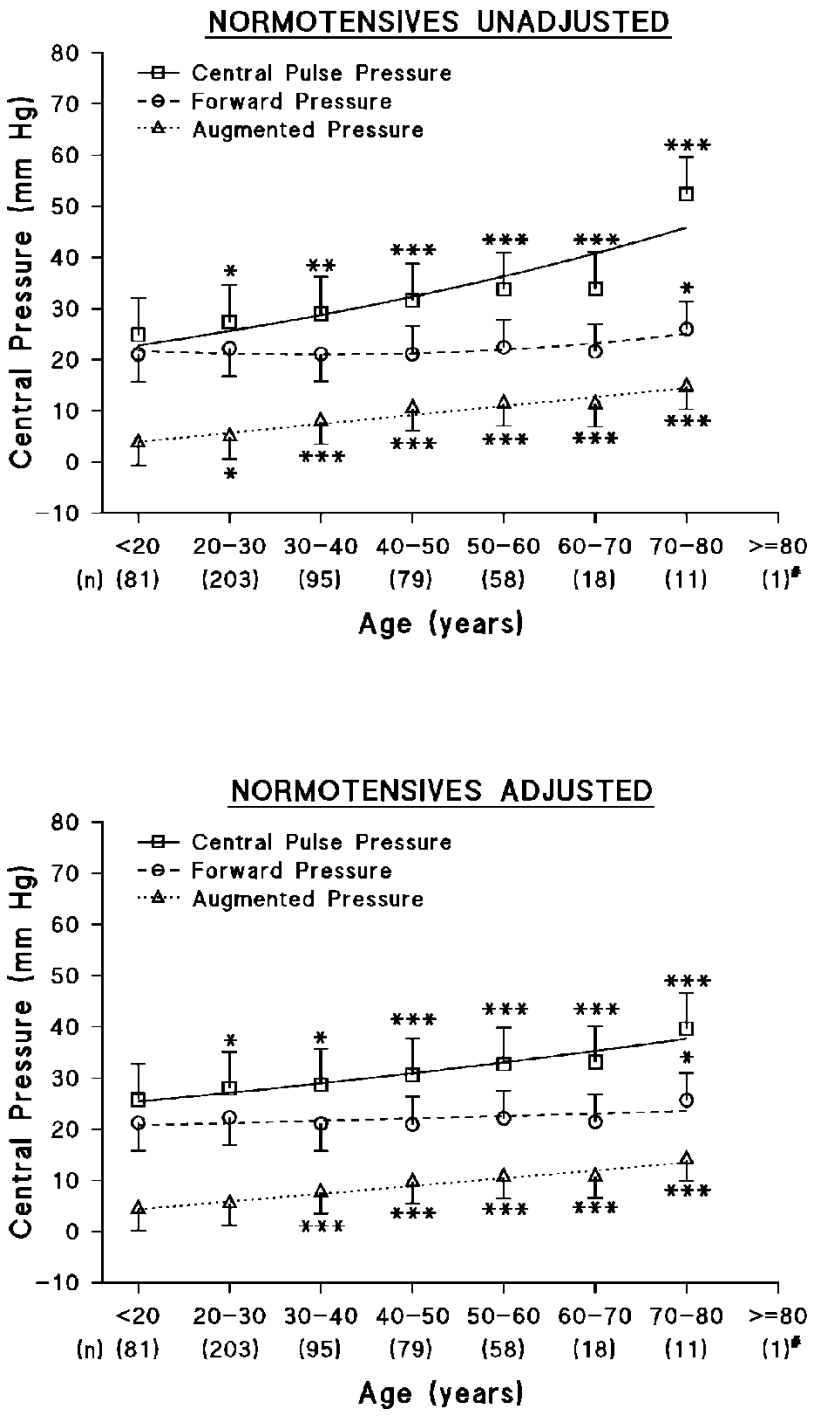


Figure 2.8. Changes in central (aortic) pulse pressure (PPc), and the forward (P1) and the augmented (AP) pressure components across the adult lifespan in normotensive participants. Unadjusted (upper panel) and mean arterial pressure and sex-adjusted (lower panel) data are shown. Probability values are further adjusted for the non-independence of family members. *p<0.05; **p<0.005; ***p<0.0001 vs reference sample<20 years of age. See Table 2 for equations that describe the relationships and Figure 2 for a comparison of the quantitative impact of age on central pressures across the adult lifespan. # only one participant was >80 years of age, hence data point not plotted.

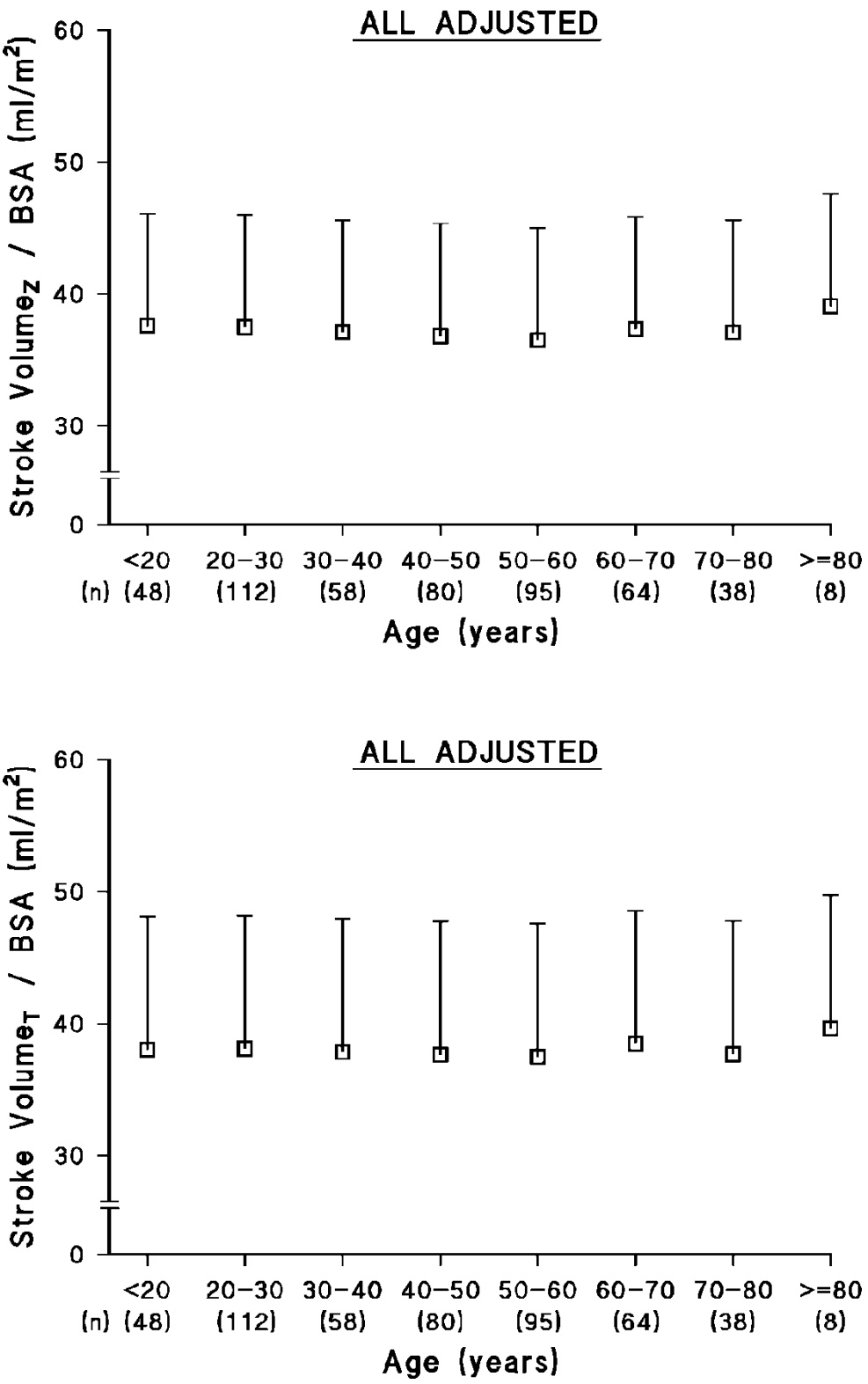


Figure 2.9. Stroke volume indexed for body surface area across the adult lifespan. SV_T, stroke volume indexed to body surface area according to Teicholtz method; SV_Z, SV indexed to body surface area according to Z-derived method. No differences were noted in SV at increasing ages.

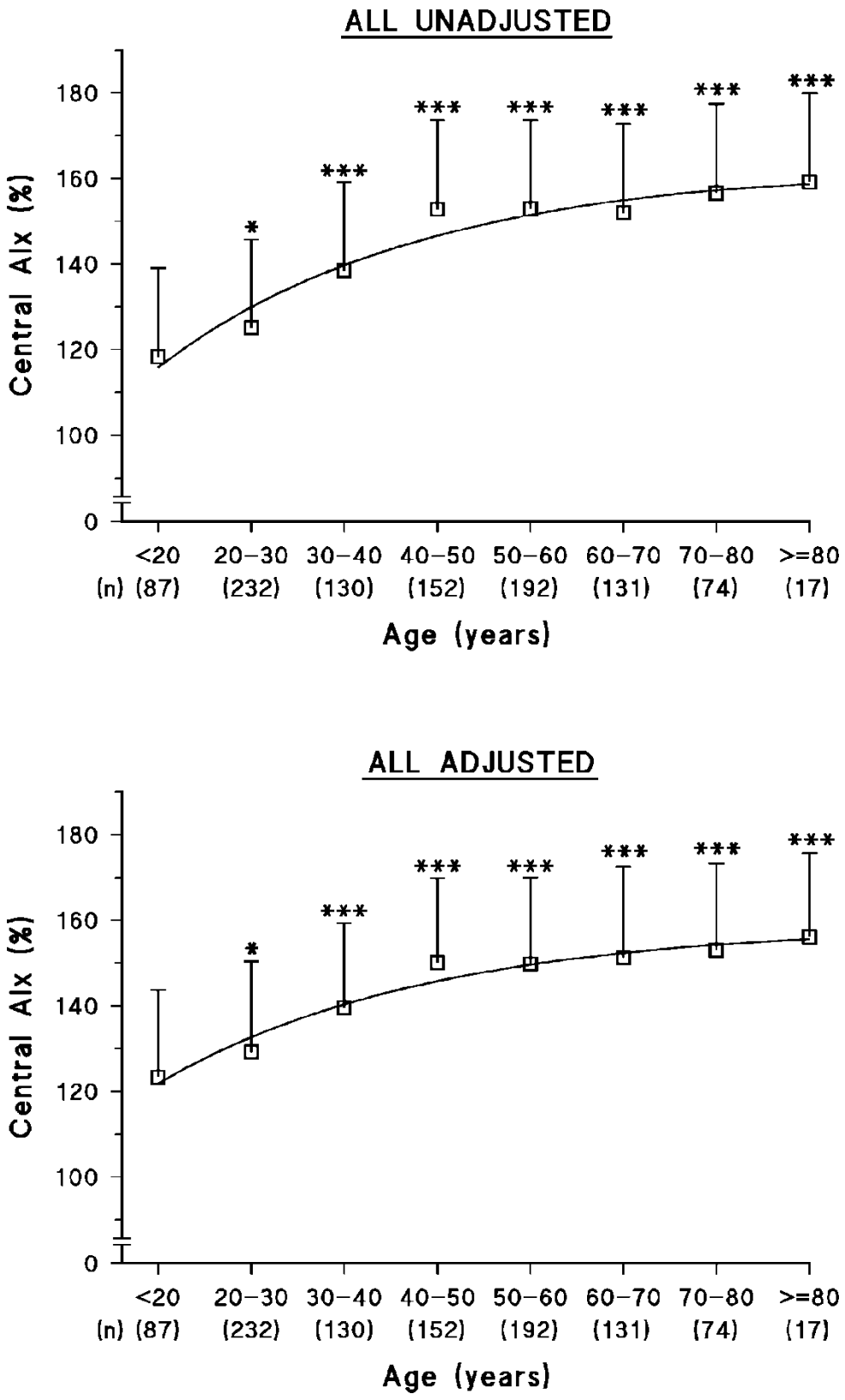


Figure 2.10. Changes in central augmentation index (AIx) across the adult lifespan. Unadjusted and mean arterial pressure, treatment for hypertension and sex-adjusted data are shown. Probability values are further adjusted for the non-independence of family members. *p<0.05; ***p<0.0001 vs reference sample<20 years of age.

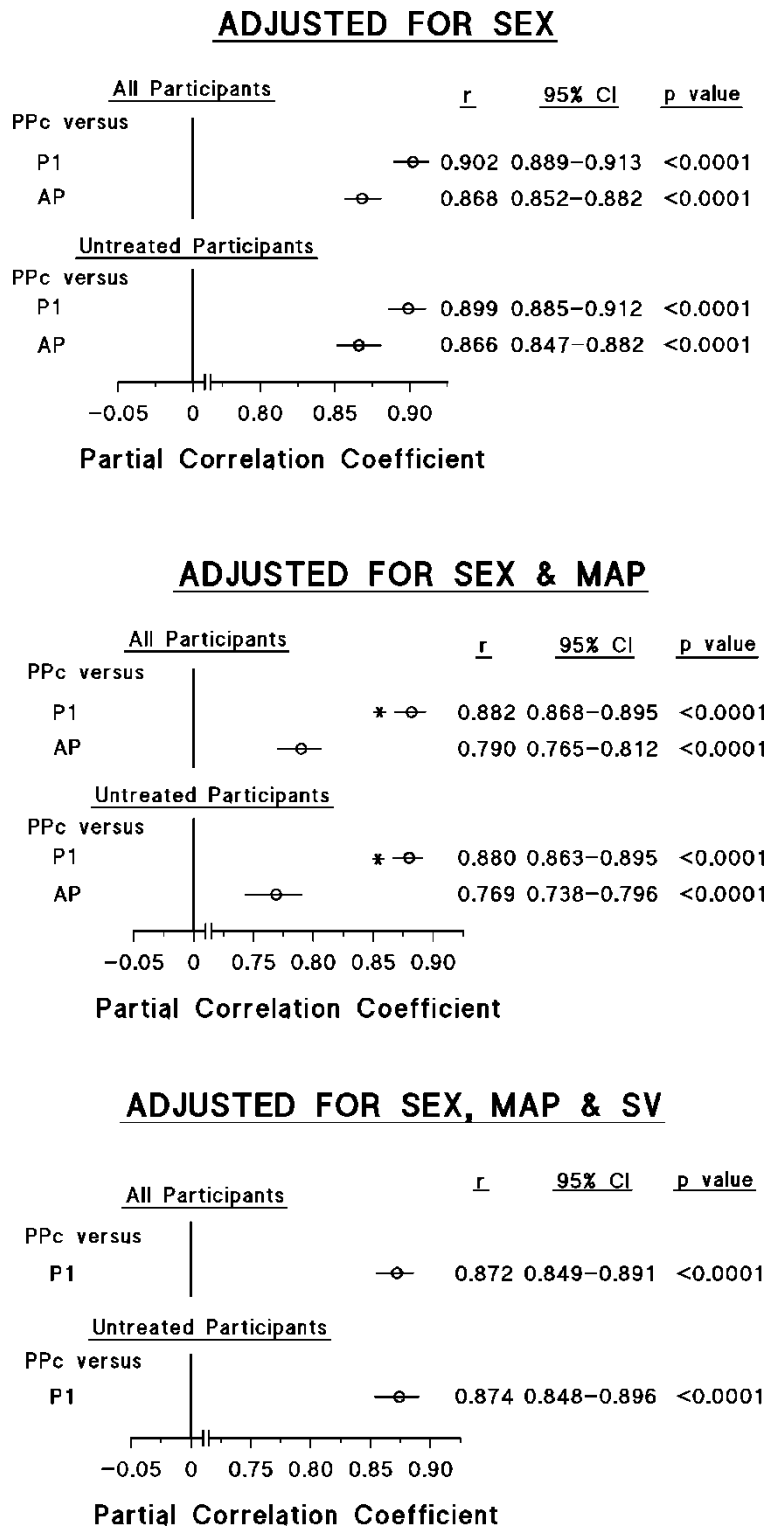


Figure 2.11. Multivariable adjusted relationships between central pulse pressure (PPc) and the forward (P1), or augmented (AP) pressures in all participants and in untreated participants. Both sex-adjusted, sex and mean arterial pressure (MAP)-adjusted and sex, MAP and stroke volume (SV)-adjusted data are shown. Probability values are further adjusted for the non-independence of family members. *p<0.01.

was stronger than the relationship between AP and PPc (Figure 2.11). Further adjustments for stroke volume failed to modify the P1-PPc relationship (Figure 2.11). In untreated participants, the relationship between P1 and PPc was similarly as strong as the relationship between AP and PPc (Figure 2.11).

2.3.6 Alternative haemodynamic determinants of PPc and the pressure components.

In the whole group independent of MAP and other confounders (age, sex, heart rate, weight, height, regular smoking, regular alcohol intake, diabetes mellitus/HbA1c>6.1%, treatment for hypertension); aortic PWV was associated with PPc (partial $r=0.17$, $p<0.0001$), P1 (partial $r=0.17$, $p<0.0001$), and AP (partial $r=0.10$, $p<0.01$). In addition, reflective wave transit time was independently related to PPc (partial $r=-0.08$, $p<0.01$) and AP (partial $r=-0.23$, $p<0.0001$). Similar relationships were noted in untreated participants (data not shown).

2.4 Discussion

The main findings of the present study are as follows: In 1015 participants of a randomly selected community sample of black African descent, 37.7% of whom had uncontrolled hypertension, independent of distending pressures (MAP) and sex, across the adult lifespan for every one SD increase in age, AP and P1 increased to a similar extent. Furthermore, with appropriate adjustors, AP and P1 contributed equally to variations in PPc.

In largely normotensive samples AP has previously been demonstrated to contribute more than P1 toward age-related increases in PPc across the adult lifespan (McEniery et al 2005, Cecelja et al 2009, Namasivayam et al 2009). Indeed, in normotensives, AP and P1 have previously been shown to contribute ~15 mm Hg and ~5 mm Hg respectively to age-related increases in PPc (Namasivayam et al 2009). Whether the contribution of P1 is similarly insubstantial in community or population samples with a high prevalence of uncontrolled hypertension, where hypertensive-related changes in the structure of the aorta may substantially increase P1 (O Rourke 1970, Mitchell et al 2003, McEniery et al 2005,Mitchell et al 2008), has not previously been evaluated. In the present study conducted

in a community sample with a high prevalence of uncontrolled hypertension, I show that P1 begins to increase at 50-60 years of age, which is in contrast to increases in P1 occurring only over 60 years of age in largely normotensive samples (Namasivayam et al 2009). Moreover, in the present study P1 and AP contributed to an equivalent extent to age-related increases in PPc and to variations in PPc, with P1 increasing by 18.0 mm Hg and AP by 21.5 mm Hg across the adult lifespan. These data therefore lend support for therapeutically targeting both AP and P1 when attempting to reduce the potential excess cardiovascular risk related to PPc (Safar et al 2002, Roman et al 2007, Williams et al 2006, Pini et al 2008, Wang et al 2009, Vlachopoulos et al 2010).

Using carotid tonometry with simultaneous flow measurements, which allow for accurate separation of the forward and reflected pressure components, alternative studies have not confirmed an important contribution of the reflected wave, (which contributes toward AP), to either variations in PPc (Mitchell et al 2010), or to cardiovascular outcomes (Mitchell et al 2010). However, these data are difficult to reconcile with evidence for a role of the reflective wave in contributing to variations in PPc (Segers et al 2007) and to cardiovascular outcomes (Wang et al 2010) in other studies using similar measurements that allow for separation of the forward and reflected waves. Furthermore, it is difficult to explain the well-recognised acute effects that nitrates have on AIc (Kelly et al 2001), if not through changes in wave reflection. Nevertheless, as I could not separate the forward and reflected waves I cannot discount the possibility that continued age-related increases in AP could represent an impact of age-related increases in the forward wave on AP (law of conservation of energy). However, when comparing the relative contribution of age-related increases in AP towards PPc, up until P1 begins to increase (thus discounting the impact of age-related increases in P1 on AP), to that of the contribution of P1 across the adult lifespan, the contribution of AP was still equivalent to P1.

Although AP increased linearly across the adult lifespan, as previously reported on (McEniery et al 2005) age-AIc relations more closely followed a power function. This observation has recently been attributed to the fact that two relationships (PPc and AP), even

if they are linear, with similar slopes but different intercepts, when combined will derive a non-linear function (Namasivayam et al 2010). Hence, the dissimilarity between age-AIc and age-AP relationships should not be taken to indicate that they are measuring different physiological information.

The potential mechanism of increases in P1, AP and hence PPc require consideration. Age-related increases in AP or P1 and the contribution of AP or P1 to PPc in the present study were largely independent of aortic distending pressures (MAP, which shifts the pressure-volume relationship to a higher point without producing structural aortic changes) and stroke volume (which may affect P1). Hence, the contribution of P1 to PPc can only be attributed to structural aortic changes that modify characteristic impedance. Importantly, recent evidence indeed suggests that the structural aortic changes that may influence characteristic impedance and hence P1 may occur earlier in life than previously thought (Redheuil et al 2010). Furthermore the contribution of AP to PPc may occur through a variety of mechanisms not explored in the present study, including a possibility that it is largely due to the Windkessel or reservoir function of the aorta (Davies et al 2010), but which are nevertheless not through increased distending pressures.

The strengths of the present study are as follows: I evaluated a randomly selected community sample with a high prevalence of uncontrolled hypertension as opposed to the largely normotensive samples previously studied (Mitchell et al 2004, McEniery et al 2005, Cecelja et al 2009, Namasivayam et al 2009, Mitchell et al 2010). Furthermore, in contrast to some studies where limited age ranges were evaluated (Mitchell et al 2004, Segers et al 2007)) central BP was assessed across the adult lifespan starting from 16 years of age and extending to >80 years of age. Moreover, I employed measurement techniques which have shown independent relations between central BP, AP or AIc and cardiovascular outcomes beyond brachial BP (London et al. 2001, Safar et al 2002, Chirinos et al 2005, Weber et al 2005, Williams et al 2006, Roman et al 2007, Pini et al 2008, Wang et al 2009, Vlachopoulos et al 2010. Last, I measured stroke volume to separate the component of P1 that is

determined by stroke volume, thus enabling me to draw conclusions regarding structural aortic changes that determine P1.

The cross-sectional design is a limitation of the present study and hence does not allow conclusions to be drawn regarding cause and effect with respect to age-related associations with central pressures. Second, as a consequence of the high prevalence of obesity (43%), I could not accurately perform carotid tonometry together with aortic flow measurements which would have allowed for separation of the forward and reflected pressure waves. Therefore, I cannot discount the possibility that continued age-related increases in AP could represent an impact of the forward wave on AP. However, when comparing the relative contribution of age-related increases in AP up until P1 begins to increase, to that of the contribution of P1 across the adult lifespan, the contribution of AP was still equivalent to P1. My inability to separate the forward and reflected waves is also an imperfect approach to identifying the magnitude of the forward wave (the peak of the wave is often masked by AP). However, this approach is the same as that used by previous investigators describing a dominant role of AP over P1 in largely healthy populations (McEniery et al 2005, Cecelja et al 2009, Namasivayam et al 2009) and if anything I have biased against the results of the present study (which show a considerably greater role for P1 than previously noted (McEniery et al 2005, Cecelja et al 2009, Namasivayam et al 2009) by underestimating the peak pressure of the forward wave. Third, I did not measure the aortic structural changes that may contribute toward PPc. However, as I was able to show that independent of MAP; P1, but not stroke volume accounted for enhanced age-related increases in PPc, these effects can only be attributed to aortic structural changes. Fourth, inherent in non-invasive measurements of aortic BP are calibration errors. In this regard calibration of the radial waveform from brachial BP measurements ignores amplification of BP from brachial to radial arteries. Fifth, the inflection point of the central waveform is not a reliable marker of reflected wave transit time. Hence I could not accurately assess the contribution of reflected wave transit time to the effects of AP on PPc. However, this was not a focus of the present study. Last, the present study was conducted in one ethnic group, and

ethnic differences in AIc have been described (Shiburi et al 2006). Hence further studies in communities of alternative ethnic origins with a high prevalence of uncontrolled hypertension are required.

The clinical implications of the present study require consideration. As AP and P1 contribute to an equivalent extent to age-related increases in PPc and variations in PPc across the adult lifespan in a community sample with a high prevalence of uncontrolled BP, the present study provides support for the notion that both P1 and AP are important targets for therapeutic intervention. In this regard, although there is little evidence to support beneficial effects of current antihypertensive agents on the structural aortic changes that characterise P1, there is good evidence for beneficial effects of some agents on AP (Zieman et al 2005, Laurent et al 2007). The present study suggests that therapeutic approaches that decrease P1 independent of distending pressures require development.

In conclusion, the present study shows that in a community of African ancestry with a high prevalence of hypertension, independent of distending pressures, P1 and AP contribute to an equivalent extent to age-related increases in PPc across the adult lifespan, and P1 and AP are similar in the ability to account for variations in PPc. The present study therefore provides further support for the view that although AP may be an important target for therapy, the caveat is that to normalise PPc, therapy may also be required that targets the structural aortic changes that determine P1. Further studies using techniques that separate the aortic forward and reflected pressure waves are required to more accurately ascertain the relative contribution of AP and P1 to PPc in communities with a high prevalence of hypertension. Furthermore the potential pathophysiological mechanisms responsible for the contribution of P1 to age-related increases in PPc across the adult lifespan have yet to be identified. In this regard, in subsequent chapters of the present thesis I have evaluated whether salt intake, inflammatory changes and genetic mechanisms may in-part explain this effect.

