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Systemic Blood Flow in Hypertension in a Community of African Ancestry

Keneilwe Nkgola Mmopi

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the requirements for the degree of Doctor of Philosophy.

Johannesburg, South Africa.

2021

ABSTRACT

Hypertension is a particularly important cause of death and disability in groups of African descent, an effect thought to be consistent with the evolutionary theory of antagonistic pleiotropy. In this regard, groups originating from equatorial countries are hypothesized to have evolved to survive fluid losing states through a genetic predisposition to renal fluid retention. Although this prevents fluid loss in hot climates, with aging and excess Na^+ intake, fluid accumulation may result in hypertension through an enhanced intravascular volume. However, because of the absence of measurable increases in systemic blood flow in primary hypertension, this hypothesis is not universally accepted. Nevertheless, studies to determine the hemodynamic basis of hypertension in this ethnic group are markedly limited. In the present thesis, in a cross-sectional community-based study conducted across the full adult lifespan (aged 16 years and above). I therefore evaluated the hemodynamic basis of hypertension in a group of African descent living in South Africa (SOWETO).

As renal fluid retention is thought to increase blood pressure (BP) in response to Na^+ intake, I first determined the relationship between 24-hour urinary Na^+ and K^+ excretion (an index of salt intake) and systemic hemodynamics in 581 randomly selected community participants from SOWETO. Using multivariate adjusted regression analysis, I showed that an abnormal salt intake (indexed by a high Na^+/K^+) is independently associated with systolic BP (SBP) and pulse pressure (PP) through an enhanced proximal aortic characteristic impedance (resistance to flow in a pulsatile system in the absence of wave reflection [Zc]), but not through changes in peak aortic flow (the product of maximum velocity and the largest diameter in the left ventricular outflow tract in early systole) [Q]). The associations of salt intake with Zc were not accounted for by variations in aortic root diameter and translated into elevated PP and SBP values through an enhanced early central systolic (determined by P_{QxZc}) and hence forward travelling pressure wave (the wave generated from the interaction between maximal flow and impedance to flow in the proximal aorta). These findings therefore suggest that the adverse effects of a high salt intake on BP in groups of African ancestry are through chronic changes in the proximal aorta (aortic stiffness) rather than through an impact of salt intake on renal fluid retention.

Although salt intake is not associated with systemic blood flow in groups of African ancestry, this does not exclude a possible alteration in the pressure natriuresis relationship produced by renal effects enhancing systemic blood flow. I therefore determined the age-related

hemodynamic correlates of blood pressure (BP) across the adult lifespan in 824 participants from the community sample of African ancestry in SOWETO. In multivariate linear regression analysis, independent of confounders, a strong positive association between age and stroke volume (SV), cardiac output (CO) and peak aortic Q, but not with systemic vascular resistance (SVR) was noted. Age relations with pulse pressure were as strongly determined by aortic Q as by Zc or total arterial compliance (TAC). SV, CO and Q accounted for a marked proportion of age-related increases in BP as determined from both office and ambulatory (24-hour) measurements. Consistent with flow effects on renin secretion, increases in systemic flow accounted for age-related decreases in plasma renin concentrations and were correlated with age-related increases in plasma aldosterone concentrations and the aldosterone-to-renin ratio. Thus, from young adulthood, strong age-related increases in systemic flow account for increases in BP in Africa, and these effects explain low-renin hypertension.

The extent to which increases in systemic flow across the adult age range account for most forms of primary hypertension (systolic and diastolic as well as isolated systolic hypertension) in Africa is unknown. I therefore subsequently aimed to determine the extent to which effects of systemic blood flow contribute to untreated or inadequately controlled systolic and diastolic as well as isolated systolic hypertension in 725 participants from the SOWETO community. Using multivariate adjusted analysis of covariance, I showed that independent of confounders, compared to those with a normotensive BP, uncontrolled systolic and diastolic hypertension was as strongly associated with Q, SV or CO as with SVR, Zc and TAC. Moreover, independent of confounders, as compared to normotensives, uncontrolled isolated systolic hypertension was more strongly associated with Q, SV and CO than with SVR, but less than with TAC and similar to Zc. Thus, in groups of African ancestry living in Africa, hypertension due to increases in either systolic or diastolic BP is as strongly associated with increases in systemic flow (SV, CO and Q) as with arterial and arteriolar effects (Zc, TAC, SVR).

In conclusion, in the present thesis I provide evidence published in the high impact journals *Hypertension* (2 papers) and *Journal of Hypertension* (1 paper) to support the evolutionary theory of antagonistic pleiotropy and a shifted pressure natriuresis relationship as a cause of primary hypertension in groups of African descent living in Africa. In this regard, I show that although habitual salt intake does not cause primary hypertension through alterations in systemic blood flow, that age-related increases in systemic blood flow are pathognomonic of most low-renin forms

of primary hypertension at a community level. These data therefore provide the missing evidence (increases in systemic blood flow) to substantiate the importance of a shifted pressure natriuresis relationship as a cause of primary hypertension proposed by Guyton and colleagues close to 50 years ago.

DECLARATION

I, Keneilwe Nkgola Mmopi declare that the work contained in this thesis is my own unaided work. It is being submitted for the degree of Doctor of Philosophy in the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. The work contained in this thesis has not been submitted for any degree or examination in this university, or any other university.



….. …….

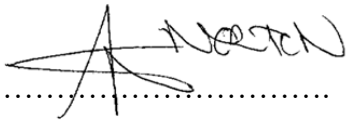
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I certify that the studies contained in this thesis have the approval of the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg. The clearance certificate number is **M170773**.



…. ……

Keneilwe Nkgola Mmopi Signed on …22nd …. day of …February.... 2021



…….

Prof. Gavin R. Norton (Supervisor)

Signed on ..22nd ..day of …February…2021



……

Prof. Angela J. Woodiwiss (Supervisor)

Signed on…22nd ..day of ..February..2021

DEDICATION

In loving memory of my late father Akanyang P. Mmopi (1917- 2012) and my late mother Ipotseng K. Mmopi (1933- 2006). I also dedicate this thesis to my children Obakeng, Zandile, Zanele and Tshwaragano. Thank you for your immense support which has enabled me to pursue this dream and complete the journey.

PUBLICATIONS

1. **Mmopi, K. N.**, Norton, G. R., Bello, H., Libhaber, C. D., Peters, F., Sareli, P., Peterson, V. R., & Woodiwiss, A. J. (2021). Contribution of Systemic Blood Flow to Untreated or Inadequately Controlled Systolic-Diastolic or Isolated Systolic Hypertension in a Community Sample of African Ancestry. *Journal of Hypertension,* 39: 526-537.
2. Woodiwiss, A. J., **Mmopi, K. N.*,** Peterson, V., Libhaber, C., Bello, H., Masiu, M., Da Silva Fernandes, D., Tade, G., Mthembu, N., Peters, F., Sareli, P., & Norton, G. R. (2020). Distinct Contribution of Systemic Blood Flow to Hypertension in an African Population Across the Adult Lifespan. *Hypertension,* 76: 410-419.
   NB: *equal contribution as first author.
3. **Mmopi, K. N.,** Norton, G. R., Bello, H., Libhaber, C., Masiu, M., Da Silva Fernandes, D., Sareli, P., Peterson, V., & Woodiwiss, A. J. (2020). Increased Aortic Characteristic Impedance Explains Relations Between Urinary Na^+/K^+ and Pulse or Systolic Blood Pressure. *Hypertension,* 75:1260-1270.

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LIST OF ABBREVIATIONS

α	Alpha
ACEI	Angiotensin Converting Enzyme Inhibitor
AGT	Angiotensinogen
AJW	Angela Jill Woodiwiss
AP	Augmented Pressure
ARB	Angiotensin Receptor Blocker
ARR	Aldosterone-to-Renin Ratio
AIx	Augmentation Index
BMI	Body Mass Index
BP	Blood Pressure
BSA	Body Surface Area
CCB	Calcium Channel Blocker
CI	Confidence Interval
Cl	Chloride
CO	Cardiac Output
CV	Cardiovascular
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
DM	Diabetes Mellitus
EDV	End diastolic Volume
EF	Ejection Fraction
ENaC	Epithelial Sodium Channel
ET-1	Endothelin-1
FWA	Forward Wave Amplitude
GBD	Global Burden of Disease
HbA1c	Glycated Hemoglobin
HCTZ	Hydrochlorothiazide
HR	Heart Rate
HT	Hypertension

IDH	Isolated Diastolic Hypertension
ISH	Isolated Systolic Hypertension
K^+	Potassium
LV	Left Ventricle
MAP	Mean Arterial Pressure
MBP	Mean Blood Pressure
MR	Mineralocorticoid Receptor
Na^+	Sodium
Na-K-2Cl	Sodium Potassium Chloride
NT	Normotensive
OR	Odds Ratio
Pb	Wave Reflection Pressure/ Backward Wave Pressure
Pf	Forward Wave Pressure
Pi	Incident Wave/ Inflection Point
PP	Pulse Pressure
PWV	Pulse Wave Velocity
P_{QxZc}	Peak Product of Flow and Characteristic Impedance
Q	Aortic Flow
RAAS	Renin-Angiotensin-Aldosterone-System
RAP	Right Atrial Pressure
RDA	Recommended Daily Allowance
RM	Reflection Magnitude
SBP	Systolic Blood Pressure
SD	Standard Deviation
SEM	Standard Error of Mean
SHEP	Systolic Hypertension in the Elderly Program
SOWETO	South West Township of Johannesburg
SP	Systolic Pressure
STOP	Swedish Trial in Old Patients with Hypertension
SV	Stroke Volume
SVR	Systemic Vascular Resistance

Syst-diast HT	Systolic-Diastolic Hypertension
TAC	Total Arterial Compliance
TPR	Total Peripheral Resistance
USA	United States of America
WHO	World Health Organization
Zc	Characteristic Impedance

PREFACE

Cardiovascular disease is one of the most important causes of morbidity and mortality in the world today. Approximately 17 million people die from cardiovascular disease annually, representing 31% of all global deaths. Whilst in the previous century cardiovascular disease was mainly a disease of the developed world, presently, of all deaths caused by cardiovascular disease, 80% now occur in low-to-middle income countries. In Africa, hypertension is a particularly important cause of cardiovascular disease but the exact nature of the problem has not been clearly identified. Hypertension is more prevalent and often associated with greater target organ damage among groups of African descent than in other ethnic groups, and those of African ancestry often present with the complications of hypertension at a comparatively younger age. One of the proposals that explain ethnic differences in hypertension is that hypertension in groups of African ancestry represents an evolutionary change with distinct biological effects.

Groups of African origins frequently develop a low-renin, volume-dependent form of hypertension hypothesized to be mediated by a genetic predisposition to renal fluid retention resulting in a shifted pressure natriuresis relationship. However, the original proposal by Arthur Guyton and colleagues a half century ago that a shifted pressure natriuresis relationship is a fundamental cause of primary hypertension is not universally accepted and is presently often viewed with skepticism. In this regard, a central argument against this proposal is that the predicted increases in systemic blood flow that should occur with hypertension associated with renal mechanisms are too small to be measured. This renders the hypothesis untestable. However, there are limited data describing the hemodynamic basis of hypertension in groups of African descent. Therefore, in this present thesis I explored the potential hemodynamic determinants of hypertension across the full adult lifespan in an African community living in South Africa.

This present thesis comprises of a series of semi-independent data chapters (chapters 2 to 4), each having its own introduction, methods, results and discussion sections. In the introduction to this thesis (chapter 1) I critically review the available evidence on the determinants of hypertension in groups of African origins and argue in favor of conducting the studies that have been included in this present thesis. The conclusions chapter of the thesis (chapter 5) consolidates the findings of the data presented, underscoring the novelty of and giving perspective to these findings based on what is presently known and understood in the field. In support of the present

thesis, the data presented in chapters 2 and 3 have been published in the journal *Hypertension* (Mmopi et al., 2020; Woodiwiss and Mmopi et al., 2020) and the data presented in chapter 4 in *Journal of Hypertension* (Mmopi et al., 2021).

CHAPTER 1- INTRODUCTION:

Hypertension in groups of African Ancestry Living in Africa: A Case for Antagonistic Pleiotropy?

1.1 Introduction

With an improved general access to better healthcare in many countries, there has been a world-wide trend for associated increases in the lifespan. Concurrent technological advancements and increasing global access to these technologies have nevertheless resulted in marked changes in lifestyle. As a consequence of an increasing aged population and lifestyle adjustments, the burden of disease in the world has evolved. In this regard, death from non-communicable disease now surpasses that of infectious disease (McAloon et al., 2016). Indeed, in 2012, 36 out of the 56 million deaths that occurred worldwide were attributed to non-communicable diseases (Aryal et al., 2015). Where previously in low and middle-income countries, including most countries in Sub-Saharan Africa, infectious diseases and diseases of poverty were the main causes of death and disability, non-communicable diseases are now considered to be equally as important (Allen, 2017). Of the non-communicable diseases, cardiovascular disease is by far the most important cause of morbidity and mortality in the world. Approximately 17 million people die from cardiovascular disease per year representing 31 % of all global deaths (McAloon et al., 2016) and the estimate is that in the current year cardiovascular disease will account for 25 million deaths globally (Yusuf et al., 2001). Whilst in the previous century, cardiovascular disease was mainly a disease of the developed world, presently, of all deaths caused by cardiovascular disease, 80% now occur in low-to-middle income countries (Mendis et al., 2007). Importantly, in 2013, cardiovascular disease accounted for 1 million deaths in Sub-Saharan Africa alone (Keates et al., 2017).

Several well established risk factors account for cardiovascular disease, but hypertension is that single risk factor that accounts for most population attributable risk of cardiovascular disease (Chobanian et al., 2003; Heidenreich et al., 2011). In this regard, at least one in every five cardiovascular deaths are thought to be attributed to the presence of hypertension (Chobanian et al., 2003). In 2017 alone, hypertension accounted for 10.4 million deaths worldwide (GBD 2017 Risk Factor Collaborators, 2018). The relatively greater impact of hypertension as opposed to alternative risk factors for cardiovascular diseases is in part attributed to the fact that as often cited, hypertension is the most prevalent risk factor affecting approximately 20-30% of the world's population (Kearney et al., 2005). In the present thesis I examined the potential hemodynamic determinants of hypertension related to renal mechanisms in a group of African ancestry living in

South Africa. My interest in this topic was driven by several factors. In this regard, as shall be highlighted in the present chapter, hypertension is a particularly important cause of cardiovascular disease in groups that have originated from Africa, but now live elsewhere. Moreover, it has become increasingly evident that hypertension is a major cause of cardiovascular disease in groups of African descent living in Africa. As will be argued, the presence of hypertension in groups of African ancestry, particularly those living in North America or the Caribbean, may in part be attributed to several renal mechanisms that occur more frequently in these groups. As shall be highlighted these renal effects are thought to represent the impact of antagonistic pleiotropy, an evolutionary theory arguing that some genes that render survival advantage during the early years of life may ultimately become disadvantageous and cause disease in later years (Williams, 1957; Byars & Voskarides, 2020; Kamo et al., 2016; Mitteldorf et al., 2019). This theory has several implications regarding the management of hypertension that shall be discussed. However, as shall be argued in the present chapter, although the potential for this theory is sound, there was, prior to the present work, little evidence to support the hemodynamic changes that should occur when renal mechanisms are responsible for sustaining primary as opposed to just the less common, secondary forms of hypertension. Moreover, there was, prior to the present work, a belief that if this effect is indeed tenable, that it was specific to those whose ancestors were victims of the Slave trade and not to those of African descent living in Africa. There was nevertheless significant evidence lacking in this regard and this prompted me to perform the work described herein.

The present chapter is designed to highlight the importance of hypertension in groups of African descent either living in North America, the Caribbean or in Africa, and the possible renal mechanisms that may contribute to these effects. In so doing I will underscore the evidence or lack thereof that these renal mechanisms translate into the hemodynamic changes that should occur if they are critical determinants of sustained primary hypertension. I will also review the evidence to suggest that antagonistic pleiotropy may apply not only to populations of African ancestry living only in North America, or the Caribbean, but on the African continent as well. The importance of understanding this issue from a therapeutic perspective will then be highlighted and the missing evidence that prompted the work described herein, outlined.

1.1.1 Importance of hypertension in groups of African ancestry: Prevalence

When living in high-income countries, groups of African ancestry are frequently reported to have a higher prevalence of hypertension than alternative ethnic groups. In this regard, African Americans show a higher age-adjusted prevalence of hypertension than alternative groups. Indeed, in the United States of America (USA) in 2006, among individuals aged 20 years or more, where 33% of the total population were estimated to have hypertension, non-Hispanic blacks (many of whom originate from ancestors brought as slaves from Africa) had the highest age-adjusted prevalence (44.4% men and 43.9% women), as compared to non- Hispanic whites (34.1% men and 30.3% women), and Hispanics (23.1% men and 30.4% women) (Lloyd-Jones et al., 2009). These racial differences have not changed in the recent past. In this regard, in the USA in the period 2009 to 2012, the age-adjusted prevalence of hypertension was 44.9% and 46.1% among non-Hispanic black men and women, respectively; as compared to 32.9% and 30.1% among non-Hispanic white men and women, respectively; and 29.6% and 29.9% among Hispanic men and women, respectively (Mozaffarian et al., 2015). These ethnic differences begin at an early age. Indeed, higher blood pressures (BPs) in groups of African ancestry as compared to alternative groups are noted from a very young age. In this regard, in the USA in 1999–2000, in children aged 8 to 17 years, systolic BP (SBP) levels were 2.9 and 1.6 mm Hg higher in non-Hispanic black boys and girls, respectively, than in age-matched non-Hispanic whites (Muntner et al., 2004). Although groups of African ancestry living in Africa may have prevalence rates of hypertension that are lower than alternative populations in Europe (Cooper et al., 2005), and cross-continental studies show an escalating gradient of hypertension prevalence in populations of African ancestry, being lowest in Africa, intermediate in the Caribbean, and highest in the urban Midwestern USA (Cooper et al., 1997), this is more likely to reflect differences in the extent of urbanisation between these regions. Indeed, data from within populations of Africa show striking rural to urban BP gradients that predictably track directly with Western lifestyles (Agyemang et al., 2006). Nevertheless, a higher prevalence of hypertension has been noted among African Americans as opposed to residents derived from Africa (Wilson et al., 1991) where 42.8% of USA-born African Americans but only 27.4% of foreign-born black Africans have hypertension (Brown et al., 2017). These data are nevertheless inconsistent with the 40-60% prevalence rates of hypertension noted to occur in adults of African ancestry living in urbanised areas in South Africa (Norton et al., 2008; Sliwa et

al., 2008; Stewart et al., 2008). Moreover, with increasing urbanisation and the adoption of western lifestyles, prevalence rates of hypertension in Africa are escalating at a considerable rate with striking prevalence rates noted in many African countries despite the low degree of urbanisation (Twagirumukiza et al., 2011). Even low-income African countries affected by under-nutrition and food insecurity show a considerable prevalence of hypertension ranging from 13-16 % of the adult population (Price et al., 2018). Whether temporal effects of prolonged urban living are required to translate into high blood pressures in those at risk, requires consideration.

1.1.2 Importance of hypertension in groups of African ancestry: Cardiovascular damage

Hypertension is well recognized as being responsible for the excess cardiovascular events often reported to occur in groups of African ancestry as compared to alternative ethnic groups. In this regard, hypertension exacts an exceedingly high death toll from groups of African ancestry and this has been well documented in groups of African ancestry living in North America. Indeed, in 2005, the death rate from hypertension (per 100 000 population) in the USA was 51.0 in black men and 40.9 in black women as compared to 15.1 in white men and 15.1 in white women, (Centers for Disease Control, 2008). Hypertension may play a particularly important role in the development of heart failure and stroke in groups of African ancestry in the USA. Indeed, during 20 years of follow-up in those aged 18-30 years at initial recruitment, the cumulative incidence of systolic heart failure was greater among African Americans (1.1% in women and 0.9% in men) than among whites (0.08% in women and 0% in men; $p=0.001$ for blacks versus whites) and these differences were directly related to the increased burden of hypertension in African Americans (Bibbins-Domingo et al., 2009). As with many prospective studies of a similar nature, an elevated BP in this study remained a significant risk factor for systolic heart failure throughout the study ($p<0.001$) (Bibbins-Domingo et al., 2009).

With respect to stroke, which is strongly determined by hypertension, a marked ethnic disparity in age-adjusted stroke risk has been reported in large studies in the USA. Indeed, an age and sex-adjusted excess risk of ischaemic stroke occurs in those of African ancestry as compared to whites (risk ratio=1.51) (Howard et al., 2006). This ethnic disparity is particularly notable over a younger age where the risk over the ages 45 to 54 years, is 4.02 times greater in those of African ancestry (Howard et al., 2006). Importantly, between 45 and 64 years of age approximately 40%

of the excess stroke risk in those of African descent is attributable to traditional stroke risk factors, with levels of systolic BP (SBP) accounting for approximately one half of this effect (Howard et al., 2006). Notably, the impact of BP was greater in those of African ancestry than in whites where for each 10 mm Hg increase in SBP, the increased stroke risk in whites was 8%, whilst in African Americans it was 24% (Howard et al., 2006). The ethnic disparity that exists for stroke has been noted for all major types of ischaemic stroke with a 5.85 times greater risk for intracranial atherosclerotic stroke, a 3.18 times greater risk for extracranial atherosclerotic stroke, a 3.09 times greater risk for lacunar stroke, and 1.58 times greater risk for cardioembolic stroke, if of African as opposed to European ancestry (White et al., 2005). Importantly, lacunar strokes are strongly determined by hypertension and hence ethnic disparities in lacunar stroke risk are likely to reflect differences in the impact of hypertension. In this regard, ethnic disparities in hypertension-specific stroke types have been reported in other studies. Indeed, in one study the adjusted relative risk of stroke because of small vessel disease (lacunar stroke) in patients of African as compared to European ancestry was 2.94 (Markus et al., 2007). Moreover, the age-adjusted relative risk of intracerebral haemorrhage, which is also a hypertension-specific small vessel disorder, among those of African as compared to European ancestry has been reported to be 2.4 for men and 3.2 for women (Sacco et al., 1998) with a very high risk noted between 35 and 54 years of age (Flaherty et al., 2005).

A central question is whether hypertension exacts the same toll in groups of African ancestry living in Africa? In this regard, the impact of hypertension on cardiovascular disease may be equally as important in groups of African ancestry living in Africa as in other parts of the world. Importantly, the age at which those from sub-Saharan Africa present with their first myocardial infarction or alternative cardiovascular event (stroke or heart failure) is about two decades earlier than any other global region (Yoruk et al., 2018), particularly higher income countries in Western Europe and in North America (Yuyun et al., 2020). In South Africa alone, estimates are that hypertensive heart disease is the second and stroke the fifth most common causes of death in South Africa (Pillay-Van Wyk et al., 2014). In urban communities of African ancestry in South Africa, hypertension may account for up to a third of all of those presenting with heart failure (Stewart et al., 2008), and hypertension is strongly associated with myocardial infarction (Steyn et al., 2005), and is the major risk factor for strokes (Connor et al., 2009; O'Donnell et al., 2010).

1.2 Blood pressure control in groups of African ancestry: Possible explanations?

The excess cardiovascular risk attributed to hypertension in groups of African ancestry may be accounted for by several factors. In this regard, BP control is critical to the development of events. Over several decades, a poor BP control in groups of African ancestry has been highlighted. In this regard, population studies performed in sub-Saharan Africa show that levels of hypertension diagnosis, treatment and control are <40%, <35% and 10-20% respectively (Yuyun et al., 2020). In contrast, in North America >80% of patients are aware of their diagnosis, >70 % are receiving treatment and >55% have adequate BP control (Yuyun et al., 2020). The corresponding figures for Western Europe are similarly 60-70%, 50% and 30-40% respectively (Yuyun et al., 2020). A poor control of hypertension in sub-Saharan Africa can be in part associated with shortage of health care workers and health centers, and lack of health insurance leading to poor adherence to medication. Although the limited BP control in groups of African ancestry living in Africa has frequently been attributed to non-biological and non-biomedical factors, there is sound evidence that even when several of these factors are accounted for, that BP control is worse in groups of African descent living in other countries. In the present section, I will describe the non-biological factors that have been considered and the evidence that suggests that these factors may not fully account for an excess of uncontrolled hypertension in groups of African ancestry.

A poor BP control has generally been attributed in most ethnic groups to a lack of awareness and as indicated above this is often cited as a reason for poor BP control in groups of African ancestry as compared to alternative ethnic groups. However, over the period 1999–2004, large nationally representative surveys in the USA demonstrated an awareness of the presence of hypertension of 81.8% in non-Hispanic black women and 67.8% in non-Hispanic black men as compared to 73.4% in non-Hispanic white women, and 70.4% in non-Hispanic white men (Cutler et al., 2008). In the assessment of BP control and associated factors over the period 1988–1994 in the USA, non-Hispanic black hypertensive men nevertheless had the lowest BP control rates, with only 16.6% showing BP control (Cutler et al., 2008). Over the subsequent period that this was assessed (1999–2000) these control rates had increased dramatically to 29.9% (Cutler et al., 2008), an effect that can only be attributed to less patient or physician inertia rather than biological factors. Nevertheless, over the period 1999–2004 in the USA, among drug-treated hypertensive African

Americans, BP control rates remained low (45%) (Giles et al., 2007). Similarly, in more recent reports of nationally representative surveys of BP control in the USA conducted over prolonged periods (1988-2008), the proportion of treated patients with controlled hypertension among African Americans was similar to that for Hispanics but lower than in white Americans (Egan et al., 2010). These ethnic differences in control rates could not be attributed to differences in either awareness (as indicated above), treatment (higher among African Americans), nor the use of non-pharmacological/ behavioural management, a lack of availability of health-insurance, economic barriers, or a limited access to health insurance (Bosworth et al., 2006). Several non-biological factors may nevertheless still explain a reduced BP control rate in groups of Africa ancestry. In this regard, medication non-adherence may occur because of a lack of education resulting in beliefs, such as for example that hypertension can be cured or that antihypertensive medication only need be taken when experiencing symptoms. Moreover, patient-physician race-discordance may play an important role in contributing to patient understanding and hence medication adherence. Nevertheless, the possibility that ethnic differences in therapeutic responses may also play an equally important role should be considered. What is the evidence that responsiveness to therapy may differ between ethnic groups?

It is well recognised that groups of African ancestry have a reduced BP response to several antihypertensive drug classes. In this regard, there is an attenuated average BP reduction to monotherapy with angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin- receptor blockers (ARBs) in groups of African ancestry as compared with whites (Wright et al., 2005; Mokwe et al., 2004; Sehgal, 2004; Saunders et al., 1990). The lack of effects of these agents is thought to be largely explained by suppression of the renin-angiotensin-aldosterone system (RAAS) as a consequence of excess fluid retention following alterations in renal function, changes which will be discussed in later sections. These limited effects of ACEIs are not only evident in African Americans, but in groups living in Africa. Indeed, the lack of effect of ACEIs in groups of African ancestry living in South Africa may be so marked, that monotherapy fails to produce significant BP lowering on 24-hour monitoring over prolonged periods (Norton et al., 2008; Sareli et al., 2001). In contrast, diuretic responses have been reported to be enhanced in groups of African ancestry as compared to those of white patients (Preston et al., 1998; Chapman et al., 2002). Differential responses to antihypertensive drug classes may explain differences in the outcomes of the three large hypertension trials conducted on different continents. In the Antihypertensive and

Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) conducted in the USA, in which 40% of participants were of African ancestry, the thiazide-like diuretic chlorthalidone was the most successful drug class employed at reducing events, even as compared to an ACEI (ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group, 2002). In contrast, in the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) in which only 2.4% of participants had African ancestors, amlodipine to which the ACEI perindopril was added was more effective at preventing events than atenolol to which a thiazide diuretic was added (Dahlöf et al., 2005). These differences may nevertheless be attributed to increased central arterial pressures produced by the heart rate reducing effects of atenolol (through harmonic effects on the magnitude of reflected waves), effects not detected at the peripheral pulse (Farasat et al., 2008; Papaioannou et al., 2008; Wilkinson et al., 2002; Xiao et al., 2018). Nevertheless, in the second Australian National Blood Pressure trial (ANBP2), in which <2% of participants were of black African ancestry, ACEIs were more effective at preventing events than diuretics (Wing et al., 2003). Thus, when employing ACEIs in those of European ancestry, the impact on events is greater than that of a diuretic, whereas when employing a diuretic in those of African ancestry, the impact on events is greater than that of an ACEI. In addition to an attenuated BP response to ACEIs or ARBs, groups of African ancestry have also been demonstrated to show a reduced BP response to atenolol therapy (Gupta et al., 2010). The importance of a role for BP responses to therapy in mediating events is highlighted by data to show that in the USA in groups of African descent there is a limited response to several drug classes including ACEIs and atenolol within, as opposed to outside of the stroke belt (a geographic region where stroke occurs commonly) (Cushman et al., 2000). Thus, drug responsiveness may be an important cause of events in groups of African origins. These differential effects of different drug classes in different ethnic groups may account for the higher prevalence of refractory hypertension (uncontrolled BP despite the use of 3 or more antihypertensive classes) (Flack et al., 2010) in blacks although one cannot exclude the possibility of more non-adherence to therapy, a common finding in refractory hypertension, or several alternative factors also highlighted as key determinants of refractoriness to antihypertensive medication. As differences in therapeutic responses to antihypertensive agents in groups of African as compared to European ancestry may in part account for a limited BP control and hence an excess in cardiovascular events, the question arises as to how these effects may occur. What then are the possible biological differences that account for hypertension in groups of African ancestry?

1.3 Hypertension in those of African ancestry: Is this a case for antagonistic pleiotropy?

Hypertension in groups of African ancestry is often cited as an adaptive change that now represents a maladaptation. In this regard, as stated in the introduction to this chapter, antagonistic pleiotropy is an evolutionary theory arguing that some genes that render survival advantage during early years, may ultimately become disadvantageous and cause disease in later years (Williams, 1957; Byars & Voskarides, 2020; Kamo et al., 2016; Mitteldorf et al., 2019). With respect to hypertension in groups of African ancestry there are several arguments posed. The central hypothesis to all of the arguments put forward is that groups of African ancestry develop more hypertension through the fingerprint of natural selection. In this regard, a survival advantage is conferred by selectively retaining genetic variants that promote salt and water retention. In this regard through fluid retention, renal mechanisms are frequently cited as being central to long-term BP control and these effects are hypothesized to ultimately translate into sustained increases in BP in primary hypertension. As will be discussed this theory was originally proposed by Arthur Guyton and colleagues a half century ago (Guyton et al., 1972), but as will also be highlighted this is not a universally accepted hypothesis today (Kurtz et al., 2016, Beard, 2013). How could the selection of fluid retaining genes have occurred?

One theory proposed is that the severe conditions on ships transporting slaves from Africa to the Caribbean and to the USA, resulted in a very high mortality rate (Wilson & Grim, 1991). Mortality was likely due to dehydration from vomiting (seasickness and disease), diarrhoea (disease), and sweating (heat) (Wilson & Grim, 1991). It is argued that those that survived the journey were therefore theoretically genetically able to better retain fluid (decreased sweat loss and increased reabsorption of sodium and water in the kidneys). That an ability to survive slave ships may have contributed to a genetically determined natural ability to retain fluid in African Americans is supported by genetic differences that exist between African Americans and black African populations (Tishkoff et al., 2009). Furthermore, as described in aforementioned sections, generally the prevalence of hypertension is lower in black Africans as compared to African Americans (see above). This would suggest that a dramatic event could have occurred that resulted in natural selection in this case in favor of renal fluid retention, but only in those whose ancestors were victims of slavery. Thus, African Americans, but not black Africans would demonstrate these phenotypic effects and be predisposed to hypertension. As previously argued however, differences

in hypertension prevalence rates between African Americans and black Africans may nevertheless simply represent differences in the extent of urbanisation or the duration of exposure to an urbanised and industrialised existence rather than genetic differences in the renal handling of sodium and fluid. It certainly appears that a limited ability to respond to ACEIs or ARBs as monotherapy is a shared phenotype in groups of African descent irrespective of geographic region. Thus, whilst the genetic predisposition is present, external forces determine the extent of the BP effect. If the impact of slavery is indeed the process of natural selection that has rendered African Americans susceptible to hypertension, then black Africans living in Africa in an industrialised and urbanized existence should show much lower prevalence rates of hypertension. As indicated in aforementioned sections, this is however, not the case with prevalence rates of hypertension in adults living in cities in Africa often cited as 40-60% (Norton et al., 2008; Sliwa et al., 2008). Thus, if antagonistic pleiotropy is indeed a mechanism that explains hypertension in groups of African descent irrespective of where they are living, alternative selection processes need to be sought.

An alternative theory to the impact of slavery on survival has been proposed for the potentially high prevalence of hypertension in groups of African ancestry. This theory also supports the notion of antagonistic pleiotropy (Young et al., 2005). This theory similarly suggests that a survival advantage is conferred by selectively retaining genetic variants that promote renal salt and water retention, but in this case, the genetic selection is in favor of an ability to survive very hot environments (Young et al., 2005) which are largely around the equator. This process of natural selection would allow for a more effective hunter-gatherer existence (physical activities) in hot environments often with limited access to water. Migrations out of Africa by groups that ultimately settled in European countries or other areas could have decreased selective pressure on systems that promote renal fluid retention and could have led to the characteristics observed today in those of European ancestry. Alternatively, genetic drift could have expunged genetic determinants of these systems in Europeans. As African Americans originated from West African countries close to the equator, a genetic selection in favor of a better physical performance in hot environments may explain a genetic susceptibility to retain sodium and water in the kidney and hence to develop hypertension. As most groups of African ancestry living in Southern Africa originate from those individuals involved in the Bantu migration from central Africa thousands of years ago (Patin et al., 2017), as long as natural selection for renal fluid retention occurred before

the Bantu migration, then populations of African ancestry living in Southern Africa would also be susceptible to hypertension. As groups of African ancestry in most areas of the world today appear to be more at risk for the adverse effects of hypertension, the latter theory is perhaps more tenable, although African Americans may be at an even greater risk through further effects of slavery. As an understanding of the pathophysiology of hypertension is essential to identify appropriate targets for management, the question is what is it that explains the impact of a genetic predisposition to renal fluid retention on BP in groups of African ancestry?

1.4 The role of renal mechanisms in hypertension

The kidney is a critical determinant of sodium (Na^+) and water balance, both of which are nutrients essential for survival. Although the kidney filters very large amounts of both nutrients, it reabsorbs greater than 99% of the filtered load in the renal tubules. This allows for large variations in the daily consumption of both nutrients to occur, with a marked ability to retain these nutrients when too little are consumed or when marked losses are present (such as in hot environments). Fluid retention in the kidney is intricately linked to Na^+ retention and hence generally increases in Na^+ reuptake in the renal tubules (reabsorption following filtration) determine renal fluid uptake. The exception is in the collecting duct where water uptake occurs independent of Na^+.