CHAPTER 3

Does the Aortic Forward Pressure Component Account for Central Blood Pressure Effects on Left Ventricular Mass Index in the General Population?

Abstract

Background. Aortic pulsatile pressures (PP) predict cardiovascular outcomes and damage beyond distending and peripheral pressures. The relative role of the forward (P1) and the augmented (AP) (reflected) pressure components of central PP toward cardiovascular damage in communities with a high prevalence of uncontrolled hypertension is uncertain.

Aim. To evaluate the independent relationship between P1 or AP and left ventricular mass index in a community sample with a high prevalence of uncontrolled hypertension.

Methods and results. In 503 randomly selected participants from a community sample (mean age=43.8±18.3 years), with a high prevalence of uncontrolled hypertension (25.9%), applanation tonometry and SphygmoCor software was employed to determine central arterial PP, P1, AP, and augmentation index (AI). Left ventricular mass indexed to height$^{1.7}$ (LVMI) was determined with echocardiography. With central and brachial artery PP included in the same regression model, central ($p<0.01$), but not brachial ($p=0.92$) PP was independently associated with LVMI. With adjustments for confounders including an index of distending pressures (mean arterial pressure-MAP), P1 (partial $r=0.16$, $p=0.001$) and AP (partial $r=0.10$, $p<0.05$) were independently associated with LVMI.

Conclusions. In a community sample with a high prevalence of uncontrolled hypertension, both the forward and the augmented pressure components are independently associated with LVMI.

3.1 Introduction

Age-induced increases in central aortic pulse pressure (PP) or indices of the reflected pressure component of aortic PP (augmentation index or augmentation pressures) are more closely associated with cardiovascular damage and outcomes than blood pressure (BP) measured at the brachial artery (Saba et al 1993, London et al 2001, Nurnberger et al 2002, Hayashi et al 2002, Weber et al 2004, Ueda et al 2004, Weber et al 2005, Chirinos et al 2005, Williams et al 2006, Hashimoto et al 2007, Safar et al 2002, Roman et al 2007, Wang et al 2009). In normotensive samples these aging effects are largely attributed to increases in augmentation pressure (AP) which account for most of the age-related increases in central BP across the lifespan (Namasivayam et al 2009,Cecelja et al 2009). These findings have important implications as a number of vasoactive drugs can modify AP, whilst the comparative weight of evidence for drugs aimed at targeting the mechanisms of increases in the forward pressure component (P1) of aortic PP independent of distending pressures is somewhat limited (Zieman et al 2005, Laurent et al 2007). However, the contribution of P1 to age-related increases in aortic BP has generated considerable controversy.

Increases in P1 and the factors that determine P1 (aortic stiffness and diameter) have generally been thought to contribute only toward increases in aortic PP in elderly patients (Namasivayam et al 2009, Mitchell et al 2004), or in patients with systolic hypertension (Mitchell et al 2003, Mitchell et al 2008). However, as demonstrated in chapter 2 of the present thesis, I have provided evidence to show for the first time that in a community with a high prevalence of uncontrolled BP, the contribution of P1 to age-related increases in central PP is equivalent to the contribution of AP across the adult lifespan. Whether this contribution of P1 translates into cardiovascular damage requires further study. In the present study I therefore assessed the relative contribution of P1 and AP independent of distending pressure to variations in left ventricular mass index (LVMI), a well recognized target organ change in hypertension, in an urban, developing community with a high prevalence of uncontrolled hypertension.

3.2 Methods

3.2.1 Study participants.

The present study design and a description of the participants recruited have been outlined in chapter 2, page 47 of the present thesis. Of the participants recruited as part of the main study, 503 had echocardiographic measurements as part of a sub-study.

3.2.2 Clinical, demographic and anthropometric assessments.

A standardized questionnaire was administered to obtain demographic and clinical data as previously described (Norton et al 2008, Woodiwiss et al 2009). Details of the questionnaire are provided in chapter 2, pages 47-48 of the present thesis.

Height and weight were measured with participants standing, wearing indoor clothing and no shoes. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Participants were identified as being overweight if their body mass index (BMI) was ≥25 kg/m^2 and obese if their BMI was ≥30 kg/m^2.

3.2.3 Conventional blood pressure

A trained nurse-technician measured conventional (brachial) blood pressure (BP) using a standard mercury sphygmomanometer. Details of the measurements are provided in chapter 2, page 48 of the present thesis. In the present study only 1.79% of visits had fewer than the planned BP recordings. The frequency of identical consecutive recordings was 0.40% for systolic BP and 1.01% for diastolic BP. The occurrence of BP values recorded as an odd number was 0.02%. Of the 4962 systolic and diastolic BP readings, 29.77% ended on a zero (expected =20%). Hypertension was defined as a mean BP≥140/90 mm Hg (Chobanian et al 2003, Williams et al 2004, Mancia et al 2007) or the use of antihypertensive medication.

3.2.4 Laboratory (blood) tests.

Standard laboratory blood tests of renal function, liver function, blood glucose, haematological parameters, and percentage glycated haemoglobin (HbA_{1C}) were performed. Details of the blood tests are provided in chapter 2, page 49, of the present thesis. Diabetes

mellitus or inappropriate blood glucose control was defined as the use of insulin or oral hypoglycaemic agents or an HbA_{1C} value greater than 6.1% (Bennett et al 2007).

3.2.5 Pulse wave analysis

Central BP, PP, P1, AP and augmentation index (AIc) were estimated using techniques previously described (Shiburi et al 2006). Details of the pulse wave analysis are provided in chapter 2, pages 49-53 of the present thesis.

3.2.6 Left ventricular mass index

Two-dimensional guided M-mode echocardiography was performed with a Hewlett Packard-5500 (Palo Alto) recorder coupled to a 2.5 MHz transducer to determine short axis internal dimension measurements as described in chapter 2, pages 53 and 55 of the present thesis. In addition to measuring left ventricular (LV) internal diameters at end diastole, septal (anterior wall) and posterior wall thickness at end diastole were also measured. Left ventricular mass (LVM) was derived according to an anatomically validated formula: LVM = 0.8 x [1.04 (LVEDD + IVS +PWT) ^{3} – (LVEDD) ^{3}] + 0.6g, where LVEDD = LV internal diameter, IVS =LV septal thickness, PWT = posterior wall thickness, all measured at diastole in centimeters (Devereux et al 1986). Intra-observer variability was assessed on 29 subjects in whom repeat echocardiographic measurements were performed within a two week period of the initial measurements. The Pearson's correlation coefficients for LV end diastolic diameter, septal wall thickness and posterior wall thickness were 0.76, 0.94 and 0.89 (all $p<0.0001$) respectively, and the variances (mean % difference ± SD) were 0.12±5.95%, -0.77±4.47% and 0.67±5.57% respectively. In addition, no significant differences between repeat measurements were evident on paired t-test analysis ($p=0.99$, $p=0.42$ and $p=0.48$ respectively).To adjust for the influence of body size on LVM, LVM was indexed to height$^{1.7}$ (LVM index-LVMI) (Chirinos et al 2010) and to height$^{2.7}$ when identifying left ventricular hypertrophy (LVH) (LVMI ≥ 51g/m$^{2.7}$) (Nunez et al 2005).

3.2.7 Data analysis

For database management and statistical analysis, SAS software, version 9.1 (SAS Institute Inc., Cary, NC) was employed. Data are expressed as mean±SD unless otherwise

stated. Regression analysis with relevant confounders included in the regression models was used to determine independent relations between central BP and LVMI with adjustments for distending pressure (mean arterial pressures-MAP), heart rate, sex, presence of diabetes mellitus or impaired glucose control, antihypertensive therapy, regular alcohol intake, and smoking status. For the derivation of probability values, further adjustments were made for non-independence of family members using the mixed procedure as outlined in the SAS package.

3.3 Results

3.3.1 Characteristics of study participants

Table 3.1 gives the demographic, clinical and haemodynamic characteristics of the study group and of the participants not included in the echocardiography sub-study. The mean age of the participants was 43.8±18.3 and more women than men participated in the study. In general the study group had a high BMI, with ~67% of participants being either overweight (~25%) or obese (~43%). Of the participants 47.4% were hypertensive and 24% were receiving antihypertensive medication, with the majority of these receiving diuretic monotherapy (87.5%). Of the study group 22.7% were either receiving medication for diabetes mellitus or had an impaired blood glucose control (HbA_{1C}>6.1%). A relatively low proportion of participants reported smoking or a regular intake of alcoholic beverages. No differences were noted in the demographic and clinical characteristics between the study group and the participants not included in the echocardiography sub-study (Table 3.1). 23.5% of participants had LV hypertrophy.

Table 3.1. Characteristics of study participants as compared to participants not included in the echocardiography sub-study.

	Study group (n=503)	Not included (n=512)
Sex (% female)	65.8	65.8
Age (years)	43.8±18.3	43.9±18.4
Body mass index (kg/m^2)	29.3±7.7	29.6±8.4
Waist circumference (cm)	90.0±15.9	90.1±16.7
% overweight/obese	24.5/42.9	21.7/42.2
% with central obesity	50.1	51.9
Regular tobacco intake (% subjects)	13.9	15.2
Regular alcohol intake (% subjects)	21.3	23.1
% with diabetes mellitus or HbA_{1C}>6.1%	22.7	23.1
% with treated hypertension	23.9	23.4
% with untreated hypertension	23.5	21.7
% women postmenopausal	44.7	45.1
Conventional SBP/DBP (mm Hg)	131±23/84±13	130±23/84±13
Conventional pulse pressure (mm Hg)	46±15	45±16
Conventional pulse rate (beats/min)	64±12	65±11
Central SBP/DBP (mm Hg)	122±24/85±13	120±23/85±13
Mean arterial pressure (mm Hg)	101±17	100±16
Central pulse pressure (mm Hg)	37±15	35±15
Forward pressure (P1) (mm Hg)	26±9	24±8
Augmented pressure (AP) (mm Hg)	11±8	11±8
Central augmentation index (AIc) (%)	27±13	28±12
Left ventricular mass index (g/m^2)	88.2±27.8	-
% with left ventricular hypertrophy	23.5	-

HbA_{1C}, glycosylated haemoglobin; BP, blood pressure; SBP, systolic BP; DBP, diastolic BP

3.3.2 Relationship of central and brachial artery PP or SBP with LVMI independent of distending pressure

Figure 3.1 shows the multivariable adjusted correlation coefficients (partial r values) for the relationships between central aortic PP (PPc) or systolic BP and LVMI and the relationships between brachial artery PP or systolic BP and LVMI before and after adjustments for mean arterial pressure (MAP). Figure 3.2 shows the multivariable adjusted LVMI values at increasing quartiles of central aortic or brachial PP before and after adjustments for MAP. Both brachial artery and central aortic SBP and PP were independently related to LVMI and these relationships survived adjustments for MAP (Figure 3.1). Moreover, multivariable adjusted LVMI increased across quartiles of either central or brachial artery PP either before or after adjustments for MAP (Figure 3.2). However, after adjustments for MAP, LVMI in the second and third quartiles for brachial PP failed to show significant increases as compared to the first quartile of PP (Figure 3.2).

3.3.3 Relationship between central PP or systolic BP and LVMI beyond brachial PP or systolic BP.

Figure 3.3 shows the independent relationship (multivariable adjusted) between central aortic PP or systolic BP and LVMI after adjustments for brachial artery systolic BP or PP as well as the independent relationship between brachial artery systolic BP or PP after adjustments for central aortic systolic BP or PP. The relationship between central aortic systolic BP or PP and LVMI survived adjustments for brachial artery systolic BP or PP. However, the relationship between brachial artery aortic systolic BP or PP and LVMI failed to survive adjustments for central systolic BP or PP.

3.3.4 Relationship between the pressure components of central PP and LVMI independent of distending pressures

Table 3.2 shows the standardized β-coefficients (slopes) and the partial r values for the relationships between the forward (P1) or augmented (AP) pressure components of central PP, or aortic augmentation index (AIc) and LVMI independent of distending pressures (MAP) as well as a number of confounders. Both P1 and AP were significantly

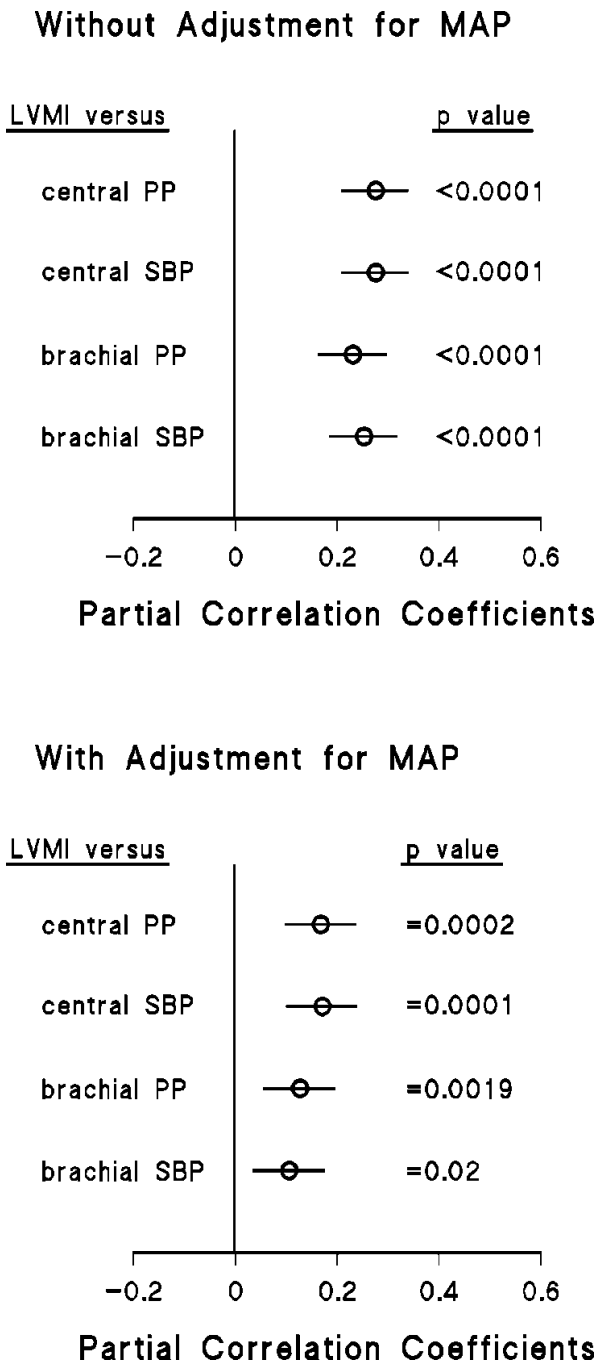


Figure 3.1. Effect of adjustments for distending pressures (mean arterial pressure-MAP) on the multivariable adjusted correlation coefficients (partial r values) for the relationships between central aortic pulse pressure (PP) or systolic blood pressure (SBP) and LVMI and the relationships between brachial artery PP or SBP and LVMI. Other adjustments include sex, presence of diabetes mellitus or impaired glucose control, antihypertensive therapy, regular alcohol intake, smoking status and heart rate where appropriate. For the derivation of probability values, further adjustments were made for non-independence of family members.

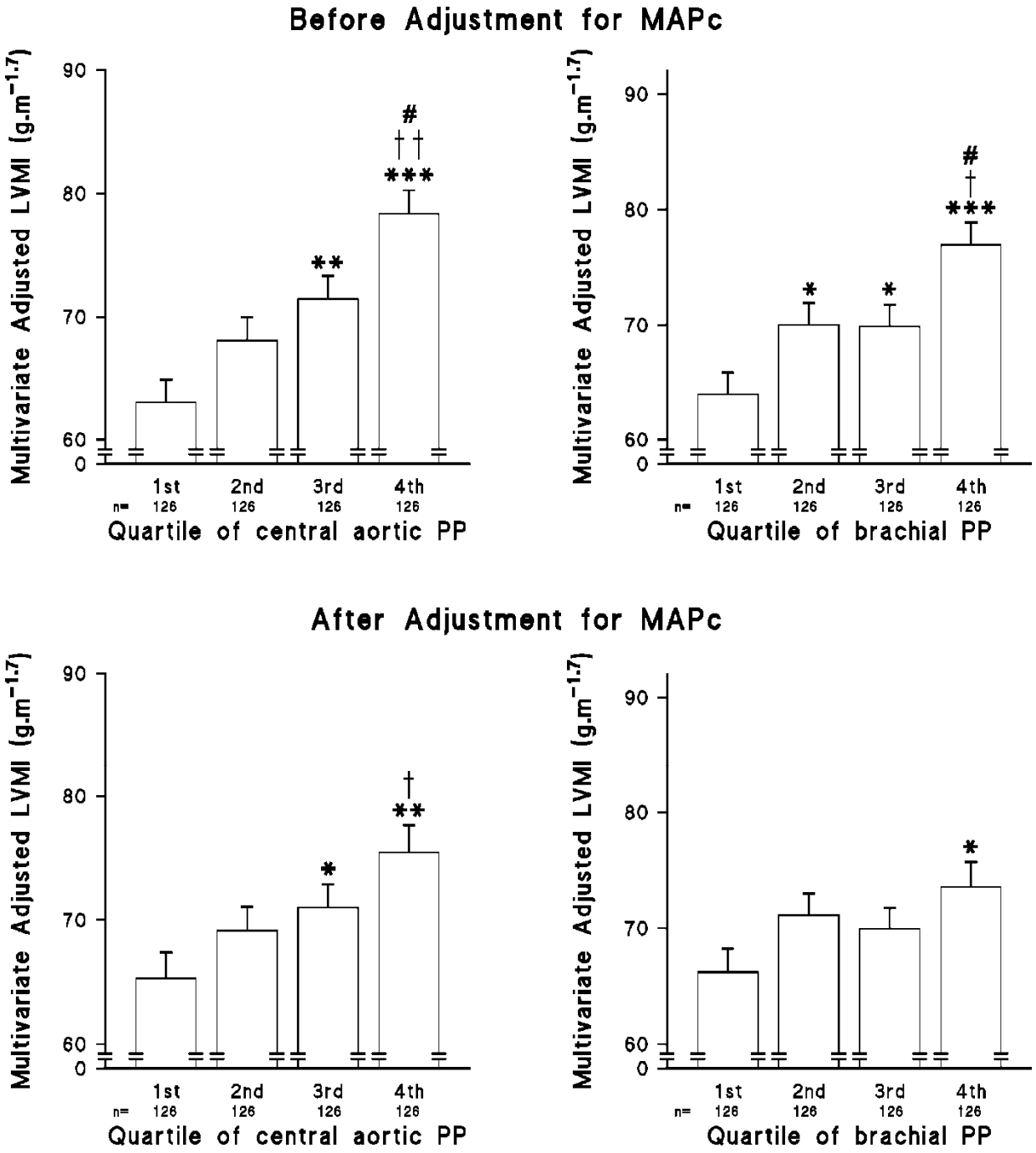
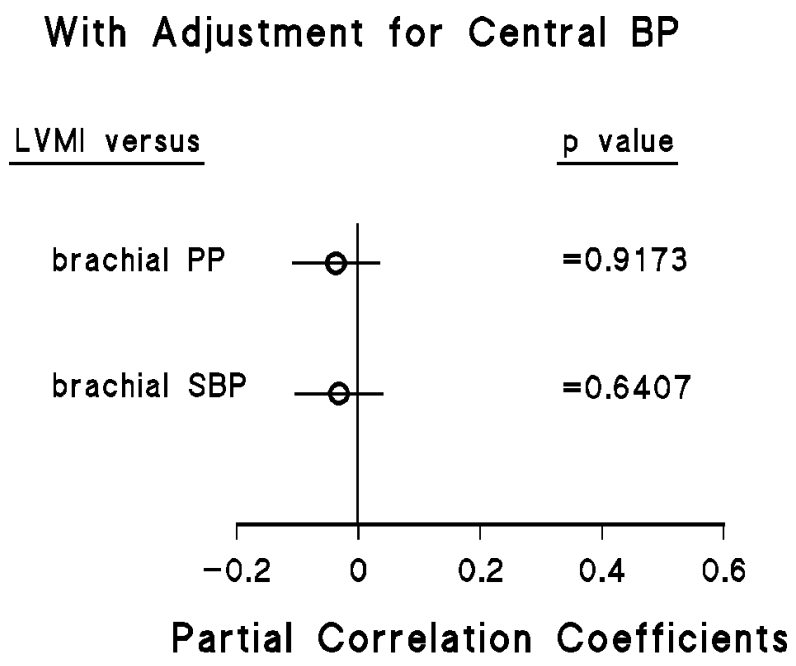


Figure 3.2. Effect of adjustments for distending pressures (mean arterial pressure-MAP) on the multivariable adjusted LVMI values across quartiles of central aortic or brachial pulse pressure (PP). Other adjustments include sex, presence of diabetes mellitus or impaired glucose control, antihypertensive therapy, regular alcohol intake, smoking status and heart rate where appropriate. For the derivation of probability values, further adjustments were made for non-independence of family members. * $p<0.05$; ** $p<0.005$; *** $p<0.0001$ versus 1st quartile; † $p<0.01$; †† $p<0.005$ versus 2nd quartile; # $p<0.01$ versus 3rd quartile.



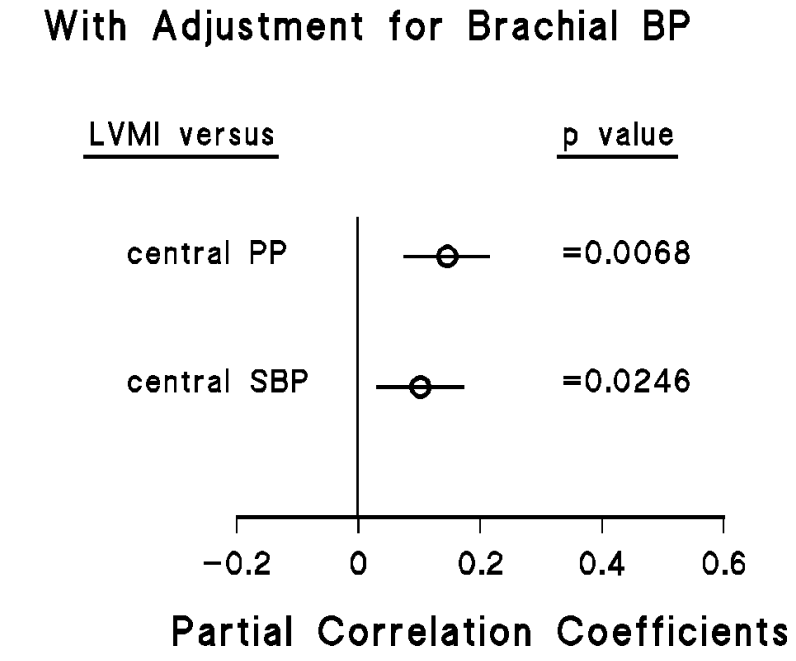


Figure 3.3. Relationship between aortic BP and left ventricular mass index (LVMI) after adjustments for brachial artery pressures (lower panel) and relationship between brachial BP and LVMI after adjustments for aortic pressures (upper panel). PP, pulse pressure; SBP, systolic BP. Other adjustments include sex, presence of diabetes mellitus or impaired glucose control, antihypertensive therapy, regular alcohol intake, smoking status and heart rate. For the derivation of probability values, further adjustments were made for non-independence of family members.

Table 3.2. Relationships between the forward (P1) or the augmented (AP) pressure components of central pulse pressure, or central augmentation index (AIc) and left ventricular mass indexed to height$^{1.7}$ (LVMI) independent of mean arterial pressure-MAP) and other confounders.

LVMI versus	β-coefficient*†±SEM	partial r †	confidence interval	p value
	With the forward pressure component (P1) in the regression model			
P1	0.16±0.04	0.17	0.08 to 0.25	=0.001
Male sex	-0.15±0.04			<0.001
DM or HbA_{1C}>6.1%	0.17±0.04			<0.0001
MAP	0.15±0.05			<0.001
Body weight	0.29±0.04			<0.0001
	With the augmented pressure (AP) in the regression model			
AP	0.13±0.06	0.10	0.01 to 0.18	<0.05
Male sex	-0.19±0.04			<0.0001
DM or HbA_{1C}>6.1%	0.16±0.04			0.0001
MAP	0.13±0.06			<0.05
Body weight	0.27±0.04			<0.0001
	With central augmentation index (AIc) in the regression model			
AIc	0.01±0.05	0.01	-0.08 to 0.10	0.45
Male sex	0.21±0.05			<0.0001
DM or HbA_{1C}>6.1%	0.17±0.04			<0.0001
MAP	0.21±0.05			<0.0001
Body weight	0.26±0.04			<0.0001

*Standardized β-coefficient. † Heart rate, smoking status, regular alcohol intake and treatment for hypertension were also included in the regression models.

associated with LVMI independent of MAP and other confounders (Table 3.2). However, no independent relationship between aortic augmentation index (AI) and LVMI was noted beyond MAP and additional confounders (Table 3.2).

To account for the strong negative relationship between AP and height ($r=-0.22$, $p<0.0001$) and AIc and height ($r=-0.28$, $p<0.0001$), I also evaluated the relationship between non-indexed LVM and AP or AIc, with both log-transformed height (to account for the non-linear relationship between height and LVM) and height (to account for the linear relationship between height and AP or AIc) in the model. In these analyses, AP and AIc were independently associated with LVM (AP, partial $r=0.19$, $p<0.0001$; AIc, partial $r=0.11$, $p<0.005$). Furthermore, P1 was independently associated with LVM (partial $r=0.22$, $p<0.0001$) and the P1-LVM relationship was equally as strong as the AP-LVMI relationship.

3.4 Discussion

The main finding of the present study are that in a randomly selected community sample of African ancestry, both the forward (P1) and the augmented (AP) pressure component of PPc were associated with LVMI independent of MAP and other confounders. Moreover, in the present study, central systolic BP or PP were related to LVM beyond systolic BP or PP measured at the brachial artery.

Although there is now increasing evidence to suggest that central PP contributes toward cardiovascular outcomes (London et al 2001, Safar et al 2002, Chirinos et al 2005, Williams et al 2006, Roman et al 2007, Wang et al 2009) and target organ changes (Lekakis et al 2004, Shaman et al 2005, Roman et al 2007, Wang et al 2009, Roman et al 2010) beyond brachial artery pressures, the pressure components of central PP that contribute toward the adverse effects of central PP on the cardiovascular system have generated recent debate. As in normotensive samples AP accounts for most of the age-related increases in central BP across the lifespan (Namasivayam et al 2009, Cecelja et al 2009) and P1 or the factors that determine P1 (aortic stiffness and diameter) contribute toward

increases in aortic PP only in the elderly (Mitchell et al 2004, Namasivayam et al 2009) or in patients with systolic hypertension (Mitchell et al 2003, Mitchell et al 2008), the role of P1 as a mediator of age-related changes in aortic BP across the lifespan has been considered to be less important than AP. However, as demonstrated in chapter 2 of the present thesis, I have provided evidence to show that in a community with a high prevalence of uncontrolled blood pressure both P1 and AP contribute to central BP across the adult lifespan. The present study extends this finding by demonstrating that independent of distending pressure P1 contributes toward a substantial proportion of the variability of LVMI in a community sample with a high prevalence of uncontrolled hypertension. As LVMI is a well-accepted strong independent risk factor for cardiovascular disease, the present study therefore suggests that therapeutically targeting the factors that influence P1 independent of distending pressures may be an important consideration across-the adult lifespan.

The role of P1 as a determinant of LVMI is in keeping with a previous study that has reported on relationships between measures of arterial stiffness which could contribute toward P1 and LV structural changes (Roman et al 2000). However, in contrast to the present study, in that study (Roman et al 2000) the relationships between the pressure components of central PP and LVMI were not assessed.

The independent relationship between AP and LVMI independent of distending pressures in the present study, is in-keeping with previous studies demonstrating a relationship between late systolic augmentation of central waveforms (Saba et al 1993), or arterial wave reflections (Hashimoto et al 2008) and LVMI and the closer relationship between augmentation pressures and regression of LVMI during the treatment of hypertension (Hashimoto et al 2007). In this regard, as compared to the effect of early systolic loading on LVH, late systolic pressure augmentation is thought to be responsible for more extensive LVH (Kobayashi et al 1996).

Although not a primary goal of the present study it is important to compare the results of correlations between LVMI and central versus brachial artery PP and systolic BP with results obtained in alternative studies. Recent data suggest that the relationships between

central aortic systolic BP or PP and LVMI exceed those of brachial artery systolic BP or PP and LVMI (Wang et al 2009, Roman et al 2010). In the present study I could not show statistically different relationships (partial r) between central aortic systolic BP or PP and LVMI versus brachial artery systolic BP or PP and LVMI. However, the partial r values for relations between LVMI and central and brachial systolic BP or PP values in the present study were very similar to those noted in a previous study (Wang et al 2009). In the previous studies showing differences in the relationships between central aortic systolic BP or PP and LVMI as compared to those of brachial artery systolic BP or PP and LVMI (Wang et al 2008, Roman et al 2010), markedly greater study sample sizes were evaluated (n=1272 [Wang et al 2009] and n=2585 [Roman et al 2010]) than those employed in the present study (=503). Thus, it is likely that I was not statistically powered to show differences in relationships between central aortic systolic BP or PP and LVMI versus brachial artery systolic BP or PP and LVMI. Nevertheless, I was able to show that when aortic BP values were considered in the same regression models with brachial BP values, that relationships between aortic BP and LVMI survived, whilst relations between brachial artery BP did not. Thus, central PP and systolic BP did show relations with LVMI independent of brachial artery BP values, whilst the converse was not noted.

The limitations of the present study are as follows: As the present study is a cross-sectional design, intervention studies targeting P1 independent of distending pressures are required to establish cause-effect relationships between the forward component and LVMI. Moreover, longitudinal studies are required to identify whether P1 predicts increases in LVMI independent of distending pressure. Although the use of a transfer function accurately estimates central aortic pressures, the reconstructed waveform underestimates the central augmentation pressure because of inaccuracies in identifying the end of the first systolic shoulder (Chen et al 1997). While both flow and pressure parameters are required to accurately separate the forward and backward pressure components, this approach is difficult with non-invasive measurements subject to errors created by turbulent flow in the ascending aorta. Nevertheless, further studies are required with wave separation analysis to

address the issue of the relative role of the forward and reflected pressures as factors involved in mediating increases in LVMI.

The clinical implications of the present study require consideration. In Chapter 2 of the present thesis, I noted that both AP and P1 contributed to increases in PPc across the adult lifespan in a community sample with a high prevalence of uncontrolled blood pressure. In the present study I have demonstrated that in this community sample P1 also contributes toward LVMI independent of distending pressures. Thus, together, these lines of evidence suggest that therapeutic strategies that target P1 may be required across the adult lifespan to produce adequate risk reduction. This has important implications as a number of vasoactive drugs can modify AP, whilst the comparative weight of evidence for drugs aimed at targeting proximal aortic structure and hence P1 is somewhat limited (Zieman et al 2005, Laurent et al 2007). Further studies are therefore required to assist in understanding the mechanisms responsible for the increases in P1 that occur in uncontrolled hypertension. Thus, in subsequent chapters of the present thesis I explored a number of possible mechanisms that could account for increases in both P1 and AP and hence in central BP independent of distending pressures.

In conclusion, the present study suggests that the forward pressure component contributes to variations in LVMI. Therefore, in contrast to the recently held view that targeting the forward pressure component is only necessary in the elderly and that antihypertensive therapy in younger age groups should rather focus on modifying the augmented pressure component (Namasivayam et al 2009, Cecelja et al 2009), the present study suggests that targeting the forward pressure component is likely to be necessary to reduce the cardiovascular risk attributed to increases in central aortic pressures independent of distending pressures across the lifespan. In order to achieve this goal in subsequent studies conducted as part of the present thesis I explored a number of possible mechanisms that could account for age-related increases in both the forward and the augmented pressure component and hence central PP independent of distending pressure. These studies were conducted in order to guide potential future clinical intervention studies.

CHAPTER 4

Relationship between Urinary Salt Excretion and Central Aortic Haemodynamics Independent of Steady State Pressure in the General Population

Redelinghuys et al. *Hypertension*. 2010;56:584-590.

Abstract

Background. Although central (aortic) pulse pressure (PPc) (dynamic pressure) is strongly related to distending pressures (mean arterial pressure [MAP]), central PP predicts cardiovascular outcomes and damage beyond MAP. However, there is uncertainty as to whether modifiable risk factors for hypertension contribute to PPc and its determinants, independent of distending pressure.

Aim. To determine whether indices of salt intake are associated with central dynamic pressures (PP) and its determinants independent of distending pressure (MAP).

Methods and results. In 635 randomly recruited participants with 24-hour urine samples, I assessed the independent relationship between urinary sodium (Na^+) or potassium (K^+) excretion and central PP, the forward (P1) and reflected (aortic augmentation pressure-AP) pressure components of PPc, central augmentation index (AIc), and the determinants of central pressure waves including aortic pulse wave velocity (PWV), effective reflecting distance (ERD), and reflective wave transit time (RWTT). Central haemodynamics were determined using applanation tonometry of the carotid, femoral and radial arteries. With adjustments for potential confounders including age, sex and body mass index, urinary Na^+/K^+ was independently associated PPc ($p<0.0001$), P1 ($p<0.0005$), AP ($p<0.0001$), and AIc ($p<0.005$). With further adjustments for MAP, urinary Na^+/K^+ was independently associated with PPc ($p=0.005$), P1 ($p<0.05$), AP ($p<0.005$), and AIc ($p<0.05$). Similar outcomes were noted with adjustments for central diastolic BP. In contrast to the MAP-independent relationships between urinary Na^+/K^+ and both PPc and its pressure components, the relationship between urinary Na^+/K^+ and brachial PP did not survive adjustments for brachial MAP. The independent relationship between urinary Na^+/K^+ and central dynamic pressures could not be accounted for by similar relationships between urinary Na^+/K^+ and aortic PWV, ERD, or RWTT, independent of static pressures.

Conclusion. In a population of African ancestry, urinary salt excretion is related to central PP independent of distending BP. The central effects of salt intake are attributed to increases in P1 and AP, but not to aortic PWV. Hence, modifying salt intake could influence

cardiovascular risk through effects on central PP as well as both the central forward and augmented pressure components independent of distending pressure (MAP or central diastolic BP) or aortic PWV.

4.1 Introduction

As discussed in the first chapter of the present thesis, pulse pressure (PP) predicts cardiovascular outcomes beyond other measures of blood pressure (BP) including measures of distending pressure such as mean arterial pressure (MAP) (Benetos et al 1997, Benetos et al 1998, Verdecchia et al 1998, Franklin et al 1999, Millar et al 1999, Chae et al 1999, Lee et al 1999, Domanski et al 1999, Blacher et al 1999, Vaccarino et al 2000, Glynn et al 2000, Franklin et al 2001, Domanski et al 2001). Moreover, central PP may be more closely associated with cardiovascular outcomes than peripheral PP (London et al 2001, Safar et al 2002, Chirinos et al 2005, Williams et al 2006, Roman et al 2007, Wang et al 2009). Thus contemporary notions of the adverse actions of BP are viewed in terms of distending effects, indexed by MAP, and dynamic or pulsatile effects, indexed by PP, with central PP receiving the most attention. The effects of PP independent of MAP on cardiovascular outcomes (Benetos et al 1997, Benetos et al 1998, Verdecchia et al 1998, Franklin et al 1999, Millar et al 1999, Chae et al 1999, Lee et al 1999, Domanski et al 1999, Blacher et al 1999, Vaccarino et al 2000, Glynn et al 2000, Franklin et al 2001, Domanski et al 2001) are particularly impressive considering the close relationship between MAP and PP. Developing strategies that decrease PP, particularly central PP, without necessarily influencing MAP, is therefore of considerable interest and in this regard understanding the mechanisms responsible for these changes is of importance.

Age has been identified as the major determinant of PP, with increases in the augmented (reflected) pressure component (AP) contributing to most of the age-related increases in central PP in normotensives across the lifespan (Cecelja et al 2009, Namasivayam et al 2009). Moreover, AP or the augmentation index (an index of wave reflection) have been demonstrated to be independently related to cardiovascular target organ changes or cardiovascular outcomes in a variety of clinical populations (Saba et al 1993, London et al 2001, Nurnberger et al 2002, Hayashi et al 2002, Weber et al 2004, Ueda et al 2004, Weber et al 2005, Chirinos et al 2005, Williams et al 2006, Hashimoto et al 2007).

However, in chapter 2 of the present thesis I have demonstrated that AP is not the only pressure component that contributes toward central aortic PP. Indeed, in a community sample with a high prevalence of uncontrolled hypertension, the forward pressure component (P1) made a substantial contribution to age-related increases in central PP and systolic BP and to the variability in central PP independent of distending pressures. Furthermore, in Chapter 3 of the present thesis I have shown that P1 contributes significantly toward increases in left ventricular mass index. Thus, in order to develop appropriate preventative or therapeutic measures, the pathophysiological mechanisms responsible for the variability of both AP and P1 independent of distending pressures need to be identified. In this regard, hypertension may increase P1 and hence central PP either as a consequence of damage to large vessels, or through the pathophysiological mechanisms responsible for hypertension. What has not received due consideration is the possibility that salt intake may influence central BP independent of distending pressures.

Although a number of studies suggest that reductions in sodium (Na^+) intake, and increases in potassium (K^+) intake influence BP and the risk for hypertension (The Trials of Hypertension Prevention Collaborative Research Group 1997, Whelton et al 1997, Whelton et al 1998, He et al 2000, Sacks et al 2001, Vollmer et al 2001, Jurgens et al 2008), whether salt intake modifies central PP, the forward and augmented pressure components and their determinants independent of MAP is uncertain. This question is particularly pertinent for groups of African descent in whom the response to modifications in salt intake may be more profound than in other ethnic groups (Sacks et al 2001, Jurgens et al 2008). Therefore, I aimed to assess whether independent of MAP urinary indexes of Na^+ and K^+ intake are associated with central (aortic) PP, and the component forward and augmented pressures and their determinants, in a population sample of African ancestry with an average Na^+ and K^+ intake that differs markedly from the recommended daily allowance (Maseko et al 2006).

4.2 Methods

4.2.1 Study participants.

The present study design and a description of the participants recruited have been outlined in chapter 2, page 47 of the present thesis. Of the 1015 participants with central haemodynamic assessments, 635 participants had 24-hour urinary samples that met with pre-specified quality control criteria previously described (Maseko et al 2006). Eighty three of these participants were too obese to obtain acceptable femoral pulse waves to determine carotid-femoral (aortic) pulse wave velocity (PWV).

4.2.2 Clinical, demographic and anthropometric assessments.

A standardized questionnaire was administered to obtain demographic and clinical data as previously described (Norton et al 2008, Woodiwiss et al 2009). Details of the questionnaire are provided in chapter 2, pages 47-48 of the present thesis.

Height and weight were measured with participants standing, wearing indoor clothing and no shoes. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Participants were identified as being overweight if their body mass index (BMI) was ≥25 kg/m^2 and obese if their BMI was ≥30 kg/m^2.

4.2.3 Conventional blood pressure

A trained nurse-technician measured conventional (brachial) blood pressure (BP) using a standard mercury sphygmomanometer. Details of the measurements are provided in chapter 2, page 48 of the present thesis. In the present study only 1.89% of visits had fewer than the planned BP recordings. The frequency of identical consecutive recordings was 0.16% for systolic BP and 0.96% for diastolic BP. The occurrence of BP values recorded as an odd number was 0.02%. Of the 6242 systolic and diastolic BP readings, 29.37% ended on a zero (expected =20%). Hypertension was defined as a mean BP≥140/90 mm Hg (Chobanian et al 2003, Williams et al 2004, Mancia et al 2007) or the use of antihypertensive medication.

4.2.4 Laboratory (blood) tests.

Standard laboratory blood tests of renal function, liver function, blood glucose, haematological parameters, and percentage glycated haemoglobin (HbA_{1C}) were performed. Details of the blood tests are provided in chapter 2, page 49 of the present thesis. Diabetes mellitus or inappropriate blood glucose control was defined as the use of insulin or oral hypoglycaemic agents or an HbA_{1C} value greater than 6.1% (Bennett et al 2007).

4.2.5 Pulse wave analysis

Central BP, pulse pressure, P1, AP, aortic pulse wave velocity (PWV), augmentation index (AIc), the effective reflecting distance (ERD) and the reflective wave transit time (RWTT) were estimated using techniques previously described (Shiburi et al 2006). Details of the pulse wave analysis are provided in chapter 2, pages 49-53 of the present thesis.

4.2.6 Urinary electrolyte excretion rates.

Urine samples were obtained over a period of at least 24-hours after disposing of urine obtained immediately prior to the collection period. Urine Na^+, K^+, and creatinine concentrations were measured and 24 urine Na^+ and K^+ excretion rates calculated from the product of urine volume and urine electrolyte concentration. Creatinine clearance was determined from the product of urine volume and urine creatinine concentration/plasma creatinine concentration. I determined the quality of the urine samples by constructing regression models between 24-hour urine creatinine and body weight and 24-hour urine volume and age in sex-specific groups. Using the 95% confidence intervals for each group, I deemed a 24-hour urine sample to be acceptable if 24-hour urine creatinine (mmol) was >3.5 and <35 for males and >3.5 and <30 for females. Urine samples with volumes <300 ml/day were regarded to be incomplete urine collections and thus not of sufficient quality to be included in data analysis. These methods of quality control are standard approaches and have been published elsewhere (Kuznetsova et al 2004).

4.2.7 Data analysis.

For database management and statistical analysis, SAS software, version 9.1 (SAS Institute Inc., Cary, NC) was employed. Data are expressed as mean±SD unless otherwise

stated. Regression analysis with relevant confounders included in the regression models was used to determine independent relations between urinary electrolyte concentrations and central BP and haemodynamics. I adjusted for age, sex, BMI, presence of diabetes mellitus or impaired glucose control, antihypertensive therapy, regular alcohol intake, smoking status, and MAP (where appropriate) as relevant confounders. For the derivation of probability values, further adjustments were made for non-independence of family members using the mixed procedure as outlined in the SAS package. As the majority of treated hypertensives were receiving thiazide diuretic therapy, which in the initial phase of therapy may affect urinary electrolyte excretion rates, sensitivity analysis was conducted in participants not receiving diuretics.

4.3 Results

4.3.1 Characteristics of the participants.

Table 4.1 gives the demographic, clinical and haemodynamic characteristics of the study group. The mean age of the participants was 45.1±18.4 and more women than men participated in the study. In general the study group had a high BMI, with ~67% of participants being either overweight (~24%) or obese (~43%). Approximately one quarter of the sample was receiving antihypertensive therapy and the majority of treated participants (21.3% of the total sample) were receiving thiazide diuretics. Of the participants, 23% regularly consumed alcohol, 14.2% were tobacco smokers, and 25.5% had diabetes mellitus or an HbA_{1C}>6.1%. Of the participants not receiving diuretic therapy only 4.2% were receiving alternative antihypertensive agents and they were younger, had a lower BMI, a lower BP, PP, AP, effective reflecting distance and aortic PWV (data not shown). The average 24-hour urinary Na^+ excretion rate was well above the recommended daily allowance (RDA) for Na^+ intake of 65 mmol/day, with most of the study group (68%) ingesting more than the RDA for Na^+ intake. All participants had 24-hour urinary K^+ excretion rates less than the RDA for K^+ intake of 120 mmol/day. The general characteristics of participants who

Table 4.1. Demographic, clinical, anthropometric and haemodynamic characteristics of study sample.

	n=635
Sex (% female)	65.2
Age (years)	45.1±18.4
Body mass index (kg/m^2)	29.8±8.2
Regular tobacco intake (%)	14.2
Regular alcohol intake (%)	23.0
% with diabetes mellitus or HbA_{1C}>6.1%	25.5
% treated for hypertension	24.6
% receiving thiazide diuretic	21.3
24-hour urinary Na^+ (mmol)	105.4±72.9
24-hour urinary K^+ (mmol)	28.1±18.7
24-hour urine volume (ml)	1382±724
Urinary Na^+/K^+	4.3±2.2
Urinary Na^+/creatinine	14.0±8.3
Urinary K^+/creatinine	3.7±1.9
Conventional SBP/DBP (mm Hg)	132±23/85±13
Conventional mean arterial pressure	102±17
Conventional pulse pressure (mm Hg)	47±16
Pulse rate (beats/min)	65±12
Central SBP/DBP (mm Hg)	124±24/86±13
Mean arterial pressure (mm Hg	102±17
Central pulse pressure (mm Hg)	38±16
Forward pressure component(P1) (mm Hg)	26±10
Augmentation pressure (AP) (mm Hg)	12±9
Augmentation index (%)	28±13
Pulse pressure amplification (mm Hg)	9.4±6.8
Effective reflecting distance (cm)	37.0±15.6
Reflected wave transit time (msec)	106±13
Aortic pulse wave velocity (m/sec) (n=552)	7.0±3.0

Data expressed as mean ± SD unless otherwise stated. HbA_{1C}, glycosylated haemoglobin; BP, blood pressure; SBP, systolic BP; DBP, diastolic BP.

did not have 24-hour urinary samples that met with pre-specified quality control were no different from the characteristics of the participants in the study group (Table 4.2).

4.3.2 Relationships between urinary indexes of salt intake and steady state or pulsatile BP.

As shown in Table 4.3, with adjustments for age, BMI, sex, treatment for hypertension, smoking status, regular alcohol intake, and diabetes mellitus or an HbA_{1C}>6.1%, an independent relationship between the ratio of urinary Na^+-to-K^+ (urinary Na^+/K^+) and either central or conventional PP was noted. Importantly, the independent relationships between urinary Na^+/K^+ and PPc were noted both before and after adjustments for MAP, whereas the relationships between urinary Na^+/K^+ and conventional PP were abolished with adjustments for MAP (Table 4.3). Similar outcomes were noted after adjustments for diastolic BP rather than MAP (data not shown). Urinary Na^+/K^+ was also independently associated with conventional and central systolic BP, but no independent relationships with diastolic BP were noted (Table 4.3).

In sex-specific analyses, a relationship between urinary Na^+/K^+ and PPc was noted after additional adjustments for MAP and other confounders both in men (partial r=0.16, p<0.05) and in women (partial r=0.12, p<0.05). Similarly, sensitivity analysis conducted in participants not receiving diuretic therapy revealed an independent relationship between urinary and PPc before and after adjustment for MAP, whereas the independent relationship between urinary Na^+/K^+ and conventional PP was abolished after MAP was added as a confounder (Table 4.3). In participants not receiving diuretic therapy urinary Na^+/K^+ was also independently associated with systolic BP, but no independent relations with diastolic BP were noted (Table 4.3).

In contrast to the independent relationships between urinary Na^+/K^+ and BP, neither 24-hour urinary Na^+ nor 24-hour urinary K^+ excretion were independently associated with conventional or central systolic BP, diastolic BP, or PP (p>0.05 for all). Further, although urinary K^+ corrected for urine creatinine concentrations was modestly associated with central PP and central systolic BP (p<0.05 for both) and urinary Na^+ excretion corrected for urine

Table 4.2. Comparison of the general characteristics of study participants with and without quality urine samples.

	With n=635	Without n=336
Sex (% female)	65.2	64.8
Age (years)	45.1±18.4	43.1±18.3
Body mass index (kg/m^2)	29.8±8.2	28.8±7.9
Regular tobacco intake (%)	14.2	13.1
Regular alcohol intake (%)	23.0	21.5
% with diabetes mellitus	8.8	7.4
% with hypertension	43.6	40.6
% treated for hypertension	24.6	22.4

Data expressed as mean ± SD unless otherwise stated.

Table 4.3. Multivariable adjusted partial correlation coefficients (partial r) and 95% confidence intervals for independent relationships between the ratio of urinary Na^+ and K^+ concentrations (urinary Na^+/K^+) and blood pressures in all participants (n=635) and participants not receiving diuretic therapy (n=500).

	All participants (n=635)			Participants not receiving diuretic therapy (n=500)		
Urinary Na^+/K^+ versus	partial r*	confidence intervals	p value†	partial r*	confidence intervals	p value†
Without adjustments for mean arterial pressure						
Central SBP	0.17	0.09-0.24	**<0.0001**	0.16	0.07-0.25	**<0.0005**
Conventional SBP	0.12	0.04-0.20	**<0.005**	0.09	0.01-0.18	**<0.05**
Central DBP	0.07	-0.01-0.14	=0.09	0.07	-0.04-0.14	=0.16
Conventional DBP	0.07	-0.01-0.15	=0.08	0.07	-0.05-0.13	=0.30
Central PP	0.18	0.10-0.26	**<0.0001**	0.18	0.10-0.27	**<0.0001**
Conventional PP	0.11	0.03-0.18	**<0.01**	0.10	0.01-0.18	**<0.05**
With adjustments for mean arterial pressure						
Central PP	0.14	0.06-0.21	**=0.005**	0.14	0.05-0.23	**<0.005**
Conventional PP	0.07	-0.01-0.15	=0.07	0.05	-0.04-0.13	=0.26

*Determined from regression analysis with adjustments for age, body mass index, sex, diabetes mellitus or an HbA_{1C}>6.1%, smoking status, regular alcohol intake, and treatment for hypertension. †Probability values are further adjusted for non-independence of family members. Significant p values are indicated in bold type.

creatinine concentrations was modestly associated with central PP and systolic BP, ($p<0.05$ for all) these relations did not survive adjustments for MAP.

4.3.3 Relationships between urinary indexes of salt intake and PP amplification.

With adjustments for age, BMI, sex, treatment for hypertension, smoking status, regular alcohol intake, and diabetes mellitus or an $HbA_{1C}>6.1\%$, no independent relationship between urinary Na^+/K^+ and PP amplification was noted (partial $r=-0.03$, $p=0.49$).

4.3.4 Relationships between urinary indexes of salt intake and P1, AP, AIc and their determinants.

With adjustments for potential confounders, urinary Na^+/K^+ was independently associated with AP, P1, and AIc, but not with aortic PWV, reflected wave transit time (RWTT), or the effective reflecting distance (ERD) (Table 4.4). The independent relationships between urinary Na^+/K^+ and AP, P1 and AIc were noted both before and after adjustments for MAP (Table 4.4). Similar outcomes were noted after adjustments for central diastolic BP rather than MAP (data not shown). The independent relationship between urinary Na^+/K^+ and P1 was no different from the relationship between urinary Na^+/K^+ and AIc ($p=0.98$ for comparison of the partial r values after adjustments for MAP).