As demonstrated a half century ago by Guyton and colleagues (Guyton et al., 1972), if BP increases so too does renal arterial pressure, and through several changes that decrease tubular Na^+ reabsorption (Ivy & Bailey, 2014), a natriuresis and diuresis occurs which reduces intravascular volume and returns BP to normal. This effect is termed a pressure natriuresis. Because of the importance of this renal change, Guyton and colleagues (1972) argued that even in primary hypertension, an increase in BP can only be sustained if, through renal mechanisms, the pressure natriuresis response is impaired. This argument has generated the notion that the kidneys are fundamental determinants of all hypertension, not only secondary forms of hypertension (Ivy & Bailey, 2014; Hall, 2016). Several abnormalities of the renal tubules may account for a shift in the pressure natriuresis relationship and an increased renal fluid retention with consequent sustained increases in BP in primary hypertension (Ivy & Bailey et al., 2014). Although a pressure natriuresis is produced by alterations in proximal tubular sites, changes in Na^+ reabsorption anywhere along the tubule may alter the pressure natriuresis relationship (Ivy & Bailey, 2014). Importantly, the

intricate relationship between fluid retention and Na^+ reuptake in the renal tubules translates into a close relationship between Na^+ intake and BP. Indeed, an acute elevation in BP occurs with increasing Na^+ intake in a proportion of any population (Williams et al., 1987; Falkner, 1988; Weinberger, 1996). This effect is termed "salt sensitivity". The sensitivity of BP increases to salt intake has been demonstrated to predict the development of chronic increases in BP, and normotensives with this trait are more likely to develop hypertension (Williams et al., 1987; Svetkey et al., 1997). Thus, the impact of a salt load on BP is often employed to identify the capacity of changes in the kidney to determine BP.

1.4.1 Renal mechanisms that may explain hypertension in groups of African ancestry

Salt sensitivity is frequently reported as being more common in hypertensives of African as opposed to European ancestry and is also present, albeit to a lesser degree, in normotensives of African as compared to European ancestry (Wright et al., 2003; Williams et al., 1987; Weinberger, 1996). The question that arises is what are the mechanisms that explain these ethnic differences in the BP response to salt loading? Although no direct evidence has been obtained to identify the exact renal mechanisms responsible for an increased salt sensitivity in groups of African ancestry, there is sound indirect evidence that provide clues. What is this evidence?

A clue to the renal mechanism that may explain an excess salt-sensitivity in groups of African ancestry comes from the consistent observation that groups of African descent excrete less potassium (K^+) in their urine than do whites (Luft et al., 1977, 1979*a*, 1979*b*; Berenson et al., 1979; Watson et al., 1980; Langford et al., 1991; Veterans Administration Cooperative Study Group on Antihypertensive Agents, 1987; Pratt et al., 1989, 2002; Voors et al., 1983; Wong et al., 2003). Total urinary K^+ excretion largely reflects K^+ intake, but the difference in urinary K^+ excretion between African as compared to European ancestry may not only result from a lower intake of K^+ in those of African descent. Indeed, after K^+ supplementation (in some instances well beyond the recommended daily allowance), daily urinary K^+ excretion is still markedly lower in those of African as compared to European ancestry (Langford et al., 1991; Voors et al., 1983; Wong et al., 2003). Even after receiving the same K^+ intake (80 mmol/d) after 15 days in a metabolic unit, participants of African ancestry consistently excrete less urinary K^+ than do participants of European descent (Luft et al., 1979*b*). It is difficult to reconcile these observations

with the idea that the lower urinary K^+ excretion in those of African ancestry is caused only by a deficit in K^+, particularly when total body K^+ is actually higher in those of African as compared to European ancestry (He Qing et al., 2003). In this regard, K^+ transport is tightly linked to Na^+ reabsorption in the distal tubules and cortical collecting ducts. Three Na^+ transport systems operate downstream to the proximal tubule that are directly or indirectly linked to K^+ transport. They are the amiloride-sensitive, epithelial Na^+ channel (ENaC), the thiazide-sensitive sodium chloride cotransport (Na-Cl cotransport), and the sodium potassium- chloride cotransport (Na-K-2Cl cotransport), which is sensitive to loop diuretics such as furosemide. Which of these three transport systems could be involved?

The hallmark of disorders that cause an increase in the activity of the ENaC is an increase in the urinary excretion of K^+ (Warnock, 2002). Because groups of African ancestry excrete less K^+ in the urine than do those of European ancestry, it has been argued that it is unlikely that sodium sensitivity in those of African descent is the result of an increase in the activity of the ENaC. In this regard, treatment with amiloride for 1 week has been noted to cause a reduction in BP in white but a paradoxical increase in BP in black participants (Pratt et al., 2002), and direct mineralocorticoid stimulation (i.e., treatment with fludrocortisone) fails to reduce the difference in urinary K^+ excretion between participants of African as opposed to European ancestry (Wong et al., 2003). However, in contrast, in an alternative study, after stimulation of the ENaC with 9-α fludrocortisone a rise in BP and increase in body weight (from fluid retention) was noted in those of African descent, but not in those of European ancestry (Tu et al., 2014). Furthermore, genetic variations in ENaC have been demonstrated to associate with BP or the response to therapy in groups of African ancestry (Spence & Rayner, 2018). Until data with fludrocortisone can be reconciled, and a mechanism for a reduced rather than increased BP response to amiloride in groups of African ancestry provided, and the decrease rather than increased urinary excretion of K^+ explained, the role of ENaC as a dominant factor causing increases in BP in groups of African ancestry remains uncertain. What of the thiazide-sensitive Na-Cl cotransport system?

A primary increase in the activity of the thiazide-sensitive Na-Cl cotransport system may result in a decreased K^+ excretion. This is explained by an increased Na^+ and water reabsorption and hence a reduced tubular flow to more distal tubular segments that are the main site of ENaC and K^+ excretion. This would decrease ENaC activity and reduce K^+ excretion. Through a reduced ENaC activity, an increase in the activity of the Na-Cl cotransport system may also decrease the

response to amiloride. An increase in the activity of the Na-Cl cotransport system would also increase renal responses to thiazide diuretics. In this regard, differences in K^+ excretion and responses to amiloride between groups of African descent and European descent have been discussed and importantly there is indeed a heightened sensitivity of BP to thiazide diuretics (Preston et al., 1998; Chapman et al., 2002), but not in the urinary excretion of Na^+ and K^+ (Ripley et al., 2000). In this regard, an increased Na-Cl cotransport activity should be associated with a greater excretion of Na^+ and K^+ in response to thiazide diuretics. Although there are several reasons why this may be compensated for by downstream alterations in ENaC activity, until this issue is fully resolved, one cannot conclude at present that a primary increase in Na-Cl cotransport activity is a determinant of an enhanced sodium sensitivity in groups of African ancestry. What of the Na-K-2Cl cotransport system?

The furosemide-sensitive Na-K-2Cl cotransport system functions primarily in the thick ascending limb of Henle's loop, where it is central to the counter-current exchange mechanism that determines the ability to maximally concentrate urine. Importantly, after furosemide treatment differences in urinary K^+ excretion between groups of African and European ancestry are partially normalized (Luft et al., 1979*a*). Thus, this transporter system may be more active in groups of African ancestry, an effect that would encourage Na^+ reabsorption and diminish renal K^+ excretion. A primary increase in the activity of the Na-K-2Cl cotransporter, by raising medullary hypertonicity, would augment the capacity to better withstand water loss from extrarenal sources. A corresponding compensatory decline in ENaC activity coupled with diminished tubular flow distally, could further decrease renal K^+ excretion. However, even without a decrease in ENaC activity, K^+ excretion would be attenuated. These data would suggest that furosemide should act as an effective antihypertensive in groups of African ancestry. However, there are no data to support this notion (most likely because the metabolic side effects and associated hypokalaemia caused by this agent prevents its possible use in uncomplicated hypertension being explored) and hence a role for the Na-K-2Cl cotransport system as a mediator of hypertension in groups of African ancestry, also remains speculative. Although excellent data have been obtained in carefully controlled studies conducted in largely African Americans, few studies have compared difference in renal tubular handling of salt and water in groups of African ancestry living in Africa with alternative groups. What is this evidence?

1.4.2 Renal differences in groups of African ancestry living in Africa as compared to alternative ethnic groups

To the best of my knowledge only one reasonably large study employing rigorous approaches has compared renal Na^+ handling in groups of African ancestry living in Africa with alternative ethnic groups (Belgians) (Bochud et al., 2009). In that study (Bochud et al., 2009), using lithium clearance techniques, the authors demonstrated a greater proximal tubular Na^+ reabsorption in groups of African ancestry living in South Africa as compared to Belgians. As data in African Americans supports an enhanced distal tubular Na^+ reabsorption, it is possible that the renal mechanisms responsible for hypertension, if renal mechanisms are indeed responsible for hypertension in these groups, are quite distinct. Thus, if antagonistic pleiotropy explains hypertension in groups of African ancestry living in Africa, it is possible that the origins (selection pressures) are indeed quite distinct from that in African Americans even if the overall effect on BP are the same.

1.4.3 Impact of renal effects in groups of African ancestry on the renin-angiotensin system

Amongst some of the major classes of agents employed to manage hypertension in current times are those that block the renin-angiotensin-aldosterone system (RAAS), including ACEIs and ARBs. As indicated in aforementioned sections, many hypertensives of African ancestry respond poorly to ACEIs or ARBs when employed as monotherapy. There are several reasons why this may occur but an important reason often cited is that renin release from the kidney is decreased in those with salt-sensitive hypertension who consume excess Na^+ and that this effect may occur with or without extracellular fluid volume expansion. Indeed, an excess Na^+ intake is well recognized as decreasing renin release and hence RAAS activity (Wisgerhof et al., 1978; Marks et al., 1979; Griffing et al., 1990; Fisher et al., 1999). A decreased RAAS activity in-turn promotes Na^+ excretion partly by decreasing aldosterone concentrations. Thus, in some measure through compensatory decreases in aldosterone concentrations, an excess Na^+ intake may not increase BP. Clinical studies nevertheless suggest that despite the decreases in plasma renin activity that occurs as a consequence of the BP response to salt intake, in salt-sensitive individuals, reductions in

plasma aldosterone concentrations may be attenuated (Wisgerhof et al., 1978; Marks et al., 1979; Griffing et al., 1990; Fisher et al., 1999). Furthermore, preclinical studies suggest that salt-induced increases in BP depend on attenuated decreases in plasma aldosterone concentrations (Makhanova et al., 2008). Indeed, as demonstrated in genetically modified animals (Makhanova et al., 2008), when aldosterone concentrations are insufficiently attenuated relative to renin in the presence of a Na^+ load, the Na^+ load subsequently increases BP. In a more recent study by our group (Scott et al., 2011), conducted in South Africans of African ancestry, a relationship between urinary Na^+/K^+ (an index of abnormalities in salt intake) and BP was noted only in participants with an aldosterone-to-renin ratio (ARR) above, but not below the median for the sample, where aldosterone concentrations are maintained at higher levels, despite Na^+ intake-induced decreases in renin concentrations. These data were obtained in a community sample in a group of African origins and provided the first evidence to show that an attenuated aldosterone suppression in salt-sensitive, low renin states is essential for maintaining an elevated BP with abnormalities in salt intake at a community level. There are several mechanisms that may explain this effect.

An increased adrenal sensitivity to angiotensin II may occur in salt-sensitive hypertension (Wisgerhof et al., 1978; Marks et al., 1979; Fisher et al., 1999) an effect that could attenuate the suppression of aldosterone relative to that of renin in the presence of a Na^+ load. Nevertheless, because circulating angiotensinogen concentrations may approximate the Michaelis-Menten constant for renin, in the presence of a low renin concentrations, cleavage of angiotensinogen into angiotensin I is thought to be a rate-limiting step in RAAS activation (Gould & Green, 1971). Thus, in the presence of high-Na^+, low-K^+ diets, increases in circulating angiotensinogen concentrations may also maintain RAAS activation despite suppression of plasma renin release. In this way angiotensinogen and, hence the RAAS may contribute to BP responses to salt intake. Indeed, some studies (Hunt et al., 1998; Norat et al., 2008; Iso et al., 2000; Yamagishi et al., 2004) suggest that genetic variation of the angiotensinogen gene is associated with alterations in BP in response to variations in Na^+ intake. Moreover, in more recent work, our group provided evidence in a community of African ancestry living in Africa that in the context of a high-Na^+ and low-K^+ diet (indexed by urinary Na^+/K^+), which suppresses renin release (resulting in a negative relationship between renin and BP), angiotensinogen is an important determinant of aldosterone concentrations and hence of SBP (Michel et al., 2012).

There are several potential alternative mechanisms that may explain an enhanced aldosterone production despite a suppressed renin release in groups of African descent. In this regard, endothelin-1 (ET-1) enhances angiotensin II and aldosterone production, but in *in-vitro* studies, ET-1 inhibits renin release (Ergul et al., 1996). In those with low-renin primary hypertension, ET-1 concentrations associate with plasma aldosterone levels (Letizia et al., 1997). Moreover, plasma concentrations of ET-1 are higher in black than white hypertensives (Ergul et al., 1996) and normotensives (Treiber et al., 2000). Notably, intensive antihypertensive therapy in blacks with uncontrolled hypertension decreases ET-1 levels (Ergul et al., 1998).

Thus, in summary, a common feature of hypertension in groups of African ancestry is the low renin state, often referred to as "low renin hypertension", mediated by renal effects (with or without extracellular fluid volume expansion). Importantly however, there are now several lines of evidence to suggest that when renin release is inhibited by an abnormal salt intake, that downstream aldosterone production, although decreased, is not suppressed to the same degree as renin and that an attenuated aldosterone suppression may be an essential element involved in mediating BP responses to an abnormal salt diet. These data therefore provide important insights into why despite overactive transporter activity in renal tubules in salt-sensitive hypertension, this is not fully compensated for by suppression of renin release and reductions in aldosterone concentrations.

1.5 Implications of antagonistic pleiotropy for managing hypertension in Africa?

As described in aforementioned sections, although some excellent clues as to the possible cause of an enhanced renal response to salt loading in groups of African ancestry have been provided, none of the evidence has led to the identification of a definitive target for the management of primary hypertension. Searching for the mechanisms responsible for differences in BP responses to salt loads is based on an assumption that the kidney is indeed central to the development of primary hypertension. As will be indicated in subsequent discussion, the central role of the kidney as a cause of primary hypertension suggested by Arthur Guyton and colleagues over a half century ago (Guyton et al., 1972) has indeed been questioned, the reasons for which will be further explained in later sections. Assuming that the kidney is indeed central to the genesis of primary hypertension, is there a case for antagonistic pleiotropy contributing to hypertension in

Africa? It is very possible that different selective pressures have produced quite distinct renal changes in those whose ancestors were victims of slavery versus those whose ancestors have always resided in Africa. Nevertheless, the importance of salt-sensitivity in groups of African ancestry has contributed to the notion that modifications in salt intake are essential in hypertensives of African ancestry and that diuretic agents (thiazide or thiazide-like diuretics) should form the mainstay of therapy in this ethnic group. This view is held for groups of African ancestry no matter where they reside. Moreover, our understanding of the impact of renal changes in salt-sensitive individuals on renin release has contributed to an awareness that RAAS blockers which do not target aldosterone effects directly (ACEI and ARBs which result in aldosterone escape when used for prolonged periods) have limited efficacy in groups of African descent when employed as monotherapy. However, the importance of diuretics as antihypertensive agents in groups of African ancestry has more recently been challenged, data which suggest that antagonistic pleiotropy mediated by sustained renal adaptations may not be as important as previously suggested in groups of African descent. What is this evidence?

More recent data (Ojji et al., 2019) suggest that thiazide diuretic agents may be less effective in combination with other agents (ACEI and thiazide diuretic) than combinations of largely vasodilators (ACEI and calcium channel blocker) in groups of African descent. These data were nevertheless obtained in African populations (Ojji et al., 2019) and hence if the salt-sensitive trait is a consequence of slavery, this finding may not be unexpected as the same trait would not be present in those living in Africa. However, if antagonistic pleiotropy occurred as a consequence of living in a hot environment (around the equator) with limited access to water, then these data would apply to any group of African ancestry. The *proviso* may nevertheless be that slavery may have produced further selective pressures and different renal mechanisms and hence that diuretic effects may have still been different. The use of thiazide as opposed to thiazide-like diuretics such as chlorthalidone, which is the diuretic demonstrated to reduce mortality in the ALLHAT study also has to be questioned.

The central issue raised by the recent data suggesting that vasodilators are equally as effective as thiazide diuretics combined with vasodilators in African populations (Ojji et al., 2019) is whether salt sensitivity is therefore an essential mechanism responsible for increases in BP in groups of African ancestry, at least those living in Africa? The relevance of the question relates to whether a reduction in extracellular fluid volume is required or whether targeting vascular changes

should be the primary approach with little need for reducing extracellular fluid volume. In this regard, the essential question is what is the ultimate goal of the renal effects that prevent fluid loss? For this to constitute antagonistic pleiotropy these effects must be beneficial in the first instance. That is, they are designed to maintain blood volume and hence systemic blood flow and hence increases in blood flow should characterize the effects produced when they are active. It is only with a reduction in blood flow that fluid loss constitutes a state where survival is not possible. If this is indeed the case, the essential feature that should characterize renal effects on BP is an increased extracellular volume and hence systemic blood flow. In this circumstance, adequate reductions in BP are best achieved by decreasing extracellular fluid volume and hence diuretics should indeed constitute the cornerstone of therapy (if they are indeed able to return blood volume and hence flow to normal). The fact that diuretics are no better than vasodilators in managing BP in groups of African ancestry (Ojji et al., 2019) suggests that either flow-dependent mechanisms do not occur or that the diuretics employed do not target these flow-dependent mechanisms. The fundamental question is therefore are these renal effects really a form of antagonistic pleiotropy in groups of African ancestry living in Africa, which ultimately cause hypertension through an enhanced extracellular volume and hence systemic blood flow? Insights into this question can only be derived from studies assessing the hemodynamic determinants of salt-sensitivity and hypertension in groups of African ancestry. The following section therefore reviews these data, and in the process highlights the missing evidence on this topic that prompted me to perform the work described in the present thesis.

1.6 Are renal effects central to the development of primary hypertension?

Guyton's original hypothesis that renal changes resulting in a shifted pressure natriuresis relationship is central to the development of primary hypertension (Guyton et al., 1972) is not universally accepted. In this regard, there are several criticisms to the logic of this hypothesis and it is often considered to be a tautological viewpoint (Beard, 2013; Kurtz et al., 2016). As argued by several antagonists to the hypothesis (Beard, 2013; Kurtz et al., 2016), the essential feature that should define hypertension caused by alterations in renal tubular function and a shifted pressure natriuresis relationship is an increased intravascular volume and hence an enhanced systemic blood flow. In this regard, as shall be highlighted, there is little evidence to suggest that systemic flow is

increased in primary hypertension, even in salt-sensitive individuals. Although protagonists argue that vascular responses to a high flow, such as autoregulation, causes an increased systemic vascular resistance returning flow to near normal levels (Hall, 2016), the only way that the vascular response can be maintained is if flow is increased (Kurtz et al., 2016). Protagonists simply argue that the increased flow is too small to be measured, but as indicated by antagonists, this suggests that the hypothesis can never be tested (Kurtz et al., 2016).

The same criticisms apply to the logic of salt-sensitivity, which as described above is thought to be determined by renal responses driven by a shift in the pressure natriuresis relationship. Indeed, in contrast to what would be expected from an enhanced renal fluid retention, an increased total peripheral resistance (TPR) (or systemic vascular resistance [SVR]) is the main hemodynamic effect that accompanies a salt challenge in salt-sensitive hypertension (Campese et al., 1993). In this regard, as demonstrated in circulating cells employed as surrogates of vascular smooth muscle, chronic effects of renal fluid retention in response to a salt load result in a greater intracellular Na^+ concentration in groups of African ancestry as compared to alternative groups (Love et al., 1953; Cooper et al., 1993). This theoretical accumulation of intracellular Na^+ in smooth muscle stimulates activity of the Na^+/Ca^{2+} exchanger and thus produces a higher intracellular Ca^{2+} concentration with a consequent enhanced contractile effect. Thus, the long-term effect on BP of an increased salt intake in those with salt sensitivity may be a vascular response. Authors of these papers however fail to explain how renal changes produce cellular changes in the vasculature. Indeed, any vascular response including an autoregulatory response to fluid retention would require a continued stimulus, and in the case of renal fluid retention, this would be an increased systemic flow. The argument that has always been given is that extracellular fluid volume and systemic flow do indeed increase for a period, but eventually long-term changes to the vasculature (broadly termed remodelling) maintain increases in SVR without the need for the renal mechanisms.

More recently, it has also been suggested that extra-renal tissues play an essential role in salt homeostasis. Indeed, storage of large quantities of Na^+ in the skin and muscles but without any associated water retention, may occur and this extra-renal sodium storage is closely associated with hypertension (Hofmeister et al., 2015). Sodium homeostasis cannot be achieved without the involvement of extra-renal mechanisms because 70-80% of extracellular fluids are found in the interstitial space, therefore these fluids are not directly controlled by renal Na^+ and water excretion

(Titze et al., 2015). Dermal vasculature is therefore proposed to function as a 'kidney-like' counter-current system, with the lymph capillaries functioning as a tubular-like drainage system. This counter-current mechanism enables the skin to control its electrolyte micro-environment, thereby creating a hyperosmolar biological barrier which prevents fluid loss (Hofmeister et al., 2015). When blood vessels are chronically embedded in this hyperosmotic medium, this could result in vascular inflammation and hypertension. Thus, salt-sensitivity may have little to do with renal mechanisms and the renal hypothesis of "salt-sensitivity" may therefore not hold. If this is indeed the case, then why would one even consider the use of diuretic agents when a higher than normal BP is not driven by an accumulation of extracellular fluid volume, but rather by a vascular response (either vascular remodelling or an inflammatory response in the vasculature)? The best approach would be the use of any agent that relaxes vascular smooth muscle. The counter-argument in this instance is that the diuretic is required as the vasodilator will reduce BP, and this will again lead to an enhanced fluid retention if a shifted pressure-natriuresis response exists. If this were indeed the case however, fluid retention should increase BP once again and hence oppose the beneficial effect of the vasodilator. All of these arguments ultimately appear circular with no real solution provided as to what the fundamental cause of the increased BP is and how best to target this increase. A central question that emerges from the aforementioned is therefore what is the hemodynamic factor that drives sustained and long-term increases in BP in groups with a high prevalence of salt-sensitive hypertension, including groups of African ancestry?

As with the increased BP that accompanies the effect of a salt load, hypertension in groups of African ancestry is more often reported as being associated with increases in SVR and aortic stiffness and not stroke volume (SV) or cardiac output (CO) (Heffernan et al., 2008; Din-Dzietham et al., 2004). In this regard, groups of African ancestry are frequently cited as having a greater vasoconstrictor response or a reduced vasodilator response to vasoactive substances. Indeed, a chronically elevated SVR is thought to be a major contributor to the higher rates of hypertension in African Americans compared to Caucasian Americans (Hill et al., 2016). In South Africa, groups of African as opposed to European ancestry show worse profiles in cardiovascular, oxidative and inflammatory parameters which promote increases in SVR as compared to whites (Mokhaneli et al., 2016). At similar BP levels, SVR is greater among blacks, and even modest increases in vascular tone markedly increase SVR (Mokhaneli et al., 2016). Groups of African ancestry show a greater degree of vascular hypertrophy and a narrower lumen diameter than in

those of European ancestry, even in normotensives (Hill et al., 2016). Groups of African ancestry show greater vasoconstrictive responses to a wide range of environmental, physical and psychosocial stressors than alternative ethnic groups, and these may contribute to the pathogenesis of hypertension (Taherzadeh et al., 2010; Treiber et al., 2000). As compared to normotensive whites, normotensive blacks have an enhanced vascular reactivity to sympathetic stimulation, an attenuated response to vasodilators and generally a reduced capability to vasodilate (see Table 1.1), impaired endothelial vascular function leading to a reduced nitric oxide bioavailability in the vascular wall, and a relatively narrower lumen diameter (Taherzadeh et al., 2010). When compared to other ethnic groups, blacks show a greater association between an increased vascular resistance and increased oxidative injury to the vessel wall, independent of the hypertensive status (Mokhaneli et al., 2016). This oxidative injury may play a role in early vascular changes leading to reduced arterial elasticity and development of atherosclerosis. Furthermore, blacks show significant hypertrophy of the systemic microvasculature which may possibly contribute to an elevated SVR that is seen in the early stages of hypertension (Hill et al., 2016; Taherzadeh et al., 2010). Evidence from twin studies and other related individuals suggest a significant genetic influence on SVR (Hill et al., 2014) and hence many of these ethnic differences in the vasculature may be attributed to genetic differences. The importance of vascular changes and SVR in groups of African as opposed to European ancestry suggests that a shifted pressure-natriuresis curve in this ethnic group may indeed result in vascular changes (SVR and aortic stiffness). The debate as to whether targeting volume (diuretics) or vascular (vasodilators) mechanisms therefore continues with suggestions that combinations of vasodilators may offer an equally effective approach to treatment as combinations of diuretics and vasodilators (Ojji et al., 2019). The question is therefore whether one even needs a diuretic, as the sustained effects on BP are purely vascular whilst the renal mechanisms are simply a trigger to initiate rather than sustain pressure effects (a temporary increase in extracellular fluid volume occurs which then returns to normal). However, the relative contribution of vascular versus volume mechanisms to hypertension in this ethnic group has not been evaluated. Similarly, the evidence to support dominance of vascular hemodynamic changes in volume-dependent populations has not accounted for more contemporary views on the hemodynamic determinants of BP across the full adult age. What is the current evidence for an

Table 1. 1 Ethnic differences in vasodilator response in normotensives

Author	Age (years)/ N		Baseline MAP (mmHg)		Vasodilator response		P value	Stimuli for vasodilation
	White	Black	White	Black	White	Black		
Lang et al., 1995	32.9 ±5.6 N =13M	31.3 ±8.0 N =9M	85 ±6	87 ±8	↑↑	↑	<0.001	Isoproterenol
Cardillo et al., 1998	49 ±2 N =7M, 7F	42 ±2 N =6M, 7F	86 ±3	83 ±3	↑↑	↑	=0.03	SNP
Cardillo et al., 1999	52 ±2 N =10M, 9F	43 ±2 N =10M, 8F	84 ±2	86 ±2	↑↑	↑	1. <0.001 2. <0.001 3. =0.006	1. Acetylcholine 2. SNP 3. Isoproterenol
Stein et al., 2000	28.3 ±1.9 N =10M	29.9 ±2.4 N =10M	92.6 ±3	96.8 ±3	↑↑	↑	=0.008	Isoproterenol
Gainer et al., 2001	32.0 ±2.3 N =8M, 6F	29.9 ±2.3 N =8M, 6F	84.1 ±1.8	86.2 ±1.8	↑↑	↑	=0.004	Bradykinin
Rosenbaum et al., 2002	30.1 ±1.9 N =11M, 8F	31.3 ±1.9 N =11M, 10F	81.6 ±1.3	87.4 ±1.9	↑↑	↑	1. =0.037 2. =0.035	1. Bradykinin 2. SNP

Data shown are mean ±SD, MAP = mean arterial pressure, N = number of participants, M = male, F = female, SNP = sodium nitroprusside, ↑ blunted response, ↑↑ good response.

impact of abnormalities in salt intake in salt-sensitive populations on the hemodynamic determinants of BP considered in the context of more contemporary views? Moreover, what is the current evidence for the hemodynamic determinants of BP in groups of Africa ancestry across the full adult age range? Is this evidence consistent with the possibility that an increase in extracellular volume (determined by renal mechanisms) and hence systemic blood flow contribute to increases in BP? Before discussing these issues, I will first describe the present understanding of the hemodynamic determinants of BP.

1.7 Importance of the pulsatile component of blood pressure

More contemporary views of increases in BP recognize the importance of the pulsatile nature of BP. In this regard, the impact of BP on cardiovascular end organs is now viewed as effects mediated by a steady component of BP, best indexed by mean arterial pressure (MAP), and a pulsatile component, best indexed by pulse pressure (PP). For convenience, the adverse effects of the steady component of BP are determined largely from diastolic BP (DBP) measurements, whereas the deleterious effects of pulsatile load are identified largely from systolic BP (SBP) measurements. Although a century of research has heralded repeated evidence for a role of DBP (and thus MAP) as a cause of cardiovascular disease, it is only in the latter half of the 20th century until present times that the importance of SBP (or PP) beyond DBP or MAP has been identified. What is the evidence for an important role of the pulsatile nature of BP as a cause of cardiovascular disease?

Several lines of evidence support the view that PP mediates cardiovascular disease beyond the steady component of BP. In this regard, independent of MAP, PP is a strong predictor of coronary artery disease (Franklin et al., 1997, 1999; Madhavan et al., 1994; Alderman et al., 1998; Benetos et al., 1997, 1998), stroke (Darné et al., 1989) and total cardiovascular events in both hypertensive patients and in the general population. In addition, isolated systolic hypertension (ISH), which is associated with a widened PP and often a lower than normal DBP, predicts heart failure (Mitchell et al., 2010*b*), coronary events (Mitchell et al., 1997), and CV mortality (Chirinos et al., 2005; Pini et al., 2008; Jankowski et al., 2008; Roman et al., 2007; Lin et al., 2016; Safar et al., 2002). Moreover, in the Systolic Hypertension in the Elderly Program (SHEP), a large randomised, double-blind, placebo-controlled trial involving elderly patients (age ≥ 60 years) with

ISH (SBP ≥ 160 mm Hg and DBP < 90 mm Hg) and thus a widened PP, antihypertensive treatment produced a reduction in strokes by 36%, myocardial infarction by 27%, all cardiovascular events by 32% and total mortality by 13% (Perry et al., 2000). Moreover, the Systolic Hypertension in Europe (Syst-Eur) (Staessen et al., 1997) and Syst-China (Wang et al., 2000) trials showed similar benefits from treatment of ISH. Furthermore, the Swedish Trial in Old Patients with Hypertension (STOP), involving much older patients (70-84 years of age), with an SBP between 180 and 230 mm Hg, who had striking increases in PP, achieved robust reductions in cardiovascular morbidity and mortality subsequent to antihypertensive treatment (Dahlöf et al., 2005). Although there is no question as to the importance of pulsatile load as a cause of marked cardiovascular damage, the past two decades has heralded ongoing debate as to the relative role of various determinants of pulsatile load as causes of cardiovascular damage. What then is the contemporary view of the factors that determine PP and which subsequently drive increases in SBP?

1.8 Contemporary views of the hemodynamic determinants of blood pressure

While the hemodynamic determinants of the steady component of BP are best described using Ohm's Law (pressure [P]=flow [F] x resistance to flow [R]) where MAP=CO x SVR, assuming that right atrial pressure =0 mm Hg, and CO =SV x heart rate, the hemodynamic determinants of PP are now well recognised as being far more complex. Importantly, the pulse produced in peripheral arteries and detected at the brachial pulse is generated in the proximal aorta and transmitted outward. What then generates the central arterial pulse? As indicated in Figure 1.1, the aortic (central arterial) pulse is the composite of several waveforms, with the two dominant waveforms being the incident or forward travelling pressure wave, the peak of which is labelled as Pf and a reflected or backward travelling pressure wave, the peak of which is labelled as Pb in the figure (Hughes et al., 2013; Hashimoto & Ito, 2009; Murgo et al., 1980; Nichols et al., 2011). What are the determinants of these two pressure waves?

1.8.1 Determinants of the incident or forward travelling pressure wave

The incident or forward travelling pressure wave is produced by the ejection of blood from the left ventricle (LV) into the aorta (SV), and ventricular ejection generates aortic flow (Q). The

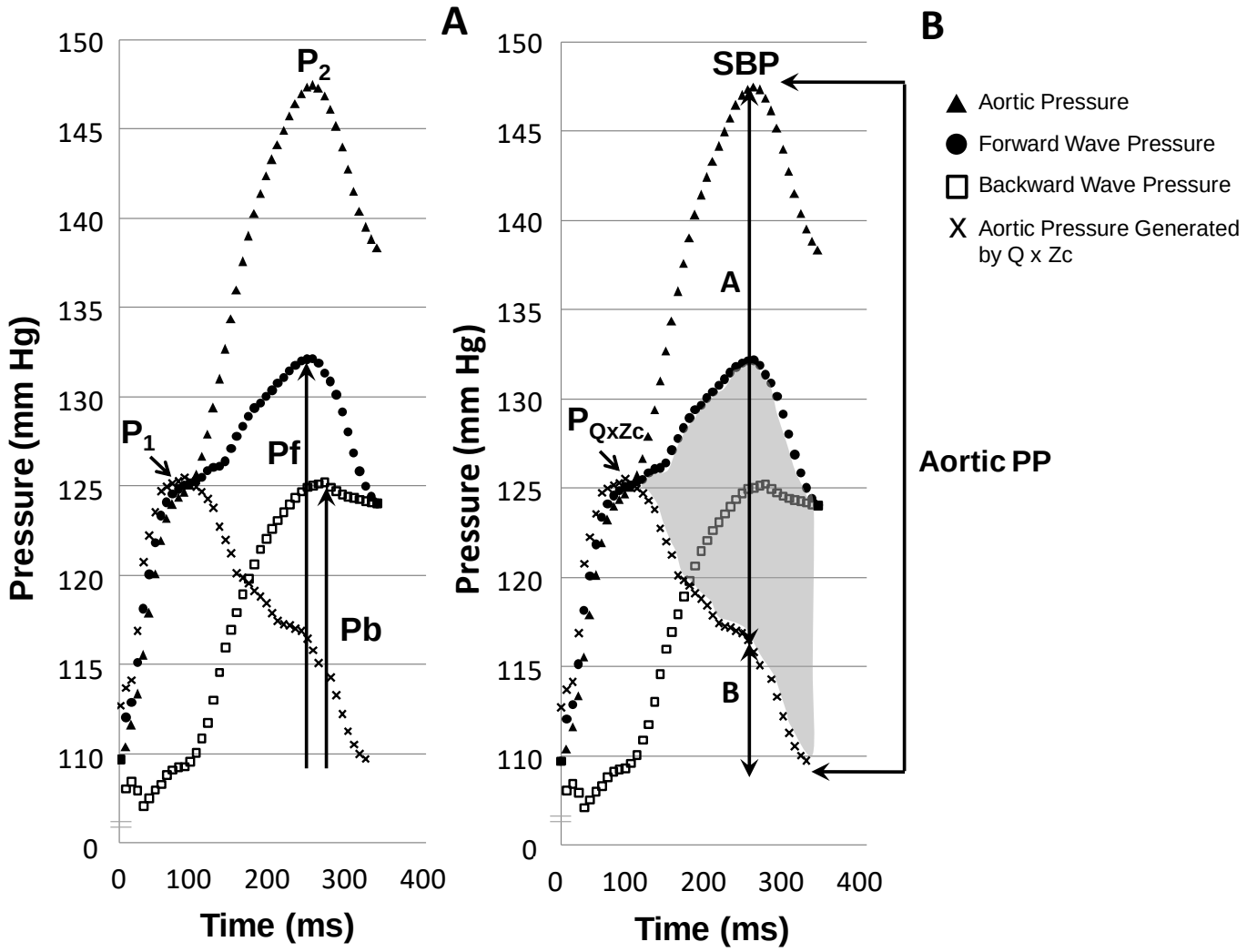


Figure 1. 1 Wave components of aortic pulse pressure (PP) showing the forward and backward wave pressures (panel A) superimposed on the aortic pressure wave and the pressure waves generated by the product of flow (Q) and characteristic impedance (Zc) and wave re-reflection (panel B). The shaded area in panel B shows that component of the forward pressure wave that represents wave re-reflection. Pb, peak backward wave pressure; Pf, peak forward wave pressure; P_1, first systolic shoulder; P_2, second systolic shoulder; P_{QxZc}, peak pressure generated by the product of aortic flow (Q) and characteristic impedance (Zc); SBP, systolic blood pressure; A, reflected + re-reflected wave pressures; B, pressure generated by the product of Q and Zc at peak PP.

incident or forward travelling pressure wave is therefore in part determined by SV and hence several cardiac effects (contractility, Frank-Starling mechanisms mediated by alterations in preload and diastolic function) as well as afterload to the LV. Although peak aortic flow is determined mainly by the impact of SV, it may also be influenced by several additional factors including ejection duration (an increased ejection duration may be associated with a lower Q although SV may be unchanged). Importantly, during the ejection of blood from the LV, Q interacts with the proximal aorta to generate pressure during aortic flow. How does this occur?