Sensitivity analysis conducted in participants not receiving diuretic therapy revealed that with adjustments for potential confounders, urinary Na^+/K^+ was independently associated with AP, P1, and AIc, but not with aortic PWV, reflected wave transit time, or the effective reflecting distance (Table 4.4). The independent relationships between urinary Na^+/K^+ and AP, P1 and AIc in participants not receiving diuretic therapy were noted both before and after adjustments for MAP (Table 4.4) and similar outcomes were noted after adjustments for diastolic BP rather than MAP (data not shown).

Neither 24-hour urinary Na^+, 24-hour Na^+ corrected for creatinine concentrations, nor 24-hour urinary K^+ excretion rates were independently associated with either P1, AP, AIc, aortic PWV, reflected wave transit time, effective reflecting distance or stroke volume (data not shown). Although urinary K^+ corrected for urine creatinine concentrations was modestly associated with P1 ($p<0.05$) this relationship did not survive adjustments for MAP.

Table 4.4. Multivariable adjusted partial correlation coefficients (partial r) and 95% confidence intervals for independent relationships between the ratio of urinary Na^+ and K^+ concentrations (urinary Na^+/K^+) and central haemodynamics in all participants and participants not receiving diuretic therapy.

	All participants (n=635)			Participants not receiving diuretic therapy (n=500)		
Urinary Na^+/K^+ versus	partial r*	confidence intervals	p value†	partial r*	confidence intervals	p value†
Without adjustments for mean arterial pressure						
Forward pressure component (P1)	0.14	0.06-0.22	**<0.0005**	0.14	0.06-0.23	**<0.001**
Augmentation pressure (AP)	0.18	0.10-0.26	**<0.0001**	0.19	0.10-0.27	**<0.0001**
Central augmentation index	0.13	0.05-0.21	**<0.005**	0.14	0.05-0.23	**<0.005**
Aortic PWV	-0.04	-0.12-0.05	0.54	-0.09	-0.18-0.01	0.13
ERD	-0.05	-0.13-0.04	0.42	-0.09	-0.19-0.00	0.11
RWTT	-0.05	-0.13-0.03	0.2	-0.05	-0.13-0.04	0.32
With adjustments for mean arterial pressure						
Forward pressure component (P1)	0.10	0.02-0.17	**<0.05**	0.10	0.01-0.19	**<0.05**
Augmentation pressure (AP)	0.14	0.06-0.21	**<0.005**	0.15	0.06-0.23	**<0.005**
Central augmentation index	0.10	0.02-0.17	**<0.05**	0.11	0.02-0.20	**<0.05**
Aortic PWV	-0.07	-0.15-0.02	0.18	-0.13	-0.22-0.03	0.06
ERD	-0.07	-0.16-0.01	0.15	-0.13	-0.22-0.03	0.06
RWTT	-0.03	-0.11-0.05	0.44	-0.03	-0.11-0.06	0.58

(n=552 for PWV and ERD analysis in all participants, n=435 for PWV and ERD in participants not receiving diuretic therapy).

*Determined from regression analysis with adjustments for age, body mass index, sex, diabetes mellitus or an HbA_{1C}>6.1%, smoking status, regular alcohol intake, and treatment for hypertension. †Probability values are further adjusted for non-independence of family members. PWV, pulse wave velocity; ERD, effective reflecting distance; RWTT, reflective wave transit time. Significant p values are indicated in bold type.

4.3.5 *Survival of the relationship between urinary Na^+/K^+ and AP or AIc with adjustments for potential determinants*.

To identify the potential factors that contribute toward the relationship between urinary Na^+/K^+ and AP or AIc, I evaluated the impact of adjustments for a number of potential factors on these relationships. As indicated in Table 4.5, adjustments for the neither aortic PWV, RWTT, nor the ERD affected the relationship between urinary Na^+/K^+ and either AP or AIc. Sensitivity analysis conducted in participants not receiving diuretic therapy revealed similar outcomes (Table 4.6).

4.4 Discussion

The novel findings of the present study are as follows: in a randomly selected community sample of African ancestry, salt intake as indexed by urinary Na^+/K^+ was associated with the dynamic (PP) component of BP for central but not conventional measurements independent of MAP or diastolic BP and other confounders. The relationship with PPc could be reproduced both in women and in men and in participants not receiving diuretic therapy, the predominant antihypertensive employed in this population. The relationship between urinary Na^+/K^+ and PPc was accounted for by the augmented (AP) and the forward (incident) (P1) pressure components, but not by arterial stiffness (as indexed by aortic pulse wave velocity), the reflected wave transit time, or the site of wave reflection.

Although there are reports indicating that salt intake is associated with brachial artery PP (Hajjar et al 2001, du Cailar et al 2004, Buyck et al 2009) and that alterations in salt intake modify PP (He et al 2005, He et al 2009), in only one study was the relationship demonstrated after adjustments for MAP and this analysis was sex and age-specific (du Cailar et al 2004). Moreover, in none of these studies was central PP evaluated (Hajjar et al 2001, du Cailar et al 2004, He et al 2005, Buyck et al 2009, He et al 2009), and the central

Table 4.5. Multivariable adjusted partial correlation coefficients (partial r) and 95% confidence intervals for the independent relationships between the ratio of urinary Na^+ and K^+ concentrations (urinary Na^+/K^+) and aortic augmentation pressure and aortic (central) augmentation index (AIc) before and after adjustments for potential determinants in all participants.

Urinary Na^+/K^+ versus	partial r*	confidence intervals	p value†
Aortic augmentation pressure (AP) with adjustments for			
Covariates as listed below* (n=635)	0.18	0.10-0.26	<0.0001
Covariates* + aortic PWV (n=552)	0.18	0.09-0.26	<0.0001
Covariates* + ERD (n=552)	0.17	0.09-0.25	<0.0001
Covariates* + RWTT (n=635)	0.17	0.10-0.25	<0.0001
Aortic augmentation index (AIc) with adjustments for			
Covariates as listed below* (n=635)	0.13	0.05-0.21	<0.005
Covariates* + aortic PWV (n=552)	0.12	0.04-0.20	<0.01
Covariates* + ERD (n=552)	0.12	0.03-0.20	<0.01
Covariates* + RWTT (n=635)	0.12	0.04-0.20	<0.005

*Determined from regression analysis with adjustments for age, body mass index, sex, diabetes mellitus or an HbA_{1C}>6.1%, smoking status, regular alcohol intake, and treatment for hypertension. †Probability values are further adjusted for non-independence of family members. PWV, pulse wave velocity; ERD, effective reflecting distance; RWTT, reflective wave transit time.

Table 4.6. Multivariable adjusted partial correlation coefficients (partial r) and 95% confidence intervals for the independent relationships between the ratio of urinary Na^+ and K^+ concentrations (urinary Na^+/K^+) and aortic augmentation pressure and aortic (central) augmentation index (AIc) before and after adjustments for potential determinants in the participants not receiving diuretic therapy.

Urinary Na^+/K^+ versus	partial r*	confidence intervals	p value†
Aortic augmentation pressure (AP) with adjustments for			
Covariates as listed below* (n=500)	0.19	0.10-0.27	<0.0001
Covariates* + aortic PWV (n=435)	0.21	0.12-0.30	<0.0001
Covariates* + ERD (n=435)	0.20	0.11-0.29	<0.0001
Covariates* + RWTT (n=500)	0.18	0.10-0.27	<0.0001
Aortic augmentation index (AIc) with adjustments for			
Covariates as listed below* (n=500)	0.14	0.05-0.23	<0.005
Covariates* + aortic PWV (n=435)	0.14	0.04-0.23	<0.05
Covariates* + ERD (n=435)	0.13	0.04-0.23	<0.05
Covariates* + RWTT (n=500)	0.13	0.05-0.22	<0.01

*Determined from regression analysis with adjustments for age, body mass index, sex, diabetes mellitus or an HbA_{1C}>6.1%, smoking status, regular alcohol intake, and treatment for hypertension. †Probability values are further adjusted for non-independence of family members. PWV, pulse wave velocity; ERD, effective reflecting distance; RWTT, reflective wave transit time.

haemodynamic mechanisms responsible for this effect were not identified. One study has demonstrated that a low Na^+ diet reduces carotid PP and systolic BP in 12 patients with isolated systolic hypertension, but failed to adjust for MAP (Gates et al 2004). As diastolic BP was also reduced in that study (Gates et al 2004), MAP is likely to have also decreased. Furthermore, in that study (Gates et al 2004), whether the impact of changes in Na^+ intake was more marked for carotid as compared to brachial PP was not evaluated. An alternative study (Starmans-Kool et al, 2010 in-press) published on-line after the results of the present study were published (Redelinghuys et al 2010) similarly demonstrated that an increased Na^+ intake in 10 normotensive participants increased carotid BP and the backward pressure component despite producing modest effects on brachial BP. However, again, the authors of this study failed to adjust for distending pressures. The present study clearly extends the information provided by these studies by demonstrating a strong MAP-independent relationship between a urinary index of salt intake and central PP in a relatively large randomly selected community sample in both men and women and the central haemodynamic changes that may explain this relationship. Importantly, these independent relations were noted for central PP, but the relations with conventional (brachial) PP did not achieve significance.

The relationship between an index of Na^+ intake and systolic, but not diastolic BP in the present study, although in contrast to a number of studies showing relationships of indexes of Na^+ intake with both systolic and diastolic BP (Intersalt Cooperative Research Group 1988, Smith et al 1988, Jurgens et al 2008), is nevertheless in keeping with data obtained in 4919 participants of the SU.VI.MAX study where Na^+ intake was independently related to PP, but not to other measures of BP (Buyck et al 2009).

Consistent with the stronger relations noted between urinary Na^+/K^+ and BP than between 24-hour Na^+ excretion rates and BP in the Intersalt (Intersalt Cooperative Research Group 1988) and the Scottish Heart (Smith et al 1988) studies, in the present study urinary Na^+/K^+, but not 24-hour Na^+ excretion was associated with PP and SBP. Although speculative, this effect could be explained by the relationship that exists between Na^+ intake

and K^+ excretion in groups of African ancestry where urinary K^+ excretion decreases on a high Na^+ diet in salt-sensitive individuals (Price et al 2002). Reduced K^+ urinary excretion may occur as a consequence of an enhanced activity of the Na-K-2Cl co-transporter in the thick ascending limb of the renal tubule (Aviv et al 2004). Consequently, it is possible that Na^+ excretion is more likely to be associated with an increase in BP in those individuals whose K^+ excretion decreases, thus indicating the presence of an active Na-K-2Cl co-transporter in response to Na^+ intake. However, it is also possible that a decreased K^+ excretion despite a similar K^+ intake could be accounted for by an increased K^+ excretion via the gastro-intestinal tract (Barlow et al 1986).

The mechanism that explains the relationship between urinary Na^+/K^+ and central PP independent of MAP is through an impact on P1 as well as AP. In this regard independent of distending pressures AP is largely determined by wave reflection. The features of wave reflection that influence AP include the timing of wave reflection (earlier waves may increase the chance of the forward and reflected wave coinciding), the site of wave reflection (if the site is closer to the central arteries, the chance is increased that the reflected wave may return earlier), the speed of wave conduction (a greater speed increases the chances of earlier reflection) and the magnitude of wave reflection. In the present study, urinary Na^+/K^+ was not related to RWTT, ERD or aortic PWV. Hence the relationship between urinary Na^+/K^+ and either central PP, AP, or the augmentation index may not be explained by changes in the timing or site of wave reflection, nor in the speed of wave conduction. Consequently, I propose that the relationship between urinary Na^+/K^+ and AP is in-part mediated by the magnitude of wave reflection, possibly by altering vascular smooth muscle tone in medium-sized or more distal arteries, a mechanism recently proposed as mediating age-induced changes in central PP (Cecelja et al 2009).

With respect to the potential mechanisms that could explain the relationship between urinary Na^+/K^+ and P1 independent of distending pressures, dietary Na^+ restriction has been demonstrated to decrease indices of arterial stiffness, independent of distending pressures (Seals et al 2001, Gates et al 2004). However, it is unlikely that these effects could be

attributed to aortic structural changes as only short-term dietary Na^+ restriction was studied (Seals et al 2001, Gates et al 2004).

Irrespective of the mechanisms that explain the relationship between urinary Na^+/K^+ and AP or P1 independent of MAP, these findings have important clinical implications as unlike age, salt intake is a modifiable risk factor for cardiovascular disease. Hence, if population-wide decreases in salt intake could be achieved, a considerable reduction in cardiovascular disease at a population level could occur by decreasing the dynamic component of central BP.

The lack of relationship between indices of Na^+ and K^+ intake or urinary Na^+/K^+ and aortic PWV in the present study is in apparent contrast to the decrease in aortic PWV noted in response to a reduction in salt intake in a group of African ancestry as previously demonstrated (He et al 2009). However, in that study (He et al 2009), diastolic BP also decreased in response to a reduction in salt intake, a change that could have contributed to changes in large artery stiffness and hence aortic PWV through decreases in distending pressures only. In contrast, in the present study no relationship between urinary indexes of salt intake and diastolic BP was noted.

The limitations of the present study include the cross-sectional nature of the study and hence conclusions regarding causality of the relations cannot be drawn. Moreover, I assessed 24-hour urinary excretion rates only once and this assessment is subject to inaccuracies in urine collection and does not account for daily variations in salt intake. However, the mean 24-hour urine volumes noted in the present study are higher than those reported on in 23 of 52 sites of the Intersalt study (Intersalt Cooperative Research Group 1988), and the electrolyte excretion rates in the present study are the same as that reported on in an alternative study conducted in the same population group and region in South Africa (SOWETO, Johannesburg) (Barlow et al 1982) as the present study, and in other “salt-sensitive” populations (Liu et al 2009). Moreover, assuming there was a degree of inaccuracy of urine collection; under these circumstances I am likely to have underestimated the impact of salt intake on PP independent of MAP. Despite the convincing independent relations

between urinary Na^+/K^+ but not urinary Na^+ excretion rate and central haemodynamics, the explanation provided for this conundrum that urinary K^+ excretion decreases on a high Na^+ diet in salt-sensitive individuals (Price et al 2002) nevertheless remains speculative. This hypothesis requires further substantiation by assessing central haemodynamics and PP whilst modifying Na^+ and maintaining K^+ intake. It is also important to note that an additional limitation of the present study as underscored in previous Chapters of the present thesis is that although the use of a transfer function and radial tonometry accurately estimates central aortic pressures, the reconstructed waveform underestimates the central augmentation pressure and augmentation index (Chen et al 1997). Again however, this underestimation is likely to have biased against the outcomes of the present study.

In conclusion, in the present study I show that urinary Na^+/K^+ ratios are independently associated with central, but not brachial PP independent of MAP and that the central PP effect is mediated by both forward and augmented pressure effects, but not through changes in aortic PWV. Thus, the present study suggests that alterations in salt intake may modify cardiovascular risk at a population level through an impact on central dynamic BP independent of distending pressures, effects which are not readily detected by brachial artery BP measurements. The present study also suggests that salt intake may in-part explain the contribution of P1 to PPc in a community sample with a high prevalence of uncontrolled hypertension as demonstrated in chapter 2. However, alternative mechanisms should also be sought. In the following 2 chapters, I investigated the possibility that alternate potential mechanisms may also contribute to central PP and its pressure components.

CHAPTER 5

Are C-Reactive Protein (CRP) Concentrations Independently Related to Central Aortic Haemodynamics in a Community with a High Prevalence of Risk-Related CRP?

(Redelinghuys et al. *Am J Hypertens* 2011;24:1094-1101.)

Abstract

Background. The independent contribution of low-grade inflammation to central aortic pressures and the determinants thereof in populations with a high prevalence of risk-related high sensitivity C-reactive protein (hs-CRP) concentrations is uncertain.

Aim. To evaluate whether increases in hs-CRP concentrations can independently account for age-related increases in central systolic blood pressure (BP), pulse pressure (PP), its component pressures (forward [P1] and augmented [AP] pressures), or the determinants of central PP including aortic pulse wave velocity (PWV), effective reflecting distance (ERD) and reflected wave transit time (RWTT) in a community sample of African ancestry, 57% of whom had hs-CRP concentrations >3 mg/l (high risk).

Methods and results. Central aortic haemodynamics (determined using applanation tonometry at the radial, carotid and femoral arteries and SphygmoCor software) and serum ultrasensitive-CRP concentrations were assessed in 836 randomly recruited participants of African ancestry from an urban developing community. Log hs-CRP was strongly correlated with age, distending pressures as indexed by mean arterial pressure (MAP), indices of adiposity, central systolic BP, central PP, AP, P1, PWV, and ERD ($p<0.0001$ for all). However, in multivariable models with adjustments for confounders, hs-CRP was not independently associated with central systolic BP, central PP, AP, P1, PWV, or ERD ($p>0.10$ for all) and multivariable adjusted central haemodynamic variables were similar in participants with low, intermediate, or high risk hs-CRP concentrations. Moreover, with adjustments for confounders, hs-CRP was not independently associated with increases in central systolic BP, PP, P1, AP, aortic PWV or ERD either in normotensives or hypertensives, men or women, lean or overweight/obese, or before or after 60 years of age.

Conclusion. In a large community-based sample with a high prevalence of risk-related hs-CRP concentrations, chronic inflammation as indexed by hs-CRP, does not independently account for age-related increases in central aortic PP, the component pressures, or the determinants of central PP.

5.1 Introduction

As underscored in previous chapters of the present thesis, despite the close relationship between pulse pressure (PP) and MAP, PP predicts cardiovascular outcomes beyond MAP (Benetos et al 1997, Benetos et al 1998, Verdecchia et al 1998, Franklin et al 1999, Millar et al 1999, Chae et al 1999, Lee et al 1999, Domanski et al 1999, Blacher et al 1999, Vaccarino et al 2000, Glynn et al 2000, Franklin et al 2001, Domanski et al 2001). Moreover, central PP may be more closely associated with cardiovascular outcomes than peripheral PP (London et al 2001, Safar et al 2002, Chirinos et al 2005, Williams et al 2006, Roman et al 2007, Wang et al 2009). Thus contemporary notions of the adverse actions of BP are viewed in terms of distending effects, indexed by MAP, and dynamic or pulsatile effects, indexed by PP, with central PP receiving the most attention. Developing strategies that decrease PP, particularly central PP, without necessarily influencing MAP, is therefore of considerable interest and in this regard understanding the mechanisms responsible for these changes is of importance.

Previous studies have indicated that increases in the augmented (reflected) pressure component (AP) contribute to most of the age-related increases in central PP in normotensives across the lifespan (Cecelja et al 2009, Namasivayam et al 2009). Moreover, AP or the augmentation index (an index of wave reflection) have been demonstrated to be independently related to cardiovascular target organ changes or cardiovascular outcomes in a variety of clinical populations (Saba et al 1993, London et al 2001, Nurnberger et al 2002, Hayashi et al 2002, Weber et al 2004, Ueda et al 2004, Weber et al 2005, Chirinos et al 2005, Williams et al 2006, Hashimoto et al 2007). However, in chapter 2 of the present thesis, as compared to normotensives, I have demonstrated that AP is not the only pressure component that contributes toward central aortic PP. Indeed, in a community with a high prevalence of uncontrolled blood pressure, the forward pressure component (P1) and AP contributed equivalently to increases in central PP independent of distending pressures. Furthermore, in Chapter 3 of the present thesis I have shown that both P1 and AP contribute

significantly toward increases in left ventricular mass index. Thus, in order to develop appropriate preventative or therapeutic measures, the pathophysiological mechanisms responsible for both AP and P1 independent of distending pressures need to be identified. In this regard, the contribution of P1 to increases in central BP in uncontrolled hypertensives may occur either as a consequence of damage to large vessels caused by hypertension, or through the pathophysiological mechanisms responsible for hypertension. In Chapter 4 I show that salt intake could account for some of the variability in central BP in this community through effects on both AP and P1. However, the role of alternative factors also requires consideration. In this regard, inflammation may modify central aortic haemodynamics independent of distending pressures.

A causal relationship between inflammatory states induced by vaccinations and reversible increases in aortic stiffness, as indexed by pulse wave velocity (PWV), has been demonstrated (Vlachopoulos et al 2005). Moreover, several studies have shown relationships between the inflammatory marker, high sensitivity C-reactive protein (hs-CRP) and central haemodynamic variables in randomly selected participants largely free of inflammatory or cardiovascular disease (Kampus et al 2004, Yasmin et al 2004, Kullo et al 2005, Duprez et al 2005, Saijo et al 2005, Nagano et al 2005, Andoh et al 2006). However, in contrast to these previous studies conducted in small study samples (n=78-427) (Kampus et al 2004, Yasmin et al 2004, Kullo et al 2005, Duprez et al 2005, Andoh et al 2006), in a large (n=2409) community-based study, Schnabel et al (2008) were unable to show independent relationships between hs-CRP concentrations and central PP, P1 or aortic pulse wave velocity (PWV). However, in that study (Schnabel et al 2008) the median values for hs-CRP concentrations were well below the threshold for a high cardiovascular risk (Pearson et al 2003). The role of hs-CRP as a potential determinant of central aortic haemodynamics in communities with a significant prevalence of high risk hs-CRP concentrations is therefore uncertain. In the present study I thus evaluated whether hs-CRP concentrations are associated with age-related increases in central BP, its component pressures, and potential determinants, namely aortic PWV, reflected wave transit time (RWTT) and effective reflecting

distance (ERD), independent of distending pressure and other confounders in a large community-based sample with a high prevalence of risk-related hs-CRP concentrations.

5.2 Methods

5.2.1 Study participants.

The present study design and a description of the participants recruited have been outlined in chapter 2, page 47 of the present thesis. Of the 1015 participants with central haemodynamic assessments, 836 participants had both serum hs-CRP measurements and pulse wave analysis. One hundred and seventeen of these participants were too obese to obtain acceptable femoral pulse waves to determine carotid-femoral (aortic) pulse wave velocity (PWV).

5.2.2 Clinical, demographic and anthropometric assessments.

A standardized questionnaire was administered to obtain demographic and clinical data as previously described (Norton et al 2008, Woodiwiss et al 2009). Details of the questionnaire are provided in chapter 2, pages 47-48 of the present thesis.

Height and weight were measured with participants standing, wearing indoor clothing and no shoes. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Participants were identified as being overweight if their body mass index (BMI) was ≥25 kg/m^2 and obese if their BMI was ≥30 kg/m^2.

5.2.3 Conventional blood pressure

A trained nurse-technician measured conventional (brachial) blood pressure (BP) using a standard mercury sphygmomanometer. Details of the measurements are provided in chapter 2, page 48 of the present thesis. In the present study only 2.75% of visits had fewer than the planned BP recordings. The frequency of identical consecutive recordings was 0.37% for systolic BP and 1.71% for diastolic BP. The occurrence of BP values recorded as an odd number was 0.01%. Of the 8163 systolic and diastolic BP readings, 29.35% ended on a zero (expected =20%). Hypertension was defined as a mean BP≥140/90 mm Hg

(Chobanian et al 2003, Williams et al 2004, Mancia et al 2007) or the use of antihypertensive medication.

5.2.4 General laboratory (blood) tests.

Standard laboratory blood tests of renal function, liver function, blood glucose, haematological parameters, and percentage glycated haemoglobin (HbA_{1C}) were performed. Details of the blood tests are provided in chapter 2, page 49 of the present thesis. Diabetes mellitus or inappropriate blood glucose control was defined as the use of insulin or oral hypoglycaemic agents or an HbA_{1C} value greater than 6.1% (Bennett et al 2007).

5.2.5 Pulse wave analysis

Central BP, pulse pressure (PPc), P1, AP, aortic pulse wave velocity (PWV), the effective reflecting distance (ERD) and the reflective wave transit time (RWTT) were estimated using techniques previously described (Shiburi et al 2006). Details of the pulse wave analysis are provided in chapter 2, pages 49-53 of the present thesis.

5.2.6 Ultrasensitive C-reaction protein

Ultrasensitive-CRP (hs-CRP) (range 0.05-170mg/l) was measured using an immunoturbidimetric assay performed on Olympus OSR 6185 reagent (Olympus Diagnostics, Lismeehan, Ireland) (inter- and intra-assay coefficients of variation of 1.3% and 0.4%, respectively) and analysed using the Olympus AU640e Chemistry Immunoanalyser (Olympus, United Kingdom). Briefly the serum sample obtained from each participant was mixed with a 0.05% suspension of latex particles coated with goat antihuman CRP antibodies, in the presence of a biological buffer. The presence of CRP in the serum sample caused the antibodies to aggregate, resulting in a light scattering proportional to the CRP concentration in the serum. The presence of the aggregate was detected by reading the absorbance at 800nm.

An hs-CRP concentration <1 mg/l was considered to be related to a low cardiovascular risk, 1-3 mg/l to moderate cardiovascular risk, and a concentration >3 mg/l to high cardiovascular risk (Pearson et al 2003).

5.2.7 Data analysis.

Database management and statistical analyses were performed with SAS software, version 9.1 (The SAS Institute Inc., Cary, North Carolina, USA). Data from individual subjects were averaged and expressed as mean±SD. To identify the independent relationship between hs-CRP and central aortic haemodynamics, linear regression analysis was performed with age, sex, BMI, the presence or absence of diabetes mellitus or an abnormal blood glucose control, antihypertensive therapy, regular alcohol intake, smoking status, MAP and heart rate (where appropriate) included in the models. As hs-CRP was not normally distributed, it was logarithmically transformed to more closely reflect a normal distribution when performing linear regression analysis. Furthermore, multivariable adjusted central aortic haemodynamics were compared between participants with hs-CRP concentrations in low, intermediate or high risk categories. To ensure that the results of the analysis could not be attributed to any specific characteristic of the community studied, sensitivity analysis was performed separately before and after 60 years of age, in normotensives and hypertensives, in men and in women and in lean and overweight/obese categories.

The confounders included in the multivariable analysis performed in different age groups included age, sex, BMI, presence of diabetes mellitus or impaired glucose control, antihypertensive therapy, regular alcohol intake, smoking status, mean arterial pressure (MAP), and heart rate (where appropriate).

For the derivation of probability values, further adjustments were made for non-independence of family members using the mixed procedure as outlined in the SAS package.

5.3 Results

5.3.1 Characteristics of the participants.

Table 5.1 outlines the clinical, demographic, and haemodynamic characteristics of the study participants. More women (64.2%) than men participated and the mean age of the

participants was 43.2±18.0 years. A high percentage of participants were either overweight (23.9%) or obese (41.5%). Hypertensive participants made up 44.4% of the study population, 21.9% were receiving antihypertensive medication of which only 36.6% had a controlled BP. Of the participants 24.4% were either receiving medication for diabetes mellitus or had an impaired blood glucose control (HbA_{1C}>6.1%), 13.4% reported smoking and 23.3% reported a regular intake of alcoholic beverages. High risk hs-CRP concentrations (>3 mg/l) were noted in 57% of the participants.

5.3.2 General factors associated with log hs-CRP.

Age (r=0.36, p<0.0001), all adiposity indices (Figure 5.1), and both peripheral (r=0.20, p<0.0001) and central (r=0.20, p<0.0001) MAP were correlated with log hs-CRP. In multivariable regression analysis, the associations between mean skin-fold thickness or waist-to-hip ratio and log hs-CRP were abolished after adjustment for either BMI or waist circumference (data not shown). However, both BMI and waist circumference retained independent relationships with log hs-CRP when adjusting for alternative adiposity indices with the independent relationships between BMI and log hs-CRP being similar to the independent relationships between waist circumference and log hs-CRP (data not shown). Therefore for all further analyses, I adjusted for BMI as an index of adiposity.

5.3.3 Relationship between log hs-CRP and central BP.

Log hs-CRP concentrations were correlated with central SBP, central PP, AP and P1 (Figure 5.2). However, in multivariable models, no independent relationship between log hs-CRP and central SBP, PPc, P1 or AP was noted (Table 5.2). Moreover, although unadjusted central SBP, PPc, AP and P1 values gradually increased in participants across low, intermediate and high risk hs-CRP concentrations, multivariable adjusted central SBP, central PP, AP and P1 were similar in participants with low, intermediate and high risk hs-CRP concentrations (Figure 5.3).

Table 5.1. Demographic, clinical, anthropometric and haemodynamic characteristics of study sample. n=836

Sex (% female)	64.2
Age (years)	43.2±18.0
Body mass index (kg/m^2)	29.3±7.8
Waist circumference (cm)	89.7±16.0
Skinfold thickness (cm)	
Triceps	1.7±1.1
Subscapular	2.4±1.3
Mean	2.1±1.1
% overweight/obese	23.9/41.5
Regular tobacco intake (% subjects)	13.4
Regular alcohol intake (% subjects)	23.2
% with diabetes mellitus or HbA_{1C}>6.1%	24.4
% with treated hypertension	21.9
% with untreated hypertension	22.5
% women postmenopausal	42.8
Hs-CRP concentration (mg/l)	3.4±3.7 (median=3.7, range=0.1-194.9)
% low/moderate/high risk hs-CRP	18.0/25.2/56.7
Conventional SBP/DBP (mm Hg)	130±22/84±13
Conventional pulse pressure (mm Hg)	45.5±14.8
Conventional pulse rate (beats/min)	64.2±11.6
Central SBP/DBP (mm Hg)	121±23/85±13
Mean arterial pressure (mm Hg)	101±16
Central pulse pressure (mm Hg)	36±15
Forward pressure component (P1) (mm Hg)	25±9
Augmentation pressure (AP) (mm Hg)	11±8
Central augmentation index (%)	27±13.
Aortic pulse wave velocity (m/sec) (n=719)	6.8±2.7
Effective reflecting distance (cm) (n=719)	35.8±14.5
Reflective wave transit time (msec)	105.8±13.1

Data expressed as mean ± SD unless otherwise stated. HbA_{1C}, glycosylated haemoglobin; CRP, C-reactive protein; BP, blood pressure; SBP, systolic BP; DBP, diastolic BP.

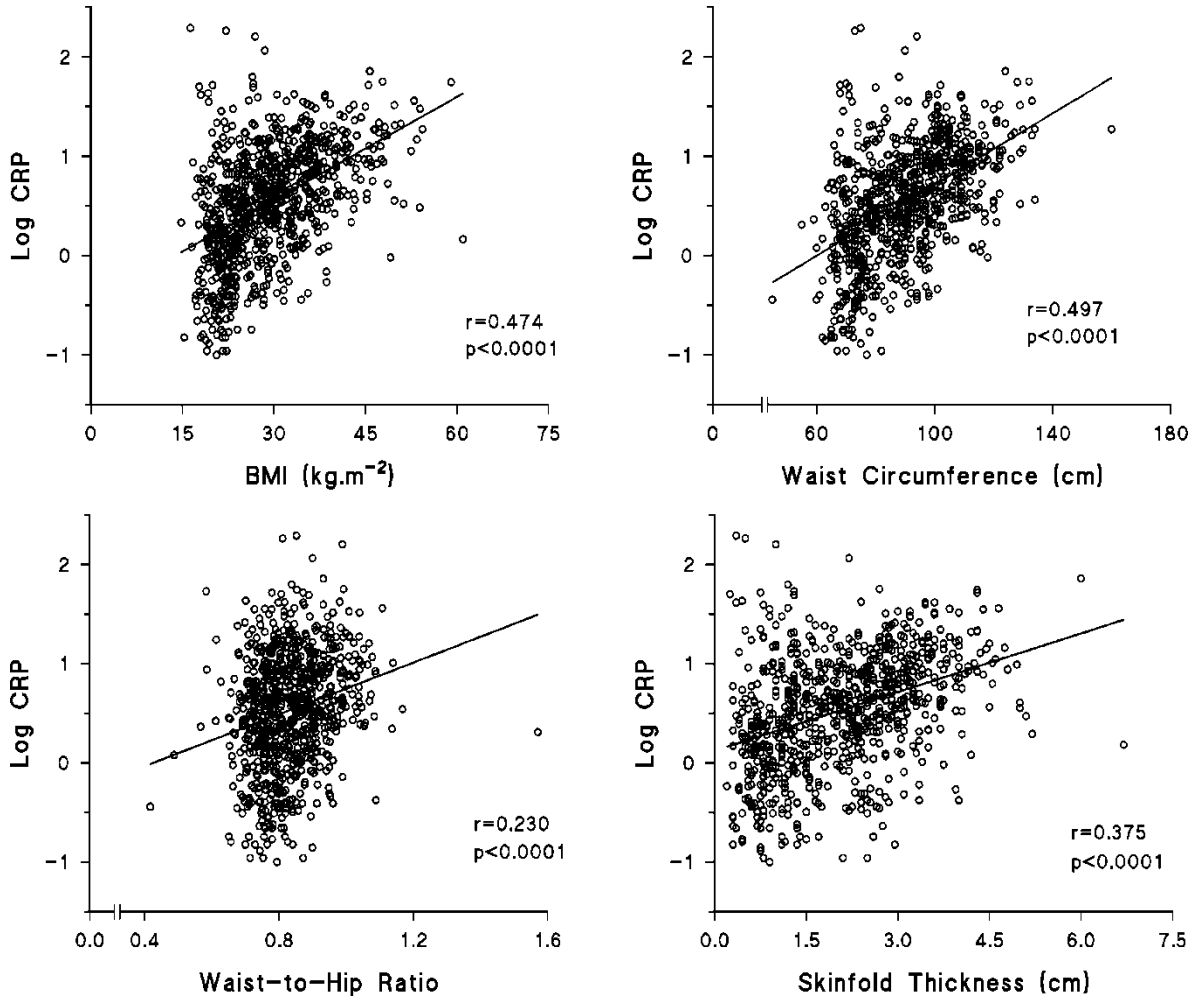


Figure 5.1. Bivariate relationships between log hs-CRP and indices of adiposity in study participants (n=836). BMI, body mass index.

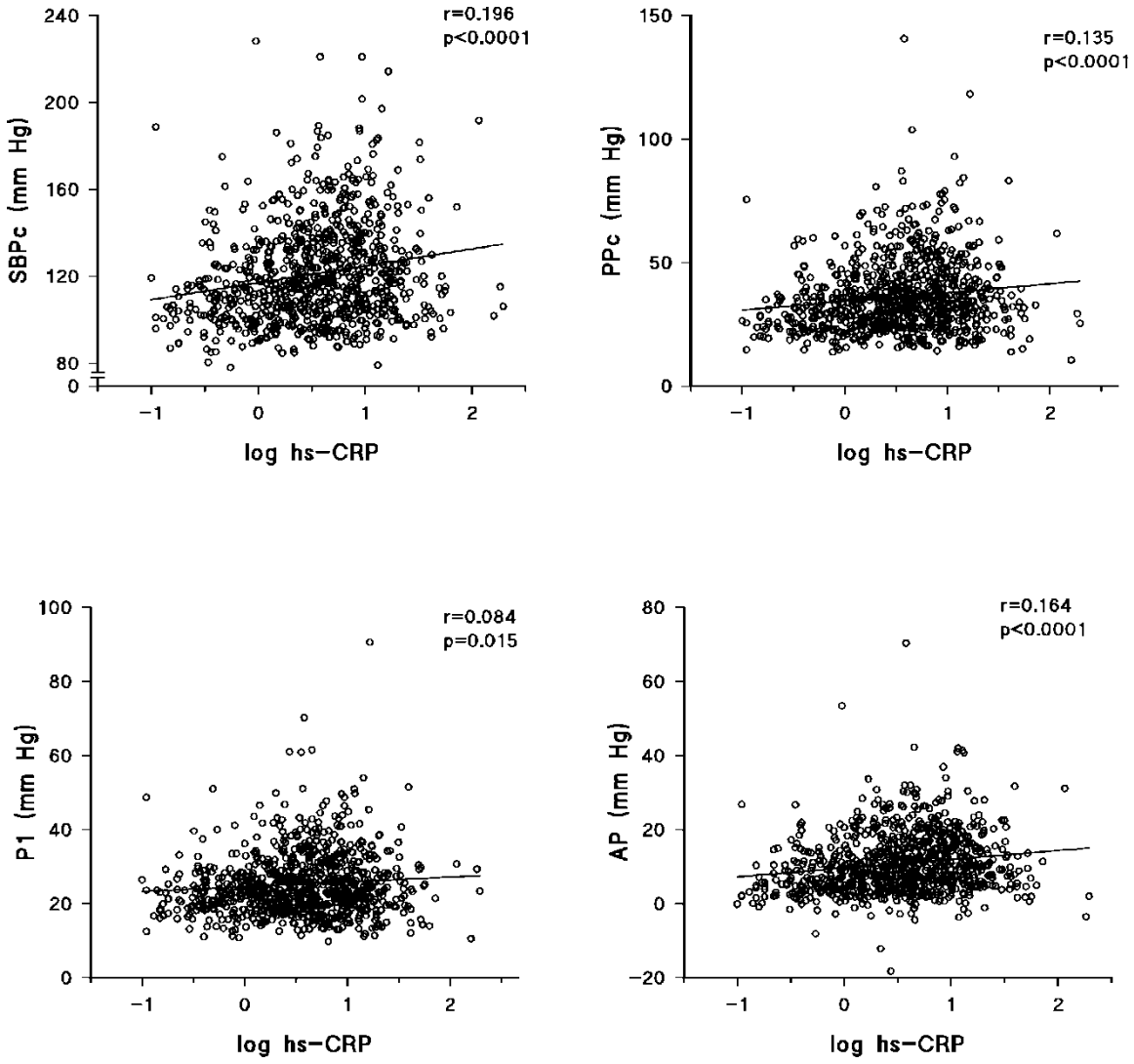


Figure 5.2. Bivariate relationships between log hs-CRP and central aortic blood pressures (BP) in study participants (n=836). SBPc, central systolic BP; PPc, central pulse pressure; P1, forward pressure component; AP, augmentation pressure.

Table 5.2. Partial correlation coefficients (r) and 95% confidence intervals for the independent relationships between log hs-CRP and central blood pressure (BP) and its pressure components in all participants (n=836).

Log hs-CRP versus	partial r*	confidence intervals	p value†
Central systolic BP	-0.03	-0.10-0.04	0.42
Central pulse pressure	-0.04	-0.11-0.02	0.22
Forward pressure (P1)	-0.05	-0.12-0.02	0.12
Augmentation pressure (AP)	-0.03	-0.09-0.04	0.53

*Determined from regression analysis with adjustments for age, sex, body mass index, presence of diabetes mellitus or impaired glucose control, antihypertensive therapy, regular alcohol intake, smoking status, heart rate and mean arterial pressure. †Probability values are further adjusted for non-independence of family members.

5.3.4 Relationship between log hs-CRP and the determinants of central BP.

Log hs-CRP concentrations were also correlated with log PWV and ERD, but not with RWTT (Figure 5.4). However, in multivariable models, no independent relationships between log hs-CRP and PWV or ERD were noted (Table 5.3). Moreover, although unadjusted PWV and ERD values gradually increased in participants across low, intermediate and high risk hs-CRP concentrations, multivariable adjusted PWV, ERD, and RWTT were similar in participants with low, intermediate and high risk hs-CRP concentrations (Figure 5.5).

5.3.5 Relationship between log hs-CRP and central BP and its determinants in subgroup analysis.

No independent relationships between log hs-CRP and central BP or the determinants of central BP were noted in hypertensive or normotensive participants (Table 5.4), in men or women (Table 5.5), or in obese or lean participants (Table 5.6).

5.3.6 Relationship between log hs-CRP and central BP or its determinants in age-specific categories.

As indicated in Table 5.7, no independent relationships between hs-CRP and central aortic haemodynamics were noted before or after 60 years of age.

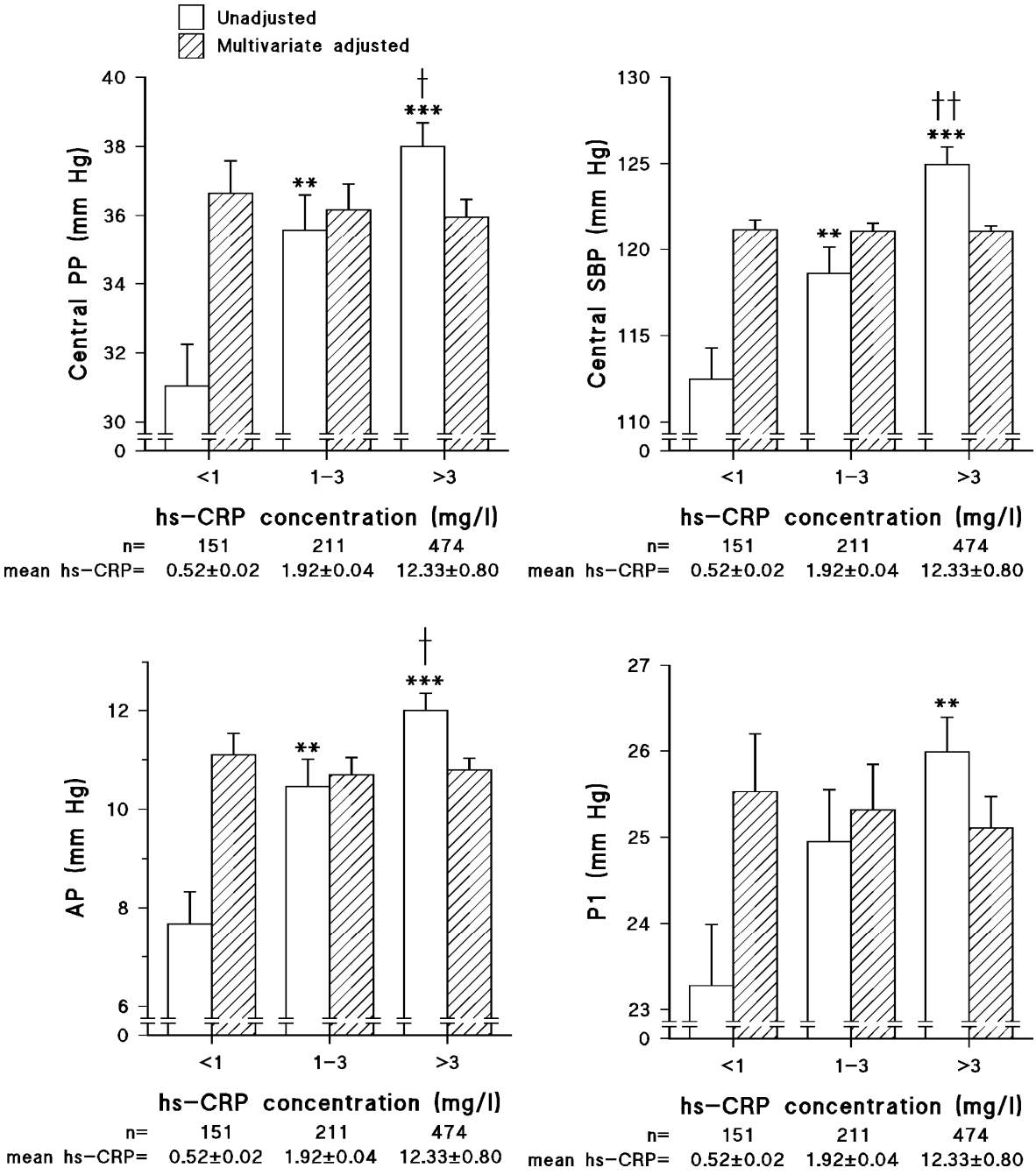


Figure 5.3. Unadjusted and multivariable adjusted central aortic blood pressures (BP) in study participants with low (<1 mg/l), moderate (1-3 mg/l) and high (>3 mg/l) risk hs-CRP concentrations. SBPc, central systolic BP; PPc, central pulse pressure; P1, forward pressure component; AP, augmentation pressure. Adjustments are as given in Table 5.2 legend. *p<0.05, **p<0.01, ***p<0.0001 versus low risk category. †p<0.05, ††p<0.001 versus moderate risk category.

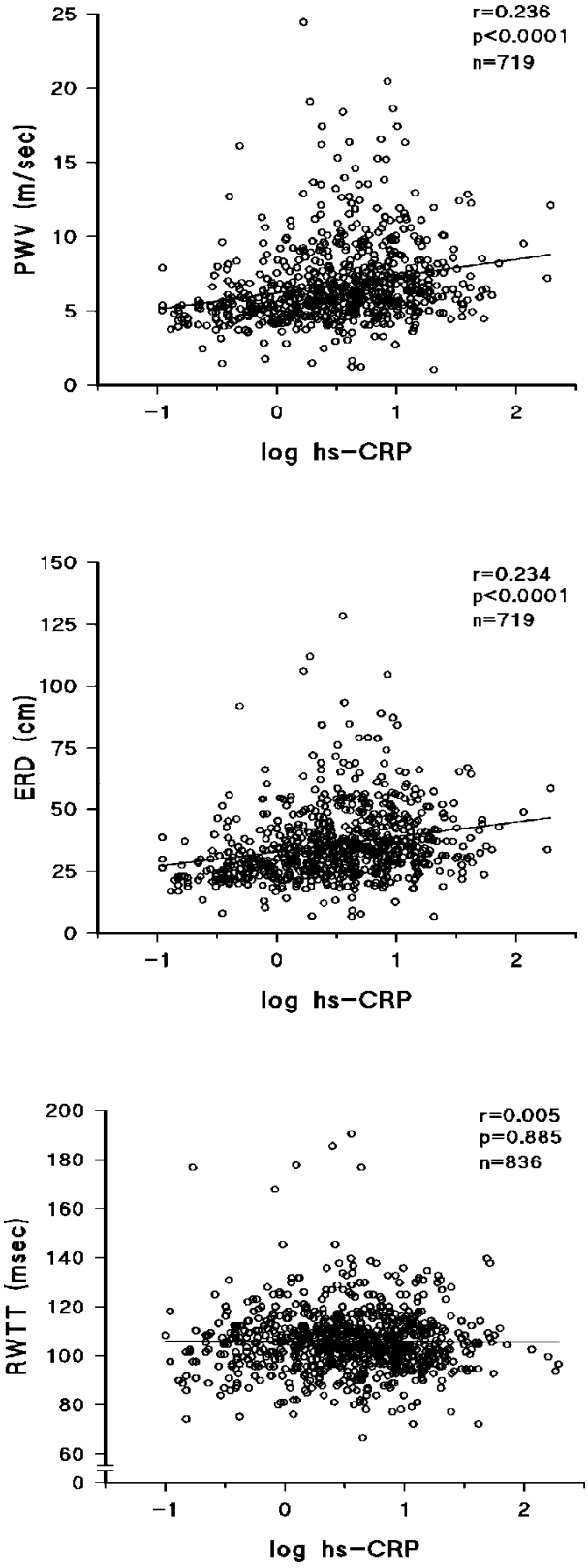


Figure 5.4. Bivariate relationships between log hs-CRP and the determinants of central aortic blood pressure in study participants (n=836). PWV, pulse wave velocity; ERD, effective reflecting distance; RWTT, reflective wave transit time.

Table 5.3. Partial correlation coefficients (r) and 95% confidence intervals for the relationships between log hs-CRP and the determinants of central blood pressure in all participants (n=836).

Log hs-CRP versus	partial r*	confidence intervals	p value†
Aortic pulse wave velocity (n=719)	-0.03	-0.10 to 0.04	0.43
Effective reflecting distance	-0.02	-0.09-0.06	0.71
Reflected wave transit time	0.00	-0.07 to 0.07	0.99

*Determined from regression analysis with adjustments for age, sex, presence of diabetes mellitus or impaired glucose control, body mass index, antihypertensive therapy, regular alcohol intake, smoking status, heart rate and mean arterial pressure. †Probability values are further adjusted for non-independence of family members.

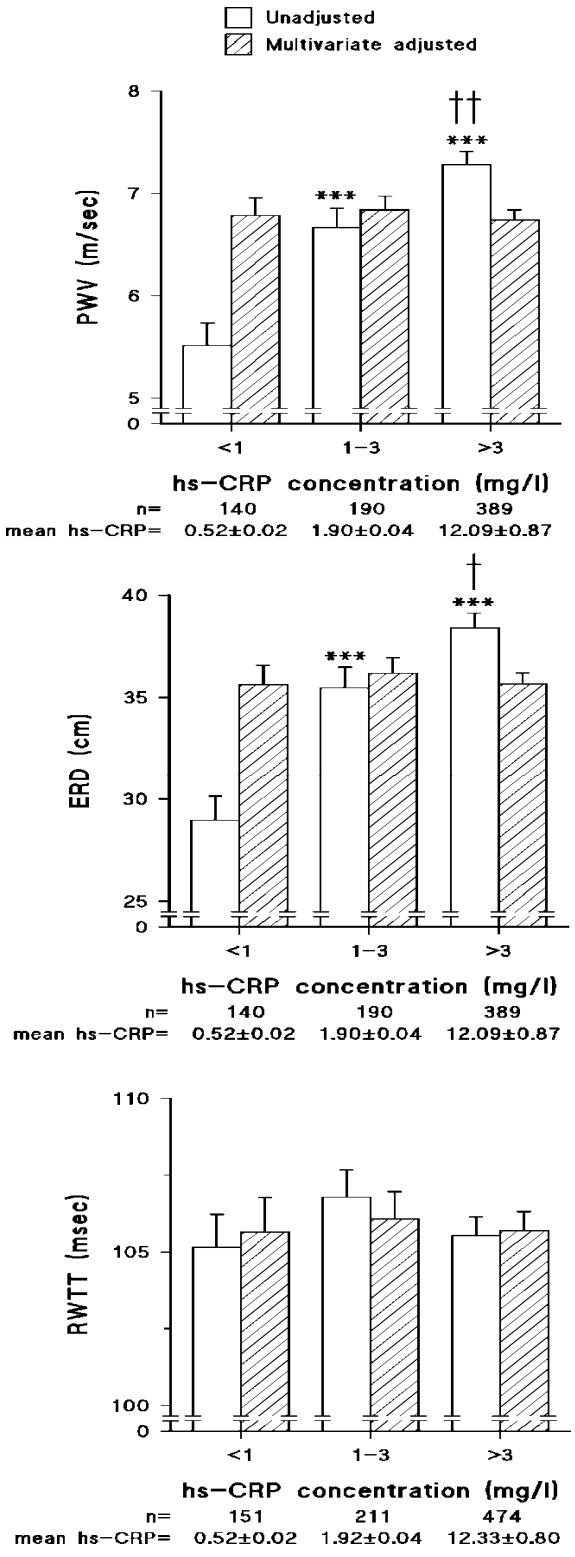


Figure 5.5. Unadjusted and multivariable adjusted aortic pulse wave velocity (PWV), effective reflecting distance (ERD) (n=719) and reflective wave transit time (RWTT) (n=836) in study participants with low (<1 mg/l), moderate (1-3 mg/l) and high (>3 mg/l) risk hs-CRP concentrations. Adjustments are as given in Table 5.2 legend. ***p<0.0001 versus low risk category. †p<0.05, ††p<0.005 versus moderate risk category.