The mechanism by which the properties of the proximal aorta generate pulsatile pressures is often likened to the effects noted in fire-hose systems employed to fight fires prior to when the world mechanised. These fire-fighting systems were referred to as "windkessels", a German word for "air chambers" (Salvi, 2012; Laurent et al., 2006). A "windkessel" was coupled to an intermittent pump employed to provide water to fight fires. The pump in its own right would generate intermittent flow if simply connected to a hose. This of course would be a hopelessly ineffective manner of delivering water to fight fires. Indeed, steady rather than intermittent flow was required to act as an effective delivery system. To generate steady flow from a pump that was intermittent, two approaches could be employed. Either use high resistance hosepipes (e.g. dampening of pulsatility occurs as one goes down the arterial system), which of course according to the equation F=P/R was not an option as this would limit flow. Thus, in order to provide a steady flow of water without having to use high resistance hosepipes, the water was exposed to compressed air in a tank (windkessel) (Salvi, 2012). The air acted as a cushion and hence allowed for dampening of pressures thus reducing the pulsatility of flow. According to the "windkessel" model as applied to the cardiovascular system, an analogy popularized by Otto Frank (Westerhof et al., 2015), the role of large elastic proximal arteries (i.e. the aorta and its main branches) include a cushioning function, which dampens high pressure pulsatility generated by intermittent ventricular ejection (Safar et al., 2003). This "windkessel effect", was attributed to the properties of a compliant arterial system with the capacitance to accommodate an increase in blood volume and pressure generated by ejection of blood from the LV. Thus, during systole, about 50% of the stroke volume and 10% of the energy accumulates in the aorta as it expands during ejection (London & Pannier, 2010). During diastole, the stored energy in the aorta then generates elastic recoil, which creates the force to produce flow during this phase of the cardiac cycle, thus ensuring continuous blood flow even in the diastolic period (London & Pannier, 2010). When arteriosclerosis of central vessel occurs

(mainly the aorta), an effect produced by most major risk factors, including hypertension itself, several changes generate an increase in aortic stiffness or a decrease in elasticity and hence aortic compliance (C = stroke volume (SV)/ PP (Chemla et al., 1998; Stergiopulos et al., 1999). As such, according to the "windkessel model", when arteriosclerosis reduces aortic elasticity and hence decreases arterial compliance (C) (or increases stiffness), it reduces the cushioning effect of the aorta and increases pulsatility with a subsequent widening of PP and consequent increases in SBP (Alfie et al., 1999). However, as shall be discussed, the function of central arteries as a "windkessel" has considerably expanded over the years to now incorporate several additional concepts. What are these additional concepts?

There is an erroneous assumption that large vessels, such as the aorta, offer little resistance to flow. This assumption nevertheless only applies to steady flow and not to pulsatile flow as during pulsatile flow, flow rate is extremely high with peaks of 300-400 mls/sec achieved. Indeed, pulsatile flow rate in the aorta is so marked during ejection that despite its large diameter, the aorta offers a high resistance to flow. Resistance to flow in a pulsatile system is called impedance. In essence, the cushioning effect of the aorta prevents impedance to flow. In contrast, arteriosclerotic-mediated increases in aortic stiffness determine an increased characteristic impedance to flow (impedance to flow in a pulsatile system in the absence of wave reflection), abbreviated as Zc (Nichols et al., 2011). The pressure generated during ventricular ejection follows the usual formula P=F x R, but in this instance PP=Aortic Q x Zc (Nichols et al., 2011). Thus, current thinking is that increases in Zc in the proximal aorta are what determines the impact of increases in aortic stiffness on PP and hence SBP. Indeed, the incident pressure wave is largely determined by the product of Q and Zc (Figure 1.1). Importantly, however, Zc is the resistance to flow in a pulsatile system and hence it is not only stiffness of central arteries that determine Zc, but also the diameter of the aorta. Generally, however, as highlighted above, aortic diameter is so large, that it is often perceived as having a limited impact on Zc. Nevertheless, as will be discussed, several lines of evidence suggest that changes in diameter of the aorta may influence Zc.

Importantly, compliance (SV/PP) discussed in the aforementioned paragraphs, is determined not only by proximal aortic stiffness, but also by compliance of the arterial system further downstream including much smaller vessels whose function is not strongly influenced by central arterial changes and which are often modified by vasoconstrictor effects. Thus, compliance is better termed total arterial compliance (TAC) and although influenced by central aortic

dysfunction, TAC is also strongly determined by vascular tone of arteries downstream from the aorta. Importantly, therefore, a distinction between total compliance provided by large arteries and that purely attributable to small peripheral arteries cannot be clearly drawn, as through increases in stiffness large arteries can impose resistance whilst smaller vessels can also provide compliance albeit limited (Chirinos & Segers, 2010). Consequently, contemporary views incorporate the notion that pulsatile load is driven by several vascular factors including TAC and proximal aortic Zc, both of which are determined by aortic stiffness. However, of the two, Zc is the only one of these mechanical factors that is determined by central arterial properties alone. What is it that alters the mechanical properties of the proximal aorta to determine Zc and hence pulsatile load?

Over a period of years pulsatile load on the aorta causes fatigue, thinning and fragmentation of the elastin lamellae of central arteries, as well as increasing collagen concentrations and promoting calcium deposition in large arteries (O'Rourke & Nichols, 2005; Nichols et al., 2011). Furthermore, glycation of elastin (Jankowski, 2015), and cross-linking of collagen molecules by advanced glycation end products (Smulyan et al., 2016) follows increases in oxidative stress. The elastin component of the arterial wall is degraded by a number of enzymes including matrix metalloproteinase-9 (MMP-9), matrix metalloproteinase-2 (MMP-2), and serum elastase activity (Wallace et al., 2005). Indeed, circulating MMP-9 concentrations associate with aortic stiffness even in apparently healthy young people (Wallace et al., 2005). Moreover, aortic stiffness and elastance activity are influenced by genetic variation in the MMP-9 gene (McEniery et al., 2006). Importantly, a number of factors contribute toward alterations in vascular MMPs, increases in vascular collagen accumulation, cross-linking of elastin or collagen molecules, and calcification of the vascular wall to promote an increased stiffness of the aorta. However, this subject goes well beyond the scope of the present thesis and hence will be discussed in relevant sections only. What of the contribution of backward travelling pressure waves to PP and hence SBP?

1.8.2 Determinants of the backward travelling pressure wave

During ventricular ejection, the forward travelling pressure wave, as with any oscillating wave (such as a sound wave) is transmitted throughout the systemic arterial tree. When oscillating pressure waves reach points of bifurcation or significant tapering, the impedance mismatch (e.g. a change from a very compliant aorta to less compliant peripheral artery) results in wave reflection

and the oscillating reflected pressure wave travels back up the arterial tree to the proximal aorta. Several points of impedance mismatch occur along the arterial tree resulting in the generation of multiple oscillating reflected waves. These oscillating reflected pressure waves travel sufficiently rapidly that they meet with the oscillating forward travelling pressure wave (where once generated, is sustained for a few moments, sufficiently long that reflecting waves have time to return to meet it) at various points along the arterial tree. As with any oscillating wave (sound waves being a key example), the forward and reflected waves are often timed so that at points where antinodes overlap, summation of the two waves will occur. Although innumerable reflected pressure waves are generated at multiple points in the arterial tree, many will return to the aorta and summate together to generate what appears to be a single reflected wave (backward wave) in the proximal aorta the peak of which is labelled Pb in Figure 1.1.

Importantly, at a peripheral level, as reflected waves do not have far to travel before encountering forward waves, reflected wave antinodes will occur close to forward wave antinodes, thus producing maximal summation and generating a pressure wave which appears as a single pulsatile wave. These reflected waves are nevertheless determined more by the characteristics of the bed that the artery supplies and not by multiple waves reflecting off many arterial sites and summating in the central aorta. Nevertheless, in contrast to peripheral sites where maximal overlap of forward and reflected wave antinodes are likely, closer to the proximal aorta reflected waves will have had further to travel before encountering forward waves. Thus, the chances of reflected wave antinodes being synchronised with forward wave antinodes, will decrease and significantly less summation will occur generating a pressure wave, which appears as two pulsatile waves with a first and second systolic shoulder (Figure 1.1). In children and adolescents, the reflected wave(s) travels sufficiently slowly that it only summates with the forward wave in diastole, an effect that enhances DBP and thus coronary perfusion pressures, thus contributing to increases in coronary flow during exercise. As the reflected wave(s) occurs in diastole at this age, it does not contribute to central arterial PP and SBP. However, from early adulthood, reflected waves arrive sufficiently early in the central aorta that summation with the forward wave markedly augments central arterial PP and SBP (Figure 1.1). Thus, it is now recognised that wave reflection is a major determinant of central arterial PP and SBP. What are the determinants of the central arterial reflected wave?

Through Newton's Laws of Motion (inertial effects and for every action there is an equal and opposite reaction), increases in forward wave pressures usually result in increases in backward

wave pressures. However, increases in wave reflection are also attributed to alterations in the vasculature, with increases in arteriolar tone and more proximal arterial vessel tone producing marked effects on wave reflection. Moreover, mainly through harmonic effects on oscillating waves, decreases in heart rate increase the amplitude of the reflected wave by reducing the wave frequency (Xiao et al., 2018). In addition, more recently, the role of wave re-reflection as a determinant of forward travelling pressures waves has been highlighted (Phan et al., 2016). In this regard, the peak of the forward travelling pressure wave shows an excess of pressure beyond that identified from the product of Q and Zc (Figure 1.1) (Phan et al., 2016). This excess pressure is theoretically generated by re-reflected or "rectified waves" reflecting off structures in the proximal aorta and this pressure adds to the forward travelling pressure wave. Importantly, the amplitude of re-reflected waves will be determined by the extent of wave reflection, which as indicated, involves changes in distal arterial structure and function rather than in the aorta *per se*. Thus, a portion of the forward travelling pressure wave is generated by factors that have little to do with either Q or Zc.

1.9 Relative contribution of the determinants of pulsatile load to increases in blood pressure in different ethnic groups

There is ongoing debate as to the relative impact of various determinants of pulsatile load to PP and hence SBP. In this regard, some studies conducted in populations largely of European ancestry (United Kingdom and Australia) suggest that increases in backward wave pressures contribute more than forward wave pressures to age-related increases in PP and hence SBP (Cecelja et al., 2009; Namasivayam et al., 2009, 2016). These data are nevertheless inconsistent with alternative studies also conducted in populations of largely European ancestry (Framingham Heart Study) demonstrating that forward travelling pressure waves are more important determinants of age-related increases in PP and hence SBP (Mitchell et al., 2010*a*). Work from our group has provided evidence to show that in groups of African ancestry living in South Africa, when considering the relative contribution of forward and backward wave pressures to variations in PP *per se* irrespective of age, backward wave pressures are far more important than forward wave pressures (Booysen et al., 2015), but when considering age-related effects, forward wave pressures determine most of the age-related increases in PP that occur (Hodson et al 2017). As

forward wave pressures are strongly determined by the product of Zc and Q, the possibility arises that through the renal mechanisms previously described, fluid retention may have increased resulting in an enhanced Q. Is there evidence to support a role for increases in Q as a determinant of hypertension in any population, but more importantly, in populations of African ancestry?

The Framingham Heart Study, a community sample of largely European ancestry attributed age-related increases in PP and SBP to an enhanced Zc and not to increases in aortic Q (Mitchell et al., 2010*a*). This effect was not associated with decreases in aortic root diameter (which increased with age), but was associated with age-related increases in carotid-femoral (aortic) pulse wave velocity (PWV), an index of stiffness across the full length of the aorta (Mitchell et al., 2010*b*). The age-related increases in Zc were therefore attributed to an enhanced aortic stiffness. Nevertheless, it is argued that age-related aortic dilatation may increase rather than decrease Zc, by transferring hemodynamic load to stiffer collagen fibres (O'Rourke & Nichols, 2005). However, using magnetic resonance imaging of the aorta, a reduced diameter of the aorta has been demonstrated to associate with an increased PP in older individuals above the age of 70 years (Torjesen et al., 2014). Irrespective of the cause of the increased Zc however, age-related increases in PP in populations of European ancestry is more likely to reflect an impact of aging and risk factors on arteriosclerosis, rather than an effect of renal mechanisms increasing plasma volume, SV and hence Q. Additional studies support the view that age-related increases in PP are attributed to increases in forward travelling pressure waves through an enhanced aortic Zc and not to aortic Q (Segers et al., 2007; Torjesen et al., 2014). These data are further supported by findings in studies such as the Dallas Heart Study showing that hypertension-related increases in PP and SBP are attributed to an enhanced forward wave pressure through an impact on aortic stiffness and Zc (Goel et al., 2017). Nevertheless, ISH has been associated with an enhanced SV and hence increases in PP may also be attributed additionally to Q (McEniery et al., 2005, Pasierski et al., 1991). Moreover, there is some data in a small study sample to suggest that SV increases over an early adult age, but that at 50-60 years of age, when aortic stiffness begins to increase, SV decreases possibly in response to a higher impedance to flow (Alfie et al 1999). However, prior to the work performed in the present thesis, there were no studies that had determined whether hypertension is associated with an increased aortic Q or Zc in populations of African ancestry either living on or outside of the African continent. The fact that age-related increases in forward wave pressures previously reported by our group in a group of African ancestry living in Africa (Hodson et al.,

2017) appeared very similar to that reported on by the Framingham Heart Study investigators (Mitchell et al., 2010*a*), nonetheless suggested that similar mechanisms drive the forward wave pressure effects. As the Framingham Heart Study investigators demonstrated a contribution of aortic Zc and not Q to age-related increases in PP and hence SBP, it is possible that in Africa, the same mechanisms may apply. What is the evidence that suggested that Q may indeed play a role despite the similarity of findings in Africa as compared to in the Framingham Heart Study? In this regard, data from studies evaluating relations between salt intake and hemodynamic changes in groups of African descent living in South Africa provided a further stimulus to explore this question. What were these findings and the potential implications thereof?

1.10 Salt intake and pulsatile load

The World Health Organization (WHO) recognizes Na^+ as a major culprit in the genesis of hypertension, and therefore recommends a salt intake of less than 5g/ day for adults aged 16 years and over in order to reduce the risk of CV events. Several population studies and meta-analysis show that generally, global Na^+ intake is above the limit recommended by WHO, including South African treated and untreated hypertensives who have Na^+ intakes of 6.55 ±3.16 and 5.96 ±2.87 g/day respectively (Maseko et al., 2006). For example, global Na^+ intakes (g/day) range from 9.82 ±n/a in Italy, and 10.20 ±n/a in Benin (Thout et al., 2019), 5.84 ±2.61 in Ghana, 5.55 ±3.87 in Western Samoa, 3.18 ±1.77 in Cameroon, and 8.42 ±3.16 in France (Brown et al., 2009). As highlighted in previous sections the adverse effects of Na^+ intake could be particularly important in groups of African ancestry whom are well-recognised as being salt-sensitive. In this regard, those of African descent show a greater magnitude of the blood pressure increase with Na^+ loading (Aviv et al., 2004; Morris et al., 1999; Wright et al., 2003). One of the major BP changes that is often associated with an abnormal Na^+ intake is an increased SBP, an effect attributed in part to an impact on PP often beyond the steady component of BP (MAP and DBP) (du Cailar et al., 2004; Buyck et al., 2009; Redelinghuys et al., 2010). These relationships have been demonstrated in several ethnic groups, but importantly, also in a study conducted by our research group, in a community sample of African ancestry living in Africa (Redelinghuys et al., 2010). These relationships have nevertheless been assumed to be through an impact of volume load produced

by renal mechanisms in salt-sensitive individuals on blood volume, SV and hence Q. However, the evidence to support this notion is limited.

As demonstrated by our research group (Redelinghuys et al., 2010), in those of African ancestry living in South Africa, salt intake is associated with an enhanced incident or forward travelling pressure wave. As highlighted above however, this relationship may nevertheless be accounted for by either increases in blood volume and hence aortic Q, or by proximal aortic Zc. Moreover, as highlighted in previous paragraphs, PP, in addition to the effects of proximal aortic Q and Zc, may also be strongly determined by wave reflection and wave re-reflection. In this regard, salt intake is also associated with an enhanced aortic augmented pressure, an index of wave reflection, in a community of African ancestry living in South Africa (Redelinghuys et al., 2010) and wave reflection is determined by vascular tone often unrelated to SVR, rather than by proximal aortic Q. However, the exact mechanisms responsible for relationships between salt intake and PP have not been identified. Thus, whether the hemodynamic basis of salt effects on pulsatile load in groups of African ancestry living in Africa is through an increased SV and hence proximal aortic Q (flow-dependent mechanisms) as compared to the vascular changes that increase aortic Zc and reflected wave function (vascular mechanisms often beyond SVR), is unknown. This is a particularly important question for the following reasons.

Although intervention studies show that reductions in Na^+ intake, and increases in K^+ intake decrease SBP (The Trials of Hypertension Prevention Collaborative Research Group, 1997; Whelton et al., 1997, 1998; He et al., 2000; Sacks et al., 2001; Vollmer et al., 2001; Jurgens et al., 2017), these effects could simply be attributed to a decrease in distending pressures (MAP), rather than through effects on blood volume and hence Q or on wave reflection. In this regard, MAP is the determinant of passive distention of the aorta, which does not modify the aortic pressure-volume relationships, but rather shifts the point on the exponential aortic pressure-volume curve. By shifting the point to a lower value on the exponential curve by decreasing MAP, a reduction in arterial stiffness occurs through passive effects and not through structural changes in the aorta. If the impact of salt intake on PP is not through increases in Q, but rather through Zc or wave reflection, there may be a residual adverse effect of salt intake on pulsatile load that may not be readily reversed and which may not be readily detected at the peripheral pulse. Indeed, when aortic Zc increases, forward wave pressures may increase dramatically, whilst because of the impedance mismatch between central and peripheral vessels, increases in brachial PP may go undetected

(Motau et al., 2018). Furthermore, when wave reflection is enhanced, through differences in the impact of reflected waves centrally as opposed to peripherally, brachial PP and the adverse effects thereof may also not be detected at the brachial pulse (Bello et al., 2020). Indeed, relations between salt intake and PP are closer for central as opposed to peripheral PP (Redelinghuys et al., 2010). Consequently, further work is required to determine the exact hemodynamic mechanisms responsible for relationships between salt intake or age and PP in groups of African ancestry.

1.11 Summary of problem statement

Groups of African ancestry have a higher prevalence of hypertension and less BP control than alternative ethnic groups. These differences translate into a particularly high toll paid in cardiovascular events caused by hypertension in groups of African descent. Although a poor BP control has repeatedly been attributed to a lack of awareness and several non-biological factors in this ethnic group, even when these factors are controlled for, prevalence rates are higher and BP control worse in groups of African descent. Thus, biological factors must be considered to in part contribute to ethnic differences in hypertension. What is it that has been proposed as being somewhat distinct about hypertension in this ethnic group? In this regard, individuals of African ancestry are more likely to show an increase in BP in response to a Na^+ load (salt-sensitivity), and hypertension in this group is therefore more likely to be associated with a suppressed RAAS. The consequence is a particular sensitivity of BP to some diuretics, but a limited ability of ACEI and ARBs to effectively lower BP. These differences are thought to be through a renal adaptation designed to retain Na^+ and hence water in the face of a salt and water losing state (hot environment with no access to water). This renal phenotype has been attributed to antagonistic pleiotropy, where through natural selection, survival on slave ships or survival as a hunter-gatherer when living around the equator, depended on this renal phenotype. Theoretically, whilst this renal phenotype allows for survival in those performing physical activity in hot and water-restricted environments, with aging it results in fluid retention and a high BP and thus a decreased chance of survival (antagonistic pleiotropy). However, several lines of evidence suggest that these changes are not distinct enough from other ethnic groups to explain either the high prevalence of hypertension or a poor BP control. What are the limitations of existing evidence and what evidence is missing?

The more recent focus of antihypertensive therapy in the world today has been on targeting vascular tone with calcium channel blockers (CCBs), ACEIs or ARBs (although targeting renal mechanisms, the impact of ACEIs and ARBs is primarily on the vasculature), the so-called newer classes of agents, while agents that target primarily renal mechanisms (diuretics) are considered to be older agents. This assumes that vascular effects rather than increases in systemic blood flow mediated by renal fluid retention are the cause of the high BP. In this regard, hypertension in groups of African ancestry has indeed only ever previously been associated with a high SVR and aortic stiffness, and not with increases in SV, CO or aortic flow. Moreover, more recent evidence demonstrates that a CCB and ACEI combination is equally as effective as a CCB and diuretic combination in groups of African ancestry, thus challenging the notion that targeting primarily renal mechanisms, at least with the diuretic employed (thiazide rather than thiazide-like), has no special properties. Moreover, salt-sensitivity has been demonstrated to cause an increased SVR and not SV or CO, a change explained by vascular alterations increasing resistance to flow and hence BP, and limiting increases in flow to unmeasurable levels. The inability to measure increases in systemic flow in salt-sensitive individuals has been criticised as being evidence counter to the importance of renal mechanisms and a shifted pressure natriuresis relationship as a cause of primary hypertension. Consequently, the "distinct" biological nature of hypertension in groups of African descent may simply be another example of a mechanism that primarily increases vascular tone and thus targeting vascular mechanisms is all that is required. However, using more contemporary approaches to assessing the hemodynamic basis of hypertension, several findings from our group in the recent past have raised the possibility that in Africa at least, increases in systemic blood flow may be an important change. First, our group has demonstrated that age-related increases in forward wave pressures, which are determined by the product of Q and Zc are important determinants of increases in BP in South Africa. Second, we have demonstrated that urinary indices of salt intake in this same community are associated with BP largely through an impact on PP, effects in part attributed to relations with forward (incident) wave pressures. Thus, the possibility exists that in groups of African ancestry living in Africa, part of the hemodynamic basis of hypertension may be distinct from alternative population groups, in that systemic blood flow may play a central role. If this is indeed the case, this would provide the first evidence to support the hypothesis of Guyton and colleagues posed half a century ago (Guyton et al., 1972) that a shifted pressure natriuresis relationship produced by renal tubular effects is central to the

pathogenesis of not only secondary forms of hypertension which are generally uncommon, but also primary hypertension. However, in none of the studies exploring the hemodynamic basis of hypertension in groups of African ancestry did the investigators assess measures of systemic blood flow (Q, SV and CO) across the full adult lifespan. Moreover, in none of these studies did the investigators evaluate all of the vascular changes that account for hypertension including Zc or TAC. Thus, a full appreciation of the more contemporary views of the hemodynamic basis of hypertension in groups of African ancestry has not been obtained. Consequently, in the present thesis, through the evaluation of all determinants of BP in keeping with contemporary views, I assessed the hemodynamic basis of hypertension across the full adult lifespan in a community sample of African ancestry living in South Africa.

1.12 Hypothesis

I hypothesized that in groups of African ancestry living in Africa, changes in systemic blood flow may be the predominant hemodynamic factor determining BP and hypertension.

1.13 Aims

In a large community sample of African ancestry living in South Africa sampled across the full adult age range, I therefore aimed to determine:

1) The relative contribution of systemic blood flow (peak aortic flow or Q) versus arterial changes (Zc and backward wave effects) to relations between urinary indices of salt intake and PP. These data have recently been published in the American Heart Association journal, *Hypertension* (Mmopi et al., 2020) and will be described and discussed in detail in chapter 2 of the present thesis.
2) The relative contribution of systemic blood flow (Q, SV and CO) versus arterial changes (SVR, Zc, TAC) to age-related BP (SBP, PP, MAP and DBP) differences across the adult lifespan and relations with RAAS activity. These data have also recently been published in *Hypertension* (Woodiwiss and Mmopi [equal contribution] et al., 2020) and will be described and discussed in detail in chapter 3 of the present thesis.

3) The relative contribution of systemic blood flow (Q, SV and CO) versus arterial changes (SVR, Zc, TAC and backward wave pressures) to systolic-diastolic, isolated systolic and isolated diastolic hypertension. These data have been accepted for publication in *Journal of Hypertension* (Mmopi et al., 2021) and will be described and discussed in detail in chapter 4 of the present thesis.

CHAPTER 2

Increased Aortic Characteristic Impedance Explains Relations Between Urinary Na^+/K^+ and Pulse or Systolic Blood Pressure.

The data in this chapter have been published in *Hypertension* as follows:

Keneilwe N Mmopi, Gavin R Norton, Hamza Bello, Carlos Libhaber, Mohlabani Masiu, Daniel Da Silva Fernandes, Pinhas Sareli, Vernice Peterson, & Angela J Woodiwiss. (2020). Increased aortic characteristic impedance explains relations between urinary Na^+/K^+ and pulse or systolic blood pressure. *Hypertension*, 75: 1260-1279.

2.0 Abstract

Background: Alterations in sodium (Na^+) relative to potassium (K^+) intake increase systolic blood pressure (SBP), effects in part attributed to enhanced pulsatile loads (pulse pressure, PP) beyond steady-state pressures (MAP). Whether this effect is through reversible changes (increases in blood volume and hence aortic flow [Q] or wave reflection [Pb]), or potentially irreversible structural changes in the proximal aorta, is unknown.

Methods: In 581 black South Africans, I determined 24-hour urinary Na^+ and K^+ excretion and aortic function from central aortic pressure (radial pulse wave analysis [SphygmoCor software]), velocity and diameter measurements. Proximal aortic function was assessed from characteristic impedance (Zc).

Results: Beyond MAP and additional confounders, urinary Na^+/K^+ was independently associated with Zc ($p<0.005$), but not peak aortic Q ($p=0.30$) or alternative aspects of Q or ejection volume. Although age was strongly associated with proximal aortic diameter, no independent relations between urinary Na^+/K^+ and aortic diameter were noted ($p=0.17$). Relations between urinary Na^+/K^+ and Zc translated into independent relations with early systolic pulsatile pressures (QxZc [P_{QxZc}]) and aortic forward wave pressures, but not Pb. Moreover, neither reflected wave magnitude ($p=0.92$), nor aortic pulse wave velocity were independently associated with urinary Na^+/K^+. In product of coefficient mediation analysis, the independent relations between urinary Na^+/K^+ and peak aortic or brachial PP or SBP were accounted for by Zc and P_{QxZc}.

Conclusions: Abnormalities in Na^+/K^+ intake determine PP or SBP beyond MAP mainly through potentially irreversible impacts on proximal aortic impedance rather than readily modifiable increases in aortic flow (blood volume) or wave reflection.

Key words: Salt intake, pulse pressure, characteristic impedance, aortic flow, wave reflection.

2.1 Introduction

Abnormalities in dietary salt intake, as indexed by increases in urinary sodium (Na^+) excretion, decreases in urinary potassium (K^+) excretion or increases in urinary Na^+/K^+, are well-recognised as being associated with blood pressure (BP) (Intersalt Cooperative Research Group, 1988; Smith et al., 1988; Hajjar et al., 2001; Buyck et al., 2009; Liu, 2009). Intervention studies show that reductions in Na^+ intake, and increases in K^+ intake decrease BP (The Trials of Hypertension Prevention Collaborative Research Group, 1997; Whelton et al., 1997, 1998; He et al., 2000; Sacks et al., 2001; Vollmer et al., 2001; Jurgens et al., 2017). As a consequence of these findings (Intersalt Cooperative Research Group, 1988; Smith et al., 1988; Hajjar et al., 2001; Buyck et al., 2009; Liu, 2009; The Trials of Hypertension Prevention Collaborative Research Group, 1997; Whelton et al., 1997, 1998; He et al., 2000; Sacks et al., 2001; Vollmer et al., 2001; Jurgens et al., 2017), all major guidelines recommend that changes in salt intake should constitute part of lifestyle changes for the management of hypertension (Williams et al., 2018; Whelton et al., 2018; National Institute for Health and Care Excellence, 2019). One of the major BP changes with an abnormal salt intake is an increased systolic BP (SBP) an effect attributed in part to an impact on pulse pressure (PP). Importantly, the impact on PP is often beyond steady pressure effects (mean arterial pressure [MAP] and diastolic BP) (du Cailar et al., 2004; Buyck et al., 2009; Redelinghuys et al., 2010). In this regard, it is well recognised that PP accounts for most of the risk related to increases in SBP (Darné et al., 1989; Rudnichi et al., 1997; Madhavan et al., 1994; Mitchell et al., 1997; Celis et al., 2001). However, the mechanism(s) responsible for the effect of salt intake on PP are unclear. Hence, whether the impact of salt intake on PP is readily reversible, is uncertain.

Both central arterial and peripheral PP and hence SBP are mediated by increases in early systolic pressures (forward or incident wave pressures, Figure 1.1). These pressures are generated by the product of aortic flow (Q) and increases in proximal aortic characteristic impedance to flow (Zc) (resistance to flow in a pulsatile system in the absence of reflected waves) (Celis et al., 2001; Nichols et al., 2011; Mitchell et al., 2010*a*; Segers et al., 2007). Importantly, increases in Zc are driven by structural changes in the proximal aorta which alter stiffness and diameter. In this regard, salt intake is associated with an enhanced incident wave pressure (Redelinghuys et al., 2010), an effect that may be mediated by either increases in blood volume and hence peak Q, or by Zc.

Although volume overload (and hence Q) is readily reversible by modifying salt intake, there is no convincing evidence for the reversibility of structural aortic changes responsible for increases in Zc. Central (but not peripheral) arterial PP and the forward travelling pressure wave are in addition to the effects of Q and Zc, also strongly determined by wave reflection and re-reflection (Phan et al., 2016). In this regard, salt intake is also associated with an enhanced aortic augmented pressure, an index of wave reflection (Redelinghuys et al., 2010). As wave reflection is modified by arteriolar tone (Nichols et al., 2011), the possibility also exists that this is readily reversed by encouraging modifications in dietary salt intake. However, the extent of wave reflection is strongly determined by forward wave pressures (through Newton's Laws of Motion) and hence also through potentially irreversible changes in proximal aortic structure (Nichols et al., 2011). Although there is no question that changes in salt intake reduce PP, this could be driven by reductions in distending pressures (MAP), rather than by those factors which cause increases in PP beyond MAP. Thus, modifying abnormalities in dietary salt intake may be insufficient to address all of the adverse effects thereof. However, the exact mechanisms responsible for relationships between salt intake and arterial PP have not been identified. In the present study conducted in a community of African descent, an ethnic group well-recognised as having a high prevalence of salt-sensitivity (Wright, 1988), we therefore determined, in product of coefficient mediation analyses, the extent to which increases in proximal aortic Zc, as opposed to Q or wave reflection, account for the relationships between urinary Na^+/K^+ and central and peripheral arterial PP and SBP previously demonstrated (Redelinghuys et al., 2010).

2.2 Methods

2.2.1 Study group

The present study was conducted according to the principles outlined in the Helsinki declaration. The Committee for Research on Human Subjects of the University of the Witwatersrand approved the protocol (approval number: M02-04-72 and renewed as M07-04-69, M12-04-108 and M17-04-01). Participants gave informed, written consent. The data are available from the corresponding authors upon reasonable request. The present study design has previously been described (Redelinghuys et al., 2010; Booysen et al., 2015; Norton et al., 2012). In the present

substudy 581 participants from randomly recruited (from the population census figures of 2001) families of black African descent (Nguni and Sotho chiefdoms) from the South West Township (SOWETO) of Johannesburg, South Africa, with siblings older than 16 years of age, were evaluated. These participants had both 24-hour urinary samples that met with pre-specified quality control criteria previously described (Redelinghuys et al., 2010), and high quality velocity measurements in the outlet tract necessary to determine Zc.

2.2.2 Clinical and demographic information

A questionnaire was administered to obtain demographic and clinical data (Redelinghuys et al., 2010; Wright, 1988; Booysen et al., 2015). Height and weight were measured using standard approaches and participants were considered to be overweight if their body mass index (BMI) was ≥25 kg/m^2 and obese if their BMI was ≥30 kg/m^2. Laboratory blood tests of renal function, liver function, blood glucose, hematological parameters, and percentage glycated hemoglobin (HbA1c) were performed. Diabetes mellitus (DM) was defined as the use of insulin or oral glucose lowering agents or an HbA1c value greater than 6.5%. High quality office brachial blood pressure (BP) measurements were obtained in the seated position and after 5 minutes of rest, by a trained nurse-technician using a standard mercury sphygmomanometer according to guidelines. The mean of 5 measurements obtained at least 30 seconds apart was taken as office BP. Hypertension was defined as a mean office BP≥140 mm Hg systolic or ≥90 mm Hg diastolic BP or the use of antihypertensive medication.

2.2.3 Urinary electrolyte excretion rates and circulating RAAS measurements

To determine salt intake, timed urine samples were successfully obtained over at least a 24-hour period on the same say as the BP measurements and the blood sampling as previously described (Redelinghuys et al., 2010). Urine collection started after discarding urine obtained immediately prior to the collection period. Urine Na^+ and K^+ concentrations were measured and 24-hour urine Na^+ and K^+ excretion rates calculated from the product of 24-hour urine volumes and urine electrolyte concentrations. The quality of urine samples has previously been described (Maseko et al., 2006).

As we have previously demonstrated that the circulating renin-angiotensin-aldosterone system (RAAS) is a strong determinant of the impact of salt intake on BP, I determined renin and aldosterone concentrations using previously described approaches (Scott et al., 2011; Michel et al., 2012). Blood samples were obtained in the supine position after 10 minutes of rest in the morning between 10:00 and 12:00 hours. After centrifugation, samples were stored at -70^{o}C until the time of analysis. Plasma renin concentrations were measured using an immunoradiometric technique (Renin III Generation, Cisbio International, Ceze, France). Serum aldosterone concentrations were measured using an ^{125}I radioimmunoassay (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA).

2.2.4 Pulse wave analysis

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 9.0 software (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia). The pulse wave was calibrated by manual measurement (auscultation) of brachial BP taken immediately before the recordings. The peripheral pressure waveform was converted into a central aortic waveform using a validated generalized transfer function incorporated in SphygmoCor software. 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. Carotid-femoral (aortic) pulse wave velocity (PWV) was determined from sequential waveform measurements at carotid and femoral sites using applanation tonometry and SphygmoCor software. The time delay in the pulse waves between the carotid and femoral sites was determined using an electrocardiograph-derived R wave as a fiducial point.

2.2.5 Central arterial function

Central arterial pressures were determined from central arterial pressure recordings obtained from pulse wave analysis as previously described (Redelinghuys et al., 2010; Wright, 1988; Booysen et al., 2015), with details given in the on-line supplement. Whilst central arterial pressure waveforms were obtained, aortic velocity and diameter measurements were obtained by an experienced observer (AJW) in the left lateral decubitus position using an Acuson SC2000 Diagnostic ultrasound system (Siemens Medical Solutions, USA, Inc.). Velocity waveforms were obtained in the 5-chamber view. Aortic diameter measurements were obtained just proximal to the aortic leaflets in the long axis parasternal view. The largest diameter recorded in early systole was employed to construct the flow waveform (Mitchell et al., 2010*a*). Aortic flow waveforms were generated from aortic velocity and diameter measurements. Taking care to avoid any overshoot of the image, the leading (outer) edge or the most dense, or brightest, portion of the spectral image of the velocity waveform was outlined using graphics software and using aortic diameter measurements employed to construct a flow waveform. Characteristic impedance (Zc) was calculated in the time domain as change in pressure/change in flow from the foot of the pulse wave up until 95% of peak flow (Mitchell et al., 2010*a*; Segers et al., 2017). Using Zc values and the flow and pressure waveforms, wave separation analysis was performed based on the following formulae: Forward wave pressure= (aortic PP + QxZc)/2 and Backward wave pressure= (aortic PP – QxZc)/2 (for example, see Figure 1.1) (Westerhof et al., 1972; Murgo et al., 1981). Wave separation analysis was performed in the time domain based upon the consensus that this analysis can be conducted in either the time or the frequency domain (Segers et al., 2017). The pressure wave generated by the product of Q and Zc (P_{QxZc}) was determined and the peak pressure taken as early systolic pulsatile load (Figure 1.1) (Phan et al., 2016). As several aspects of Q may contribute to PP (Vennin et al., 2017), we assessed relations between urinary indexes of salt excretion and peak aortic Q, flow rate (Q) at peak aortic PP and the volume of blood ejected up until peak aortic PP. Flow rate at peak aortic PP was determined in addition to peak aortic Q, as a significant proportion of participants had striking increases in wave reflection with peak aortic PP occurring much later than peak aortic Q (on the downslope of the flow wave). Volume of blood ejected up until peak aortic PP was determined from the product of the velocity time integral up until peak aortic PP and the aortic root area. The contribution of wave re-reflection to peak aortic PP and

forward wave pressures was determined as the difference between forward wave pressures and the pressures generated by Q x Zc at the peak of aortic PP (Figure 1.1) (Phan et al., 2016).