Table 5.4. Partial correlation coefficients (r) and 95% confidence intervals for the relationships between log hs-CRP and central blood pressure (BP) and the determinants of central BP in normotensive participants (n=465) and hypertensive participants (n=371).

Log hs-CRP versus	partial r*	confidence intervals	p value†
	In normotensive participants		
Central systolic BP	0.01	-0.08 to 0.10	0.89
Central pulse pressure	-0.01	-0.10 to 0.08	0.80
Forward component (P1)	0.03	-0.06 to 0.12	0.55
Augmentation pressure (AP)	-0.09	-0.18 to 0.01	0.07
Aortic pulse wave velocity (n=411)	0.01	-0.09 to 0.11	0.65
Effective reflecting distance (n=411)	0.04	-0.06 to 0.14	0.29
Reflected wave transit time	0.03	-0.07 to 0.12	0.59
	In hypertensive participants		
Central systolic BP	-0.02	-0.12 to 0.08	0.71
Central pulse pressure	-0.03	-0.13 to 0.07	0.58
Forward component (P1)	-0.10	-0.20 to 0.01	0.06
Augmentation pressure (AP)	0.06	-0.05 to 0.16	0.26
Aortic pulse wave velocity (n=308)	0.05	-0.07 to 0.16	0.66
Effective reflecting distance (n=308)	-0.01	-0.13 to 0.10	0.82
Reflected wave transit time	-0.06	-0.17 to 0.04	0.22

*Determined from regression analysis with adjustments for age, sex, presence of diabetes mellitus or impaired glucose control, body mass index, antihypertensive therapy, regular alcohol intake, smoking status, heart rate and mean arterial pressure. †Probability values are further adjusted for non-independence of family members.

Table 5.5. Partial correlation coefficients (r) and 95% confidence intervals for the relationships between log hs-CRP and central blood pressure (BP) and the determinants of central BP in women (n=537) and men (n=299).

Log hs-CRP versus	partial r*	confidence intervals	p value†
	In women		
Central systolic BP	-0.06	-0.14 to 0.03	0.24
Central pulse pressure	-0.08	-0.16 to 0.01	0.10
Forward component (P1)	-0.07	-0.15 to 0.02	0.11
Augmentation pressure (AP)	-0.07	-0.15 to 0.02	0.23
Aortic pulse wave velocity (n=446)	-0.05	-0.15 to 0.04	0.30
Effective reflecting distance (n=446)	-0.01	-0.10 to 0.09	0.94
Reflected wave transit time	0.05	-0.03 to 0.14	0.30
	In men		
Central systolic BP	0.02	-0.10 to 0.13	0.81
Central pulse pressure	0.01	-0.11 to 0.12	0.93
Forward component (P1)	-0.01	-0.13 to 0.10	0.79
Augmentation pressure (AP)	0.02	-0.09 to 0.14	0.62
Aortic pulse wave velocity (n=273)	-0.02	-0.14 to 0.10	0.72
Effective reflecting distance (n=273)	-0.04	-0.16 to 0.08	0.57
Reflected wave transit time	-0.07	-0.18 to 0.05	0.24

*Determined from regression analysis with adjustments for age, sex, presence of diabetes mellitus or impaired glucose control, body mass index, antihypertensive therapy, regular alcohol intake, smoking status, heart rate and mean arterial pressure. †Probability values are further adjusted for non-independence of family members.

Table 5.6. Partial correlation coefficients (r) and 95% confidence intervals for the relationships between log hs-CRP and central blood pressure (BP) and the determinants of central BP in lean participants (n=289) and overweight or obese participants (n=547).

Log hs-CRP versus	partial r*	confidence intervals	p value†
	In lean participants (BMI<25kg/m²)		
Central systolic BP	0.02	-0.10 to 0.14	0.75
Central pulse pressure	0.02	-0.10 to 0.13	0.79
Forward component (P1)	0.03	-0.09 to 0.14	0.67
Augmentation pressure (AP)	-0.02	-0.14 to 0.10	0.78
Aortic pulse wave velocity (n=274)	0.05	-0.07 to 0.17	0.34
Effective reflecting distance (n=274)	0.06	-0.07 to 0.17	0.29
Reflected wave transit time	-0.02	-0.13 to 0.10	0.77
	In overweight or obese participants (BMI≥25kg/m²)		
Central systolic BP	-0.04	-0.13 to 0.04	0.41
Central pulse pressure	-0.06	-0.15 to 0.02	0.18
Forward component (P1)	-0.08	-0.16 to 0.01	0.07
Augmentation pressure (AP)	-0.02	-0.10 to 0.07	0.70
Aortic pulse wave velocity (n=445)	-0.07	-0.16 to 0.02	0.13
Effective reflecting distance (n=445)	-0.06	-0.15 to 0.03	0.16
Reflected wave transit time	-0.01	-0.09 to 0.08	0.83

*Determined from regression analysis with adjustments for age, sex, presence of diabetes mellitus or impaired glucose control, body mass index, antihypertensive therapy, regular alcohol intake, smoking status, heart rate and mean arterial pressure. †Probability values are further adjusted for non-independence of family members.

Table 5.7. Partial correlation coefficients (r) and 95% confidence intervals for the relationships between log hs-CRP and central blood pressure (BP) and the determinants of central BP in participants either younger than 60 years (n=663) or ≥60 years (n=173).

Log hs-CRP versus	partial r*	confidence intervals	p value†
	<60 years of age		
Central systolic BP	-0.02	-0.10 to 0.05	0.72
Central pulse pressure	-0.03	-0.11 to 0.05	0.57
Forward component (P1)	-0.01	-0.09 to 0.06	0.83
Augmentation pressure (AP)	-0.05	-0.13 to 0.03	0.28
Aortic pulse wave velocity (n=568)	0.02	-0.06 to 0.11	0.35
Effective reflecting distance (n=568)	0.03	-0.06 to 0.11	0.24
Reflected wave transit time	-0.01	-0.09 to 0.06	0.77
	≥60 years of age		
Central systolic BP	0.02	-0.14 to 0.17	0.84
Central pulse pressure	-0.02	-0.17 to 0.13	0.78
Forward component (P1)	-0.10	-0.25 to 0.06	0.21
Augmentation pressure (AP)	0.04	-0.11 to 0.20	0.58
Aortic pulse wave velocity (n=151)	-0.05	-0.21 to 0.12	0.57
Effective reflecting distance (n=151)	-0.05	-0.21 to 0.12	0.56
Reflected wave transit time	-0.02	-0.17 to 0.13	0.82

*Determined from regression analysis with adjustments for age, sex, presence of diabetes mellitus or impaired glucose control, antihypertensive therapy, regular alcohol intake, smoking status, body mass index, heart rate and mean arterial pressure. †Probability values are further adjusted for non-independence of family members.

5.4 Discussion

The main findings of the present study are as follows: in an urban developing African community with a significant prevalence (57%) of high risk-related hs-CRP concentrations, despite bivariate correlations between log hs-CRP concentrations and central systolic BP, PPc, AP and P1, aortic PWV or the effective reflecting distance; with adjustments, neither hs-CRP, nor risk categories of hs-CRP (low, intermediate and high) were independently and positively associated with central aortic haemodynamic variables. The lack of independent positive relationship between hs-CRP and central aortic haemodynamic variables was noted in normotensives and hypertensives, men and women, lean and overweight/obese, and before and after 60 years of age.

In agreement with data obtained from the Framingham Heart Study conducted in 2409 participants, where no independent relationships between hs-CRP concentrations and central PP, the forward pressure component or aortic PWV were noted (Schnabel et al 2008), in the present study conducted in a relatively large community sample (n=836), I similarly could show no independent positive relationship between hs-CRP and a number of central aortic haemodynamic variables. The present study nevertheless extends the study by Schnabel et al (2008), in that I have been able to show a lack of positive independent relationship between hs-CRP concentrations and central aortic haemodynamics in a community sample with a significant prevalence (57%) of high risk-related hs-CRP concentrations. In this regard, the lack of independent relationship between hs-CRP concentrations and central PP, P1 or aortic PWV in the Framingham Study was noted in a community where the median values for hs-CRP concentrations (Schnabel et al 2008) were well below the threshold for a high cardiovascular risk (Pearson et al 2003). Furthermore, contrary to the Framingham Heart Study (Schnabel et al 2008), I have demonstrated a lack of an independent association between hs-CRP and central aortic haemodynamics, without adjustment for IL-6 in multivariate analysis, thus excluding the possibility that co-linearity

between hs-CRP and IL-6 is responsible for the elimination of hs-CRP from the multivariate model.

The present study is nevertheless in contrast to the Framingham Study in that no independent relation between hs-CRP concentrations and AP were observed, whilst in the Framingham Study, a modest independent relation between hs-CRP concentrations and AP was noted (Schnabel et al 2008). However, this independent relation in the Framingham sample was only observed after adjusting for multiple potential confounders, whilst when only adjusting for age, age^2, sex and cohort, an independent relation was absent (Schnabel et al 2008). Moreover, in that study (Schnabel et al 2008), hs-CRP contributed to only 0.69% of the variability of AP after multiple adjustments.

The present study is also in agreement with a number of previous studies that have demonstrated strong bivariate relationships between hs-CRP and a number of central aortic haemodynamic variables, or increasing unadjusted central aortic haemodynamic variables across categories of low to intermediate risk hs-CRP concentrations in cross-sectional samples of participants largely free of inflammatory or cardiovascular diseases (Kampus et al 2004, Yasmin et al 2004, Duprez et al 2005, Saijo et al 2005, Nagano et al 2005, Kullo et al 2005, Andoh et al 2006, Schnabel et al 2008). However, the present and a previous study (Schnabel et al 2008) is in contrast to many of these prior studies (Kampus et al 2004, Yasmin et al 2004, Duprez et al 2005, Saijo et al 2005, Nagano et al 2005, Kullo et al 2005, Andoh et al 2006) that showed independent hs-CRP-central aortic haemodynamic relations, in that with adjustments for confounders, the relations between hs-CRP concentrations and central aortic haemodynamic variables were abolished in the present study and in the Framingham sample (Schnabel et al 2008). Importantly, many of these prior studies (Kampus et al 2004, Yasmin et al 2004, Duprez et al 2005, Kullo et al 2005, Andoh et al 2006) that did show independent relations between hs-CRP concentrations and central aortic haemodynamic variables, significantly smaller study sample sizes (n=78-427) were employed as compared to the present (n=836) and the prior (n=2409) study by Schnabel et al (2008). It is therefore possible that these studies (Kampus et al 2004, Yasmin et al 2004,

Duprez et al 2005, Kullo et al 2005, Andoh et al 2006) are not necessarily representative of the populations sampled.

In addition to previous studies showing independent relationships between hs-CRP and central aortic haemodynamic variables in largely healthy population or community samples (Yasmin et al 2004, Kullo et al 2005, Kampus et al 2004, Duprez et al 2005, Andoh et al 2006, Saijo et al 2005, Nagano et al 2005), similar relationships have been demonstrated in participants with essential hypertension (Mahmud and Feely 2005, Pietri et al 2006, Pietri et al 2009) and in elderly participants (Mattace-Raso et al 2005, Nakhai-Pour et al 2007). It is therefore possible that although I could not show independent relations between hs-CRP and central aortic haemodynamic variables in the sample studied, that independent relationships may exist in the elderly or in hypertensives in the present community. However, no independent relationships were noted between hs-CRP concentrations and central aortic haemodynamics in either older participants (≥60 years) or in hypertensives alone. Moreover, as a high prevalence of obesity was noted in the present study, analysis was also conducted in lean and overweight/obese participants separately, with similar results noted in both groups. Moreover, as more women than men volunteered for the present study, analysis was also conducted in sex-specific groups, with similar results noted in both groups.

The limitations of the study are as follows: Only one ethnic group was studied and hence the present results may differ in other ethnic groups. Second, as highlighted in previous chapters of the present thesis, a transfer function was employed to determine an aortic pressure wave from a radial pressure wave. Although the use of a transfer function accurately estimates central aortic pressures, the reconstructed waveform underestimates the central augmentation pressure because of inaccuracies in identifying the end of the first systolic shoulder (Chen et al 1997). While both flow and pressure parameters are required to accurately separate the forward and backward pressure waves, this approach is difficult with non-invasive measurements subject to errors created by turbulent flow in the ascending aorta. However, three of the previous studies that have demonstrated an independent

relationship between hs-CRP concentrations and central aortic haemodynamics have employed the same method (Kampus et al 2004, Kullo et al 2005, Pietri et al 2009). Thus the results obtained in the present study are comparable to previous findings. Nevertheless, further studies are required with wave separation analysis to address the issue of the independent relations between hs-CRP and the forward and reflected pressure waves respectively. Third, as highlighted in previous chapters, the cross-sectional nature of the study design does not allow for inferences being drawn regarding cause and effect. Thus, the results of the present study need to be confirmed in a prospective study. Last, I have not examined the impact of other inflammatory markers on central BP. In this regard, although interleukin-6 concentrations were independently associated with aortic PWV in the Framingham Study (Schnabel et al 2008), this finding failed to translate into similar relationships with central PP, and hence the relevance with respect to BP effects is questionable.

In conclusion, the present study conducted in a relatively large study sample of randomly selected participants of African ancestry, demonstrates that in a community with a striking prevalence of high risk-related hs-CRP concentrations, hs-CRP is not independently associated with central aortic haemodynamic variables. These data question the importance of inflammatory changes as factors contributing toward large vessel changes and hence central BP. Further longitudinal studies and studies exploring relationships between alternative inflammatory markers and central aortic haemodynamics are required to confirm this notion.

CHAPTER 6

Contribution of Aortic Pulse Wave Velocity to Intra-familial Aggregation and Heritability of Central Pulse Pressure and the Pressure Components Independent of Distending Pressure.

Abstract

Background. Although central (aortic) pulse pressure (PPc) and its components predict cardiovascular damage independent of distending pressures or peripheral PP and is in-part genetically determined, the contribution of aortic pulse wave velocity (PWV) to the genetic determinants of PPc and the component waves is uncertain.

Aim. To identify the relative contribution of aortic PWV to the intra-familial aggregation and heritability of PPc and the forward and augmented pressure components independent of distending pressure.

Methods and results. Intra-familial aggregation and heritability analysis was performed on PPc, its component forward (P1) and augmented (AP) pressure components, aortic PWV, effective reflecting distance (ERD) and the reflected wave transit time (RWTT), (all determined using applanation tonometry at radial, femoral and carotid artery sites and SphygmoCor software) in 568 participants from 183 nuclear families recruited from an urban developing community in South Africa. With adjustments for confounders including distending pressures (as indexed by mean arterial pressure-MAP) and urinary Na^+/K^+, parent-child correlations were noted for all traits ($p<0.05$) except AIc, and sibling-sibling correlations for PPc, P1 and AP (partial $r=0.20$-0.43, $p<0.05$). Father-mother correlations were noted for P1 and PPc ($p<0.05$). Independent of MAP and confounders significant heritability was identified for PPc ($h^2=0.22\pm0.09$, $p<0.01$), P1 ($h^2=0.22\pm0.09$, $p<0.01$), AP ($h^2=0.34\pm0.10$, $p<0.001$), aortic PWV ($h^2=0.37\pm0.16$, $p=0.01$) and ERD ($h^2=0.29\pm0.14$, $p=0.02$). Adjusting for aortic PWV did not attenuate the heritability of P1, AP or PPc and improved the heritability estimates for P1 ($h^2=0.31\pm0.09$, $p<0.0005$) and PPc ($h^2=0.29\pm0.10$, $p<0.005$).

Conclusion. Although aortic PWV is inherited, aortic PWV cannot account for the intra-familial aggregation and heritability of central aortic PP and the forward and the augmented pressure components independent of distending pressures.

6.1 Introduction

As highlighted in previous chapters of the present thesis, pulsatile pressure (pulse pressure-PP) predicts cardiovascular outcomes beyond distending pressures, indexed by mean arterial pressure (MAP) (Benetos et al 1997, Benetos et al 1998, Verdecchia et al 1998, Franklin et al 1999, Millar et al 1999, Chae et al 1999, Lee et al 1999, Domanski et al 1999, Blacher et al 1999, Vaccarino et al 2000, Glynn et al 2000, Franklin et al 2001, Domanski et al 2001). Moreover, central aortic PP (PPc) is more closely associated with cardiovascular outcomes than peripheral PP (London et al 2001, Safar et al 2002, Chirinos et al 2005, Williams et al 2006, Roman et al 2007, Wang et al 2009). Developing strategies that decrease PPc, without necessarily influencing MAP, is therefore of considerable interest and in this regard understanding the mechanisms responsible for these changes is of importance.

Previous studies have shown that increases in the augmented (reflected) pressure component (AP) contribute to most of the age-related increases in central PP in normotensives across the lifespan (Cecelja et al 2009, Namasivayam et al 2009). Moreover, AP or the augmentation index (an index of wave reflection) have been demonstrated to be independently related to cardiovascular target organ changes or cardiovascular outcomes in a variety of clinical populations (Saba et al 1993, London et al 2001, Nurnberger et al 2002, Hayashi et al 2002, Weber et al 2004, Ueda et al 2004, Weber et al 2005, Chirinos et al 2005, Williams et al 2006, Hashimoto et al 2007). However, in chapter 2 of the present thesis, I have demonstrated that both the forward pressure component (P1) and AP contribute toward central aortic PP. Indeed, independent of distending pressure, P1 and AP contributed equally to the increases in PPc across the adult lifespan in a community sample with a high prevalence of uncontrolled hypertension. Furthermore, in Chapter 3 of the present thesis I have shown that both P1 and AP contribute significantly toward increases in left ventricular mass index. Thus, in order to develop appropriate preventative or therapeutic measures, the pathophysiological mechanisms responsible for both AP and P1 independent of distending pressures need to be identified. In this regard, the contribution of P1 to

increases in central BP across the adult lifespan in uncontrolled hypertension may occur either as a consequence of damage to large vessels produced by hypertension, or through the pathophysiological mechanisms responsible for hypertension. In Chapter 4 I show that salt intake could account for some of the variability in central BP in this community through effects on both AP and P1 but in Chapter 5 I show that low-grade inflammation as indexed by hs-CRP concentrations cannot account for variations in PPc or the component pressures. Are there alternative factors that require consideration?

Although a number of studies have demonstrated heritability of central aortic haemodynamic changes including central PP, P1, AP and indices of aortic stiffness (Mitchell et al 2005, Sayed-Tabatabaei et al 2005, Pilia et al 2006, Levy et al 2007, Seidlerova et al 2008, Cecelja et al 2009), in only a few of these studies has the heritability of the forward and reflective pressure components of aortic PP been evaluated (Mitchell et al 2005, Levy et al 2007, Cecelja et al 2009). In two studies reporting on the heritability of P1 and AP, these estimates were not adjusted for an index of distending pressures (Mitchell et al 2005, Levy et al 2007). Thus, heritability of either the forward or the reflected wave in these studies (Mitchell et al 2005, Levy et al 2007) could have been attributed to the well known role of genetic effects on distending pressures. In an alternative study (Cecelja et al 2009) the heritability estimates were adjusted for distending pressures, but were determined in one sex only. Although aortic pulse wave velocity (PWV) was noted to be inherited, in none of the aforementioned studies (Mitchell et al 2005, Levy et al 2007, Cecelja et al 2009) did the investigators assess whether aortic PWV could account for the heritability of PPc, P1 or AP independent of distending pressures. Without knowledge of the haemodynamic mechanisms responsible for the heritability of central PP, identifying the genetic determinants that may lead to novel drug development targeting central PP will remain limited. Thus, in the present study I evaluated the intra-familial aggregation and heritability of central PP, AP, P1 and aortic PWV and assessed the relative contribution to the intra-familial aggregation and heritability of central PP, AP and P1 of PWV independent of distending pressures in nuclear families of African descent.

6.2 Methods

6.2.1 Study participants.

The present study design and a description of the participants recruited have been outlined in chapter 2, page 47 of the present thesis. Of the 1015 participants with central haemodynamic assessments from 360 nuclear families, 568 participants from 183 families with 24-hour urine samples that met with pre-specified quality control criteria and had complete familial pairing were included in the present study. Aortic pulse wave velocity (PWV) could not be measured on 75 participants because they were too obese to obtain acceptable femoral pulse waves. Therefore, the overall number of participants statistically analyzed for PWV totalled 493.

6.2.2 Clinical, demographic and anthropometric assessments.

A standardized questionnaire was administered to obtain demographic and clinical data as previously described (Norton et al 2008, Woodiwiss et al 2009). Details of the questionnaire are provided in chapter 2, pages 47-48 of the present thesis.

Height and weight were measured with participants standing, wearing indoor clothing and no shoes. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Participants were identified as being overweight if their body mass index (BMI) was ≥25 kg/m^2 and obese if their BMI was ≥30 kg/m^2.

6.2.3 Conventional blood pressure

A trained nurse-technician measured conventional (brachial) blood pressure (BP) using a standard mercury sphygmomanometer. Details of the measurements are provided in chapter 2, page 48 of the present thesis. In the present study only 1.76% of visits had fewer than the planned BP recordings. The frequency of identical consecutive recordings was 0% for systolic BP and 1.07% for diastolic BP. The occurrence of BP values recorded as an odd number was 0%. Of the 5592 systolic and diastolic BP readings, 28.92% ended on a zero (expected =20%). Hypertension was defined as a mean BP≥140/90 mm Hg (Chobanian et al 2003, Williams et al 2004, Mancia et al 2007) or the use of antihypertensive medication.

6.2.4 Laboratory (blood) tests.

Standard laboratory blood tests of renal function, liver function, blood glucose, haematological parameters, and percentage glycated haemoglobin (HbA_{1C}) were performed. Details of the blood tests are provided in chapter 2, page 49 of the present thesis. Diabetes mellitus or inappropriate blood glucose control was defined as the use of insulin or oral hypoglycaemic agents or an HbA_{1C} value greater than 6.1% (Bennett et al 2007). In addition to standard laboratory blood tests, participants' blood groups (ABO and Rhesus) were evaluated to confirm Mendelian segregation.

6.2.5 Pulse wave analysis

Central pulse pressure (PPc), MAP, the forward pressure (P1), augmentation pressure (AP), aortic pulse wave velocity (PWV), the effective reflecting distance (ERD) and the reflective wave transit time (RWTT) were estimated using techniques previously described (Shiburi et al 2006). Details of the pulse wave analysis are provided in chapter 2, pages 49-53 of the present thesis.

. *6.2.6 Urinary electrolyte excretion rates*.

Urine samples were obtained over a period of at least 24-hours after disposing of urine obtained immediately prior to the collection period. Urine Na^+, K^+, and creatinine concentrations were measured. The quality of urine collection was determined using approaches employed on page 94 of the present thesis.

6.2.7 Data analysis.

Database management and statistical analyses were performed with SAS software, version 9.1 (The SAS Institute Inc., Cary, North Carolina, USA). Aortic PWV was logarithmically transformed to more closely reflect a normal distribution. Data from individual subjects were expressed as mean±SD. To evaluate differences in demographic, clinical, and anthropometric measurements between parents and offspring, means and proportions were compared using Student's unpaired t-tests and the χ^2-statistic respectively.

To assess the genetic contribution to the variability of central PP, P1 and AP, and the

potential determinants of central BP, I conducted intra-familial aggregation and heritability analyses. For intra-familial aggregation analysis, intra-familial correlations were determined from the PROC GENMOD procedure of the SAS package to determine concordance between family members. Unadjusted and multivariable adjusted correlation coefficients were determined in 71 father-mother, 331 parent-child and 116 sibling-sibling pairs, except for PWV and ERD where 52 father-mother, 256 parent-child and 92 sibling-sibling pairs were analysed. Three statistical models were analysed; model 1 was unadjusted for any confounders, model 2 was adjusted for age and sex, and model 3 was adjusted for age, sex, MAP, smoking status, regular alcohol intake, treatment for hypertension, heart rate, height, weight, urinary Na^+/K^+ ratio, and diabetes mellitus or inappropriate glucose control. A significant father-mother relationship, adjusted for potential confounding variables, represents the contribution of the shared familial environment towards the trait. A significant multivariable adjusted parent-child or sibling-sibling correlation coefficient significantly greater than zero indicates an environmental and genetic contribution towards the trait.

Heritability (h^2) of central aortic haemodynamic variables was estimated using the Statistical Analysis for Genetic Epidemiology (S.A.G.E) software (version 6.0.1) (Department of Epidemiology and Statistics, Case Western Reserve, University of Cleveland, Ohio) using the Marker-Trait Associations in Pedigree Data (ASSOC) programme (S.A.G.E. 6.0.1 [2009]). The ASSOC programme estimates by maximum likelihood, assuming a generalization of multivariable normality, familial variance components and hence heritability, assuming correlation structures described by Elston et al (1992) and the regression model described by George and Elston (1987). The ASSOC programme uses a linear regression model, in which the residual variance is partitioned into the sum of an additive polygenic component and a subject-specific random component. Heritability is the polygenic component divided by the total variance. For the heritability estimates, adjustments were made for the confounders listed in model 3.

To determine the contribution of aortic PWV to the heritability of PPc, AP and P1, intra-familial correlations and heritability estimates were further adjusted for aortic PWV.

6.3 Results

6.3.1 Characteristics of participants.

Table 6.1 shows the characteristics of the parents and their offspring in the present study sample. More women than men participated in the study. The mean age of the parents was 60.0 ± 11.0 years compared to 30.3 ± 10.5 years of the offspring. Parents had a higher BMI than offspring ($p<0.0001$). As compared to their offspring, a greater percentage of parents were overweight or obese, had hypertension or received antihypertensive treatment, and had diabetes mellitus or inappropriate blood glucose control ($p<0.0001$ for all). A greater proportion of mothers were postmenopausal as compared to their daughters ($p<0.0001$). For both peripheral BP and central haemodynamic measurements, parents had higher values than offspring ($p<0.0001$ parents vs. offspring for all measurements). There were no differences in alcohol consumption between parents and offspring ($p=0.43$) but offspring were more likely to smoke tobacco than parents ($p=0.03$). Urinary Na^+/K^+ ratio was similar in parents and offspring. The general characteristics of participants not included in the study were similar to the characteristics of the participants evaluated (data not shown).

6.3.2 Intra-familial aggregation of central haemodynamic traits.

Figure 6.1 shows the intra-familial aggregation for PPc and the pressure components. In model 1 (unadjusted) significant intra-familial aggregation was noted for AP only. In the fully adjusted models (model 3 which includes adjustments for MAP) the correlation coefficients of parent-child and sibling-sibling pairs were significant for all traits. After adjustments, the father-mother correlations were significant for PPc and P1.

Table 6.1. Demographic, clinical, anthropometric and haemodynamic characteristics of parents and offspring of the study sample.

	Parents n=256	Offspring n=312
Sex (% female)	66.0	65.7
Age (years)	60.0±11.0	30.3±10.5
Body mass index (kg/m^2)	32.7±7.7	26.9±7.7
% overweight/obese	26.6/57.4	20.8/28.5
Regular tobacco intake (% subjects)	10.9	17.6
Regular alcohol intake (% subjects)	21.5	24.4
% with diabetes mellitus or HbA_{1C}>6.1%	43.4	9.9
% with treated hypertension	41.8	7.1
% with untreated hypertension	33.6	15.1
% women postmenopausal	87.6	9.3
Conventional SBP/DBP (mm Hg)	143±24/89±13	122±20/81±12
Central SBP/DBP (mm Hg)	136±23/90±13	112±20/81±12
Mean arterial pressure (mm Hg)	109±16	95±15
Central pulse pressure (mm Hg)	46±16	31±13
Forward pressure component (P1) (mm Hg)	30±10	23±9
Augmented pressure component (AP) (mm Hg)	16±9	8±7
Central augmentation index (%)	33±10	23.±13
Aortic pulse wave velocity (m/sec) (n=493)	8.7±3.4	5.5±1.3
Effective reflecting distance (cm) (n=493)	45.9±18.0	28.7±7.9
Reflective wave transit time (msec)	106.6±13.6	104.8±13.1
Urinary Na^+/K^+ ratio	4.1±2.0	4.7±3.8

Data are expressed as mean ± SD unless stated otherwise. HbA_{1C}, glycosylated haemoglobin; BP, blood pressure; SBP, systolic BP; DBP, diastolic BP.

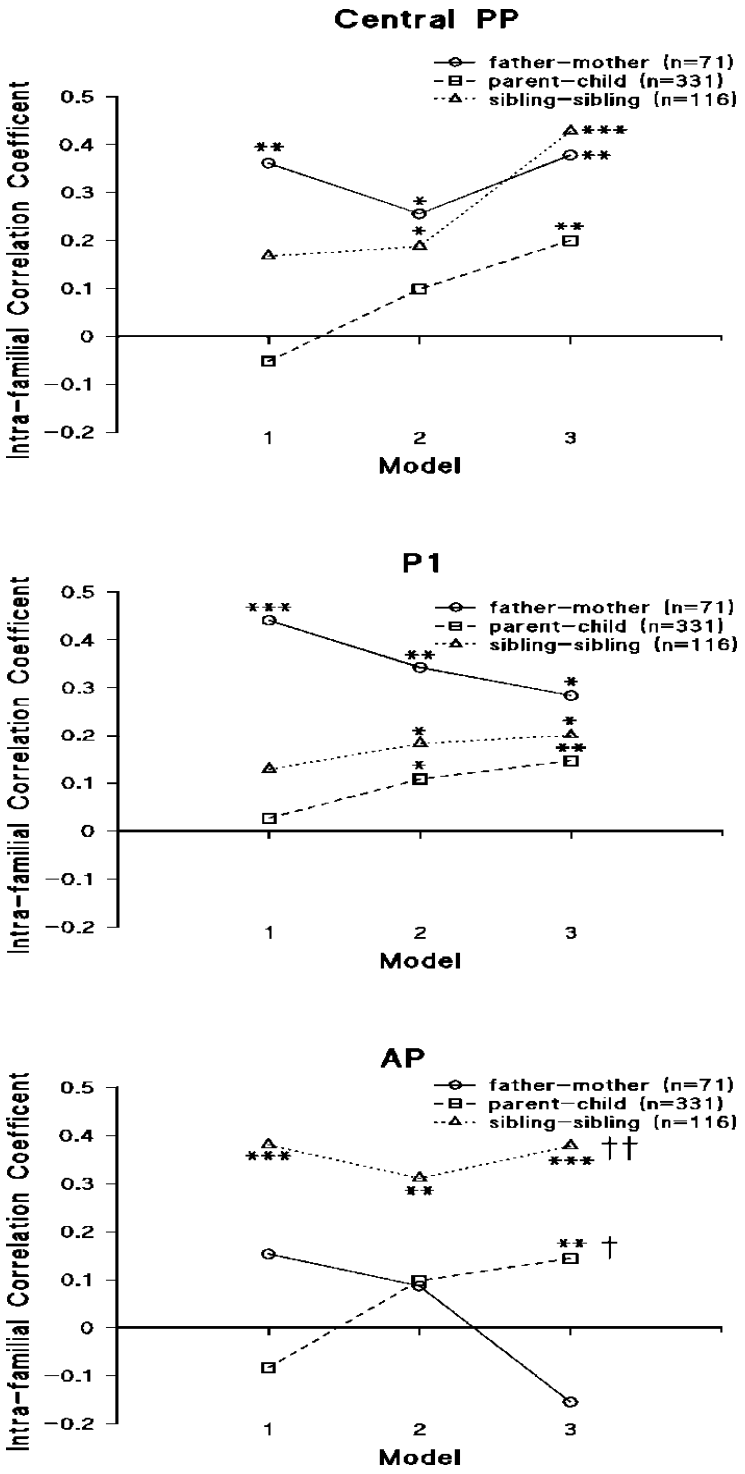


Figure 6.1. Intra-familial correlation coefficients for central pulse pressure (PPc) and the component forward (P1) and augmented (AP) pressure components. Model 1 is unadjusted. Model 2 is adjusted for age and sex. Model 3 is adjusted for age, sex, mean arterial pressure, smoking status, regular alcohol intake, treatment for hypertension, heart rate, body height, body weight, urinary sodium-to-potassium ratio, and diabetes mellitus or inappropriate glucose control. * $p<0.05$, ** $p<0.01$, *** $p<0.0001$ versus zero; † $p<0.05$, †† $p<0.0001$ versus father-mother pair.

Multivariable adjusted sibling-sibling correlations were greater than multivariable adjusted father-mother correlations for AP ($p<0.0005$) but not for P1 or PPc. Similarly, multivariable adjusted parent-child correlations were greater than multivariable adjusted father-mother correlations for AP ($p<0.05$) but not for P1 or PPc.

Figure 6.2 shows the intra-familial aggregation for the central determinants (aortic PWV, ERD and RWTT) of PPc and the pressure components. In model 1 (unadjusted) significant intra-familial aggregation was noted for ERD only. In the fully adjusted model (model 3) the correlation coefficients of parent-child pairs were significant for all traits. No multivariable adjusted sibling-sibling or father-mother correlations were significant. No multivariable adjusted sibling-sibling correlations were greater than multivariable adjusted father-mother correlations. Multivariable adjusted parent-child correlations were greater than father-mother correlations for aortic PWV ($p<0.0005$) and ERD ($p<0.005$) but not for RWTT.

6.3.3 Heritability estimates of individual central haemodynamic traits.

The heritability estimates for the individual central aortic haemodynamic traits, adjusted for potential confounders including MAP, are shown in Table 3.2. Significant heritability was noted for all traits, except RWTT. The strongest heritability estimate was for aortic PWV.

6.3.4 Impact of adjustments for aortic PWV on the intra-familial aggregation of central pulse pressure and the component pressures.

Figure 6.3 shows the impact of further adjustments for aortic PWV on the intra-familial aggregation of central PP and the component pressures. Further adjustments for aortic PWV failed to influence the multivariable adjusted correlations for PPc, P1 or AP between parents and children and between sibling pairs.

6.3.5 Impact of adjustments for aortic PWV on the heritability of central pulse pressure and the component pressures.

Figure 6.4 shows the impact of further adjustments for aortic PWV on the heritability of central pulse pressure and the component pressures. Adjustments for aortic PWV failed to

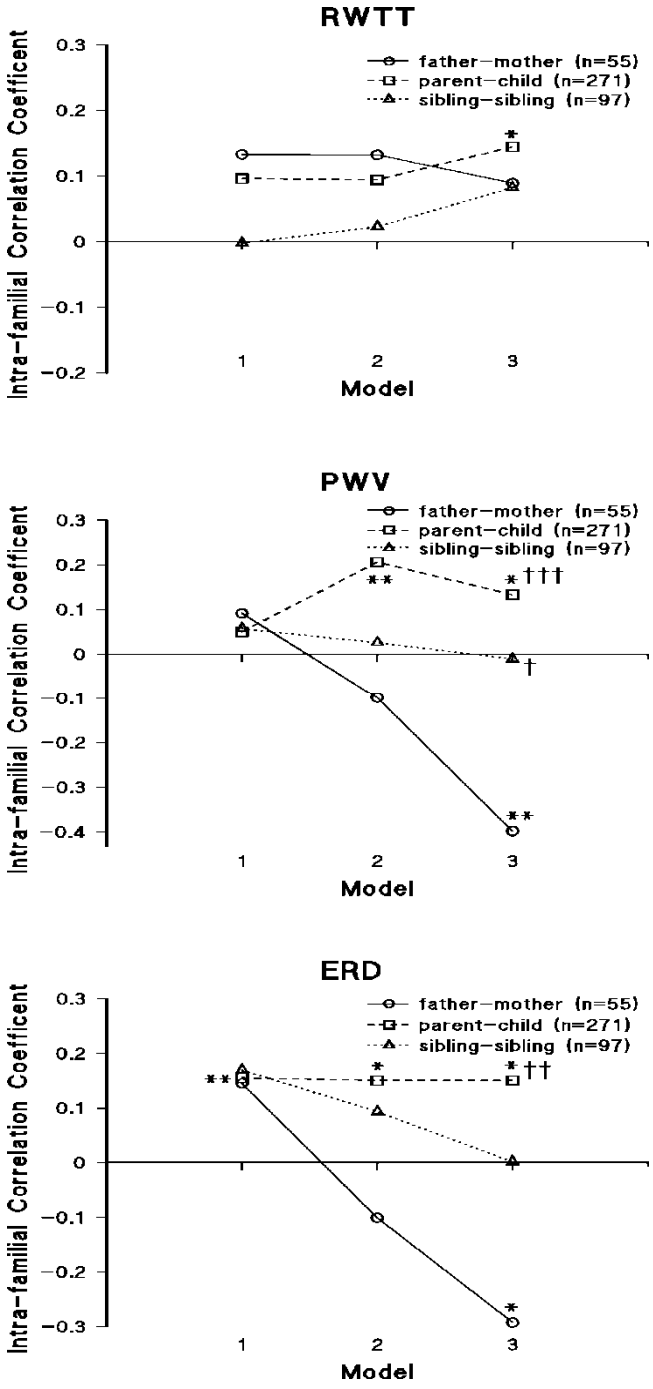


Figure 6.2. Intra-familial correlation coefficients for aortic pulse wave velocity (PWV), the effective reflecting distance (ERD) and the reflective wave transit time (RWTT). Model 1 is unadjusted. Model 2 is adjusted for age and sex. Model 3 is adjusted for age, sex, mean arterial pressure, smoking status, regular alcohol intake, treatment for hypertension, heart rate, body height, body weight, urinary sodium-to-potassium ratio, and diabetes mellitus or inappropriate glucose control. * $p<0.05$, **$p<0.01$, *** $p<0.0001$ versus zero; † $p<0.05$; †† $p<0.005$; ††† $p<0.0005$ versus father-mother pair.

Table 6.2. Multivariable adjusted heritability estimates for central pulse pressure (PP), the pressure components and their determinants.

Trait	h^2 ± SEM	p-value
Central PP	0.22±0.09	<0.01
Forward pressure (P1)	0.22±0.09	<0.01
Augmented pressure (AP)	0.34±0.10	<0.001
Central augmentation index (AIc)	0.36±0.09	<0.0001
Aortic PWV	0.37±0.16	0.01
Effective reflecting distance	0.29±0.14	0.02
Reflected wave transit time	0.07±0.08	0.21

Heritability estimates adjusted for age, sex, mean arterial pressure, regular smoking, regular alcohol intake, treatment for hypertension, heart rate, body height, body weight, urinary Na^+/K^+ ratio, and diabetes mellitus or inappropriate glucose control. h^2, heritability estimate.

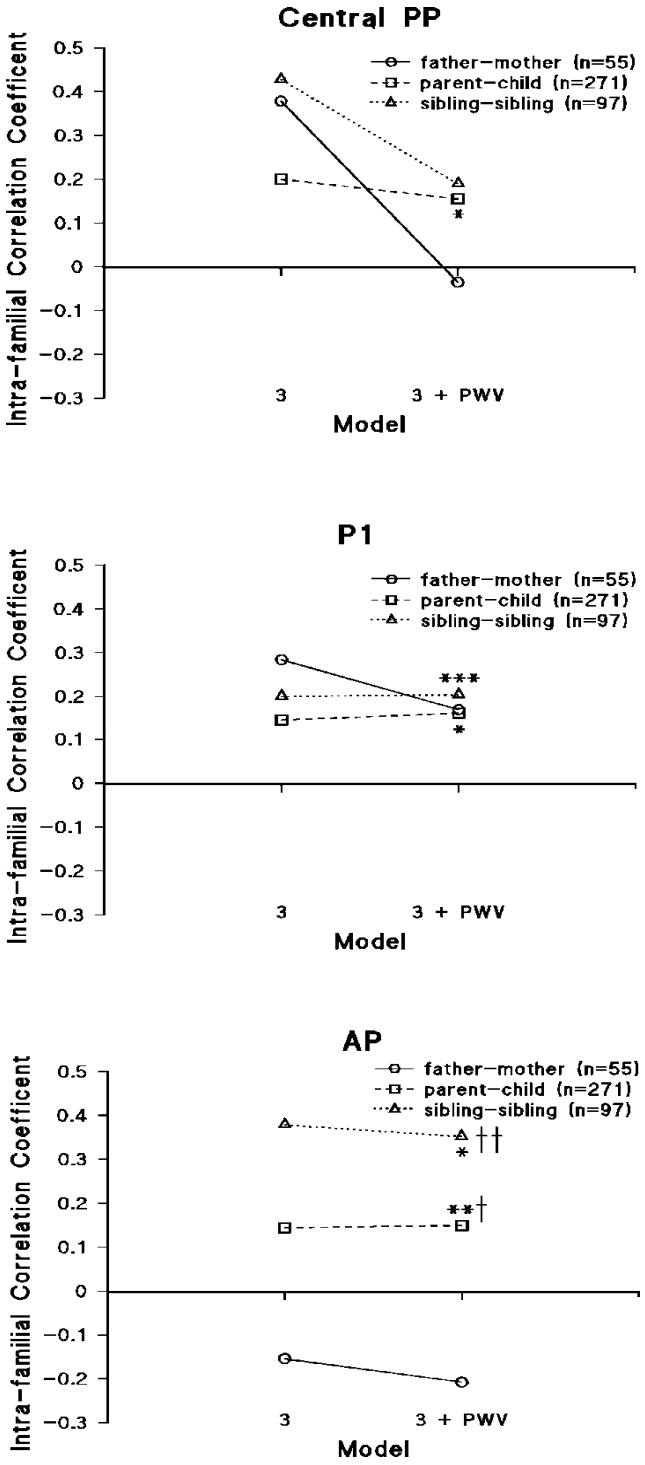


Figure 6.3. Intra-familial correlation coefficients for central pulse pressure and the component forward (P1) and augmented (AP) pressures before and after adjustments for aortic pulse wave velocity (PWV). All models are adjusted for age, sex, mean arterial pressure, smoking status, regular alcohol intake, treatment for hypertension, heart rate, body height, body weight, urinary sodium-to-potassium ratio, and diabetes mellitus or inappropriate glucose control.* $p<0.05$, **$p<0.01$, *** $p<0.0001$ versus zero; † $p<0.01$, †† $p<0.0001$ versus father-mother pair.

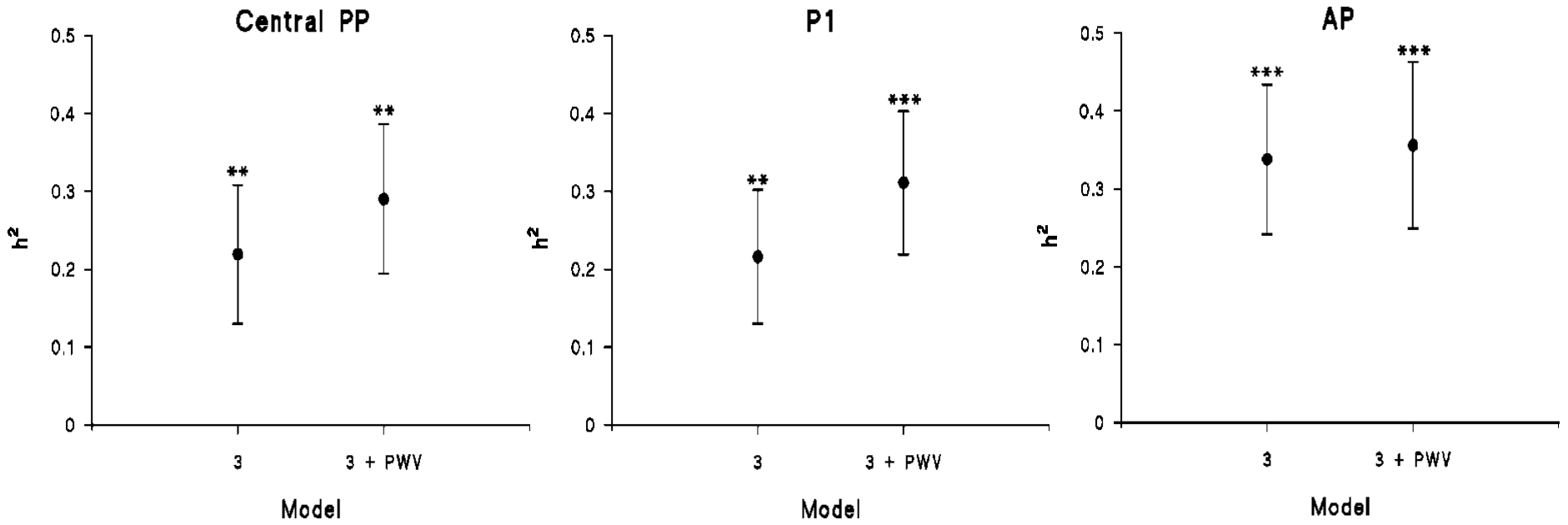


Figure 6.4. Heritability estimates (h^2) for central pulse pressure and the component forward (P1) and augmented (AP) pressure components before and after adjustments for aortic pulse wave velocity (PWV). All heritability estimates are adjusted for age, sex, mean arterial pressure, regular smoking, regular alcohol intake, treatment for hypertension, heart rate, body weight, body height, urinary sodium-to-potassium ratio, and diabetes mellitus or inappropriate glucose control. **p<0.01, *** p<0.0005 versus zero.

decrease the multivariable adjusted heritability estimates for PPc, P1 or AP and even tended to increase the heritability estimates for PPc and P1.

6.4 Discussion

The main findings of the present study are as follows: In a population sample of African ancestry, intra-familial aggregation and heritability of PPc and both the forward (P1) and the augmented (AP) pressure components of PPc were noted independent of confounders and distending pressures. Although aortic PWV showed intra-familial aggregation and heritability, adjustments for aortic PWV failed to decrease either the intra-familial aggregation or the heritability estimates of central PP, AP or P1.

Although three previous studies have reported on the heritability of the component pressures of central PP (Mitchell et al 2005, Levy et al 2007, Cecelja et al 2009), the present study extends these studies as follows: although similar to the present study conducted in 568 participants of nuclear families, in 1480 participants in a cohort of the Framingham Offspring Study, moderate degrees of heritability for central PP, P1 and AP were demonstrated, adjustments were not made for distending pressures as indexed by MAP (Mitchell et al 2005, Levy et al 2007). Thus in those studies at least (Mitchell et al 2005, Levy et al 2007), heritability could have been attributed to genetic factors that influence distending rather than dynamic (pulsatile) pressures. Indeed, as previously highlighted throughout the present thesis a major determinant of pulsatile pressures is distending pressure. Although in a twin study conducted in 496 women living in the United Kingdom (Cecelja et al 2009), markedly greater heritability estimates were noted for P1 and AP than the present and alternative (Mitchell et al 2005, Levy et al 2007) studies, in the twin study (Cecelja et al 2009) the heritability estimates were determined in one sex only.

The present study also builds on previous studies demonstrating significant heritability of the pressure components of central PP (Mitchell et al 2005, Levy et al 2007, Cecelja et al 2009) in that these studies failed to conduct statistical survival analysis (survival

of independent relationships after adjustments) to identify whether the genetic factors that account for aortic PWV also account for the intra-familial aggregation and heritability of central PP and its component pressures. In this regard, the assumption in these studies (Mitchell et al 2005, Levy et al 2007, Cecelja et al 2009) was that if a central aortic haemodynamic trait was inherited, that the genetic determinants of this trait would also contribute toward central PP. Thus, the previously demonstrated heritability of aortic PWV (Mitchell et al 2005 Sayed-Tabatabaei et al 2005, Pilia et al 2006, Levy et al 2007, Seidlerova et al 2008, Cecelja et al 2009) may be interpreted as indicating that the genetic factors responsible for aortic PWV would in-part be the same as those that determine central PP and its pressure components. In contrast to this notion, in the present study adjusting for aortic PWV which was demonstrated to be inherited, failed to attenuate the intra-familial aggregation and heritability estimates for central PP or its component pressures.

If the genetic determinants of P1 and AP and hence central PP cannot be accounted for by aortic PWV, what are the possible factors that may account for the intra-familial aggregation and heritability of central PP and the component pressures? In this regard, independent of systemic vascular resistance, P1 is determined by stroke volume, arterial stiffness and possibly aortic diameter (Segers et al 2007). However, as demonstrated in chapter 2, stroke volume was unable to account for the age-related impact of P1 on central BP. Although aortic root diameter is not a predictor of BP (Ingelsson et al 2008) and generalized aortic stiffness (as indexed by PWV) does not account for the heritability of P1, it is nevertheless still possible that aortic diameter distal to the aortic root or local ascending aortic stiffness may account for the heritability of P1. Indeed, age-related changes in aortic PWV are not paralleled by age-related changes in aortic compliance or characteristic impedance (Segers et al 2007). Further, in older women and in systolic hypertension, more direct measures of aortic stiffness are independently associated with aortic PP even when PWV is included as an adjustor in regression models (Mitchell et al 2008).

With respect to the factors that may account for the heritability of AP, as long as the reflection coefficient remains constant, AP may in-part be determined by the forward

pressure component. Thus, the same inherited factors that determine P1 may also determine the heritability of AP. However, central augmentation index, a relative index of wave reflection, was also shown to be heritable in the present thesis. In this regard, AP is also determined by wave reflection, which is in-turn accounted for by the site, speed, timing, or the amplitude of wave reflection (see Chapter 1 for an overview). In this regard, the heritability of AP was not influenced by adjustments for aortic PWV and no heritability of the reflective wave transit time was noted. However, the site of wave reflection (effective reflective distance) was shown to be inherited. Thus, although neither the speed, nor the timing of wave reflection may contribute, the site of wave reflection may in-part account for the heritability of AP. It is nevertheless possible that the heritability of AP can be attributed to genetic determinants of muscular artery tone, a change that may influence not only the site of wave reflection, but also the amplitude of the reflective waves (Cecelja et al 2009).

The higher heritability estimates for AP as compared to P1 noted in the present study sample of black African ancestry are in agreement with the higher heritability estimates for AP noted in a twin study conducted in Caucasian women living in the United Kingdom (Cecelja et al 2009) and in the Framingham Offspring cohort of largely Caucasians (Mitchell et al 2005, Levy et al 2007).