2.2.6 Data analysis

For database management and statistical analysis, SAS software, version 9.4 (SAS Institute Inc., Cary, NC) was employed. Continuous variables are expressed as mean (±SD or SEM) or median and interquartile range. Dichotomous variables are expressed as percentages. For data that were non-normally distributed, in regression analyses they were first log transformed to improve on the distribution. To determine relationships multivariate adjusted linear regression analysis was performed. To determine the contribution of the various hemodynamic parameters to the impact of urinary indexes of salt excretion on increases in BP, multivariate adjusted product of coefficient mediation analysis was performed. In regression analysis and product of coefficient mediation analysis, adjustments were for MAP, age, sex, regular alcohol intake, regular tobacco intake, body mass index (BMI), treatment for hypertension and diabetes mellitus (treatment or an HbA1c> 6.5%). In analysis with indexes of wave reflection additional adjustments for heart rate were included. Relationships were compared using z-statistics. As most treated participants were receiving thiazide diuretic agents which may influence blood volume and hence Q, sensitivity analysis was performed in those not receiving thiazide diuretic agents.

2.3 Results

2.3.1 Characteristics of the participants

Table 2.1 gives the demographic and clinical characteristics of the study group. The general characteristics of participants who did not have either 24-hour urinary samples that met with pre-specified quality control or high quality velocity assessments in the outlet tract were no different from the characteristics of the participants whose data is shown in Table 2.1 (and Table 2.2). More women than men participated. In general, a high proportion of participants were

Table 2. 1 Characteristics of sample.

Characteristic	
Number (% women)	581 (66.9)
Age (years)	46.6±18.5
Body mass index (kg/m^2)	29.4±7.6
% overweight/obese	27.0/42.3
Regular tobacco intake (% subjects)	15.0
Regular alcohol intake (% subjects)	20.0
% Diabetes mellitus or HbA_{1c}>6.5%	14.6
% Hypertension	47.2
Current antihypertensive medication (%)	28.6
Total/HDL cholesterol	3.54±1.11
Serum K^+ (mEq/l)	3.98±0.41
Urinary Na^+ excretion (mEq/24 hours)	94.0 (63.6 to 144.2)
Urinary K^+ excretion (mEq/24 hours)	25.7 (15.6 to 38.4)
Urinary (Na^+/K^+)	3.8 (2.7 to 5.2)
Plasma renin concentrations (pg/ml)	12.5 (5.5 to 32.9)
Serum aldosterone concentrations (ng/dl)	5.26 (2.66 to 8.68)
Aldosterone-to-renin ratio (ARR) (ng/dl / ng/l)	0.30 (0.10 to 0.86)
Brachial SBP/DBP (mm Hg)	129±22/83±12
Pulse rate (beats/minute)	66.7±12.1
Mean arterial pressure (mm Hg)	100±15
Brachial pulse pressure (PP)(mm Hg)	45.3±15.0
Aortic SBP (mm Hg)	120±22
Aortic PP (PPc) (mm Hg)	35.8±14.0
Aortic pulse wave velocity (PWV) (m/sec)	6.00±2.83
Characteristic impedance (Zc)($dynes.s/cm^5$)	80.1 (57.5 to 111.1)
Aortic flow (Q) (ml/sec)	299.6 (227.6 to 409.0)

Aortic flow (Q) at peak aortic PP (ml/sec)	198.5 (135.1 to 280.8)
Ejection (Ej) volume up until peak aortic PP (mls)	64.7 (46.4 to 89.6)
Aortic diameter (mm)	25.4±4.5
Maximal (max) $P_{Q \times Zc}$ (mm Hg)	25.6±8.0
Forward wave pressures (Pf) (mm Hg)	24.8±8.7
Backward wave pressures (Pb) (mm Hg)	17.6±7.8
Reflection magnitude (RM) (%)	71.6±21.0

Data are shown as mean±SD or median and interquartile range or proportions. HbA_{1c}, glycated haemoglobin; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 2. 2 Characteristics of those without high quality urine or aortic velocity assessments in the outlet tract.

Characteristic		p-value versus those with all data
Number (% women)	867 (67.0)	=1.00
Age (years)	44.2±18.2	=0.02
Body mass index (kg/m^2)	29.8±8.2	=0.34
% overweight/obese	20.5/45.4	=0.02
Regular tobacco intake (% subjects)	15.0	=1.00
Regular alcohol intake (% subjects)	21.2	=0.60
% Diabetes mellitus or HbA_{1c}>6.5%	12.5	=0.24
% Hypertension	47.4	=0.96
Current antihypertensive medication (%)	24.2	=0.07
Total/HDL cholesterol	3.46±1.19	=0.19
SBP/DBP (mm Hg)	128±22/84±13	=0.40/=0.13
Pulse rate (beats/minute)	66±11	=0.26
Mean arterial pressure (mm Hg)	100±16	=1.00
Brachial pulse pressure (PP)(mm Hg)	44.8±15.8	=0.54

HbA_{1c}, glycated hemoglobin; SBP, systolic blood pressure; DBP, diastolic blood pressure.

either overweight or obese. Although 47.2% of the sample were hypertensive, only 28.6% were receiving antihypertensive treatment, and the majority (94.0%) were receiving thiazide diuretics. 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 (73.4%) 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.

2.3.2 Hemodynamic determinants of PP and SBP

In regression analysis with either peak aortic Q, Zc and RM or maximal P_{QxZc} and RM in the same models, a similar contribution of all of the determinants of aortic PP and SBP to variations in aortic PP and SBP were noted (Table 2.3).

2.3.3 Relationships between urinary indexes of salt intake and PP and SBP

As shown in Table 2.4, with adjustments for MAP and confounders, an independent relationship between the ratio of urinary Na^+-to-K^+ (urinary Na^+/K^+) and either brachial or peak central arterial (aortic) PP or SBP was noted in all participants. Urinary Na^+/K^+ was not independently associated with DBP (partial r=0.075, p=0.09). Trend effects were noted in those not receiving diuretic therapy (Table 2.4). In contrast, neither 24-hour urinary Na^+, nor 24-hour urinary K^+ excretion were independently associated with PP (Table 2.5) or SBP.

2.3.4 Relationships between urinary indexes of salt intake and factors that potentially contribute to PP and SBP

With adjustments for potential confounders including steady-state pressures (MAP), urinary Na^+/K^+ was independently associated with Zc, maximal P_{QxZc} and forward wave pressures, but not with carotid-femoral (aortic) PWV (Tables 2.4 and 2.6). Importantly, despite the lack of significant effect of urinary Na^+/K^+ on PP or SBP in those not receiving diuretic therapy, the impact

Table 2. 3 Relative strength (partial r) of the impact of hemodynamic factors to variations in aortic pulse pressure (PP) and systolic blood pressure (SBP) in a community sample of African ancestry.

	Aortic PP		Aortic SBP	
Factor	Partial r* (95% CI)	p value†	partial r* (95% CI)	p value†
		All participants (n=581)		
Models with Q and Zc				
Peak aortic Q	0.406 (0.331 to 0.476)	p<0.0001	0.461(0.390 to 0.526)	p<0.0001
Zc	0.511 (0.444 to 0.572)	p<0.0001	0.578 (0.517 to 0.633)	p<0.0001
RM	0.319 (0.239 to 0.394)	p<0.0001	0.434 (0.361 to 0.501)	p<0.0001
Models with max P_{QxZc}				
max P_{QxZc}	0.633 (0.578 to 0.682)	p<0.0001	0.716 (0.671 to 0.755)	p<0.0001
RM	0.319 (0.239 to 0.394)	p<0.0001	0.448 (0.376 to 0.514)	p<0.0001
		No diuretic therapy (n=425)		
Models with Q and Zc				
Peak aortic Q	0.426 (0.340 to 0.504)	p<0.0001	0.494 (0.414 to 0.565)	p<0.0001
Zc	0.578 (0.506 to 0.640)	p<0.0001	0.640 (0.576 to 0.695)	p<0.0001
RM	0.384 (0.295 to 0.465)	p<0.0001	0.486 (0.405 to 0.558)	p<0.0001
Models with max P_{QxZc}				
max P_{QxZc}	0.607 (0.539 to 0.666)	p<0.0001	0.685 (0.628 to 0.734)	p<0.0001
RM	0.342 (0.251 to 0.427)	p<0.0001	0.436 (0.351 to 0.513)	p<0.0001

See table 2.1 for abbreviations. *Adjustments were for mean arterial pressure, age, sex, body mass index, regular alcohol consumption, regular tobacco use, the presence of diabetes mellitus or an HbA1c>6.5%, and antihypertensive treatment (all). †Probability values were further adjusted for non-independence of family members.

Table 2. 4 Relationships between urinary Na^+/K^+ and brachial or aortic pulse pressure (PP) or systolic blood pressure (SBP) and the possible determinants thereof.

		All (n=581)		No diuretics (n=425)	
Urinary Na^+/K^+ vs	Adjustments	partial r (95% CI)	p value	partial r (95% CI)	p value
Brachial PP	*	0.092 (0.006 to 0.176)	=0.036	0.087 (-0.012 to 0.186)	=0.087
Aortic PP	*	0.097 (0.011 to 0.181)	=0.026	0.087 (-0.012 to 0.185)	=0.086
Brachial SBP	*	0.127 (0.042 to 0.211)	=0.003	0.102 (0.003 to 0.199)	=0.043
Aortic SBP	*	0.114 (0.028 to 0.198)	=0.009	0.104 (0.005 to 0.201)	=0.040
Zc	*	0.134 (0.048 to 0.217)	=0.002	0.132 (0.033 to 0.228)	=0.009
Q	*	-0.046 (-0.131 to 0.040)	=0.30	-0.022 (-0.121 to 0.077)	=0.66
Aortic diameter	*	-0.061 (-0.147 to 0.026)	=0.17	-0.089 (-0.188 to 0.011)	=0.082
Max $P_{Qx\ Zc}$	*	0.102 (0.016 to 0.186)	=0.019	0.105 (0.005 to 0.202)	=0.039
Pf	*	0.108 (0.022 to 0.192)	=0.014	0.097 (-0.004 to 0.195)	=0.059
Aortic PWV	*	-0.019 (-0.110 to 0.071)	=0.68	-0.086 (-0.190 to 0.020)	=0.11
Pb	*	0.079 (-0.007 to 0.164)	=0.071	0.066 (-0.034 to 0.164)	=0.19
Pb	* + Max $P_{Qx\ Zc}$	0.033 (-0.054 to 0.118)	=0.46	0.039 (-0.061 to 0.138)	=0.44
RM	*	-0.004 (-0.090 to 0.082)	=0.92	0.040 (-0.060 to 0.139)	=0.43
$P_{Q\ xZc}$ at peak PP	*	0.139 (0.054 to 0.222)	=0.001	0.130 (0.032 to 0.226)	=0.009
$P_{reflect+re\text{-}reflect}$	*	0.032 (-0.099 to 0.072)	=0.46	0.004 (-0.095 to 0.103)	=0.94
$P_{reflect+re\text{-}reflect}$	* + Max $P_{Qx\ Zc}$	-0.014 (-0.014 to 0.176)	=0.75	-0.026 (-0.124 to 0.074)	=0.61
Q at peak PP	*	0.026 (-0.059 to 0.112)	=0.54	0.040 (-0.060 to 0.138)	=0.43

Ej. volume up until PP *		-0.018 (-0.105 to 0.069)	=0.68	-0.059 (-0.159 to 0.042)	=0.25
Adjusted for Zc					
Brachial PP	* + Zc	0.056 (-0.030 to 0.142)	=0.20	0.032 (-0.067 to 0.131)	=0.53
Aortic PP	* + Zc	0.068 (-0.018 to 0.153)	=0.12	0.065 (-0.035 to 0.164)	=0.20
Brachial SBP	* + Zc	0.079 (-0.007 to 0.164)	=0.071	0.075 (-0.025 to 0.173)	=0.14
Aortic SBP	* + Zc	0.082 (-0.004 to 0.166)	=0.063	0.083 (-0.016 to 0.181)	=0.099

See table 2.1 and figure 2.2 for abbreviations. *Adjustments are for variations in mean arterial pressure, age, sex, BMI, regular smoking, regular alcohol intake, and diabetes mellitus or an HbA_{1c}>6.5%, treatment for hypertension (in all), and compression wave pressures as indicated For Pb, $P_{reflect+re\text{-}reflect}$ and reflection magnitude (RM), relations were further adjusted for heart rate. Probability values are further adjusted for the non-independence of family members.

Table 2. 5 Multivariate adjusted relationships between urinary electrolyte excretion and pulse pressure (PP).

	Brachial PP		Aortic PP	
Urinary electrolyte	Partial r* (95% CI)	p value†	Partial r* (95% CI)	p value†
	All participants (n=581)			
Urinary Na^+ (mEq/24 hours)	0.069 (-0.028 to 0.165)	=0.16	0.080 (-0.016 to 0.176)	=0.10
Urinary K^+ (mEq/24 hours)	0.078 (-0.019 to 0.173)	=0.11	0.066 (-0.031 to 0.162)	= 0.18
Urinary Na^+/K^+	0.092 (0.006 to 0.176)	=0.036	0.097 (0.011 to 0.181)	=0.026
Urinary Na^+/creatinine	0.045 (-0.051 to 0.141)	=0.35	0.036 (-0.060 to 0.131)	=0.46
Urinary K^+/creatinine	0.018 (-0.078 to 0.114)	=0.72	0.002 (-0.094 to 0.098)	=0.97
	No diuretic therapy (n=425)			
Urinary Na^+ (mEq/24 hours)	0.036 (-0.077 to 0.148)	=0.53	0.052 (-0.061 to 0.164)	=0.36
Urinary K^+ (mEq/24 hours)	0.019 (-0.094 to 0.132)	=0.74	0.014 (-0.099 to 0.126)	=0.81
Urinary Na^+/K^+	0.087 (-0.012 to 0.186)	=0.087	0.087 (-0.012 to 0.185)	=0.086
Urinary Na^+/creatinine	0.021 (-0.099 to 0.140)	=0.73	0.064 (-0.048 to 0.175)	=0.26
Urinary K^+/creatinine	0.055 (-0.067 to 0.175)	=0.38	0.027 (-0.085 to 0.139)	=0.63

*Adjustments were for mean arterial pressure, age, sex, body mass index, regular alcohol consumption, regular tobacco use, the presence of diabetes mellitus or an HbA1c>6.5%, , and antihypertensive treatment (all). †Probability values were further adjusted for non-independence of family members.

Table 2. 6 Sex-specific relationships between urinary Na^+/K^+ and brachial or aortic pulse pressure (PP) or systolic blood pressure (SBP) and the possible determinants thereof.

			Women (n=389)		Men (n=192)	
Urinary Na^+/K^+ vs	Adjustments		Partial r (95% CI)	p value	Partial r (95% CI)	p value
Brachial PP		*	0.128 (0.022 to 0.230)	=0.018	0.120 (-0.032 to 0.266)	=0.12
Aortic PP		*	0.145 (0.040 to 0.246)	=0.007	0.112 (-0.041 to 0.259)	=0.15
Brachial SBP		*	0.138 (0.033 to 0.239)	=0.009	0.097 (-0.055 to 0.244)	=0.21
Aortic SBP		*	0.147 (0.042 to 0.248)	=0.006	0.117 (-0.034 to 0.262)	=0.13
Zc		*	0.117 (0.011 to 0.220)	=0.031	0.157 (0.008 to 0.299)	=0.038
Q		*	-0.026 (-0.132 to 0.080)	=0.63	-0.056 (-0.202 to 0.094)	=0.46
Aortic diameter		*	-0.039 (-0.146 to 0.069)	=0.47	-0.076 (-0.224 to 0.075)	=0.32
Max $P_{Qx\ Zc}$		*	0.108 (0.002 to 0.212)	=0.045	0.143 (-0.007 to 0.287)	=0.062
Pf		*	0.126 (0.019 to 0.229)	=0.021	0.144 (-0.008 to 0.288)	=0.062
Pb		*	0.100 (-0.006 to 0.204)	=0.065	-0.059 (-0.205 to 0.091)	=0.44
RM		*	0.017 (-0.089 to 0.122)	=0.76	0.029 (-0.119 to 0.176)	=0.70
$P_{Q\ xZc}$ at peak PP		*	0.124 (0.019 to 0.226)	=0.020	0.155 (0.006 to 0.296)	=0.040
$P_{reflect+re\text{-}reflect}$		*	0.085 (-0.021 to 0.188)	=0.113	-0.029 (-0.175 to 0.119)	=0.70
Q at peak PP	*		0.023 (-0.083 to 0.129)	=0.67	0.026 (-0.123 to 0.173)	=0.74
Volume at peak PP	*		-0.026 (-0.133 to 0.081)	=0.63	-0.009 (-0.160 to 0.144)	=0.91

See table 2.1 and figure 1.1 for abbreviations. *Adjustments are for variations in mean arterial pressure, age, BMI, regular smoking, regular alcohol intake, and diabetes mellitus or an HbA_{1c}>6.5%, and treatment for hypertension. For Pb, $P_{reflect+re\text{-}reflect}$ and reflection magnitude (RM), relations were further adjusted for heart rate. Probability values are further adjusted for the non-independence of family members.

of urinary Na^+/K^+ on Zc and P_{QxZc} was reproduced in those not receiving diuretic therapy (Table 2.4) and in sex-specific analysis (Table 2.6). These relations translated into stepwise increases in Zc and P_{QxZc} beyond confounders including steady component pressures (MAP) (Figure 2.1). Urinary Na^+/K^+-associated increases in Zc were however not accounted for by variations in aortic diameter and urinary Na^+/K^+ was not independently associated with either peak aortic Q, aortic Q at peak PP, or the volume of blood ejected up until peak aortic PP (Tables 2.4, 2.6 and figure 2.1). This is despite the strong independent relations (adjusted for MAP, sex, BMI, regular smoking, regular alcohol intake, diabetes mellitus or an HbA1c> 6.5% and antihypertensive treatment) between age and Zc (partial r=0.12, p<0.01), aortic diameter (partial r=0.15, p<0.001) and peak aortic Q (partial r=0.16, p<0.0005). Beyond the individual terms, no interactive effects between age and urinary Na^+/K^+ were independently associated with Zc (p=0.69), aortic diameter (p=0.87), peak aortic Q (p=0.23), Q at peak aortic PP (p=0.35) or the volume of blood ejected up until peak PP (p=0.93). Independent relations of urinary Na^+/K^+ with forward wave pressures were accounted for by increases in the pressures generated by the product of Q x Zc (Tables 2.4 and 2.7). Urinary Na^+/K^+ was not however, independently associated with either backward wave pressures (Pb), the pressures generated by wave reflection and re-reflection ($P_{reflect+re\text{-}reflect}$), or reflection magnitude (RM=Pb/Pf x 100) (Table 2.4 and figure 2.1).

2.3.5 Factors accounting for relationships between urinary indexes of salt intake and PP or SBP

Adjustments for Zc and maximal P_{QxZc}, but not peak aortic Q, Q at peak PP, the volume of blood ejected up until peak PP, or RM strongly reduced the relations between urinary Na^+/K^+ and aortic and brachial artery PP or SBP (Figures 2.2-2.5, Table 2.4, Table 2.7). Moreover, in product of coefficient mediation analysis, Zc and maximal P_{QxZc}, but not peak aortic Q, Q at peak PP, the volume of blood ejected up until peak PP, or RM accounted for the relations between urinary Na^+/K^+ and aortic and brachial artery PP or SBP (Figure 2.6).

Table 2. 7 Impact of adjustments for the factors that contribute to aortic pulse pressure on the strength (partial r) of the multivariate adjusted relations of urinary Na^+/K^+ and aortic pulse pressure (PP) and hence systolic blood pressure (SBP).

		All (n=581)		No diuretic therapy (n=425)	
Relationship	Adjustments	Partial r (95% CI)	p value	Partial r (95% CI)	p value
Urinary Na^+/K^+ vs aortic SBP					
	*	0.114 (0.028 to 0.198)	=0.0091	0.104 (0.005 to 0.201)	=0.040
	* + Q	0.113 (0.027 to 0.197)	=0.0097	0.104 (0.004 to 0.201)	=0.041
	* Max $P_{Qx\ Zc}$	0.061 (-0.024 to 0.147	=0.161	0.074 (-0.026 to 0.172)	=0.145
	* RM	0.118 (0.032 to 0.203)	=0.0071	0.105 (0.006 to 0.203)	=0.037
	* Q at PPc	0.114 (0.028 to 0.198)	=0.0096	0.105 (0.005 to 0.203)	=0.039
	* Vol at PPc	0.120 (0.032 to 0.206)	=0.0074	0.111 (0.009 to 0.210)	=0.033
Urinary Na^+/K^+ vs aortic PP					
	*	0.097 (0.011 to 0.181)	=0.026	0.087 (-0.012 to 0.185)	=0.086
	* + Q	0.097 (0.011 to 0.181	=0.027	0.087 (-0.013 to 0.185)	=0.087
	* Max $P_{Qx\ Zc}$	0.040 (-0.046 to 0.125	=0.36	0.051 (-0.049 to 0.150)	=0.32
	* RM	0.098 (0.012 to 0.183)	=0.026	0.084 (-0.017 to 0.183)	=0.10
	* Q at PPc	0.107 (0.019 to 0.193)	=0.018	0.098 (-0.005 to 0.198)	=0.061
	* Vol at PPc	0.097 (0.010 to 0.181)	=0.028	0.088 (-0.012 to 0.187)	=0.084

See figures 1.1 and table 2.1 for abbreviations. *Adjustments are for variations in mean arterial pressure, age, sex, BMI, regular smoking, regular alcohol intake, diabetes mellitus or an HbA_{1c}>6.5%, treatment for hypertension (in all) and aortic functional change as indicated. Probability values are further adjusted for the non-independence of family members.

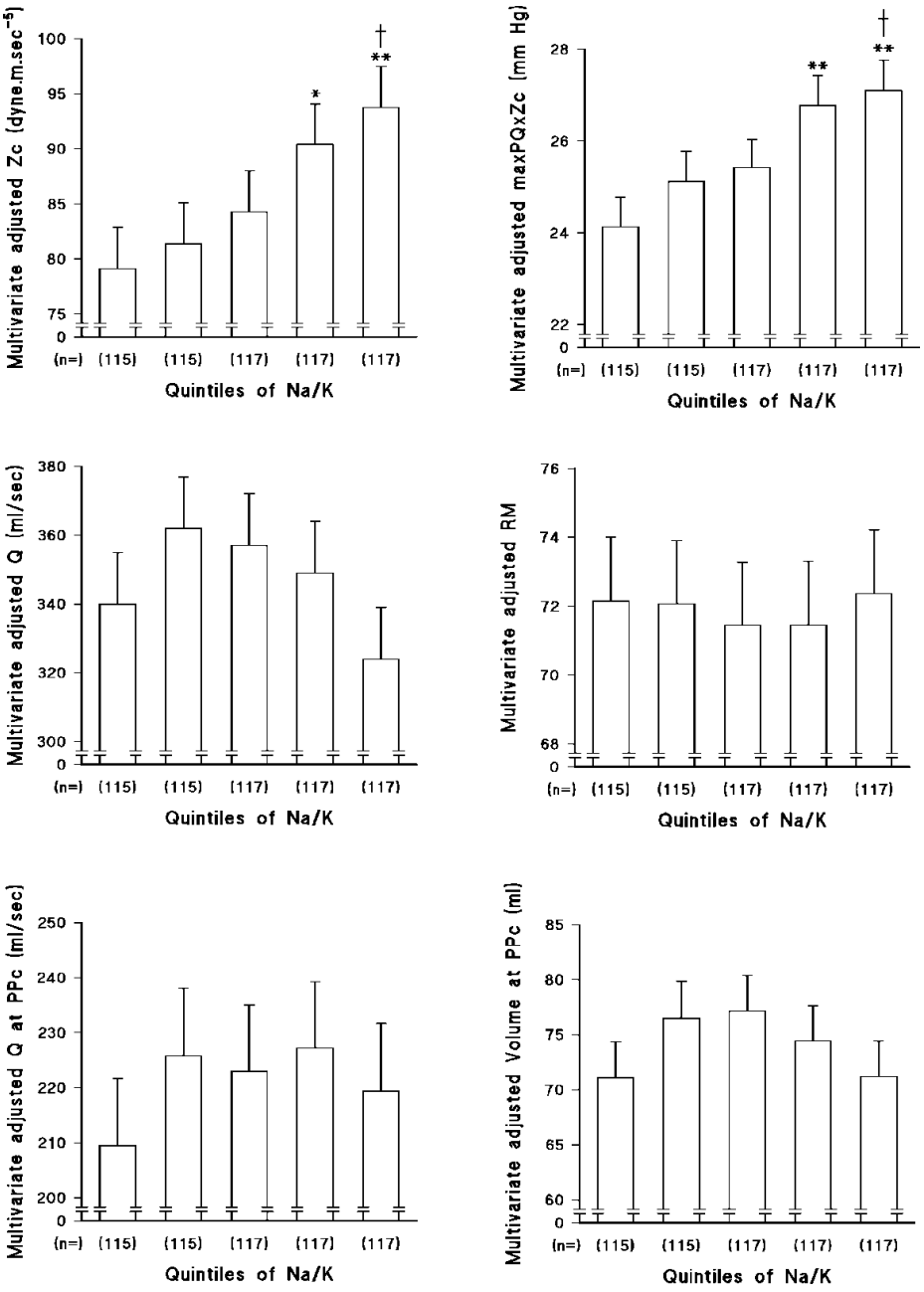


Figure 2. 1 Multivariate adjusted increases in the factors that contribute to pulse pressure (PP) and hence systolic blood pressure across incremental quintiles of urinary Na^+/K^+. Adjustments are for variations in mean arterial pressure, age, BMI, regular smoking, regular alcohol intake, diabetes mellitus or an HbA_{1c}>6.5% and treatment for hypertension. For reflection magnitude (RM),

relations were further adjusted for heart rate. See figure 1.1 and table 2.1 for abbreviations and Table 2.4 for relations. *p<0.05, **p<0.005 versus quintile 1; †p<0.05 versus quintile 2.

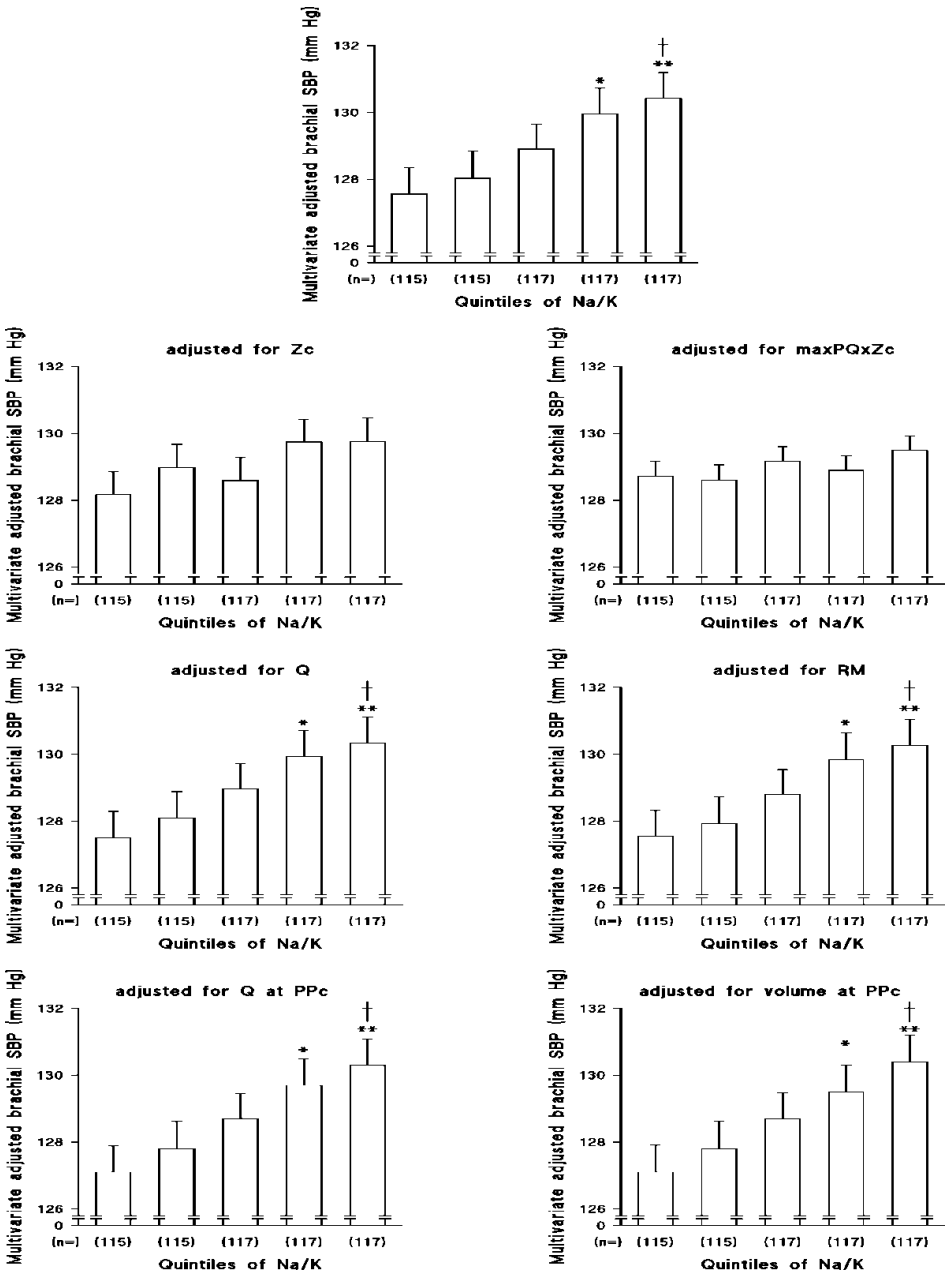


Figure 2. 2 Impact of adjustments for the factors that contribute to pulse pressure and hence systolic blood pressure (SBP) on multivariate adjusted increases in brachial SBP across incremental quintiles of urinary Na^+/K^+. Additional adjustments are for variations in mean arterial pressure, age, sex, BMI, regular smoking, regular alcohol intake, diabetes mellitus or an HbA_{1c}>6.5% and treatment for hypertension. For reflection magnitude (RM), relations were further adjusted for heart rate. *p<0.05, **p<0.005 versus quintile 1; †p<0.05 versus quintile 2.

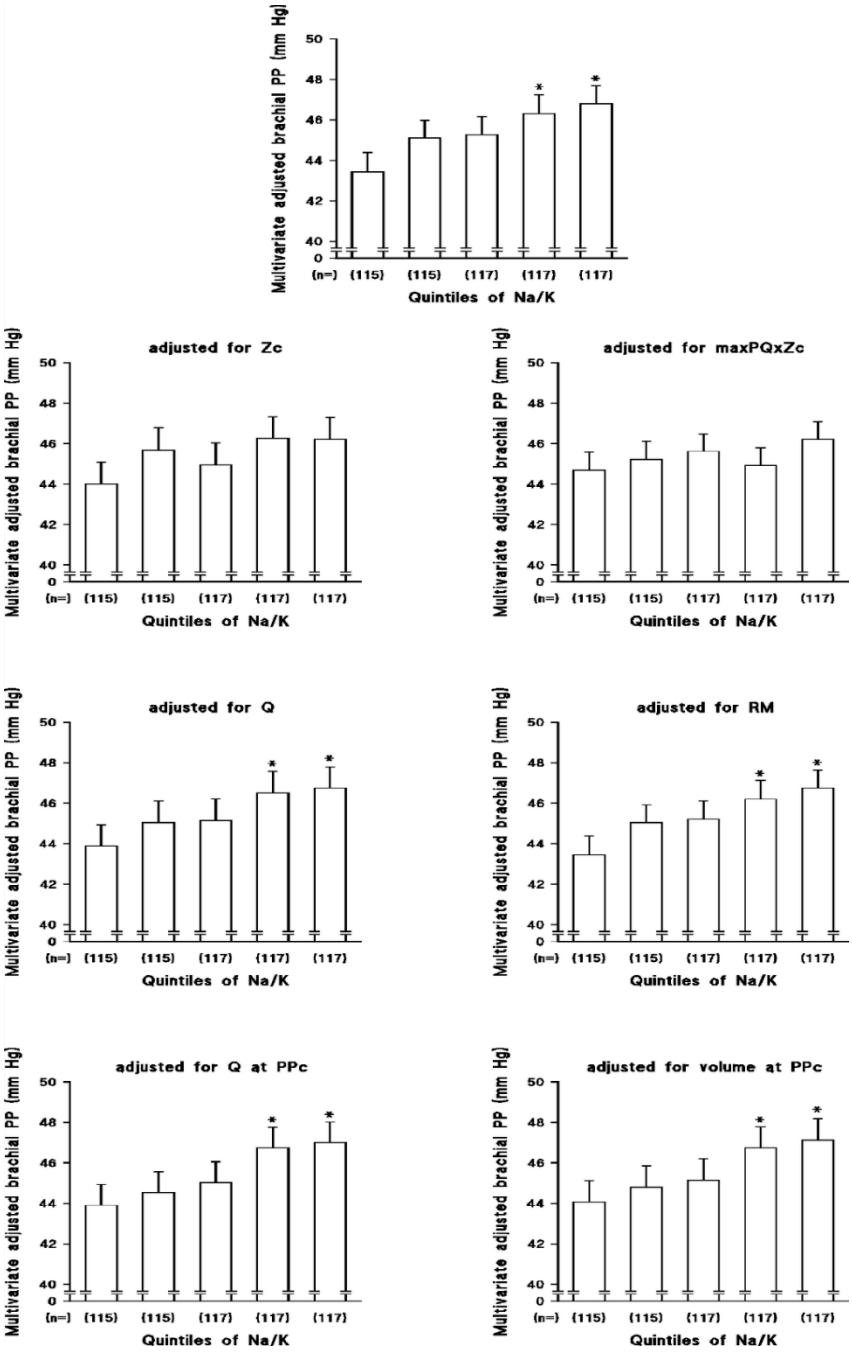


Figure 2. 3 Impact of adjustments for the factors that contribute to pulse pressure on multivariate adjusted increases in brachial pulse pressure (PP) across incremental quintiles of urinary Na^+/K^+. Additional adjustments are for variations in mean arterial pressure, age, sex, BMI, regular smoking, regular alcohol intake, diabetes mellitus or an HbA_{1c}>6.5% and treatment for hypertension. For reflection magnitude (RM), relations were further adjusted for heart rate. *p<0.05 versus quintile 1.

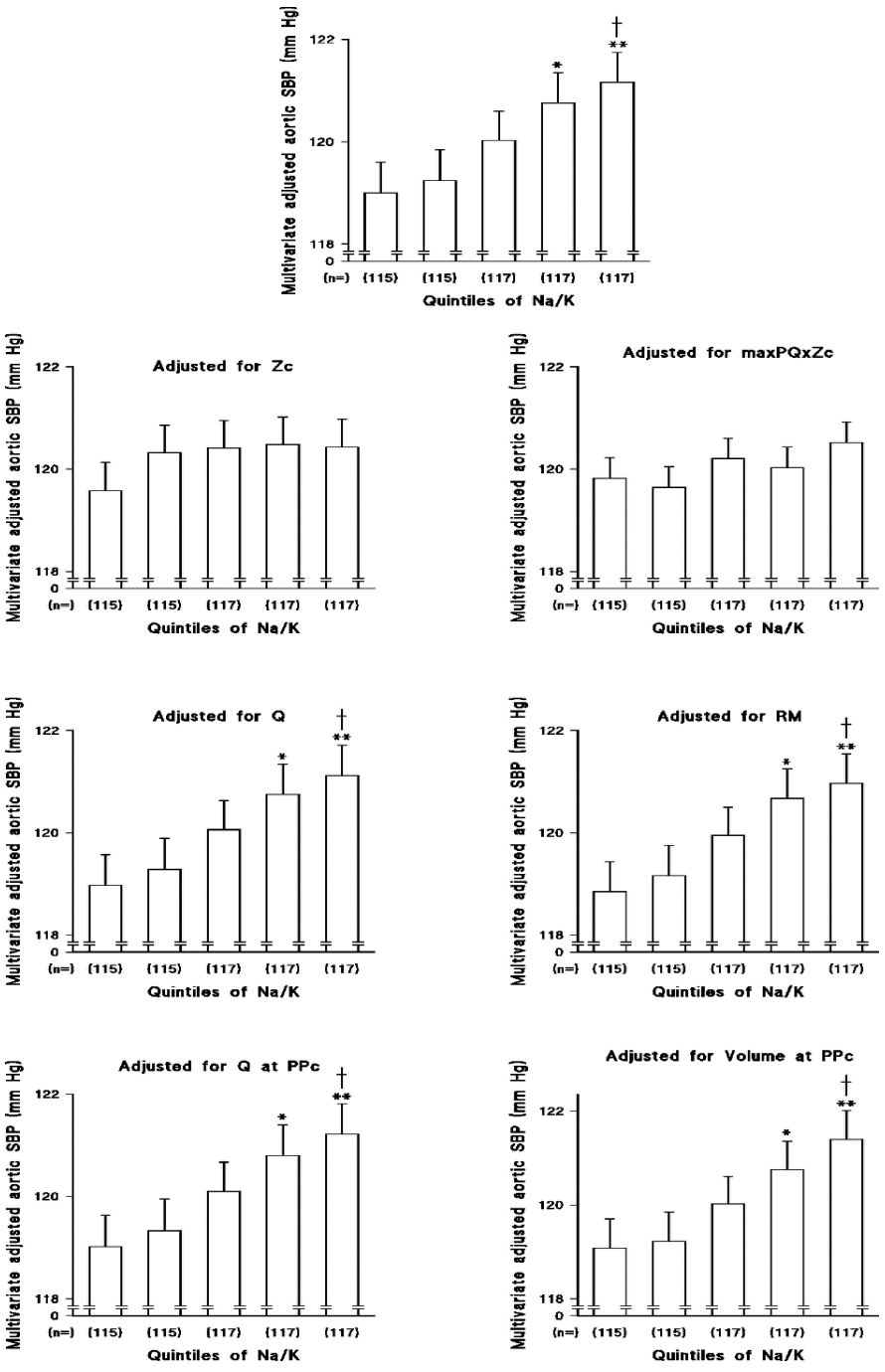


Figure 2. 4 Impact of adjustments for the factors that contribute to pulse pressure on multivariate adjusted increases in aortic systolic blood pressure (SBP) across incremental quintiles of urinary Na^+/K^+. Additional adjustments are for variations in mean arterial pressure, age, sex, BMI, regular smoking, regular alcohol intake, diabetes mellitus or an HbA_{1c}>6.5% and treatment for hypertension. For reflection magnitude (RM), relations were further adjusted for heart rate. *p<0.05, **p<0.005 versus quintile 1; †p<0.05 versus quintile 2.

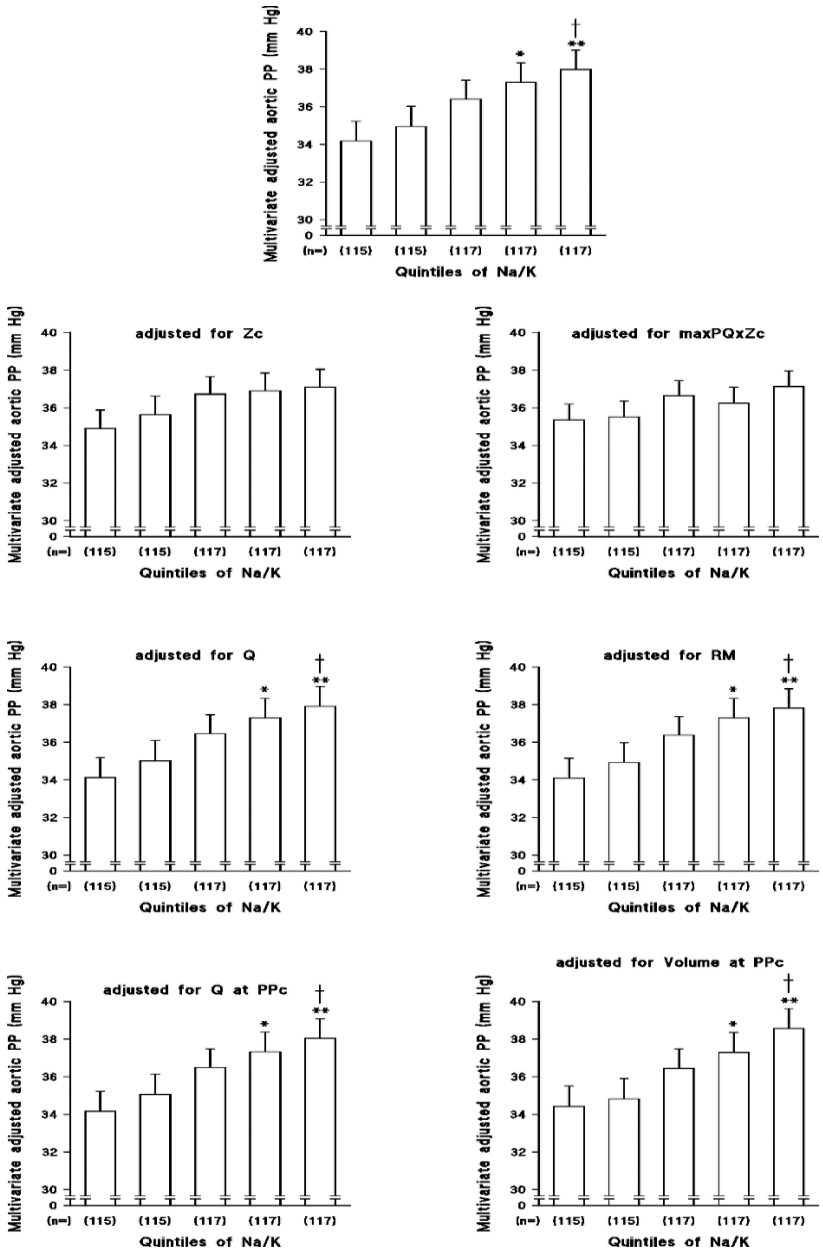


Figure 2. 5 Impact of adjustments for the factors that contribute to pulse pressure on multivariate adjusted increases in aortic pulse pressure (PP) across incremental quintiles of urinary Na^+/K^+. Additional adjustments are for variations in mean arterial pressure, age, sex, BMI, regular smoking, regular alcohol intake, diabetes mellitus or an HbA_{1c}>6.5% and treatment for hypertension. For reflection magnitude (RM), relations were further adjusted for heart rate. *p<0.05, **p<0.01 versus quintile 1; †p<0.05 versus quintile 2.

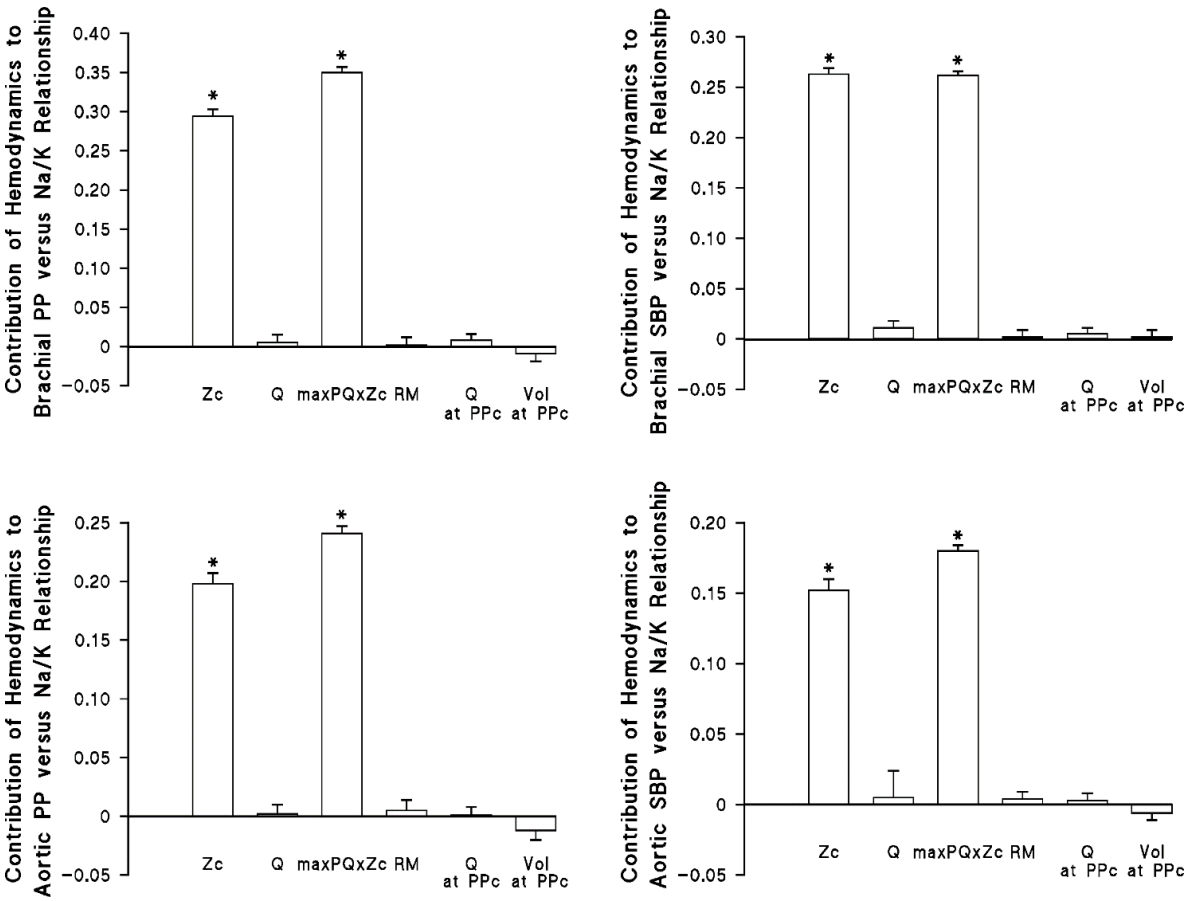


Figure 2. 6 Contribution in product of coefficient mediation analysis of the hemodynamic factors that contribute to pulse pressure (PP) and systolic blood pressure (SBP) to the impact of urinary Na^+/K^+ on PP and SBP in a community sample of African ancestry. Adjustments are for variations in mean arterial pressure, age, sex, BMI, regular smoking, regular alcohol intake, diabetes mellitus or an HbA_{1c}>6.5% and treatment for hypertension.

2.3.6 Impact of urinary indexes of salt intake on aortic function is determined by the RAAS

Whilst neither circulating renin nor aldosterone concentrations were positively and independently associated with any index of aortic function, independent of the individual terms, a strong interaction between urinary Na^+/K^+ and the aldosterone-to-renin ratio (ARR) was independently associated with both Zc and maximal P_{QxZc} (Table 2.8), but not peak aortic Q, Q at peak PP, the volume of blood ejected up until peak PP (Table 2.8), or alternative aortic functional changes. This interaction translated into a trend (p=0.07) for stepwise increase in the relationship between urinary Na^+/K^+ and Zc across tertiles of ARR (Figure 2.7).

2.4 Discussion

The main findings of the present study are as follows: In a community sample of African ancestry in South Africa, I identified the mechanisms that account for increases in both central aortic and brachial PP and hence SBP in association with the index of abnormalities in salt intake, urinary Na^+/K^+. In this regard, I show that urinary Na^+/K^+, was independently associated with proximal aortic characteristic impedance to flow (Zc) rather than to peak aortic flow (Q) or to alternative aspects of flow or ejection volume that may contribute to peak PP. The increased Zc associated with abnormalities of salt intake translated into enhanced pressures generated by the product of Zc and Q and hence forward wave pressures. Importantly, the impedance changes were not explained by differences in aortic root diameter and hence are accounted for by increases in proximal aortic stiffness, despite stiffness along the full length of the aorta (aortic PWV) showing no relationship with urinary Na^+/K^+. Independent of confounders including heart rate, urinary Na^+/K^+ was not independently associated with backward wave pressures (Pb), wave reflection + re-reflection or reflection magnitude (Pb/Pf x 100). The impact of urinary Na^+/K^+ on Zc and pressures generated by the product of Zc and Q depended to some extent on RAAS activity, showing trends for stronger effects at higher values of aldosterone-to-renin ratios.

Table 2. 8 Multivariate adjusted relationships and interactive (with urinary Na^+/K^+) relationships between circulating markers of the renin-angiotensin-aldosterone system (RAAS) and the factors that contribute to aortic pulse pressure and hence systolic blood pressure.

	All (n=581)		No diuretics (n=425)	
Relationships	partial r*	p value†	partial r*	p value†
Aortic Zc versus				
ARR#	-0.116 (-0.212 to -0.017)	=0.022	-0.104 (-0.215 to 0.011)	=0.075
Serum [aldosterone]#	-0.117 (-0.213 to -0.018)	=0.020	-0.057 (-0.170 to 0.058)	=0.33
Plasma [renin]#	0.046 (-0.052 to 0.143)	=0.36	0.011 (-0.103 to 0.125)	=0.85
ARR# x urinary Na^+/K^+	-0.113 (-0.209 to -0.014)	=0.025	-0.070 (-0.184 to 0.046)	=0.23
Aortic Q versus				
ARR#	0.084 (-0.016 to 0.181)	=0.099	0.055 (-0.059 to 0.168)	=0.34
Serum [aldosterone]#	0.092 (-0.006 to 0.189)	=0.066	0.065 (-0.048 to 0.176)	=0.26
Plasma [renin]#	-0.022 (-0.120 to 0.076)	=0.66	0.005 (-0.107 to 0.117)	=0.93
ARR# x urinary Na^+/K^+	0.112 (0.013 to 0.208)	=0.026	0.061 (-0.052 to 0.173)	=0.29
Aortic compression wave pressures (maximal P_{QxZc}) versus				
ARR#	-0.078 (-0.176 to 0.022)	=0.13	-0.042 (-0.155 to 0.073)	=0.48
Serum [aldosterone]#	-0.107 (-0.203 to -0.008)	=0.033	-0.052 (-0.164 to 0.062)	=0.37
Plasma [renin]#	0.050 (-0.049 to 0.147)	=0.32	0.004 (-0.109 to 0.116)	=0.95
ARR# x urinary Na^+/K^+	0.046 (-0.054 to 0.144)	=0.37	0.029 (-0.084 to 0.142)	=0.61
Aortic Q at peak PP versus				

ARR#	-0.018	(-0.118 to 0.082)	=0.72	-0.056 (-0.169 to 0.058)	=0.33
Serum [aldosterone]#	0.062	(-0.038 to 0.161)	=0.22	0.044 (-0.070 to 0.158)	=0.45
Plasma [renin]#	0.110	(0.010 to 0.208)	=0.030	0.146 (0.033 to 0.256)	=0.012
ARR# x urinary Na^+/K^+	0.019	(-0.081 to 0.118)	=0.71	-0.003 (-0.117 to 0.111)	=0.95
Aortic ejection volume up until peak PP versus					
ARR#	0.016	(-0.086 to 0.118)	=0.76	0.006 (-0.111 to 0.123)	=0.92
Serum [aldosterone]#	0.031	(-0.071 to 0.133)	=0.55	0.023 (-0.096 to 0.141)	=0.71
Plasma [renin]#	-0.006	(-0.108 to 0.097)	=0.91	-0.013 (-0.131 to 0.104)	=0.82
ARR# x urinary Na^+/K^+	0.080	(-0.022 to 0.181)	=0.13	0.043 (-0.075 to 0.159)	=0.47

ARR, aldosterone-to-renin ratio. #Serum [aldosterone] and ARR refer to the square root of the values and plasma renin refers to log transformed data. *Adjustments were for mean arterial pressure, age, sex, BMI, urinary Na^+/K^+ (when assessing independent effects), the presence of diabetes mellitus or an HbA1c>6.5%, regular alcohol consumption, regular tobacco use, and antihypertensive treatment (in all participants). When assessing aldosterone effects, plasma renin concentrations and diuretic use were also included in the model. When assessing renin effects, diuretic use and angiotensin-converting enzyme inhibitor use were also included in the model. When assessing interactive effects, the independent terms were included in the model. † Probability values were further adjusted for non-independence of family members.

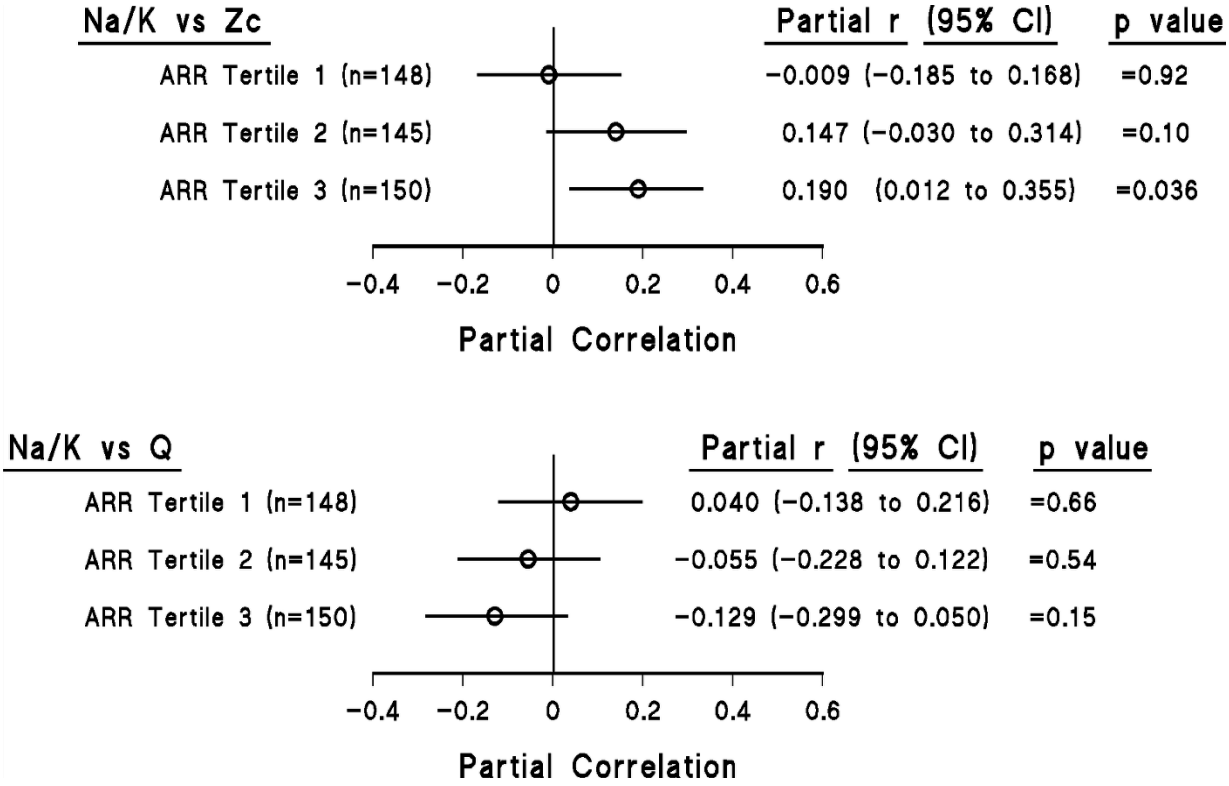


Figure 2. 7 Multivariate adjusted partial correlation coefficients (partial r) and 95% confidence intervals for the relations between urinary Na^+/K^+ and the factors that contribute to pulse pressure and hence systolic blood pressure across tertiles of the square root aldosterone-to-renin ratios (ARR). Adjustments were for mean arterial pressure, age, sex, BMI, regular alcohol consumption, regular tobacco use, the presence of diabetes mellitus or an HbA1c>6.5%, and treatment for hypertension. Probability values were further adjusted for non-independence of family members. A trend (p=0.07) for an increased partial r in tertile 3 versus tertile 1 for urinary Na^+/K^+ versus Zc was noted.

Although prior studies provide strong support for the notion that abnormalities in salt intake increase SBP in part through an impact on PP beyond steady component pressures (MAP) (du Cailar et al., 2004; Buyck et al., 2009; Redelinghuys et al., 2010), whether these adverse effects are easily reversible is uncertain. In this regard, it is well accepted that a strong determinant of PP and hence SBP is an increase in aortic stiffness (Nichols et al., 2011; Mitchell et al., 2010*a*; Segers et al., 2007), a functional change that has little convincing evidence to support its reversibility. However, abnormalities in salt intake are thought to produce effects on BP in salt-sensitive populations such as the population studied by us, largely through renal mechanisms (Aviv, Hollenberg & Weder, 2004) that may increase blood volume. These changes (through Frank-Starling effects on the left ventricle), if they indeed occurred, would potentially translate into increases in aortic flow and hence PP and SBP, and could be readily reversed by reducing Na^+ intake. An impact of abnormalities of salt intake on Zc suggests that alternative effects other than those that are readily reversible also occur. Importantly, several studies (He, Markandu & MacGregor, 2005; He et al., 2009) suggest that a reduction in Na^+ intake over relatively short periods, attenuates SBP possibly through decreases in aortic stiffness. Indeed, this has been noted in isolated systolic hypertension (He et al., 2005), a form of hypertension well-recognised as being associated with increases in aortic stiffness, or in the general hypertensive population in association with a decrease in aortic PWV (He et al., 2009), an index of aortic stiffness. However, these studies (He et al., 2005, 2009) did not exclude the impact of associated reductions in distending pressures (MAP) on aortic stiffness and hence Zc, or a possible effect of reductions in peak aortic flow or alternative flow characteristics through blood volume reduction. Thus, it is uncertain from these studies (Cooper et al., 2015; Grigorova et al., 2016) whether modifications in dietary Na^+ intake indeed target the essential determinant of increases in PP and SBP associated with abnormalities in dietary salt intake noted in the present study. In this regard, the present study is the first to show that the principle mechanism responsible for increases in PP and SBP associated with abnormalities of dietary salt intake are not through increases in aortic flow, or ventricular ejection rate or volume, but through structural changes in the proximal aorta that enhance stiffness and resistance to flow in a pulsatile system in the absence of wave reflection (proximal aortic Zc). In this regard, although increases in Zc may be readily influenced by changes in distending pressures, the essential modification responsible for increases in forward wave pressures associated with abnormalities of salt intake and hence PP and SBP are likely to be irreversible.

There are several clinical implications to the present study that warrant consideration. First, the present study suggests that the adverse effects of abnormalities of salt intake, at least in salt-sensitive populations, on BP, are mediated to a large extent by the impact of chronic rather than acute dietary salt changes. Indeed, there is no evidence to show that Zc may be altered independent of MAP with short-term interventions in dietary salt intake. Thus, salt restriction in these populations should be achieved at an early age and maintained for a significant period of time to derive benefits. As the average Na^+ intake in the present study is not particularly high (although at least half of the participants have values above the RDA), very careful control of Na^+ intake may be required to achieve these benefits. Second, the lack of relationship between abnormalities of salt intake and Q or the volume of blood ejected in the present study suggests that reversing abnormalities in salt intake is unlikely to address the increases in forward wave pressures and hence PP caused by salt intake in salt-sensitive populations. Thus, no benefits on PP are likely to be noted with short-term Na^+ reduction unless abnormalities in salt intake have increased MAP or Q as well. In this regard, it is only when abnormalities in salt intake are associated with increases in MAP that modifying changes in salt intake will reduce Zc and compression wave pressures through distending pressure effects. Importantly the present study suggests that reductions in forward wave pressures in salt-sensitive populations may require more intense decreases in MAP with conventional antihypertensive agents than usual, to achieve sufficiently low Zc-related pulsatile loads to prevent events. The exact MAP values required to achieve these effects requires further study. Third, although there is little convincing evidence for a role of peak aortic PP in mediating vascular events beyond brachial PP (Vlachopoulos et al., 2010), forward wave pressures, which are strongly determined by compression wave effects, predict vascular events beyond brachial PP (Cooper et al., 2015). These brachial BP-independent effects (Cooper et al., 2015) are likely to be accounted for by a reduced impedance mismatch between the aorta and more distal vessels and hence will not be appropriately detected with brachial BP measurements. This is likely to explain why abnormalities of salt intake noted in previous studies relate better to central arterial than brachial PP (Redelinghuys et al., 2010). Thus, the adverse effects on vascular events of Zc changes produced by abnormalities in salt intake, may in part go undetected and thus untreated. Consequently, to adequately identify and manage the chronic impact of salt intake on PP, forward wave pressure measurements may be required, particularly in those with a normal brachial BP.

Although the present study was not designed to detect the mechanisms responsible for the impact of abnormalities of dietary salt intake on Zc, they are nevertheless worthy of consideration. Importantly, these effects may be mediated by pressor effects (MAP) produced during periods when an even more abnormal salt intake may have occurred in those with habitual abnormalities of salt intake. To identify this possibility, follow-up over the full adult lifetime would be necessary. Importantly, however, abnormalities in salt intake may also be associated with several possible changes at a vascular level (including inflammatory alterations) that may encourage fibrosis or calcification even in the absence of pressor effects (Grigorova et al., 2016). Further, in the present study the relationship between urinary Na^+/K^+ and Zc tended to be more marked in those with a higher ARR. In this regard, as we had previously described (Scott et al., 2011, Michel et al., 2012), although the RAAS is inhibited in salt-sensitive hypertension, the extent to which aldosterone concentrations are suppressed (relative to renin) is attenuated. Although this effect initially suggested to us that renal mechanisms explain the impact of salt intake on SBP (Scott et al., 2011), the present study rather suggests that the adverse effect may in part be through a direct impact of abnormalities in salt intake and the RAAS on the proximal aorta. Indeed, aldosterone is well-recognised as mediating increases in aortic collagen formation (Neves et al., 2005). Alternatively, participants with attenuated suppression of aldosterone who have abnormalities of salt intake may have had, through renal effects, both systolic and diastolic hypertension for a chronic period and thus, again through BP effects *per se*, developed increases in proximal aortic stiffness. As increases in proximal aortic stiffness reduce flow during diastole, diastolic BP and MAP may have thus decreased while Zc, PP and SBP remained elevated. To identify the exact mechanism responsible for the impact of abnormalities in salt intake on proximal aortic Zc, further longitudinal studies are therefore required.

Although in the present study I show that urinary indexes of salt intake independently associate with Zc, I show no relationship with either aortic root diameter (present study) or carotid-femoral PWV (present and previous work [Redelinghuys et al., 2010]). In this regard, the principle determinants of Zc are aortic diameter and aortic stiffness (Nichols et al., 2011). Although this suggests an alternative possible effect on Zc mediated by alterations in salt intake (such as changes in blood density), despite clear age-related PWV changes (Hodson et al., 2016) I was unable to show an independent relationship between aortic PWV and Zc ($p=0.82$). These data can only be interpreted to indicate that aortic stiffness along the full length of the aorta is a poor index of

stiffness effects on Zc in the proximal aorta in the present population. Thus, it is likely that abnormalities in salt intake in the present population increase Zc through an impact of stiffness in the proximal aorta that cannot be readily detected by assessing stiffness along the full length of the aorta.

There are several limitations to the present study that warrant consideration. First, the present study was cross-sectional in design and hence no conclusions regarding causality may be drawn. In this regard, residual confounding may therefore explain relations between abnormalities of salt intake and Zc or compression wave pressures. However, as all major guidelines recommend reducing Na^+ intake, longitudinal studies with an appropriately controlled intervention (including a control group not receiving modifications in salt intake) are no longer ethically feasible. Second a half of all hypertensives were receiving low-dose diuretic therapy and hence a lack of relationship between salt intake and Q, flow characteristics or blood volume ejected up until peak PP may have been confounded by this effect. However, strong age-related increases in Q were observed and at least half of all hypertensives were not receiving antihypertensive therapy. In this regard, similar relations were noted between salt intake and aortic function in sensitivity analysis performed in those not receiving diuretic therapy. Third, in the present study, calibration of the radial waveform from brachial BP measurements ignores amplification of BP from brachial to radial arteries. Hence, aortic pressures may have been underestimated using the current approach. However, all central pressure wave components would have been similarly affected by this calibration error. Last, the present study was conducted in an ethnic group well recognised as being salt-sensitive. Our findings may therefore not be translatable to other groups.

2.4.1 Conclusions

Although it is well recognised that abnormalities of Na^+ and K^+ intake are potentially causally related to increases in PP and hence SBP beyond steady-state pressures (MAP or DBP) (du Cailar et al., 2004; Buyck et al., 2009; Redelinghuys et al., 2010), the mechanism of this effect has been assumed to be through renal mechanisms and hence principally an increase in volume load. In this regard, the impact of renal-related increases in volume load is readily rectified by modifying abnormalities in salt intake. In contrast however, in the present study conducted in an ethnic group recognised as being salt-sensitive (black African), I show that the index of salt intake,

urinary Na^+/K^+, is independently associated with proximal aortic stiffness (increased Zc without alterations in diameter) and forward wave pressures (product of Zc and flow), but not to aortic flow (the change that explains increases in PP with volume overload) or the volume of blood ejected up until peak aortic PP. Importantly, the increased Zc explained relations between urinary Na^+/K^+ and both brachial and central aortic PP and SBP. As proximal aortic stiffness-induced forward wave pressures are responsible for vascular events, and structural aortic changes are largely irreversible, the present study suggests that the adverse cardiovascular effects of an abnormal salt intake cannot be addressed by only correcting salt intake. Identifying those with enhanced forward wave pressures and initiating intense BP reduction in these individuals may be required.

CHAPTER 3

Distinct Contribution of Systemic Blood Flow to Hypertension in an African Population Across the Adult Lifespan.

The data in this chapter have been published in *Hypertension* as follows:

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3.0 Abstract

Background: Although hypertension in groups of African ancestry is volume-dependent, the relative impact of systemic flow (stroke volume [SV] and peak aortic flow [Q]) versus resistance to flow on either steady (systemic vascular resistance, [SVR]) or pulsatile (aortic characteristic impedance, [Zc]; total arterial compliance [TAC]) components of arterial load, has not been evaluated across the adult age range.

Methods: In participants of African ancestry (n=824, age=16-99years, 68.3% female), using central arterial pressure and aortic velocity and diameter measurements in the outflow tract, I determined the hemodynamic correlates of age-related increases in BP.

Results: Independent of confounders, strong positive relations between age and SV or peak aortic Q were noted ($p<0.0001$), effects associated with ventricular end diastolic volume and aldosterone-to-renin ratios. Age-related increases in MAP were associated with SV and not SVR. Although relations between age and Q began from early adulthood, initially an inverse association between age and aortic Zc ($p<0.0001$) driven by increments in aortic root diameter ($p<0.0001$) prevented an enhanced SBP and PP. When Zc began to positively relate to age ($p<0.0001$), age relations with Q translated into marked increases in forward wave pressures and hence SBP and PP. Age relations with PP were as strongly determined by Q as by Zc or TAC (0.027±0.001 vs 0.028±0.001 and 0.032±0.003 mm Hg per yearly increase in PP produced by Q, Zc and TAC, $p<0.0001$). Uncontrolled hypertension (confirmed with 24-hour BP) over the full adult lifespan was determined more by peak aortic Q, Zc and TAC than by increases in SVR ($p<0.0005$ for comparison).

Conclusion: Relationships between age and systemic blood flow contribute markedly to hypertension in groups of African origins.

Key words: Volume load, characteristic impedance, aortic flow, stroke volume, African ancestry.

3.1 Introduction

An inability to adequately control blood pressure (BP) accounted for 10.4 million deaths worldwide in 2017 (GBD 2017 Risk Factor Collaborators, 2017). Groups of African descent are well recognised as developing hypertension which is often more difficult to control. Indeed, hypertension in this ethnic group requires several drug combinations to manage BP (Sareli et al., 2001; Flack et al., 2010). Targeting distinct mechanisms of an increased BP in this ethnic group may provide more rational targets for BP control. In this regard, groups of African ancestry more frequently develop a volume-dependent, low-renin form of hypertension (Luft et al., 1991; Freis, Reda & Masterson, 1988), sensitive to diuretic therapy but with poor responses to blockade of the renin-angiotensin system (ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group, 2002). Although a renal mechanism explains an increased BP (Aviv et al., 2004), there is no evidence to support a hemodynamic effect of volume overload with subsequent increases in stroke volume (SV), or aortic flow (Q). Indeed, hypertension in this group is more often reported as being associated with increases in systemic vascular resistance (SVR) and aortic stiffness and not SV (Heffernan et al., 2008; Din-Dzietham et al., 2004). Thus, a shifted pressure-natriuresis curve may result in vascular changes (SVR) that promote volume reduction at an increased BP. The debate as to whether targeting volume (diuretics) or vascular (vasodilators) mechanisms therefore continues with suggestions that combinations of vasodilators may offer an equally effective approach to treatment as combinations of diuretics and vasodilators (Ojji et al., 2019). However, the evidence to support dominance of vascular hemodynamic changes in volume-dependent populations has not accounted for age-related effects on BP.

The adverse cardiovascular effects of hypertension are determined by increases in both the steady (indexed by mean arterial pressure [MAP]) and the pulsatile (indexed by pulse pressure [PP], systolic BP [SBP] and diastolic blood pressure [DBP]) components of BP (Darné et al., 1989; Rudnichi et al., 1997; Madhavan et al., 1994; Mitchell et al., 1997; Celis et al., 2001). Age is the strongest risk factor for hypertension and increases in PP and SBP are the prevailing BP changes with age. While increases in the steady components of pressure are thought to be determined mainly by systemic vascular resistance (SVR) with SV playing little role, the principle factors accounting for increases in PP and hence SBP have only more recently been clarified. In this regard, peak Q interacts with resistance to flow in the pulsatile component of arterial load

(characteristic impedance, Zc) to determine SBP (Nichols et al., 2011). However, most increases in SBP in groups of European ancestry are mediated by an enhanced aortic stiffness and hence Zc which augments the forward travelling wave (Mitchell et al., 2010*a*; Segers et al., 2001). Although age-related increases in SBP in groups of African descent are also strongly determined by forward wave pressures associated with increases in aortic stiffness (Hodson et al., 2016, 2017), the relative impact of Zc as compared to Q on age-related increases in SBP in this ethnic group has not been determined. Moreover, the relative impact of SV versus SVR on the steady component of BP across the full adult age range in volume-dependent populations is similarly unknown. As there is limited knowledge as to the hemodynamic determinants of age-dependent increases in BP in volume-dependent populations, in the present study I therefore compared the contribution of volume versus vascular-dependent hemodynamic correlates of age-related increases in BP across the full adult age range in a community sample in South Africa.

3.2 Methods

3.2.1 Study group

The present study was conducted according to the principles outlined in the Helsinki declaration. The Committee for Research on Human Subjects of the University of the Witwatersrand approved the protocol (approval number: M02-04-72 and renewed as M07-04-69, M12-04-108 and M17-04-01). Participants gave informed, written consent. The present study design has previously been described (Booysen et al., 2015; Norton et al., 2012; Woodiwiss et al., 2009). Briefly, we randomly recruited families (from the population census figures of 2001) of black African descent (Nguni and Sotho chiefdoms) from the South West Township (SOWETO) of Johannesburg, South Africa, with siblings older than 16 years of age, if at least one or two offspring and one or both parents were available for examination. There were no other inclusion/exclusion criteria. In the present substudy, 1062 participants had echocardiographic evaluations and central arterial assessments. 824 of these participants had high quality velocity measurements in the outflow tract (a smooth velocity waveform with a dense leading [outer] edge and a clear maximum velocity). Of this sample, 537 participants had 24-hour ambulatory BP monitoring that met with the European Society of Hypertension guidelines (longer than 14 and 7

readings for the computation of day and night means, respectively).

3.2.2 Clinical and demographic information

A questionnaire was administered to obtain demographic and clinical data (Booysen et al., 2015; Norton et al., 2012; Woodiwiss et al., 2009). Height and weight were measured using standard approaches and participants were considered to be overweight if their body mass index (BMI) was ≥25 kg/m^2 and obese if their BMI was ≥30 kg/m^2. Laboratory blood tests of renal function, liver function, blood glucose, hematological parameters, and percentage glycated hemoglobin (HbA1c) were performed. Diabetes mellitus (DM) was defined as the use of insulin or oral glucose lowering agents or an HbA1c value greater than 6.5%. High quality office brachial blood pressure (BP) measurements were obtained in the seated position and after 5 minutes of rest, by a trained nurse-technician using a standard mercury sphygmomanometer (Woodiwiss et al., 2009) according to guidelines. The mean of 5 measurements obtained at least 30 seconds apart was taken as office BP. Ambulatory 24-hour BP was determined using SpaceLabs monitors (model 90207) as previously described (Woodiwiss et al., 2009). Hypertension was defined as a mean office BP≥140 mm Hg systolic or ≥90 mm Hg diastolic BP or the use of antihypertensive medication. Uncontrolled hypertension was defined as either a mean office BP≥140 mm Hg systolic or ≥90 mm Hg diastolic BP and in those with 24-hour BP measurements that met with pre-specified criteria, a 24-hour BP≥130 mm Hg systolic or ≥85 mm Hg diastolic BP. Circulating renin and aldosterone concentrations were determined as previously described (Mitchell et al., 2012; Michel et al., 2014). Plasma renin concentrations were measured using an immunoradiometric technique (Renin III Generation, Cisbio International, Ceze, France) and serum aldosterone concentrations using an ^{125}I radioimmunoassay (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA).

3.2.3 Hemodynamics

Central arterial pressures were obtained from pulse wave analysis as previously described (Booysen et al., 2015; Norton et al., 2012). 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 9.0 software (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia). The pulse wave was calibrated by manual measurement (auscultation) of brachial BP (SBP and DBP) taken immediately before the recordings. The peripheral pressure waveform was converted into a central aortic waveform using a validated generalized transfer function incorporated in SphygmoCor software. 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. Immediately after the central arterial pressure waveforms were obtained, aortic velocity and diameter measurements were obtained by an experienced observer (AJW) with the participant in the left lateral decubitus position using an Acuson SC2000 Diagnostic ultrasound system (Siemens Medical Solutions, USA, Inc.). Velocity waveforms were obtained in the 5-chamber view. Aortic diameter measurements were obtained just proximal to the aortic leaflets in the long axis parasternal view. The largest diameter recorded in early systole was employed to construct the flow waveform (Mitchell et al., 2010*a*). Aortic flow waveforms were generated from aortic velocity and diameter measurements (Mitchell et al., 2010*a*). Taking care to avoid any overshoot of the image, the leading (outer) edge or the most dense, or brightest, portion of the spectral image of the velocity waveform was outlined using graphics software and using aortic diameter measurements employed to construct a flow waveform. Characteristic impedance (Zc) was calculated in the time domain as change in pressure/change in flow from the foot of the pulse wave up until 95% of peak flow (Mitchell et al., 2010*a*; Segers et al., 2017). Using Zc values and the flow and pressure waveforms, wave separation analysis was performed based on the following formulae: Forward wave pressure=(aortic pressure + QxZc)/2 and Backward wave pressure=(aortic pressure – QxZc)/2 (Westerhof et al., 1972; Murgo et al., 1981). Wave separation analysis was performed in the time domain based upon the consensus that this analysis can be conducted in either the time or the frequency domain (Segers et al., 2017). The peak pressures

generated by the product of peak Q and Zc (maximal P_{QxZc}) were also determined (Phan et al., 2016). Stroke volume was determined from the product of the velocity-time integral of the aortic velocity wave and aortic root diameter. Systemic vascular resistance (SVR) was calculated from mean arterial pressure (MAP, average arterial pressure for the peripheral wave form using the formula $MP = \frac{\sum_{i=T_0}^{T_F} P_i}{n}$, where T_0=start of waveform; T_F=end of waveform; P_i=pressure points, n= number of pressure points) and cardiac output (SVR=[MAP-RAP]/CO), assuming right atrial pressure (RAP) = 0 mm Hg. Cardiac output was determined from SV x heart rate. Heart rate (HR) was determined from the length (PD) of an averaged peripheral waveform captured over a 10 second period, using the formula: HR=1000/PDx60. Total arterial compliance (TAC) was calculated as SV/aortic pulse pressure. Left ventricular end diastolic volume (EDV) and LV ejection fraction (EF) were determined from the biplane Simpson approach. The impact of age on SV was determined from SV values obtained from both outflow tract velocity and aortic diameter measurements and from SV values obtained from the biplane Simpson approach.

3.2.4 Data analysis

For database management and statistical analysis, SAS software, version 9.4 (SAS Institute Inc., Cary, NC) was employed. Continuous variables are expressed as mean (±SD). Dichotomous variables are expressed as percentages. Relationships were evaluated from multivariate linear regression analysis. To determine probability values, further adjustments for non-independence of family members was performed using non-linear regression analysis (mixed procedure as defined in the SAS package). To determine the yearly contribution of the various hemodynamic parameters to age-related increases in BP, multivariate adjusted product of coefficient mediation analysis was performed. In both regression analysis and in mediation analysis, adjustments were for age (within age categories), sex, regular alcohol intake, regular tobacco intake, BMI, diabetes mellitus and/or an HbA_{1c}>6.5%. To determine the hemodynamic correlates of uncontrolled hypertension across the adult lifespan, multivariate adjusted logistic regression analysis was performed. Relationships were compared using z-statistics. As the impact of forward wave pressures depends on age,

analyses were conducted in those either less than or more than 50 years of age (the age at which the forward waves begin to increase as identified from the inflection points of age-pressure wave relations [LOESS regression analysis]). As more women than men volunteered for the present study sensitivity analysis was conducted in sex-specific groups. As antihypertensive therapy may influence the results, sensitivity analysis was also performed in those not receiving therapy.

3.3 Results

3.3.1 Participant characteristics

Table 3.1 shows the characteristics of the study sample. In general, a high proportion of participants had hypertension and obesity. Participants without high quality aortic velocity measurements in the outflow tract showed similar characteristics as those with these measures (Table 3.2). A half of all hypertensives were receiving therapy. A significant proportion of hypertensives had uncontrolled BP values despite a number of these individuals receiving antihypertensive medication.

3.3.2 Relationships between age and mean arterial blood pressures and the determinants thereof

Age was positively associated with MAP across the full adult lifespan (Figures 3.1 and 3.2); whereas heart rate was only weakly related to age on bivariate analysis ($r=0.083$, $p=0.017$) and unrelated to age on multivariate analysis (Figures 3.1 and 3.2). Across the full adult age range, independent of several confounders, age was strongly positively associated with SV and CO, but not with SVR (Figures 3.1 and 3.2) effects consistent in men and women (Table 3.3). Age relations with SV were similar irrespective of whether SV was determined from the product of the velocity-time integral and aortic root diameter or using the biplane Simpson approach (Figure 3.3). Body weight was related to age (bivariate analysis: $r=0.298$, $p<0.0001$; multivariate analysis: partial $r=0.188$, $p<0.0001$), and SV was related to body weight (bivariate analysis: $r=0.140$, $p<0.0001$; multivariate analysis: partial $r=0.107$, $p=0.002$); however SV was related to age independent of adjustors including body mass index (Figures 3.1, 3.2 and 3.3 upper panel, Table 3.3). In addition,

Table 3. 1 Participant characteristics.

Sample size (% female)	824 (68.3)
Age (years)	46.3±18.2
Body mass index (kg/m^2)	29.7±7.7
% Overweight/obese	25.0/44.5
% Hypertensive	47.3
% Treated for hypertension	27.5
% Uncontrolled office BP	35.3
% Uncontrolled 24-hour BP	22.5
% Diabetes mellitus (DM)	13.1
% Regular smokers	15.1
% Regular alcohol intake	19.8
Systolic blood pressure (SBP) (mm Hg)	128±22
Diastolic blood pressure (DBP)(mm Hg)	83±12
Mean arterial pressure (MAP) (mm Hg)	99±15
Pulse pressure (PP) (mm Hg)	46±16
Heart rate (beats/min)	68±12
24-hour SBP (mm Hg) (n=537)	119±15
24-hour DBP (mm Hg) (n=537)	73±10
Stroke volume (SV) (mls/beat)	80±24
Cardiac output (CO) (l/min)	5.4±2.4
Systemic vascular resistance (SVR)(mm Hg/l/min)	20.7±9.6
Peak aortic flow (Q) (mls/sec)	352±173
Characteristic impedance (Zc)(dynes.s/cm^5)	86.3±42.3
Total arterial compliance (TAC) (ml/mm Hg)	2.35±0.99
Maximal $P_{Q \times Zc}$ (mm Hg)	25.5±8.1
Forward travelling pressure wave (Pf) (mm Hg)	24.5±8.2
Left ventricular EDV (mls)	110±28
Left ventricular EF (%)	67.1±8.9

Plasma renin (pg/ml)	37.1 (0.2 to 507)
Serum aldosterone (ng/dl)	6.5 (undetected to 37.7)
Aldosterone/rennin (ng/dl / ng/l)	0.83 (undetected to 9.24)

Data are shown as mean±SD or interquartile ranges or proportions. Maximal P_{QxZc}, early systolic pulsatile load as determined from the peak pressure generated by the product of Zc and Q (compression wave); EDV, end diastolic volume; EF, ejection fraction.

Table 3. 2 Characteristics of participants with or without high quality velocity measures in the outflow tract.

	Without	With	p-value vs with
Sample size (% female)	238 (63.8)	824 (68.3)	=0.19
Age (years)	44.8±18.2	46.3±18.2	=0.25
Body mass index (kg/m^2)	30.2±8.0	29.7±7.7	=0.40
% Overweight/obese	18.3/48.3	25.0/44.5	=0.10
% Hypertensive	53.2	47.3	=0.10
% Uncontrolled hypertension	41.5	35.3	=0.08
% Treated for hypertension	26.3	27.5	=0.68
% Diabetes mellitus (DM)	14.2	13.1	=0.68
% Regular smokers	13.8	15.1	=0.61
% Regular alcohol intake	21.7	19.8	=0.53
Systolic blood pressure (SBP) (mm Hg)	129±22	128±22	=0.70
Diastolic blood pressure (DBP) (mm Hg)	84±13	83±12	=0.08
Mean arterial pressure (MAP) (mm Hg)	101±16	99±15	=0.26
Pulse pressure (PP) (mm Hg)	45±15	46±16	=0.67

Data are shown as mean±SD or proportions.

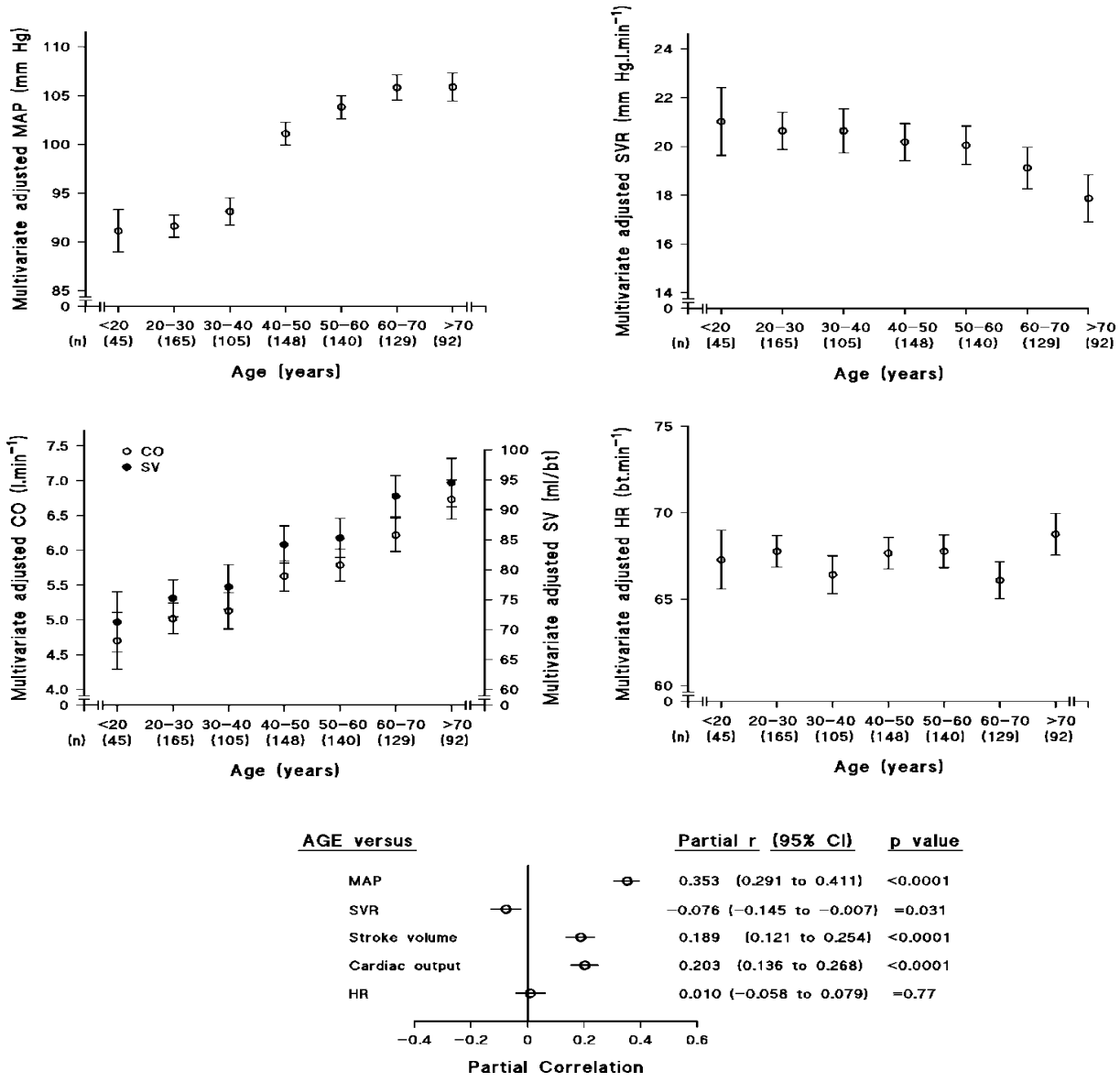


Figure 3. 1 Age-related increases in the steady components of pressure (mean arterial pressure [MAP]) and the determinants thereof across the full adult age range in a community sample of African ancestry. Data shown are multivariate adjusted values across the adult age range (upper and middle panels) and relations between age and hemodynamic variables (lower panel). Adjustments are for age within age categories, sex, regular alcohol intake, regular tobacco intake, BMI, diabetes mellitus and/or an HbA_{1c}>6.5%. Abbreviations are given in table 3.1.

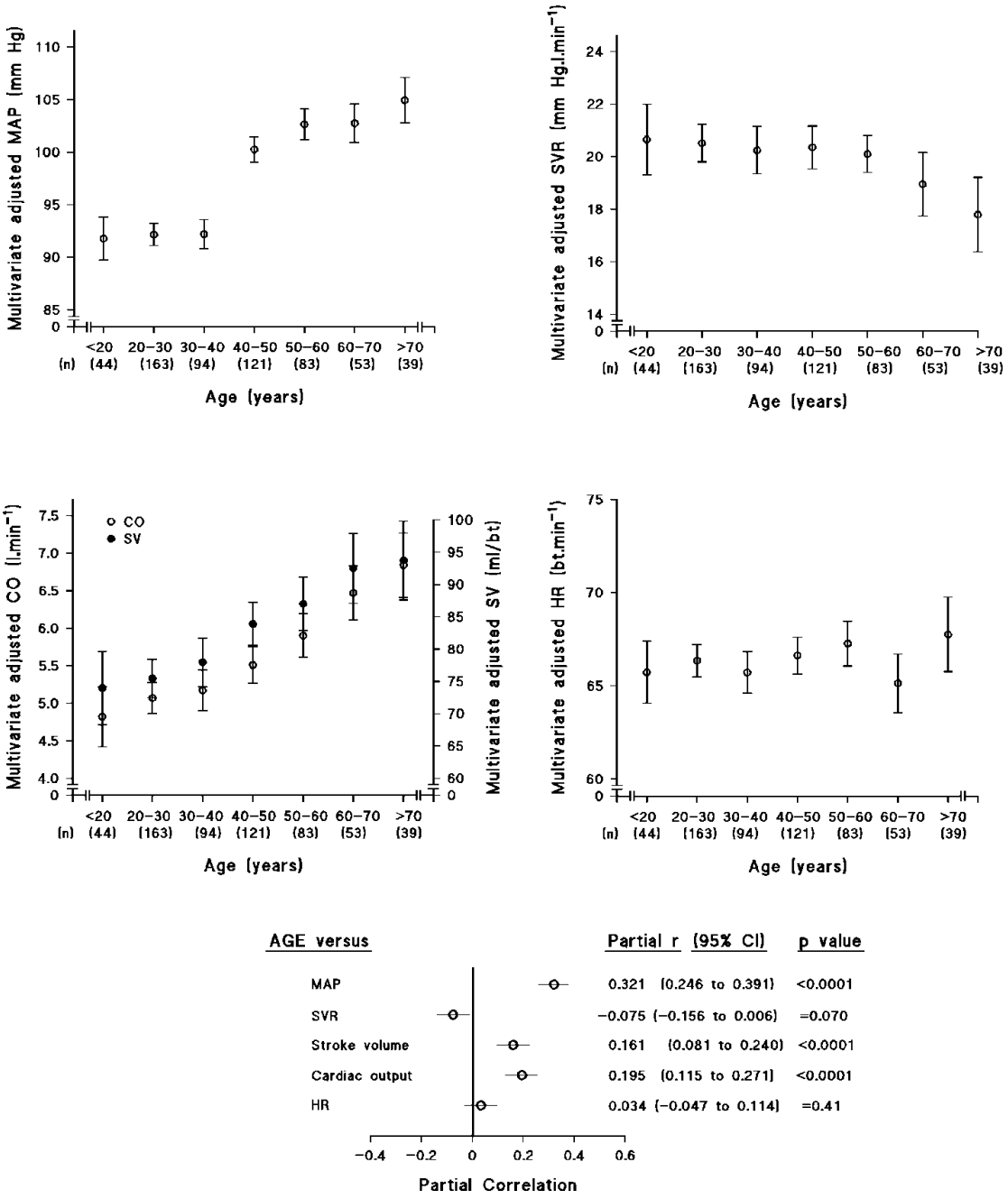


Figure 3. 2 Age-related increases in the steady components of pressure (mean arterial pressure [MAP]) and the determinants thereof across the full adult age range in participants from a community sample of African ancestry not receiving antihypertensive therapy. Data shown are multivariate adjusted values across the adult age range (upper and middle panels) and relations between age and hemodynamic variables (lower panel). Adjustments are for age within age

categories, sex, regular alcohol intake, regular tobacco intake, BMI, diabetes mellitus and/or an HbA_{1c}>6.5%. Abbreviations are given in table 3.1.

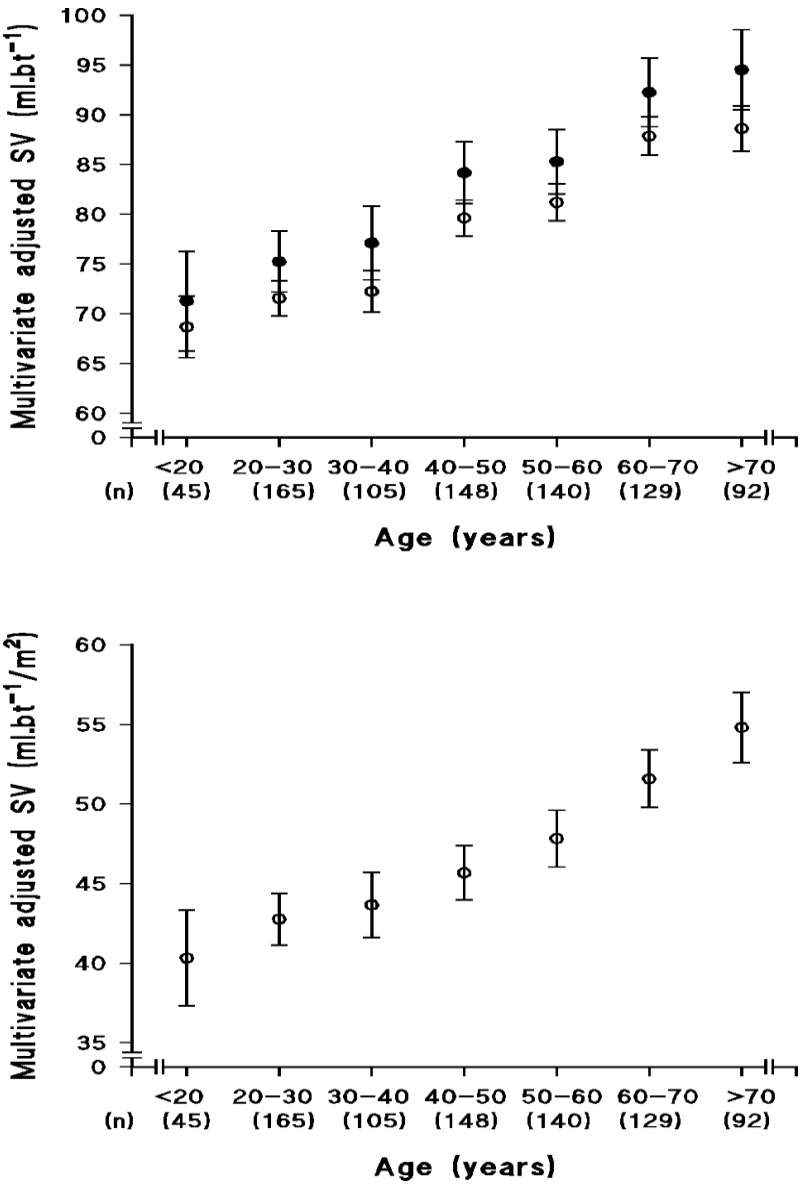


Figure 3. 3 Age-related increases in stroke volume (SV) as determined from the velocity-time integral and aortic root diameter and using the biplane Simpson approach (upper panel) and SV indexed to body surface area (BSA) (lower panel) in a community sample of African ancestry. Data shown are multivariate adjusted values across the adult age range. Adjustments are for age within age categories, sex, regular alcohol intake, regular tobacco intake, BMI (except for SV indexed to BSA), diabetes mellitus and/or an HbA_{1c}>6.5%. Abbreviations are given in table 3.1.

SV was similarly related to age when SV was indexed to body surface area (BSA) (partial r=0.183, 95%CI=0.11 to 0.249, p<0.0001) (Figure 3.3). Importantly, in mediation analysis, SV but not SVR accounted for age relationships with MAP (Table 3.4).

3.3.3 Relationships between age and pulse pressure, systolic blood pressure or diastolic blood pressure and the determinants thereof

While age was positively (directly) associated with DBP until 50 years of age, after 50 years of age this relationship decreased (Figures 3.4 and 3.5). Age was strongly associated with increases in SBP (Figures 3.4 and 3.5) and PP (Figures 3.4 and 3.5) mainly from 50 years of age and in parallel with associations between age and forward wave pressures or maximal P_{QxZc} (Figures 3.4 and 3.5). These age-related changes in SBP, DBP and PP were paralleled by positive relations between age and peak aortic Q, and inverse relations between age and total arterial compliance (TAC) across the adult lifespan and positive relations between age and Zc after 50 years of age (Figures 3.4 and 3.5). The positive associations between age and Q across the adult lifespan occurred despite the decline in TAC with age and a positive relationship between TAC and Q (partial r=0.34, p<0.0001). Similarly, the positive associations between age and Q after 50 years of age occurred despite an inverse relationship noted between Zc and Q (partial r=-0.67, p<0.0001). Importantly, the strength of the relationship between age and Q was the same as that for the relationship between age and Zc and for the relationship between age and TAC (Figures 3.4 and 3.5). Before 50 years of age forward wave pressures and maximal P_{QxZc} (Figures 3.4 and 3.5) showed no age relations despite Q showing striking relations with age (Figures 3.4 and 3.5). This is explained by inverse associations between age and Zc that were initially noted to occur, relationships explained by positive associations between age and aortic root diameters (Figures 3.4 and 3.5). Importantly, no interactions were noted between SV and aortic root diameter (partial r = -0.044 to -0.025, p-value = 0.21 to 0.48). Relations between age and pulsatile pressures and the determinants thereof were consistent in both women and in men (Table 3.3). Importantly, in product of coefficient mediation analysis, age-related increases in peak aortic Q contributed as much to the yearly increase in PP as did age-related increases in Zc (Table 3.4) and age-related decreases in TAC (Table 3.4).

Table 3. 3 Sex-specific relations between age and blood pressures (BP) and the determinants thereof across the full adult lifespan.

	Women (n=563)		Men (n=261)	
Age vs	Partial r (95% CI)	p value	Partial r (95% CI)	p value
	Steady components effects			
MAP	0.398 (0.326-0.466)	<0.0001	0.301 (0.185-0.408)	<0.0001
SVR	-0.082 (-0.165-0.002)	=0.055	-0.074 (-0.196-0.051)	=0.25
SV	0.210 (0.129-0.288)	<0.0001	0.175 (0.053-0.292)	<0.005
CO	0.191 (0.109-0.269)	<0.0001	0.225 (0.105-0.337)	<0.0005
	Pulsatile components effects			
SBP	0.467 (0.399-0.529)	<0.0001	0.339 (0.225-0.442)	<0.0001
DBP	0.279 (0.201-0.354)	<0.0001	0.169 (0.046-0.285)	<0.01
PP	0.420 (0.349-0.486)	<0.0001	0.362 (0.250-0.463)	<0.0001
Pf	0.297 (0.217-0.373)	<0.0001	0.344 (0.229-0.450)	<0.0001
Maximal P_{QxZc}	0.316 (0.239-0.389)	<0.0001	0.352 (0.240-0.455)	<0.0001
Aortic root diameter	0.170 (0.088-0.249)	<0.0001	0.232 (0.112-0.345)	<0.0005
TAC	-0.108 (-0.190 to -0.024)	=0.012	-0.186 (-0.304 to -0.062)	=0.003
Zc	0.136 (0.048-0.221)	<0.005	0.132 (0.004-0.256)	<0.05
Peak aortic Q	0.218 (0.133-0.299)	<0.0001	0.246 (0.122-0.362)	<0.0001

Adjustments are for MAP (for Pf and Zc), sex, regular alcohol intake, regular tobacco intake, BMI, diabetes mellitus and/or an HbA_{1c}>6.5%. Abbreviations are given in table 3.1.

Table 3. 4 Contribution in product of coefficient mediation analysis of the hemodynamic determinants to the age-related (yearly increase) in blood pressures (BP) across the full adult age range in a community sample of African ancestry.

	Full age range	< 50 years	≥50 years
	Yearly contribution	Yearly contribution	Yearly contribution
n=	824	463	361
Yearly contribution to age-related increases in MAP (mm Hg per year)			
SVR	-0.011±0.001***	0.0098±0.0030**	-0.031±0.005***
Stroke volume	0.036±0.002***††	0.016±0.003***	0.020±0.005***††
Heart rate	0.0007±0.0009	0.0013±0.0030	-0.003±0.005
Yearly contribution to age-related increases in PP (mm Hg per year)			
Peak aortic Q	0.027±0.001***	0.024±0.001***††	0.031±0.003***††
Zc	0.028±0.001***	-0.050±0.003***	0.066±0.004***
TAC	0.032±0.003***	0.018±0.005***††	0.039±0.006***††
MAP	0.143±0.010***	0.123±0.020***	0.110±0.042*

See table 3.1 for additional abbreviations. Adjustments are for sex, regular alcohol intake, regular tobacco intake, BMI, diabetes mellitus and/or an HbA_{1c}>6.5%. *p<0.01, **p<0.001, ***p<0.0001 for significance of contribution. †p<0.05, ††p<0.0001 versus contribution of SVR or Zc.

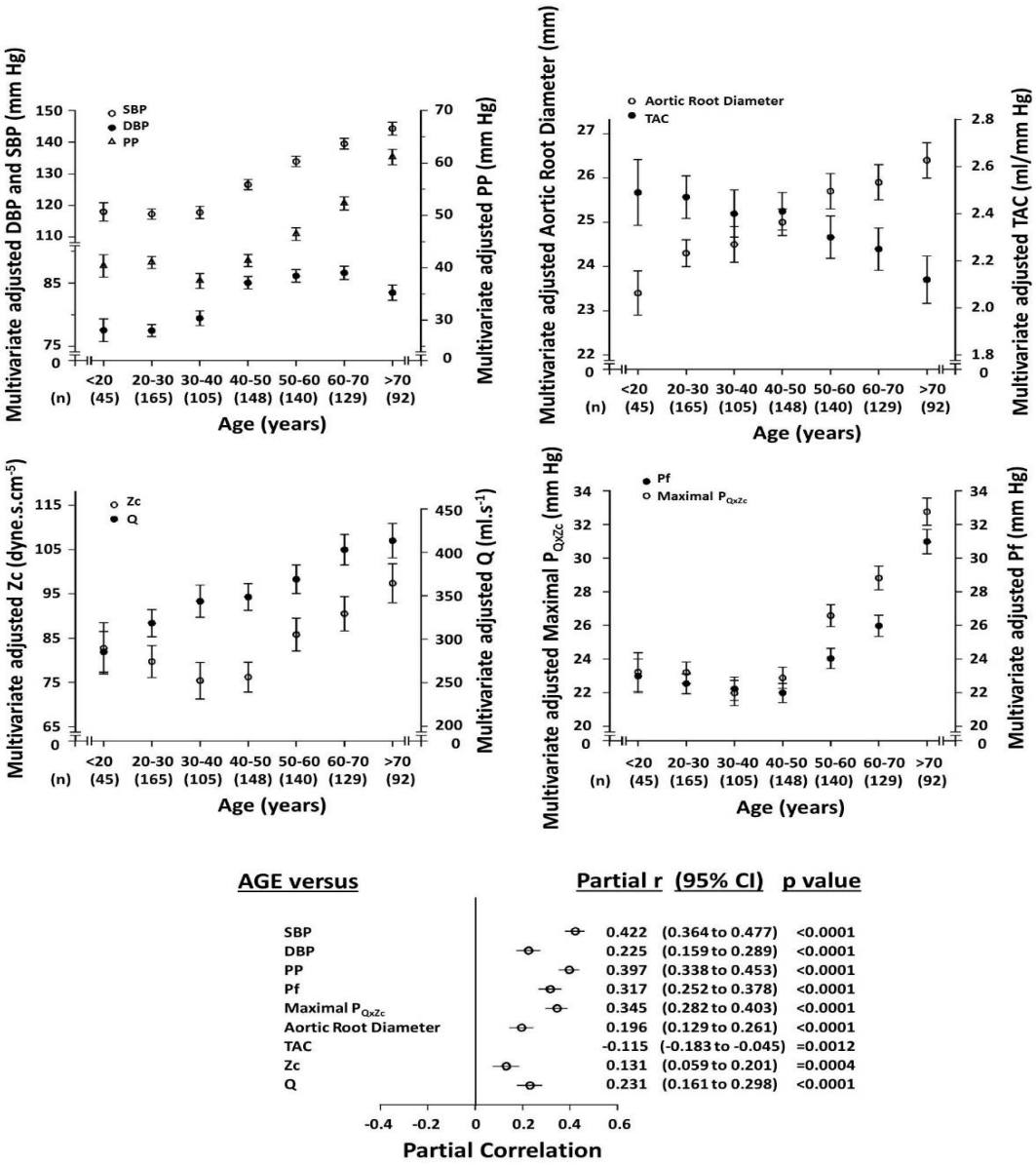


Figure 3. 4 Age-related increases in systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP) and the determinants thereof across the full adult age range in a community sample of African ancestry. Data shown are multivariate adjusted values across the adult age range (upper and middle panels) and relations between age and hemodynamic variables (lower panel). Adjustments are for age within age categories, MAP (for Pf and Zc), sex, regular alcohol intake, regular tobacco intake, BMI, diabetes mellitus and/or an HbA_{1c}>6.5%. Abbreviations are given in table 3.1.

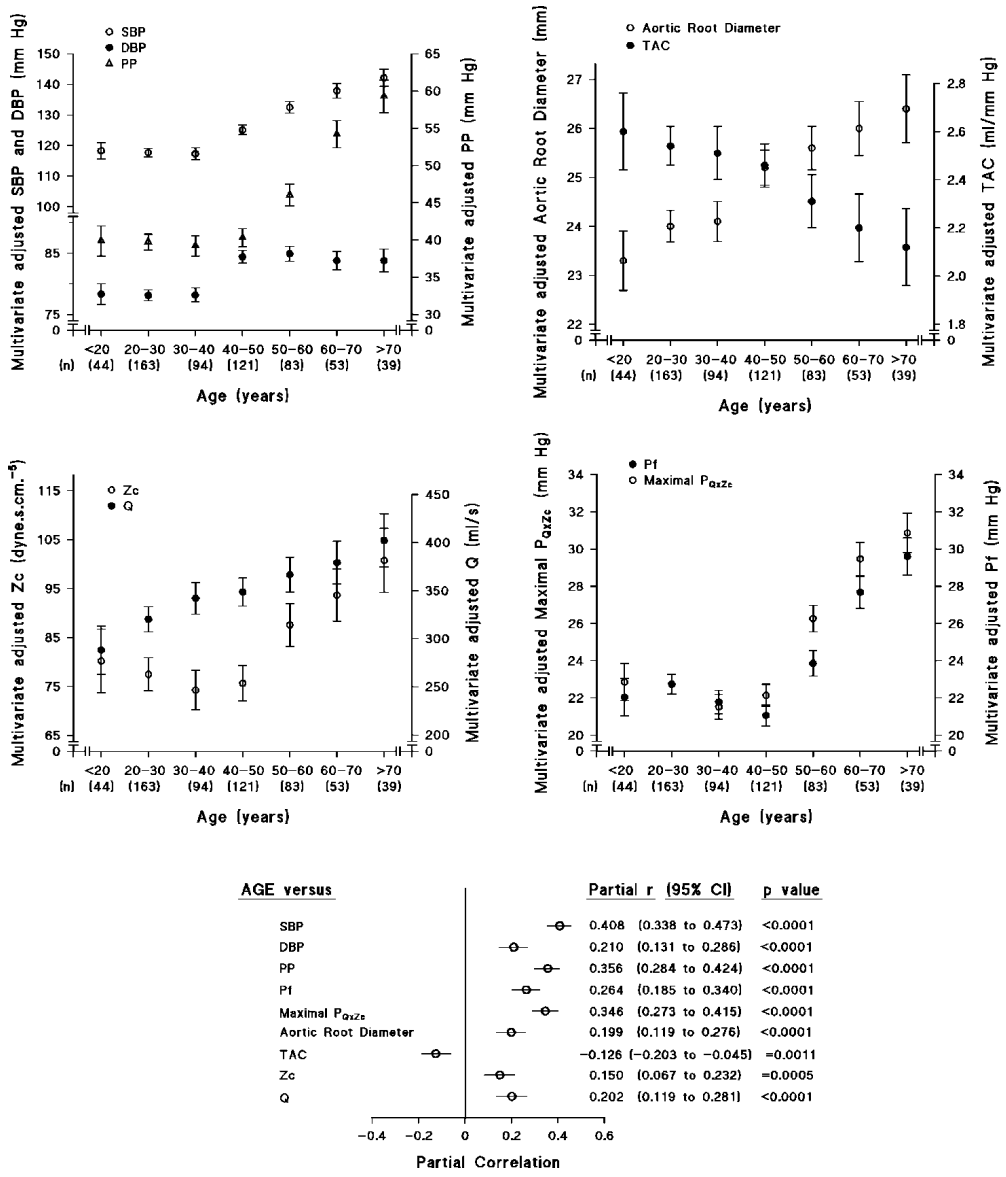


Figure 3. 5 Age-related increases in systolic blood pressure (SBP), diastolic blood pressure (DBP) and pulse pressure (PP) and the determinants thereof across the full adult age range in participants from a community sample of African ancestry not receiving antihypertensive therapy. Data shown are multivariate adjusted values across the adult age range (upper and middle panels) and relations between age and hemodynamic variables (lower panel). Adjustments are for age within age categories, MAP (for Pf and Zc), sex, regular alcohol intake, regular tobacco intake, BMI, diabetes mellitus and/or an HbA_{1c}>6.5%. Abbreviations are given in table 3.1.

3.3.4 Determinants of stroke volume or peak aortic flow

Independent of confounders, SV and peak aortic Q were independently associated with LV end diastolic volume (Figure 3.6). No independent relations between either SV or Q were noted with LV ejection fraction (Figure 3.6). Plasma renin concentrations were inversely and aldosterone concentrations and hence ARR positively associated with age (Figure 3.6). Inverse relations between SV, but not Q and renin concentrations were noted (Figure 3.6). However, ARR was positively associated with both SV and peak aortic Q (Figure 3.6).

3.3.5 Hemodynamic correlates of uncontrolled hypertension across the adult lifespan

Uncontrolled hypertension defined according to either office or 24-hour BP criteria was associated with a markedly higher age (Tables 3.5 and 3.6). However, across early adult life the presence of uncontrolled hypertension was independently associated with increases in both SVR and SV (Figure 3.7) and decreases in TAC (Figure 3.8). Moreover, in later adult life the presence of uncontrolled hypertension was independently associated with increases in Zc and peak aortic Q (Figure 3.7) and decreases in TAC (Figure 3.8). Importantly, across the full adult lifespan increases in peak aortic Q contributed as much as Zc and then the inverse of TAC (1/TAC) and markedly more than SVR to the odds of having uncontrolled hypertension (Table 3.7).

3.4 Discussion

The main findings of the present study are as follows; In a large community-based sample of African ancestry in Africa studied across the full adult age range, I show a hemodynamic correlation with age, quite distinct from alternative populations (Mitchell et al., 2008, 2010*a*; Segers et al., 2007), that contributes to increases in BP in groups of African ancestry. In this regard, I show for the first time that beyond increases in body size, a striking contribution of systemic flow in addition to vascular changes that generate resistance to flow, to age-related and hypertension-associated increases in both the steady (and hence MAP) and pulsatile (and hence PP) components

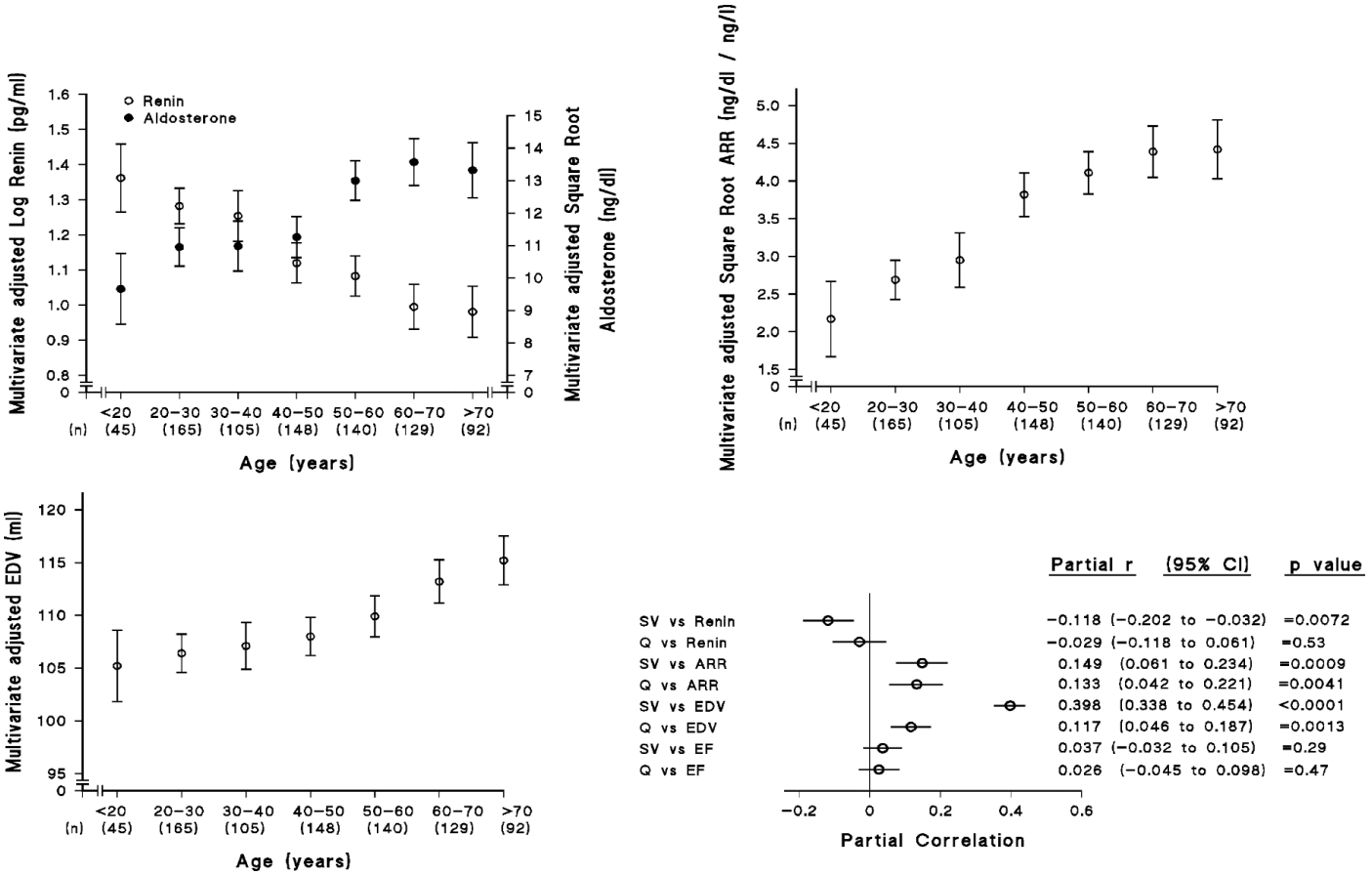


Figure 3. 6 Age-related changes in the determinants of stroke volume (SV) or peak aortic flow (Q) (upper and lower left panels) and relations between SV or Q and the determinants thereof (lower right panel) in a community sample of African ancestry. Relations between SV or Q and the determinants thereof are adjusted for age within age categories, sex, regular alcohol intake, regular tobacco intake, BMI, diabetes mellitus and/or an HbA_{1c}>6.5%. Abbreviations are given in table 3.1. p<0.0001 for all age-related effects shown except age vs EDV which was p<0.001.

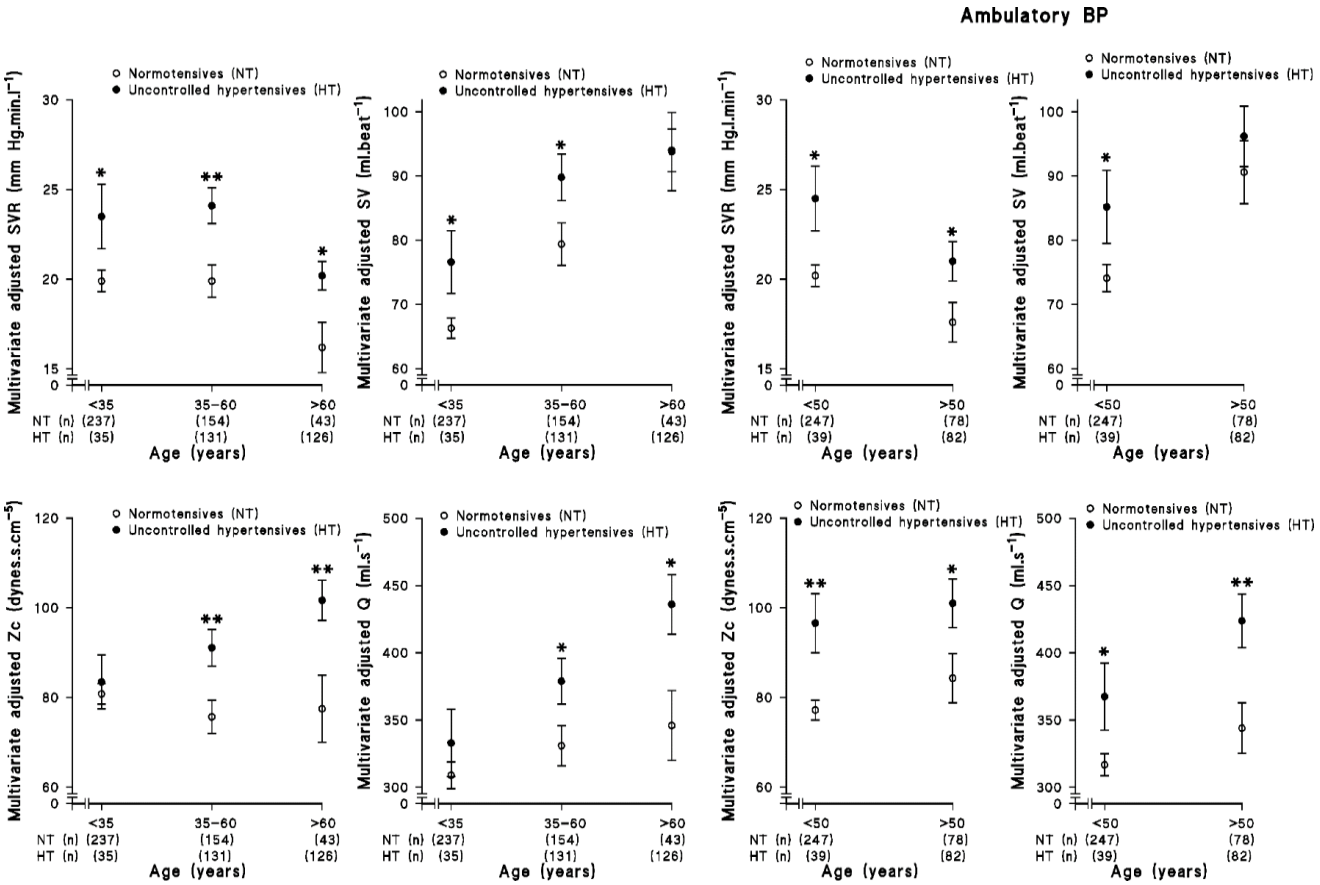


Figure 3. 7 Differences in multivariate adjusted hemodynamic determinants of blood pressure (BP) in uncontrolled hypertensives (HT) versus normotensives (NT) at different ages across the adult age range. Normotension and uncontrolled HT were defined according to office or 24-hour ambulatory BP thresholds. Adjustments are for age within age categories, sex, regular alcohol intake, regular tobacco intake, BMI, diabetes mellitus and/or an HbA_{1c}>6.5%. Abbreviations are given in table 3.1. *p<0.05, **p<0.005, for comparison with NT.

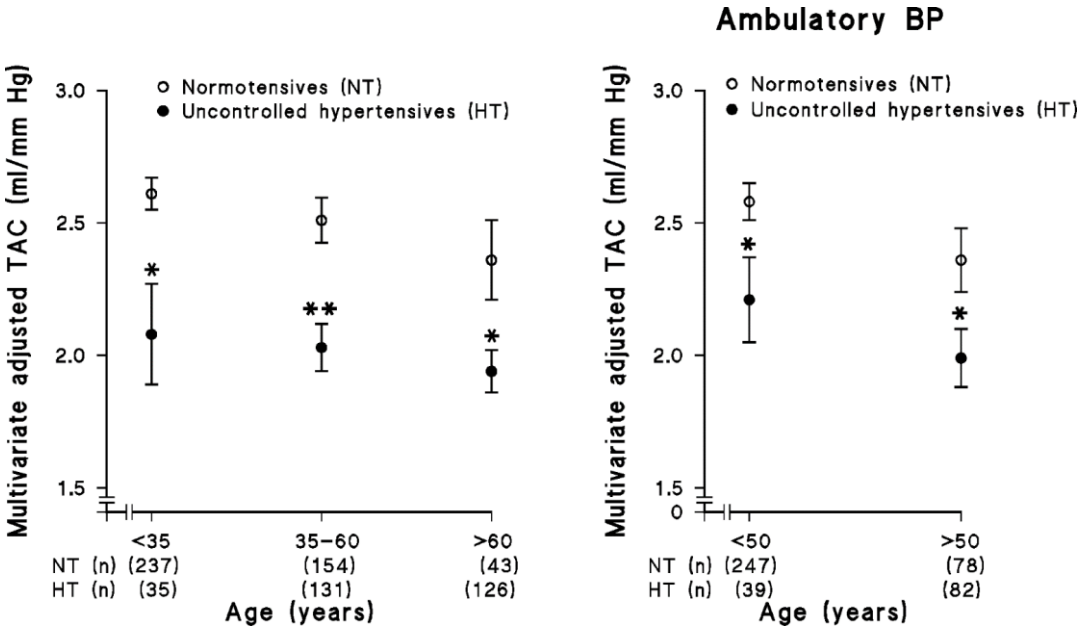


Figure 3. 8 Differences in multivariate adjusted total arterial compliance (TAC) in uncontrolled hypertensives (HT) versus normotensives (NT) at different ages across the adult age range. Normotension and uncontrolled HT were defined according to office or 24-hour ambulatory BP thresholds. Adjustments are for age within age categories, sex, regular alcohol intake, regular tobacco intake, BMI, diabetes mellitus and/or an HbA_{1c}>6.5%. Abbreviations are given in table 3.1. *p<0.05, **p<0.005, for comparison with NT.

Table 3. 5 Characteristics of normotensives and uncontrolled hypertensives.

	Normotensives	Uncontrolled hypertensives	
Sample size (% female)	434 (67.7)	291 (63.9)	=0.29
Age (years)	37.0±15.6	55.9±15.8	<0.0001
Body mass index (kg/m^2)	27.5±7.1	31.6±7.6	<0.0001
% Overweight/obese	28.1/32.0	21.6/56.4	<0.0001
% Treated for hypertension	0	44.0	<0.0001
% Diabetes mellitus (DM)	4.4	17.5	<0.0001
% Regular smokers	15.4	16.8	=0.61
% Regular alcohol intake	21.2	19.6	=0.60
Systolic blood pressure (SBP) (mm Hg)	115±11	151±20	<0.0001
Diastolic blood pressure (DBP) (mm Hg)	76±8	94±12	<0.0001
Mean arterial pressure (MAP) (mm Hg)	90±8	115±13	<0.0001
Pulse pressure (PP) (mm Hg)	39±9	56±19	<0.0001

Data are shown as mean±SD or proportions.

Table 3. 6 Characteristics of normotensives and uncontrolled hypertensives based on 24-hour blood pressure criteria.

	Normotensives	Uncontrolled hypertensives	
Sample size (% female)	325 (64.9)	121 (61.2)	=0.46
Age (years)	38.2±16.0	57.6±16.1	<0.0001
Body mass index (kg/m^2)	27.3±6.7	31.5±7.9	<0.0001
% Overweight/obese	28.3/31.7	26.5/51.2	=0.0002
% Treated for hypertension	0	41.3	<0.0001
% Diabetes mellitus (DM)	4.3	24.8	<0.0001
% Regular smokers	15.7	17.4	=0.67
% Regular alcohol intake	21.5	20.7	=0.84
24-hour SBP (mm Hg)	119±16	149±21	<0.0001
24-hour DBP (mm Hg)	78±9	91±13	<0.0001
24-hour MBP (mm Hg)	93±11	113±15	<0.0001
24-hour PP (mm Hg)	41±14	57±16	<0.0001

Data are shown as mean±SD or proportions.

Table 3. 7 Contribution of the hemodynamic determinants of blood pressure (BP) to uncontrolled office or 24-hour ambulatory BP across the full adult lifespan in a community sample of African ancestry.

Models	1 x SD effect	Odds ratios (95% CI)	p value
	Uncontrolled office BP versus NT (n= 291 vs 434)		
One SD increase in			
SVR	9.6 mm Hg/l/min	0.817 (0.611-1.084)	=0.16
Peak aortic Q	173 mls/sec	2.535 (1.903-3.425)**	<0.0001
Zc	42 dynes x s/cm^5	1.838 (1.352-2.530)**	=0.0001
1/TAC	0.27 mm Hg/ml	1.903 (1.354-2.710)**	<0.0005
One SD increase in			
SVR	9.6 mm Hg/l/min	1.809 (1.245-2.647)†	<0.005
SV	24 mls/beat	4.550 (3.287-6.423)**††	<0.0001
Zc	42 dynes x s/cm^5	1.084 (0.853-1.375)	=0.51
1/TAC	0.27 mm Hg/ml	3.235 (2.293-4.666)*††	<0.0001
	Uncontrolled 24-hour BP versus NT (n=121 vs 325)		
One SD increase in			
SVR	9.2 mm Hg/l/min	0.533 (0.328-0.829)	=0.008
Peak aortic Q	167 mls/sec	2.211 (1.516-3.255)**	<0.0001
Zc	40 dynes x s/cm^5	1.758 (1.202-2.602)**	<0.005
1/TAC	0.26 mm Hg/ml	2.291 (1.452-3.714)**	<0.0005
One SD increase in			
SVR	9.2 mm Hg/l/min	0.774 (0.455-1.257)	=0.32
SV	25 mls/beat	2.576 (1.743-3.855)**†	<0.0001
Zc	40 dynes x s/cm^5	1.186 (0.878-1.592)	=0.26
1/TAC	0.26 mm Hg/ml	3.172 (2.048-5.096)**††	<0.0001

NT, normotensives defined as no therapy and a normal BP; Uncontrolled BP, hypertensives defined as office BP above normal ranges. See table 3.1 for additional abbreviations. *$p<0.05$, **$p<0.0005$ versus relations with SVR; $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.0005$ versus relations with Zc.

of BP. Indeed, I note that across the adult age range, stroke volume (SV) and hence cardiac output (CO) account for as much of the increase in MAP in hypertension as do increases in systemic vascular resistance (SVR). Moreover, I show that from midlife to late in adult life, peak aortic flow (Q) accounts for as much of age-related and hypertension-associated increases in PP beyond the steady components of pressures as do increases in resistance to flow in the pulsatile component of arterial load (characteristic impedance, Zc and the inverse of total arterial compliance (1/TAC). In early adult life (<50 years of age) although striking positive associations between age and peak aortic Q were noted, these changes did not translate into increases in the forward travelling pressure wave or maximal P_{QxZc} and hence SBP or PP beyond the steady components of pressure. This occurred because up until 50 years of age, Zc was inversely associated with age in parallel with increases in aortic root diameter. Only once aortic stiffness was sufficiently advanced and Zc began to increase was age positively associated with SBP and PP beyond the steady components of load. Importantly, age relations with SV and peak aortic Q paralleled age-relations with aldosterone-to-renin ratio (ARR) and both SV and Q were independently associated with ARR. Moreover, peak aortic Q showed a striking impact on the presence of uncontrolled hypertension, equivalent to Zc and TAC and markedly greater than SVR.

It is well accepted that groups of African ancestry develop a volume-dependent form of hypertension (Luft et al., 1991; Freis et al., 1988), often associated with low renin concentrations but an increased ARR related in part to an enhanced angiotensinogen production (Mitchell et al., 1997; Celis et al., 2001). However, there is little evidence to show the extent to which volume-induced increases in blood flow as opposed to vascular resistance to flow contribute to increases in either the steady or pulsatile components of BP in this ethnic group. Although several studies have demonstrated that SVR but not SV account for increases in BP in hypertensives of African origin (Heffernan et al., 2008; Din-Dzietham et al., 2004), the relative impact of volume versus vascular changes across the full adult age range has not been demonstrated. In this regard, the hemodynamic correlates of BP are well recognised as changing considerably with aging. More recent evidence suggests that the age-related hemodynamic correlates of increases in SBP in groups of African ancestry (Hodson et al., 2016, 2017) closely mirror that noted in alternative populations where aortic stiffness rather than volume load drives SBP changes with aging (Mitchell et al., 2008, 2010; Segers et al., 2007). This study (Hodson et al., 2016, 2017) did not however evaluate the relative contribution of peak aortic Q, Zc and TAC to age-related increases

in BP. In the present study I provide the first evidence to show that age relations with both steady and pulsatile components of BP in groups of African ancestry across the full adult lifespan are as strongly dependent on age relations with volume-induced increases in systemic blood flow (SV and peak aortic Q) as they are on vascular resistance to flow either in the steady (SVR) or the pulsatile (aortic Zc or TAC) components of arterial load. These data are in distinct contrast to the strong dependence of age-related increases in SBP on increases in Zc (Mitchell et al., 2010*a*), and the Zc-associated decrease in Q (Q decreases when resistance to flow [Zc] in the pulsatile component increases) which may accompany increases in SBP in alternative populations (Mitchell et al., 2008). Similarly, these data are in contrast to the impact of age-related decreases in TAC on PP and the lack of impact of SV on PP in hypertensive men over 50 years of age (Alfie et al., 1999). The continuing positive age relationship with Q later in adult life noted in the present study was despite the strong inverse relationship noted between Zc and Q and the positive relationship noted between TAC and Q in the present study sample.

There are several implications to the finding of an equally important role of volume-dependent increases in peak aortic Q to PP as those mediated by Zc and TAC, and to the equally important role of increases in SV and hence CO to MAP as those mediated by SVR. Of significance, in the presence of a greater SV or Q, the presence of much smaller vascular changes (SVR or Zc and TAC) are likely to produce a more marked increase in BP. The present data therefore provide a possible explanation as to why hypertension is often more severe and hence more difficult to control in this ethnic group. With respect to the clinical importance of the contribution of Q to PP, aortic stiffness-associated increases in Zc are mediated by structural aortic changes and with no convincing evidence for reversibility. In contrast volume-dependent increases in peak aortic Q could be targeted by several interventions that reduce volume load (cardiac preload) and hence peak flow; thereby markedly reducing the impact of Zc on SBP and PP. Thus, in populations which depend more on volume load for increases in SBP and PP, the present study suggests that the adverse effects of increases in aortic stiffness may be readily prevented. As the adverse effect of increases in aortic stiffness and hence Zc on SBP and PP may be markedly pronounced if peak aortic Q is enhanced, targeting increases in Q is of critical importance. However, whether current approaches to managing BP achieve adequate reductions in Q is presently unknown. Indeed, independently targeting Q (which is a composite effect of forward and

reflected waves) may be difficult to achieve and may require differential changes to arterial compliance of the upper and lower body circulations.

With respect to the clinical importance of the contribution of SV and CO to MAP, the continued addition of vasodilators to hypertensives with an uncontrolled BP may provide little beneficial effect if volume overload and hence SV are the residual determinants of uncontrolled BP values. Indeed, in the present study uncontrolled hypertension was associated as much with increases in SV as with SVR. The present study therefore suggests that combinations of agents that reduce both SVR (vasodilators) and SV (diuretics) or heart rate (β-adrenoreceptor blockers) may be essential. Again however, whether current approaches to managing BP achieve adequate reductions in SV and CO is presently unknown.

An important finding of the present study is the volume-dependent age versus SV and Q relationship that occurred over the full adult age range as opposed to a persistent volume overload starting at an early adult age. These data suggest that the major impact of volume load on hemodynamic changes is a gradual effect produced over a prolonged period of time and not due to a premature onset of volume overload in early adult life. The present study nevertheless supports the view that renal mechanisms contribute significantly to increases in BP in groups of African ancestry even in early adult life (Aviv et al., 2004). The present findings similarly support a view that interventions to reduce volume load such as sodium restriction may play a key role in the ability to reduce BP, particularly in groups of African ancestry. What is nevertheless unclear is to what extent sodium restriction can normalise age-related increases in SV and aortic Q such as those noted in the present study, especially when part of the driving force for the volume load may be a humoral increase in aldosterone relative to renin. Further studies are warranted to identify the cause of the possible age-related volume overload and in particular, the increase in aldosterone production (or release) relative to renin. In this regard, an enhanced angiotensinogen production may in part contribute to this effect (Michel et al., 2012, 2014). However the fundamental change in renal function that triggers the volume load with aging is uncertain and the reason for the striking age relationship with ARR is unclear. Whether these changes reflect a particular susceptibility to progressive renal deterioration with age and risk factors and simultaneous adrenal changes with aging requires careful assessment.

The dependence of SBP and PP on increases in SV or Q noted in the present study population offers a potential explanation for the striking beneficial effects of diuretics on BP and

associated events in groups of African ancestry particularly in the elderly (ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group, 2002). In this regard, although SBP and PP only begin to markedly increase later in adult life in parallel with increases in Zc and decreases in TAC, the contribution of increases in Q are as striking as the increases in Zc and decreases in TAC. Nevertheless, whether diuretics decrease SV and hence Q in those with an increase in SV and Q, is unknown; exactly which diuretics (thiazide-like, thiazide, amiloride or aldosterone receptor antagonists) are able to achieve this effect is also uncertain; and the duration of therapy that would be required to achieve a therapeutic benefit in those with a high BP, is similarly unknown. In this regard, the relations between volume-dependent hemodynamic alterations and ARR suggest that one logical approach to abolish this effect is to target the impact of aldosterone.

There are several limitations to the present study that warrant consideration. First, the present study was cross-sectional in design. Hence no conclusions regarding causality can be drawn and age relations may be through residual confounding. Longitudinal studies will nevertheless require follow up from an early adult age to late in life. Despite the limitations in the study design however, the relationships between age and SV or Q and the subsequent effect on BP were remarkably consistent and hence irrespective of whether age effects on renal function are the cause, the contribution of systemic flow to hypertension is irrefutable. Second, in the present study, calibration of the radial waveform from brachial BP measurements ignores amplification of BP from brachial to radial arteries (Picone et al., 2015). Hence, aortic pressures may have been underestimated using the current approach. Thirdly, as cuff measurements underestimate SBP and overestimate DBP compared to invasive BP and these differences increase with increasing age (Picone et al., 2020), the magnitude of the impact of age on SBP and PP may have been underestimated in the current study. Hence, the yearly increases in SBP and PP produced by Q and Zc are likely to be an underestimation.

3.4.1 Conclusions

Hypertension in groups of African ancestry is thought to be distinct from alternative populations, relying more on volume load than alternative groups. However, there is little evidence to show that this effect translates into BP changes associated with increases in systemic blood

flow. In the present study I show a strong contribution of systemic blood flow to age-related and hypertension-associated increases in both steady (MAP) and pulsatile (PP, SBP and DBP) components of BP in a community sample of African descent. Indeed, I show that hypertension across the full adult age range in this ethnic group is as strongly determined by volume effects on blood flow (SV and peak aortic Q), as by increases in resistance to flow in either the steady (SVR) or the pulsatile (aortic Zc and TAC) components of arterial load. The present study therefore provides an explanation for the susceptibility of this ethnic group to BP effects even in response to small vascular changes (SVR or Zc and TAC) and highlights the possibility of developing a more rational approach to treating hypertension in groups of African ancestry. In this regard, a focus on both volume and vascular mechanisms, rather than on one or the other separately may be required to achieve adequate BP control.

CHAPTER 4

Contribution of Systemic Blood Flow to Untreated or Inadaquately Controlled Systolic-Diastolic or Isolated Systolic Hypertension in a Community Sample of African Ancestry.

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4.0 Abstract

Aims: Age-related increases in systemic blood flow (stroke volume [SV], cardiac output [CO], and aortic flow [Q]) contribute substantially to untreated or inadequately controlled (uncontrolled) blood pressure (BP) in Africa. I aimed to identify the hemodynamic determinants of uncontrolled systolic-diastolic (Syst-diast HT) versus uncontrolled isolated systolic (ISH) or diastolic (IDH) hypertension.

Methods: Using central arterial pressure and aortic outflow tract velocity and diameter measurements (echocardiography), the hemodynamic correlates of BP were determined in 725 community participants of African ancestry (19.6% uncontrolled Syst-diast HT, 9.2% uncontrolled ISH, 11.3% uncontrolled IDH).

Results: Independent of confounders, compared to those with a normotensive BP (NT), those with uncontrolled Syst-diast HT had increases in SV, CO, Q, systemic vascular resistance (SVR) and aortic characteristic impedance (Zc) and decreases in total arterial compliance (TAC) ($p<0.05$-$p<0.0001$). In multivariate regression models, uncontrolled Syst-diast HT was as strongly associated with Q, SV or CO as with SVR ($p=0.04$-$p=0.20$), Zc ($p=0.74$-$p<0.0005$) and TAC ($p=0.43$-$p<0.005$). Independent of confounders, compared to NT, those with uncontrolled ISH had increases in SV, CO, Q and Zc but not SVR, and decreases in TAC ($p<0.05$-$p<0.0001$), and those with IDH only had increases in SVR ($p<0.0001$). Uncontrolled ISH was more strongly associated with Q, SV and CO than with SVR ($p<0.0005$), but less than with TAC ($p<0.05$-$p<0.0005$).

Conclusions: In groups of African ancestry living in Africa, hypertension due to increases in either systolic or diastolic BP is as strongly associated with increases in systemic flow (SV, Q) as with arterial and arteriolar effects (Zc, TAC, SVR).

Key words: Hypertension, systemic flow, characteristic impedance, total arterial compliance, systemic vascular resistance.

4.1 Introduction

Groups of African descent are well recognised as developing hypertension which is more difficult to control, often requiring several drug combinations to manage BP (Sareli et al., 2001; Flack et al., 2010). This effect is thought to be mediated through a relatively distinct mechanism accounting for an increased BP in this ethnic group. Indeed, groups of African ancestry more frequently develop a volume-dependent, low-renin form of hypertension with limited responses to renin-angiotensin system blockers (Luft et al., 1991; Freis et al., 1988; ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group, 2002). Although a renal mechanism resulting in fluid retention explains an increased BP in this ethnic group (Aviv et al., 2004), older studies more often report increases in systemic vascular resistance (SVR) and aortic stiffness and not systemic blood flow (Heffernan et al., 2008; Din-Dzietham et al., 2004). Thus, although renal mechanisms contribute to fluid retention, through autoregulatory effects, an increased SVR may promote volume reduction through a pressure natriuresis. In distinct contrast to this notion however, more contemporary evidence obtained in a large community-based study in a group of African ancestry living in Africa, indicates a strong unique contribution of age-related increases in systemic blood flow to uncontrolled BP (Woodiwiss et al., 2020). The extent to which these increases in systemic blood flow contribute to different aspects (systolic or diastolic BP) of BP control nevertheless requires consideration.

It is well accepted that increases in systolic BP in hypertension are associated with an enhanced central arterial (aortic) stiffness (Nichols et al., 2011). An enhanced aortic stiffness increases resistance to flow in a pulsatile system in the absence of wave reflection (characteristic impedance, Zc) and decreases total arterial compliance (TAC) (Nichols et al., 2011; Yano et al., 2017). However, in addition to large artery changes contributing to an enhanced BP, increases in systemic blood flow have been demonstrated to associate with isolated systolic hypertension (ISH) at either a younger or older adult age (McEniery et al., 2005; Pasierski et al., 199). In contrast, the form of hypertension most commonly encountered over a middle age (systolic and diastolic hypertension) is associated with increases in SVR, whilst systemic flow remains unchanged (Yano et al., 2017). As hypertension often occurs over a young-to-middle age in groups of African ancestry, the question arises as to whether the impact of age-related increases in systemic blood flow on BP in Africa (Woodiwiss et al., 2020) largely affects those with ISH and less commonly

affects those with both systolic and diastolic hypertension, a more common form of hypertension in Africa. To address this question, I therefore compared the hemodynamic correlates of BP in those with untreated or inadequately controlled systolic and diastolic hypertension, ISH or isolated diastolic hypertension (IDH) with the hemodynamic correlates of BP in those with normotensive BP values. I performed this study in the community sample of African ancestry previously demonstrated to show an important contribution of increases in systemic blood flow to an uncontrolled BP (Woodiwiss et al., 2020).

4.2 Methods

4.2.1 Study group

The present study was conducted according to the principles outlined in the Helsinki declaration. The Committee for Research on Human Subjects of the University of the Witwatersrand approved the protocol (approval number: M02-04-72 and renewed as M07-04-69, M12-04-108 and M17-04-01). Participants gave informed, written consent. The present study design has previously been described (Booysen et al., 2015; Norton et al., 2012; Woodiwiss et al., 2009). In the present substudy 1062 participants from randomly recruited (from the population census figures of 2001) families of black African descent (Nguni and Sotho chiefdoms) from the South West Township (SOWETO) of Johannesburg, South Africa, with siblings older than 16 years of age, had echocardiographic evaluations and central arterial assessments. Overall 824 of these participants had high quality velocity measurements in the outflow tract and 725 of these were either normotensive or had untreated or treated but inadequately controlled hypertension.

4.2.2 Clinical and demographic information

A questionnaire was administered to obtain demographic and clinical data (Booysen et al., 2015; Norton et al., 2012; Woodiwiss et al., 2009). Height and weight were measured using standard approaches and participants were considered to be overweight if their body mass index (BMI) was ≥25 kg/m^2 and obese if their BMI was ≥30 kg/m^2. Laboratory blood tests of renal function, liver function, blood glucose, haematological parameters, and percentage glycated

haemoglobin (HbA1c) were performed. Diabetes mellitus (DM) was defined as the use of insulin or oral glucose lowering agents or an HbA1c value greater than 6.5%. Serum creatinine concentrations were measured using the Advia Chemistry systems (Siemens) with calibration traceable to isotope dilution mass spectrometry (IDMS) (Kolkenbeck-Ruh et al., 2019). The 4-variable CKD-EPI equation was employed to estimate GFR (eGFR) (Kolkenbeck-Ruh et al., 2019). High quality office brachial blood pressure (BP) measurements were obtained in the seated position and after 5 minutes of rest, by a trained nurse-technician using a standard mercury sphygmomanometer (Woodiwiss et al., 2009) according to guidelines. The mean of 5 measurements obtained at least 30 seconds apart was taken as office BP. Hypertension was defined as a mean office SBP≥140 mm Hg or DBP≥90 mm Hg. Untreated or treated but inadequately controlled hypertension was defined as either a mean office BP≥140 mm Hg systolic or ≥90 mm Hg diastolic BP in the absence or presence of antihypertensive therapy respectively. Systolic-diastolic hypertension and ISH or IDH were defined from standard approaches. In separate analyses, untreated or treated but inadequately controlled hypertension was defined as either a mean office BP≥130 mm Hg systolic or ≥80 mm Hg diastolic BP, as per the American Heart Association guidelines. Systolic-diastolic hypertension and ISH or IDH were defined from standard approaches, but using BP≥130 mm Hg systolic or ≥80 mm Hg diastolic BP as the threshold.

4.2.3 Hemodynamics

Arterial hemodynamics were obtained from pulse wave analysis as previously described (Woodiwiss et al., 2020; Booysen et al., 2015; Norton et al., 2012) and from echocardiographic aortic velocity and diameter measurements in the left ventricular outflow tract as described (Woodiwiss et al., 2020; Mitchell et al., 2010*a*). 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 9.0 software (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia). The pulse wave was calibrated by manual measurement (auscultation) of brachial BP taken immediately before the recordings. The peripheral pressure waveform was converted into a central

aortic waveform using a validated generalized transfer function incorporated in SphygmoCor software. 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. Next, aortic velocity and diameter measurements were obtained by an experienced observer (AJW) in the left lateral decubitus position using an Acuson SC2000 Diagnostic ultrasound system (Siemens Medical Solutions, USA, Inc.). Velocity waveforms were obtained in the 5-chamber view. Aortic diameter measurements were obtained just proximal to the aortic leaflets in the long axis parasternal view. The largest diameter recorded in early systole was employed to construct the flow waveform (Woodiwiss et al., 2020; Mitchell et al., 2010*a*). Aortic flow (Q) waveforms were generated from aortic velocity and diameter measurements (Woodiwiss et al., 2020; Mitchell et al., 2010*a*). Taking care to avoid any overshoot of the image, the leading (outer) edge or the most dense, or brightest, portion of the spectral image of the velocity waveform was outlined using graphics software and using aortic diameter measurements employed to construct a flow waveform. Characteristic impedance (Zc) was calculated in the time domain as change in central arterial pressure/change in flow from the foot of the pulse wave up until 95% of peak flow (Woodiwiss et al., 2020; Mitchell et al., 2010*a*; Segers et al., 2017). Stroke volume (SV) was determined from the product of the velocity-time integral of the aortic velocity wave and aortic root diameter as well as from the difference between end diastolic and systolic volumes determined from the biplane Simpson approach. Total arterial compliance (TAC) was calculated as SV/central arterial pulse pressure (PP). Systemic vascular resistance (SVR) was calculated from mean arterial pressure (MAP) and cardiac output (MAP=SVR x CO), assuming right atrial pressure = 0 mm Hg. Cardiac output was determined from SV x heart rate.

4.2.4 Data analysis

For database management and statistical analysis, SAS software, version 9.4 (SAS Institute Inc., Cary, NC) was employed. Continuous variables are expressed as mean (±SD or SEM). Dichotomous variables are expressed as percentages. Multivariate analysis of covariance was performed to compare variables (continuous data) across the groups. Multivariate adjusted logistic regression analysis was performed to determine the relative contribution of hemodynamic factors to hypertension. Odds ratios were compared using z statistics. The covariates (adjustors) included

in the multivariate regression analyses were age, sex, regular alcohol intake, regular tobacco intake, BMI, diabetes mellitus and/or an HbA1c>6.5%, renal function (eGFR), heart rate and drug class (except for in those not receiving anti-hypertensive therapy). The drug classes were defined as, low dose thiazide diuretic monotherapy, dihydropyridine type calcium channel blocker monotherapy or dual therapy with alternative agents (mostly low-dose thiazide diuretic), angiotensin converting enzyme inhibitor monotherapy or dual therapy (mostly low-dose thiazide diuretic), and other classes (including beta-blockers [one participant]) of agents. To remove the contribution of BP itself, further analyses were performed including MAP as an adjustor. To determine probability values, further adjustments for non-independence of family members was performed using non-linear regression analysis (mixed procedure as defined in the SAS package). As antihypertensive therapy may influence the results, separate sensitivity analyses was performed in those not receiving anti-hypertensive therapy (untreated) and in those receiving anti-hypertensive therapy (treated but inadequately controlled).

4.3 Results

4.3.1 Characteristics of hypertensives versus normotensives

Participants without high quality aortic velocity measurements in the outflow tract showed similar characteristics as those with these measures (Table 4.1). The characteristics of normotensives and untreated or inadequately controlled hypertensives are given in Table 4.2. Irrespective of whether hypertension was associated with increases in systolic and diastolic BP (Syst-diast HT), ISH or IDH, participants with hypertension were older, more obese and had more diabetes mellitus. Participants with ISH were older than normotensives or those with Syst-diast HT or IDH and consequently had more diabetes mellitus than those with Syst-diast HT or IDH. Irrespective of the type of hypertension, less than 50% of these individuals were receiving antihypertensive medication and they were predominantly receiving diuretics. Importantly, no differences were noted between the hypertensive groups (Syst-diast HT, ISH or IDH), in either

Table 4. 1 Characteristics of normotensives and untreated or inadequately controlled hypertensive (SBP≥140 mm Hg or DBP≥90 mm Hg) participants with and without high quality measurements in the outflow tract.

	With	Without	p-value
n=	725	238	
Age (years)	44.6±18.2	44.8±18.2	=0.94
Sex (% male)	66.2	63.8	=0.56
Body mass index (kg/m^2)	29.1±7.5	30.2±8.0	=0.14
% Overweight/obese	25.5/41.8	18.3/48.3	=0.10
% Treated for hypertension	17.7	26.3	=0.006
% Treated with thiazide diuretics	15.7	22.9	=0.04
% Diabetes mellitus (DM)	9.7	14.2	=0.054
% Regular smokers	16.0	13.8	=0.51
% Regular alcohol intake	20.6	21.7	=0.69
Systolic blood pressure (mm Hg)	130±23	129±22	=0.85
Diastolic blood pressure (mm Hg)	83±13	84±13	=0.26
Mean arterial pressure (mm Hg)	100±16	101±16	=0.74
Pulse pressure (mm Hg)	46±16	45±15	=0.52
% Normotension	59.9	46.3	=0.0005
% Isolated systolic hypertension	9.2	5.0	=0.06
% Increased systolic and diastolic BP	19.6	23.5	=0.22
% Isolated diastolic BP	11.3	14.3	=0.27
% Controlled hypertension	0	10.9	<0.0001

Data are shown as mean±SD or proportions.

Table 4. 2 Participant characteristics.

	Normotensives	Untreated or inadequately controlled hypertensives (HT)		
		Syst-diast	Isolated systolic (ISH)	Isolated diastolic (IDH)
n=	434	142	67	82
Age (years)	37.0±15.6	58.9±13.3***	63.7±13.9***†	44.5±15.1***†††‡‡
Sex (% male)	32.3	32.4	32.8	45.1
Body mass index (kg/m^2)	27.4±7.1	32.4±7.3***	30.8±7.6***	30.7±8.0***
% Overweight/obese	28.1/32.0	19.7*/63.4***	19.4/53.7**	26.8/46.3*†
Heart rate (beats/min)	66.8±12.2	67.0±11.2	68.3±12.3	67.8±13.3
Estimated GFR (ml/min/1.73m^2)	104.8±19.9	86.2±19.7***	80.9±22.5***	97.4±20.0**†††‡
Adjusted[§] estimated GFR (ml/min/1.73m^2)	98.1±16.7	98.8±16.7	98.1±17.2	97.2±16.3
% Treated for hypertension	0	47.9***	47.8 ***	34.1***
% Treated with thiazide diuretics	0	44.4 ***	40.3***	28.1***†
% Treated with 1 anti-hypertensive agent	0	26.1***	35.8***	19.5***
% Treated with 2 anti-hypertensive agents	0	15.5***	9.0*	13.4**
% Treated with 3 anti-hypertensive agents	0	5.6**	1.5	1.2
% Treated with 4 anti-hypertensive agents	0	0.7	1.5	0
% Treated with thiazide diuretic monotherapy	0	24.7***	29.9***	18.3***
% Treated with CCB mono or dual therapy	0	6.3*	7.4*	3.7*
% Treated with ACEI mono or dual therapy	0	13.4**	9.0*	8.5*

% Treated with BB mono or dual therapy	0	0	0	1.2
% Treated with other classes mono or dual therapy	0	3.5*	1.5	2.4
% Diabetes mellitus (DM)	4.4	15.5***	29.9***†	11.0*‡
% Regular smokers	5.4	18.3	14.9	15.9
% Regular alcohol intake	21.2	19.7	14.9	23.2

Data are shown as mean±SD or proportions. Syst-diast, Systolic-diastolic; dual therapy, specified anti-hypertensive agent together with alternative anti-hypertensive agents (mostly low-dose thiazide diuretic); CCB, dihydropyridine type calcium-channel blocker; ACEI, angiotensin-converting enzyme inhibitor; BB, beta-blocker. § adjusted for age, sex, BMI, regular smoking, regular alcohol and diabetes mellitus, *p<0.05, **p<0.001, ***p<0.0005 versus NT; †p<0.05,††p<0.0001 versus Syst-diast HT; ‡p<0.005, ‡‡p<0.0001 versus ISH.

the number of antihypertensive agents or the class of antihypertensive agents. Although, reduced renal function was noted in all of the hypertensive groups compared to the normotensives, these differences were no longer evident after the correction for differences in age, BMI and diabetes mellitus between the groups. In sensitivity analysis, similar differences were noted in untreated hypertensives as compared to normotensives (Table 4.3), and in treated but inadequately controlled hypertensives as compared to normotensives (Table 4.4). Furthermore, when hypertension was defined according to a threshold of ≥130/80 mm Hg similar differences were noted in untreated or inadequately controlled hypertensives as compared to normotensives (Table 4.5).

4.3.2 Blood pressures

Without or with adjustments for age, sex and standard risk factors for hypertension, participants with untreated or inadequately controlled Syst-diast HT, ISH and IDH showed marked increases in BP (except for PP in IDH) as compared to normotensives (Table 4.6). In sensitivity analysis conducted in those not receiving antihypertensive therapy, similar differences were noted in untreated hypertensives as compared to normotensives (Table 4.7), in treated but inadequately controlled hypertensives compared to normotensives (Table 4.8), and in untreated or inadequately controlled hypertensives defined according to a threshold of ≥130/80 mm Hg compared to normotensives (Table 4.9).

4.3.3 Systemic blood flow in hypertensives

Without or with adjustments for age, sex, standard risk factors for hypertension and anti-hypertensive drug class, participants with untreated or inadequately controlled Syst-diast HT and ISH but not IDH showed marked increases in SV, CO and peak aortic Q (Figure 4.1 [all participants], figure 4.2 [untreated participants]). Increases in SV and CO in participants with untreated or inadequately controlled Syst-diast HT and ISH were noted irrespective of whether expressed per body surface area or not (Figures 4.1 and 4.2). Moreover, increases in SV and CO in participants with untreated or inadequately controlled Syst-diast HT and ISH were noted irrespective of whether calculated from outflow tract velocities and diameters or left ventricular

Table 4. 3 Characteristics of untreated hypertensives (SBP≥140 mm Hg or DBP≥90 mm Hg) versus normotensives.

	Normotensives	Untreated hypertensives (HT)		
		Syst-diast	Isolated systolic (ISH)	Isolated diastolic (IDH)
n=	434	74	35	54
Age (years)	37.0±15.6	55.3±13.3***	58.7±15.3***	39.8±13.5†††‡‡
Sex (% male)	32.3	45.9*	45.7	55.6**
Body mass index (kg/m^2)	27.4±7.1	31.2±7.1***	28.0±7.5†	28.4±7.1†
% Overweight/obese	28.1/32.0	23.0/56.8***	14.3/40.0†	29.6/33.3†
Heart rate (beats/min)	66.8±12.2	66.8±11.7	65.7±13.2	64.7±8.5
Estimated GFR (ml/min/1.73m^2)	104.8±19.9	90.0±18.1***	90.8±20.3***	102.5±17.2†††‡
Adjusted§ estimated GFR (ml/min/1.73m^2)	101.6±14.6	102.3±16.3	105.6±16.0	102.0±15.4
% Diabetes mellitus (DM)	4.4	8.1	22.9***†	3.7‡
% Regular smokers	15.4	25.7	25.7	18.5
% Regular alcohol intake	21.2	27.0	22.9	31.5

Data are shown as mean±SD or proportions. Syst-diast, Systolic-diastolic. See Table 4.2 for additional abbreviations. § Adjusted for age, sex, BMI, regular smoking, regular alcohol and diabetes mellitus, *p<0.05, **p<0.005, ***p<0.0001 versus NT; †p<0.05,††p<0.005, †††p<0.0001 versus Syst-diast HT; ‡p<0.005, ‡‡p<0.0001 versus ISH.

Table 4. 4 Characteristics of treated but inadequately controlled hypertensives (SBP≥140 mm Hg or DBP≥90 mm Hg) versus normotensives.

	Normotensives	Treated but inadequately controlled hypertensives (HT)		
		Syst-diast	Isolated systolic (ISH)	Isolated diastolic (IDH)
n=	434	68	32	28
Age (years)	37.0±15.6	62.8±12.3***	69.2±9.8***†	53.6±13.9***††‡‡
Sex (% male)	32.3	17.7*	18.8	25.0
Body mass index (kg/m^2)	27.4±7.1	33.7±7.3***	33.9±6.5***	35.2±7.8***
% Overweight/obese	28.1/32.0	16.2*/70.6***	25.0/68.8***	21.4/71.4***
Heart rate (beats/min)	66.8±12.2	67.1±10.8	71.1±10.6	73.8±18.3**†
Estimated GFR (ml/min/1.73m^2)	104.8±19.9	82.3±20.5***	70.7±19.8***††	87.8±19.8***‡
Adjusted§ estimated GFR (ml/min/1.73m^2)	100.0±16.7	98.9±17.3	93.3±17.0	96.5±15.9
% Treated with thiazide diuretics	0	92.7***	84.3***	82.4***
% Treated with 1 anti-hypertensive agent	0	54.4***	75.0***	57.1***
% Treated with 2 anti-hypertensive agents	0	32.3***	18.8**	39.3***
% Treated with 3 anti-hypertensive agents	0	11.8**	3.1	3.6
% Treated with 4 anti-hypertensive agents	0	1.5	3.1	0
% Treated with thiazide diuretic monotherapy	0	51.5***	62.5***	53.6***
% Treated with CCB mono or dual therapy	0	13.2**	15.6**	10.7*
% Treated with ACEI mono or dual therapy	0	27.9***	18.8**	25.0**

% Treated with BB mono or dual therapy	0	0	0	3.6
% Treated with other classes mono or dual therapy	0	7.4*	3.1	7.1*
% Diabetes mellitus (DM)	4.4	23.5***	37.5***	25.0***
% Regular smokers	15.4	10.3	3.1	10.7
% Regular alcohol intake	21.2	11.8	6.3*	7.1

Data are shown as mean±SD or proportions. Syst-diast, Systolic-diastolic. See Table 4.2 for additional abbreviations. [§]Adjusted for age, sex, BMI, regular smoking, regular alcohol and diabetes mellitus, *p<0.05, **p<0.005, ***p<0.0001 versus NT; [†]p<0.05,[††]p<0.005, [†††]p<0.0001 versus Syst-diast HT; [‡]p<0.005, [‡‡]p<0.0001 versus ISH.

Table 4. 5 Characteristics of untreated or inadequately controlled hypertensives (SBP≥130 mm Hg or DBP≥80 mm Hg) versus normotensives.

	Normotensives	Untreated or inadequately controlled hypertensives (HT)		
		Syst-diast	Isolated systolic (ISH)	Isolated diastolic (IDH)
n=	239	291	30	165
Age (years)	35.0±14.5	54.6±16.4***	69.2±9.8***†	53.6±13.9***†††‡‡
Sex (% male)	28.5	33.3	18.8	25.0
Body mass index (kg/m^2)	26.5±6.8	31.5±7.5***	33.9±6.5***	35.2±7.8***
% Overweight/obese	27.6/27.6	23.7/55.7***	25.0/68.8***	21.4/71.4***
Heart rate (beats/min)	66.9±12.4	67.6±11.2	71.1±10.6	73.8±18.3**†
Estimated GFR (ml/min/1.73m^2)	107.2±20.0	90.7±21.1***	70.7±19.8***††	87.8±19.8***‡
Adjusted[§] estimated GFR (ml/min/1.73m^2)	98.6±17.0	99.5±17.1	93.3±17.0	96.5±15.9
% Treated for hypertension	0	38.8***	23.3**	4.8*††††‡
% Treated with thiazide diuretics	0	36.1***	23.3**	4.2*
% Treated with 1 anti-hypertensive agent	0	21.6***	23.3**	4.2*
% Treated with 2 anti-hypertensive agents	0	13.4**	0†	0†
% Treated with 3 anti-hypertensive agents	0	3.1	0	0.6
% Treated with 4 anti-hypertensive agents	0	0.7	0	0
% Treated with thiazide diuretic monotherapy	0	19.6**	23.3**	3.6
% Treated with CCB mono or dual therapy	0	5.5*	0†	0.6

% Treated with ACEI mono or dual therapy	0	10.6**	0	0.6
% Treated with BB mono or dual therapy	0	0.3	0	0
% Treated with other classes mono or dual therapy	0	2.8	0	0
% Diabetes mellitus (DM)	2.9	14.8***	37.5***	25.0***
% Regular smokers	16.3	16.2	3.1	10.7
% Regular alcohol intake	20.5	21.3	6.3*	7.1

Data are shown as mean±SD or proportions. Syst-diast, Systolic-diastolic. See Table 4.2 for additional abbreviations. [§]Adjusted for age, sex, BMI, regular smoking, regular alcohol and diabetes mellitus, *p<0.05, **p<0.005, ***p<0.0001 versus NT; [†]p<0.05,[††]p<0.005, [†††]p<0.0001 versus Syst-diast HT; [‡]p<0.005, [‡‡]p<0.0001 versus ISH.

Table 4. 6 Blood pressures in normotensives and untreated or inadequately controlled hypertensives.

	Normotensives (NT)	Untreated or inadequately controlled hypertensives (HT)		
		Syst-diast	Isolated systolic (ISH)	Isolated diastolic (IDH)
		Unadjusted		
n=	434	142	67	82
Systolic blood pressure (mm Hg)	116±11	162±18***	152±18***†	130±7***†‡
Diastolic blood pressure (mm Hg)	76±8	101±11***	81±6***†	94±4***†‡
Mean arterial pressure (mm Hg)	90±8	124±12***	107±8***†	107±4***†
Pulse pressure (mm Hg)	39±9	60±15***	71±20***†	37±9†‡
		Adjusted		
Systolic blood pressure (mm Hg)	117±15	159±14***	149±14***†	129±13***†‡
Diastolic blood pressure (mm Hg)	77±8	100±8***	80±8**†	93±8***†‡
Mean arterial pressure (mm Hg)	91±8	122±10***	106±9***†	107±9***†
Pulse pressure (mm Hg)	40±12	58±13***	68±12***†	37±12*†‡

Data shown are mean±SD. Syst-diast, Systolic-diastolic. Adjustments are for age, sex, BMI, regular smoking, regular alcohol and diabetes mellitus. *p<0.02, **p<0.001, ***p<0.0001 versus NT; †p<0.0001 versus Syst-diast HT; ‡p<0.0001 versus ISH.

Table 4. 7 Blood pressures in untreated hypertensives (SBP≥140 mm Hg or DBP≥90 mm Hg) versus normotensives.

	Normotensives	Untreated hypertensives (HT)		
		Syst-diast	Isolated systolic (ISH)	Isolated diastolic (IDH)
Unadjusted				
n=	434	74	35	54
Systolic blood pressure (mm Hg)	116±11	161±17***	153±20***†	130±7***†††‡
Diastolic blood pressure (mm Hg)	76±8	101±13***	81±6***††	94±4***†††‡
Mean arterial pressure (mm Hg)	90±8	124±13***	107±10***††	107±4***††
Pulse pressure (mm Hg)	39±9	59±14***	72±22***††	37±8†††‡
Adjusted				
Systolic blood pressure (mm Hg)	116±13	158±13***	150±13***†	129±12***†††‡
Diastolic blood pressure (mm Hg)	76±8	100±8***	81±8**††	93±8***†††‡
Mean arterial pressure (mm Hg)	91±9	122±9***	106±9***††	107±9***††
Pulse pressure (mm Hg)	40±11	57±12***	70±11***††	36±11*†††‡

Data shown are mean±SD. Syst-diast, Systolic-diastolic. Adjustments are for age, sex, BMI, regular smoking, regular alcohol and diabetes mellitus. *p<0.05, **p<0.005, ***p<0.0005 versus NT; †p<0.005,††p<0.0001 versus Syst-diast HT; ‡p<0.0001 versus ISH.

Table 4. 8 Blood pressures in treated but inadequately controlled hypertensives (SBP≥140 mm Hg or DBP≥90 mm Hg) versus normotensives.

	Normotensives	Treated but inadequately controlled hypertensives (HT)		
		Syst-diast	Isolated systolic (ISH)	Isolated diastolic (IDH)
Unadjusted				
n=	434	68	32	28
Systolic blood pressure (mm Hg)	116±11	163±18***	151±15***††	130±7***†††‡
Diastolic blood pressure (mm Hg)	76±8	100±9***	80±6**†	93±5***†††‡
Mean arterial pressure (mm Hg)	90±8	123±11***	107±6***††	107±5***††
Pulse pressure (mm Hg)	39±9	61±16***	70±17***††	38±11†††‡
Adjusted				
Systolic blood pressure (mm Hg)	117±12	159±13***	146±13***††	127±12***†††‡
Diastolic blood pressure (mm Hg)	77±8	99±8***	77±8††	92±7***†††‡
Mean arterial pressure (mm Hg)	91±8	121±9***	103±9***††	105±8***††
Pulse pressure (mm Hg)	40±10	58±11***	66±12***†	36±11†††‡

Data shown are mean±SD. Syst-diast, Systolic-diastolic. Adjustments are for age, sex, BMI, regular smoking, regular alcohol and diabetes mellitus. *p<0.05, **p<0.005, ***p<0.0005 versus NT; †p<0.005,††p<0.0001 versus Syst-diast HT; ‡p<0.0001 versus ISH.

Table 4. 9 Blood pressures in untreated or treated but inadequately controlled hypertensives (SBP≥130 mm Hg or DBP≥80 mm Hg) versus normotensives.

	Normotensives	Untreated or inadequately controlled hypertensives (HT)		
		Syst-diast	Isolated systolic (ISH)	Isolated diastolic (IDH)
Unadjusted				
n=	239	291	30	165
Systolic blood pressure (mm Hg)	110±10	150±18***	147±25***	119±7***†††‡‡
Diastolic blood pressure (mm Hg)	71±6	94±11***	72±5†††	84±5***†††‡‡
Mean arterial pressure (mm Hg)	85±7	115±13***	98±11***†††	97±5***†††
Pulse pressure (mm Hg)	38±9	55±15***	72±27***†††	35±7**†††‡‡
Adjusted				
Systolic blood pressure (mm Hg)	112±15	147±15***	144±14***	121±14***†††‡‡
Diastolic blood pressure (mm Hg)	72±9	93±9***	72±8†††	84±8***†††‡‡
Mean arterial pressure (mm Hg)	86±10	113±10***	97±9***†††	98±9***†††
Pulse pressure (mm Hg)	41±13	53±13***	69±12***†††	36±13**†††‡‡

Data shown are mean±SD. Syst-diast, Systolic-diastolic. Adjustments are for age, sex, BMI, regular smoking, regular alcohol and diabetes mellitus. *p<0.05, **p<0.005, ***p<0.0005 versus NT; †p<0.005,††p<0.0001 versus Syst-diast HT; ‡p<0.0001 versus ISH.

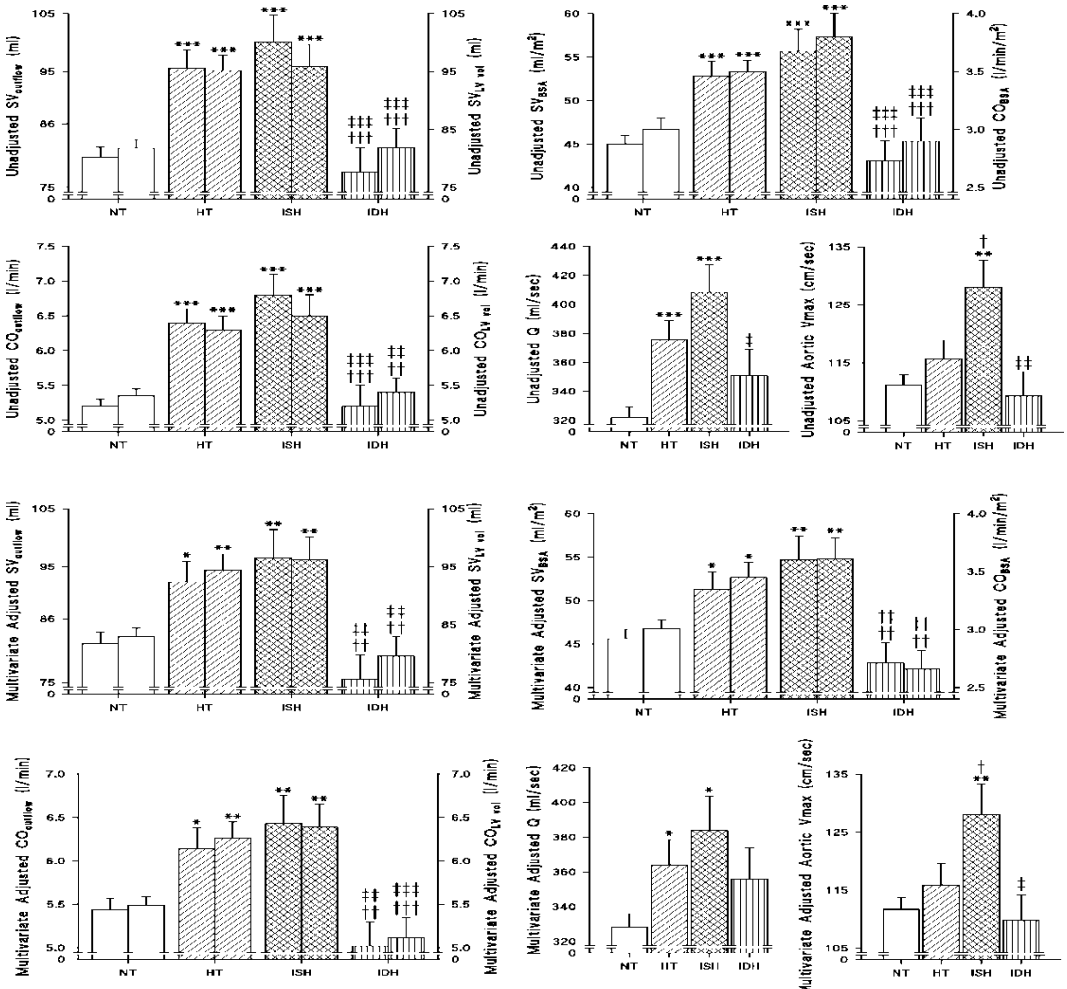


Figure 4. 1 Systemic blood flow in untreated or inadequately controlled hypertensives (SBP≥140 mm Hg or DBP≥90 mm Hg) versus normotensives in a community sample of African ancestry. Data shown are unadjusted and multivariate adjusted mean±SEM. See Table 4.2 for sample sizes. NT, normotensives; HT, untreated or inadequately controlled systolic and diastolic blood pressure; ISH, untreated or inadequately controlled isolated systolic hypertension; IDH, isolated increases in diastolic blood pressure (isolated diastolic hypertension); SV, stroke volume: SV_{BSA}, SV per body surface area: $SV_{outflow}$, SV calculated from outflow tract measurements; $SV_{LV\ vol}$, SV calculated from LV volumes; CO, cardiac output; CO_{BSA}, CO per body surface area; $CO_{outflow}$, CO where SV calculated from outflow tract measurements; $CO_{LV\ vol}$, CO where SV calculated from LV volumes; Q, peak aortic flow; Aortic Vmax, Aortic velocity employed to calculate Q. Adjustments are for age, sex, BMI, regular smoking, regular alcohol, diabetes mellitus, renal function, heart rate and drug class. *p<0.05, **p<0.005, ***p<0.0005 versus NT; †p<0.05, ††p<0.005, †††p<0.0005 versus HT; ‡p<0.05, ‡‡p<0.005, ‡‡‡p<0.0005 versus ISH.

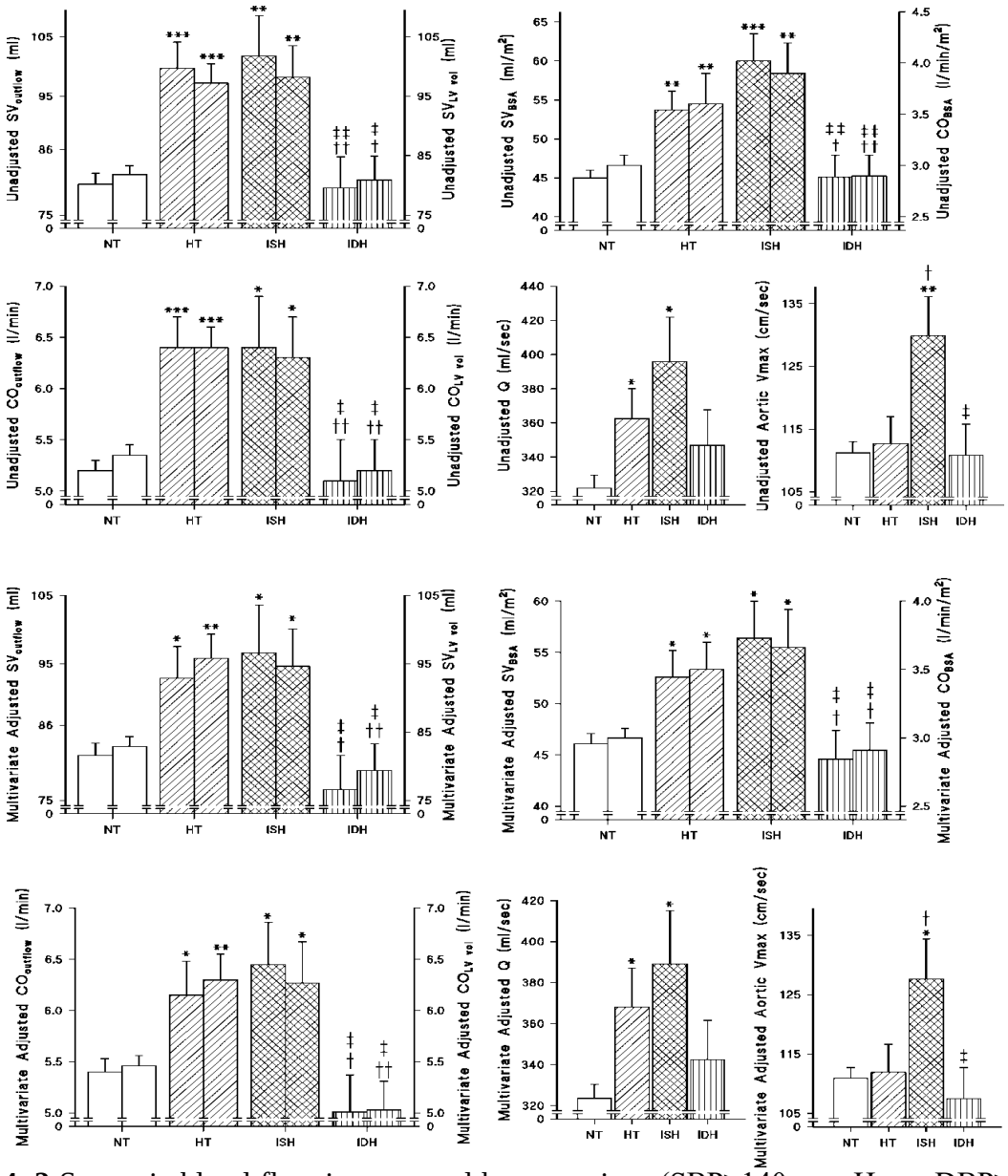


Figure 4. 2 Systemic blood flow in untreated hypertensives (SBP≥140 mm Hg or DBP≥90 mm Hg) versus normotensives in a community sample of African ancestry. Data shown are unadjusted and multivariate adjusted mean±SEM. See Table 4.3 for sample sizes and characteristics. NT, normotensives; HT, untreated increased systolic and diastolic blood pressure; ISH, untreated isolated systolic hypertension; IDH, isolated increases in diastolic blood pressure in untreated participants (isolated diastolic hypertension). Adjustments are for age, sex, BMI, regular smoking, regular alcohol, diabetes mellitus, renal function and heart rate. See Figure 4.1 for additional abbreviations. *p<0.05, **p<0.005, ***p<0.0005 versus NT; †p<0.05, ††p<0.005 versus HT; ‡p<0.05, ‡‡p<0.005 versus ISH.

volumes (biplane Simpson) (Figures 4.1 and 4.2). Although increases in Q in Syst-diast HT were associated with increases in aortic root diameter (Figures 4.3 and 4.4) but not velocity (Figures 4.1 and 4.2), increases in Q in ISH were associated with increases in velocity (Figures 4.1 and 4.2), but not aortic root diameter (Figures 4.3 and 4.4). In sensitivity analyses, similar differences in systemic flow were noted in treated but inadequately controlled hypertensives as compared to normotensives (Figure 4.5). Furthermore, similar differences in systemic flow were noted in untreated or inadequately controlled hypertensives as compared to normotensives when hypertension was defined according to a threshold of ≥130/80 mm Hg (Figure 4.6).

4.3.4 Vascular mechanisms in hypertensives

Without or with adjustments for age, sex, standard risk factors for hypertension and anti-hypertensive drug class, participants with untreated or inadequately controlled Syst-diast HT and IDH but not ISH showed marked increases in SVR irrespective of whether SV and hence CO and SVR were calculated from outflow tract velocities and diameters or left ventricular volumes (biplane Simpson) (Figure 4.3 [all participants, Figure 4.4 [untreated participants]). Moreover, without or with adjustments for age, sex, standard risk factors for hypertension and anti-hypertensive drug class, participants with untreated or inadequately controlled Syst-diast HT and ISH, but not IDH showed increases in Zc, with ISH showing the most striking increases (Figures 4.3 and 4.4). Importantly, increases in Zc in ISH were associated with an aortic root diameter that was not increased as compared to normotensives, while participants with Syst-diast HT had an increased aortic root diameter as compared to normotensives (Figures 4.3 and 4.4). Without or with adjustments for age, sex, standard risk factors for hypertension and anti-hypertensive drug class, participants with untreated or inadequately controlled Syst-diast HT and ISH, but not IDH also showed decreases in TAC irrespective of whether SV and hence TAC was calculated from outflow tract velocities and diameters or left ventricular volumes (biplane Simpson) (Figures 4.3 and 4.4). Importantly, once again participants with untreated or inadequately controlled ISH showed the most striking decreases in TAC (Figures 4.3 and 4.4). In sensitivity analyses, similar differences in the vascular determinants of BP were noted in treated but inadequately controlled hypertensives as compared to normotensives (Figure 4.7). Furthermore, similar differences in the vascular determinants of BP were noted in untreated or inadequately controlled hypertensives