The strengths of the present study are the relatively large study sample (n=568), and the fact that all intra-familial correlations and heritability estimates of central haemodynamic factors were adjusted for distending pressure and multiple confounders including a urinary index of salt intake. In this regard, as recently highlighted (Chapter 4, Redelinghuys et al 2010), this urinary index of salt intake has a greater effect on central haemodynamic factors than on pressures measured at the brachial artery. Moreover, unlike previous studies assessing the haemodynamic determinants of the heritability of central PP, the role of aortic PWV in the intra-familial aggregation and heritability estimates of central PP and its component pressures was assessed by re-estimating intra-familial correlations and heritability estimates of central PP, AP and P1 with adjustments for aortic PWV.

The potential limitations of this study are as follows: The smaller number of father-mother pairs compared to parent-child or sibling-sibling pairs may result in underestimation of the effect of the shared environment on the traits examined in intra-familial aggregation analysis. However, multivariable adjusted heritability analysis was conducted to confirm the outcomes of intra-familial aggregation. As indicated in previous chapters of the present thesis although the use of a transfer function accurately estimates central aortic pressures, the reconstructed waveform underestimates the central augmentation pressure because of inaccuracies in identifying the end of the first systolic shoulder (Chen et al 1997). To accurately separate the forward and reflected pressure waves both flow and pressure parameters are required, which is difficult with non-invasive measurements which are subject to errors created by turbulent flow in the ascending aorta. Furthermore, although sex was included as an adjustor in the analysis, I could not adjust for potential sex-environment interactions as the sample size of males was too small to identify such interactions. As such interactions may be genetically pre-determined I may therefore have over-estimated independent genetic effects on large vessel function.

To conclude, the present study demonstrates that the genetic factors that contribute to central PP are likely to involve those that modify both AP and P1 independent of distending pressures and other confounders such as age. However, although PWV is also inherited, the genetic mechanisms that underpin aortic PWV are unlikely to account for a significant proportion of the inheritance of central PP and its component pressures. Future studies aimed at identifying the potential genetically determined phenotypes that can account for a significant proportion of the inheritance of aortic PP and its pressure components are thus still required.

CHAPTER 7

Summary and Conclusions.

Over the past two decades an increasing prevalence of hypertension has been noted in both economically developed and developing countries (WHO et al 2003, Chobanian et al 2007). In South Africa, an economically developing country, the prevalence of hypertension is particularly high in urban communities of African descent (Malhotra et al 2008, Maseko et al 2010). Despite the fact that hypertension-related cardiovascular events account for a significant proportion of morbidity and mortality in both the elderly and younger age groups in urban, developing communities in Africa (Steyn et al 2005, Tollman et al 2008, Stewart et al 2008, Connor et al 2009) in these communities, hypertension is poorly managed and controlled (Steyn et al 2008, Maseko et al 2010). Consistent with a high prevalence of hypertension and poor BP control rates in communities of African ancestry, there is abundant evidence to indicate that as compared to groups of European ancestry excessive cardiovascular damage occurs in groups of African descent (Gillum 1999, Sacco et al 2001, Howard 2001, Murtaugh et al 2003, Skelton et al 2003, Lorber et al 2003, Hollar et al 2004, McGruder et al 2004, Rodriguez et al 2004, Jamerson 2004, Kizer et al 2004, Nunez et al 2005, Drazner et al 2005, Bryson et al 2006). However, there is considerable debate as to the explanation for these findings with interest in the possibility that these ethnic differences may not be completely explained by uncontrolled BP as measured at the brachial artery.

In communities of African origins in countries other than Africa, although the prevalence of cardiovascular disease and damage is higher than communities of European descent, these differences persist despite adjustments for brachial blood pressure (BP) (Gillum 1999, Sacco et al 2001, Howard 2001, Murtaugh et al 2003, Skelton et al 2003, Lorber et al 2003, Hollar et al 2004, McGruder et al 2004, Rodriguez et al 2004, Jamerson 2004, Kizer et al 2004, Nunez et al 2005, Drazner et al 2005, Bryson et al 2006).Therefore brachial BP may not accurately predict cardiovascular damage in urban communities of African descent. The possible factors that may account for excessive cardiovascular damage in these communities beyond BP measured at the brachial artery have nevertheless remained elusive. One possibility is that brachial BP may not closely reflect the extent of cardiovascular damage produced by pressures closer to target organs. In this regard, central

(aortic) BP, which has different determinants than brachial BP, is more likely to reflect the damage done to target organs produced by an increased BP. Indeed, central systolic BP and pulse pressure (PP) are stronger independent predictors of cardiovascular outcomes than brachial BP (Waddell et al 2001, London et al 2001, Safar et al 2002, Chirinos et al 2005, Williams et al 2006, Roman et al 2007, Pini et al 2008, Jankowski et al 2008, Wang et al 2009, Roman et al 2009). However, a number of outstanding questions remain with regards to the contribution of the pressure components of central PP (PPc) toward increases in PPc across the adult lifespan. In the present chapter I will summarise these issues and indicate how the findings described in the present thesis have addressed some of these outstanding questions.

The component pressures of PPc include the forward (incident) (P1) and the augmented (reflected) (AP) pressures. In this regard, a number of studies have demonstrated an independent relationship between AP or central augmentation index (AP indexed to central PP) and cardiovascular outcomes (Saba et al 1993, London et al 2001, Nurnberger et al 2002, Hayashi et al 2002, Weber et al 2004, Ueda et al 2004, Weber et al 2005, Chirinos et al 2005, Williams et al 2006, Hashimoto et al 2007), but no studies have evaluated whether similar relationships exist between P1 and cardiovascular damage or outcomes beyond distending pressures. The findings that AP contributes independently to cardiovascular outcomes are viewed in a positive light as although there is currently significant evidence to support the capacity of many currently available antihypertensive agents to decrease AP, the weight of evidence does not support a similar effect on the structural aortic changes that determine P1 (Zieman et al 2005, Laurent et al 2007). However, the contribution of P1 to increases in PPc is unclear.

Age is the strongest determinant of PPc. In largely normotensive samples whereas AP has been shown to contribute toward PPc across the adult lifespan (Segers et al 2007, Namasivayam et al 2009, Cecelja et al 2009), P1 has been demonstrated to contribute only modestly toward age-related increases in aortic PP and this effect only occurs after the age of 60 years (Mitchell et al 2004, Cecelja et al 2009, Namasivayam et al 2009). In addition P1

(Mitchell et al 2003) and the determinants of P1 (aortic stiffness and diameter) may account for aortic PP in hypertension only in older persons with isolated systolic hypertension (Mitchell et al 2008). However, there are presently no studies that have assessed the relative roles of P1 and AP as a determinant of aortic PP across the adult lifespan in communities or populations with a high prevalence of uncontrolled hypertension. Therefore, as described in chapter 2 of the present thesis I evaluated the relative contribution of the pressure components of PPc toward age-related increases in PPc and central systolic BP across the adult lifespan. This study was conducted in a community sample of African ancestry with a high prevalence of uncontrolled blood pressure.

In 1015 participants of a randomly selected community sample, in agreement with previous studies (Segers et al 2007, Namasivayam et al 2009, Cecelja et al 2009) I demonstrated that in 546 normotensive participants, AP contributes toward PPc across the adult lifespan independent of distending pressures. Moreover, also in agreement with previous studies (Mitchell et al 2004, Namasivayam et al 2009, Cecelja et al 2009), in normotensives I showed that P1 contributes only modestly toward age-related increases in aortic PP and that this effect only occurs after the age of 60 years. However, in the study described in chapter 2 I noted that in all of the 1015 participants studied, including those with hypertension, P1 and AP contributed equally to age-related PPc across the adult lifespan in a community sample with a high prevalence of uncontrolled blood pressure. The age-related increase in P1 across the adult lifespan could not be accounted for by increases in stroke volume and hence may only be attributed to aortic structural changes that determine aortic compliance or aortic diameter.

The data reported on in chapter 2 broadens our current knowledge of the mechanisms responsible for increases in aortic PP, a topic which has recently generated considerable debate (Mitchell et al 2008, O'Rourke and Nichols 2005). In this regard, as indicated in the aforementioned discussion, increases in PPc in previous studies were largely attributed to AP, a pressure component that is determined by a number of factors that can be modified acutely by many currently employed antihypertensive agents. In contrast, P1 is

determined by stroke volume and aortic structural changes. As the data in chapter 2 suggest that age-related increases in P1 in a community with a high prevalence of uncontrolled hypertension cannot be attributed to increases in stroke volume, the assumption can only be that aortic structural changes explain the contribution of P1 to increases in PPc across the adult lifespan. This study therefore suggests that modifying structural aortic changes with antihypertensive therapy may be required to achieve optimal risk reduction. The data described in chapter 2 would therefore support a focussed effort to develop therapeutic strategies to minimise structural aortic changes across the adult lifespan and not just in elderly patients with isolated systolic hypertension.

Although in chapter 2 I provide substantial evidence to support a role for P1 in contributing toward increases in PPc and systolic BP across the adult lifespan, whether this contribution translates into excessive cardiovascular damage is nevertheless uncertain. Relationships between measures of arterial stiffness, which in-part account for variations in P1, and left ventricular (LV) structural changes (Roman et al 2000) suggest that P1 could contribute toward PPc-LV mass index (LVMI) relationships. However, no studies have been conducted to formally evaluate whether aortic structural changes in hypertension account for PPc effects on LVMI. Therefore in chapter 3 of the present thesis I assessed the relative contribution of P1 and AP independent of distending pressures to variations in LVMI, a well recognized target organ change in hypertension, in 503 randomly selected participants from an urban, developing community with a high prevalence of uncontrolled hypertension. In chapter 3 I show that independent of distending pressure, both P1 and AP contribute to variations in LVMI. Thus, the aortic structural changes that determine P1, such as changes in aortic compliance or diameter are likely to explain the relationship between P1 and LVMI independent of distending pressure. These data therefore provide additional support for an important role of P1 as a cause of the adverse effects of central BP on cardiovascular target organs.

The marked increase in the contribution of P1 toward age-related increases in aortic BP independent of distending pressures across the adult lifespan in a community sample

with a high prevalence of uncontrolled hypertension as described in chapter 2, and the relationship between P1 and LVMI independent of distending pressures as described in chapter 3 could be attributed to several possibilities. First, uncontrolled hypertension may produce early aortic structural changes, the consequence being premature increments in P1 over the adult lifespan. Second, this relationship could be accounted for by the pathophysiological mechanisms responsible for hypertension such as environmental or genetic effects. To test this hypothesis I therefore considered the role of a number of factors which could influence BP, and especially aortic BP independent of mean arterial pressure. In this regard, I explored three potential mechanisms which could account for increases in P1 and AP and thus an enhanced central BP independent of distending pressures. The following paragraphs describe these three hypotheses, and underscore how the data presented in the current thesis has extended our knowledge of this field.

The role of salt intake as a determinant of central BP and the component pressures, independent of distending pressures, has not been adequately addressed. Although there are reports indicating that Na^+ intake is associated with brachial artery PP (Hajjar et al 2001, du Cailar et al 2004, Buyck et al 2009) and that alterations in Na^+ intake modify PP (He et al 2005, He et al 2009), in only one study was the relationship demonstrated after adjustments for distending pressures and this analysis was sex and age-specific (de Cailar et al 2004). The relationship between Na^+ intake and PP could be attributed to the well-recognised relationship between distending and dynamic pressures. Moreover, in none of these studies was central PP evaluated (Hajjar et al 2001, du Cailar et al 2004, He et al 2005, Buyck et al 2009, He et al 2009), and the central haemodynamic mechanisms responsible for this effect were not identified.

With respect to studies evaluating the impact of salt intake on central BP, one study has demonstrated that a low Na^+ diet reduces carotid PP and systolic BP in 12 patients with isolated systolic hypertension, but similarly failed to adjust for distending pressures (Gates et al 2004). As diastolic BP was also reduced in that study (Gates et al 2004), MAP is likely to have decreased. Hence again the relationship between salt intake and central PP in that

study (Gates et al 2004) could be attributed to the relationship between distending pressures and PP. Furthermore, in that study (Gates et al 2004), whether the impact of changes in Na^+ intake was more marked for carotid as compared to brachial PP was not evaluated. An alternative study (Starmans-Kool et al, 2010 in press) published on-line after the results of the present thesis were published (Redelinghuys et al 2010) similarly demonstrated that an increased Na^+ intake in 10 normotensive participants increased carotid BP and the backward pressure despite producing modest effects on brachial BP. However, again, the authors of this study failed to adjust for distending pressures and hence the relationship between salt intake and central PP in that study (Starmans-Kool et al, 2010 in-press) could be attributed to the relationship between distending pressures and PP. As a consequence of the uncertainty as to the effect of salt intake on central BP independent of distending pressures, as described in chapter 4 of the present thesis I evaluated the relationship between urinary indices of salt intake and PPc and the determinants of PPc independent of distending pressures and in the context of relationships with PP measured at the brachial artery. This question is particularly relevant in groups of African descent whom have been reported to have a high prevalence of salt-sensitive hypertension (Sowers et al 1988, Morris et al 1999, Sacks et al 2001, Wright et al 2003, Jurgens et al 2008).

In 635 participants with 24-hour urine collections that met with pre-specified quality control criteria, from a randomly selected community sample of African ancestry, salt intake as indexed by urinary Na^+/K^+ was associated with the dynamic (PP) component of BP for central but not brachial artery BP measurements independent of distending BP (MAP or diastolic BP) and other confounders. The relationship between urinary Na^+/K^+ and central PP could be reproduced both in women and in men and in participants not receiving diuretic therapy, the predominant antihypertensive employed in this population. The relationship between urinary Na^+/K^+ and central PP was accounted for by AP and P1, but not by aortic pulse wave velocity, the reflected wave transit time, or the site of wave reflection.

The results of the study described in chapter 4 clearly extend the information provided by prior studies on the relationship between indices of salt intake and PPc. In this

regard, unlike previous studies, the present study demonstrates a strong MAP-independent relationship between a urinary index of salt intake and PPc in a relatively large randomly selected community sample in both men and women. Moreover, the study described in chapter 4 shows that independent of distending pressures, this effect is mediated through increases in both AP and P1. Moreover, the present study indicates that these MAP-independent relations between a urinary index of salt intake and central PP could not be reproduced with PP measured at the brachial artery.

The mechanism(s) that account for the relationship between urinary indices of salt intake and PPc as described in chapter 4, warrant consideration. In this regard AP may be determined by the timing of wave reflection (earlier waves may increase the chance of the forward and reflected wave coinciding), the site of wave reflection (if the site is closer to the central arteries, the chance is increased that the reflected wave may return earlier), the speed of wave conduction (a greater speed increases the chances of earlier reflection) and the magnitude of wave reflection. However, as I have described in chapter 4, urinary salt excretion is not related to the reflected wave transit time, effective reflecting distance or aortic pulse wave velocity and adjustments for these variables failed to modify the relationship between urinary salt excretion and AP. Therefore the data from chapter 4 suggest that the relationship between urinary salt excretion and either central PP or AP may not be explained by changes in the timing or site of wave reflection, nor in the speed of wave conduction. Consequently, these lines of evidence suggest that the relationship between salt intake and central PP and AP is in-part mediated by the magnitude of wave reflection, possibly by altering vascular smooth muscle tone in medium-sized or more distal arteries, a mechanism recently proposed as mediating age-induced changes in central PP (Cecelja et al 2009).This proposed mechanism of action has important clinical implications as unlike age, salt intake is a modifiable risk factor for cardiovascular disease. Hence, I show for the first time that modifying salt intake could influence cardiovascular risk through changes in central PP and the component pressures. What are the alternate potential hypotheses which may

explain increases in central BP and the component pressures that I explored in the present thesis?

There are a number of reasons to support a hypothesis that low-grade inflammation may contribute toward aortic BP independent of distending pressures and that these increases in aortic BP may not be reflected in BP measured at the brachial artery. A potential mechanism by which low-grade inflammation may contribute towards central BP is through the development of atherosclerotic plaque which is associated with structural alterations in large vessels thus potentially changing aortic compliance and diameter and hence the pressure components of PPc. The development of atherosclerotic plaque is thought to be mediated by C-reactive protein (CRP) (Pasceri et al 2000, Pasceri et al 2001, Zwaka et al 2001). C-reactive protein may also mediate structural changes in the arterial wall through an increased production of metallo-proteinases which degrade components of the interstitium and through a loss of elastane fibres (Yasmin et al 2005) thus also modifying aortic structure and potentially aortic compliance and diameter and hence the pressure components of PPc. What is the current evidence to suggest that CRP or low-grade inflammation may potentially contribute to central BP through an action on the component pressures?

A causal relationship between acute inflammatory states induced by vaccination and reversible increases in aortic stiffness, as indexed by aortic PWV, has been demonstrated (Vlachopoulos et al 2005). However, the fact that the change in aortic PWV produced by the vaccine-related inflammation is acute and reversible (Vlachopoulos et al 2005) suggests that the increase in aortic stiffness is not mediated by structural changes in the aorta. Nevertheless, several studies have shown relationships between high sensitivity CRP (hs-CRP) and central haemodynamic variables in participants largely free of inflammatory or cardiovascular disease (Kampus et al 2004, Yasmin et al 2004, Kullo et al 2005, Saijo et al 2005, Nagano et al 2005, Duprez et al 2005, Andoh et al 2006). However, these studies (Kampus et al 2004, Yasmin et al 2004, Kullo et al 2005, Duprez et al 2005, Andoh et al 2006) were conducted in small study samples and in contrast, in a large (n=2409) community-based study, hs-CRP was not independently related to central PP, P1 or aortic

PWV and although hs-CRP was independently associated with AP, hs-CRP accounted for less than 1% of the variability of AP (Schnabel et al 2008). Importantly, in the study by Schnabel et al (2008) the median values for hs-CRP concentrations were well below the threshold for a high cardiovascular risk (Pearson et al 2003). As there are no large studies that have assessed the contribution of low-grade inflammation, as indexed by hs-CRP, to central BP in a sample with a high prevalence of risk-related hs-CRP concentrations, in chapter 5 of the present thesis I assessed this question in a community with a high prevalence of risk-related hs-CRP. Consistent with previous studies (Ford et al 1999, Visser et al 1999, Festa et al 2001), in the study described in chapter 5, the high prevalence of risk-related hs-CRP concentrations was largely accounted for by the high proportion of overweight and obese participants noted in the community.

In chapter 5 of the present thesis, I showed in 836 randomly recruited participants of African ancestry, 57% of whom had high-risk hs-CRP concentrations, that in spite of bivariate associations between log-transformed hs-CRP concentrations and central systolic BP, central PP, AP, P1, aortic PWV or the effective reflecting distance, with adjustments for confounders, neither hs-CRP, nor risk categories of hs-CRP (low, intermediate and high) were independently and positively associated with central aortic haemodynamic variables. I noted a lack of independent positive relationship between hs-CRP and central aortic haemodynamic variables in a number of discrete sub-groups including in normotensives and hypertensives, men and women, lean and overweight/obese participants, and the young or the elderly. These data question the importance of inflammatory changes as factors contributing toward large vessel changes and hence central BP. Further longitudinal studies and studies exploring relationships between alternative inflammatory markers and central aortic haemodynamics are required to confirm this notion.

Lastly, in chapter 6 of the present thesis I performed a study designed to extend our knowledge of the role of genetic factors as a non-modifiable risk factors contributing towards central haemodynamic variables. What is the current evidence to suggest that genetic factors can account for central BP changes and how does the study described in chapter 6 extend

our knowledge of this field? Although the heritability of central aortic haemodynamic changes including PPc and indices of aortic stiffness has been demonstrated (Mitchell et al 2005, Sayed-Tabatabaei et al 2005, Pilia et al 2006, Levy et al 2007, Seidlerova et al 2008, Cecelja et al 2009), only a small body of evidence is available regarding the heritability of the AP and P1 components of PPc (Mitchell et al 2005, Levy et al 2007, Cecelja et al 2009). In two of the studies reporting on heritability of P1 and AP, these estimates were not adjusted for an index of distending pressures (Mitchell et al 2005, Levy et al 2007). Thus, the heritability of AP and P1 in these studies ((Mitchell et al 2005, Levy et al 2007) could be accounted for by the well recognised genetic contribution to distending pressures. Moreover, in the alternative study (Cecelja et al 2009) the heritability estimates were determined in one sex only. Although aortic pulse wave velocity (PWV) was noted to be inherited, in none of the aforementioned studies (Mitchell et al 2005, Levy et al 2007, Cecelja et al 2009) did the investigators assess whether aortic PWV could account for the heritability of PPc, P1 or AP independent of distending pressures. Without knowledge of the haemodynamic mechanisms responsible for the heritability of central PP, identifying the genetic determinants that may lead to novel drug development targeting central PP will remain limited.

Thus, in chapter 6 I assessed the heritability of central PP, the component pressures, and aortic PWV and evaluated the relative contribution of PWV to the heritability of PPc and the pressure components of PPc, independent of distending pressures, in 568 participants from 183 nuclear families of African ancestry. I noted significant intra-familial aggregation and heritability for both AP and P1, independent of confounders and distending pressures. I also demonstrated, for the first time that although aortic PWV has a significant degree of intra-familial aggregation and heritability, adjustments for aortic PWV fails to decrease either the intra-familial aggregation or the heritability estimates of central PP, AP or P1. These data support the notion that independent of distending pressures, the genetic factors which account for increases in central PP are likely to influence both AP and P1. However, although PWV is also inherited, the genetic mechanisms that contribute to aortic PWV are unlikely to account for a significant proportion of the inheritance of central PP and its

component pressures. It is plausible that aortic diameter distal to the aortic root or local ascending aortic stiffness may account for the heritability of P1. Thus, further studies aimed at identifying the potential genetically determined phenotypes that can account for a significant proportion of the inheritance of aortic PP and its component pressures, particularly P1, need to be conducted.

The particular strengths and limitations of each of the studies described in the present thesis have been discussed in the respective chapters. However, from a general perspective, the strengths of the studies included the random recruitment of participants, the reasonably large sample sizes, and the high quality laboratory and clinical measurements. One of the major limitations is the cross-sectional study design and lack of prospective and intervention studies to confirm the data described in the present thesis. Another potential limitation is the use of a generalized transfer function to estimate central aortic pressures and the component pressures. Although central BP can be accurately estimated with this technique, the reconstructed waveform underestimates AP because of inaccuracies in identifying the end of the first systolic shoulder (Chen et al 1997). However, in chapter 2 of the present thesis I was able to show similar age-related increases in P1 and AP in normotensives as that previously reported on (Namasivayam et al 2009).

In conclusion, evidence presented in the present thesis clarifies some outstanding issues *a propos* the mechanisms that explain increases in central BP. In this regard, I provide evidence to suggest that independent of distending pressures and stroke volume, P1 accounts for a significant proportion of age-related increases in PPc across the adult lifespan in a community sample with a high prevalence of uncontrolled hypertension. Second, I provide evidence to suggest that the contribution of P1 to PPc translates into an association between P1 and LVMI. Third, I have shown that PPc and both the AP and P1 component pressures are associated with a urinary index of salt intake as well as genetic factors, but not to an index of low-grade inflammation. These findings suggest that to achieve optimal cardiovascular risk reduction in hypertension, therapeutic strategies that target the aortic structural changes responsible for P1 are likely to be required across the adult lifespan, and

that this therapy must in-part address the impact of salt intake and genetic factors, but not necessarily low-grade inflammation on PPc.

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M110244

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Ms Michelle Redelinghuys

CLEARANCE CERTIFICATE	**M110244**
PROJECT	Centreal Blood Pressure in an Urban Developing Community in South Africa (part of M070469/R14/49 Woodiwiss/Norton)
INVESTIGATORS	Ms Michelle Redelinghuys.
DEPARTMENT	School of Physiology
DATE CONSIDERED	14/02/2011
DECISION OF THE COMMITTEE*	

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 14/02/2011 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Professor Woodiwiss

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Woodiwiss/Norton

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER MO70469**
PROJECT	Gene Candidates As Determinants of Blood Pressure and Intermediary Phenotypes in Pathogenesis of Hypertension in Black S Africans
INVESTIGATORS	Profs A/G Woodiwiss/Norton
DEPARTMENT	School of Physiology
DATE CONSIDERED	07.05.09
DECISION OF THE COMMITTEE*	Approved unconditionally (refer M020472)

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 07.05.09 **CHAIRPERSON** .....
(Professors PE Cleaton-Jones, A Dhai, M Vorster, C Feldman, A Woodiwiss)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Woodiwiss A Prof

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 10th Floor, Senate House, University.
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PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 Woodiwiss/Norton et al

CLEARANCE CERTIFICATE **PROTOCOL NUMBER** M02-04-72

PROJECT Gene Candidates As Determinants of Blood Pressure And Intermediary Phenotypes In Pathogenesis of Hypertension In Black South Africans

INVESTIGATORS Prof's AJ/G et al Woodiwiss/Norton et al

DEPARTMENT School of Physiology, Wits Medical School

DATE CONSIDERED 02-04-26

UNIVERSITY OF THE WITWATERSRAND
PROF PE CLEATON-JONES
HREC (MEDICAL)
2007 -05- 09
JOHANNESBURG

DECISION OF THE COMMITTEE *

Approved unconditionally

This clearance is valid and within the WITS 5-year validity

DATE 02-05-14 **CHAIRMAN** ..... (Professor P E Cleaton-Jones)

* Guidelines for written "informed consent" attached where applicable.

c c Supervisor: Prof AJ Woodiwiss
Dept of School of Physiology, Wits Medical School

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DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES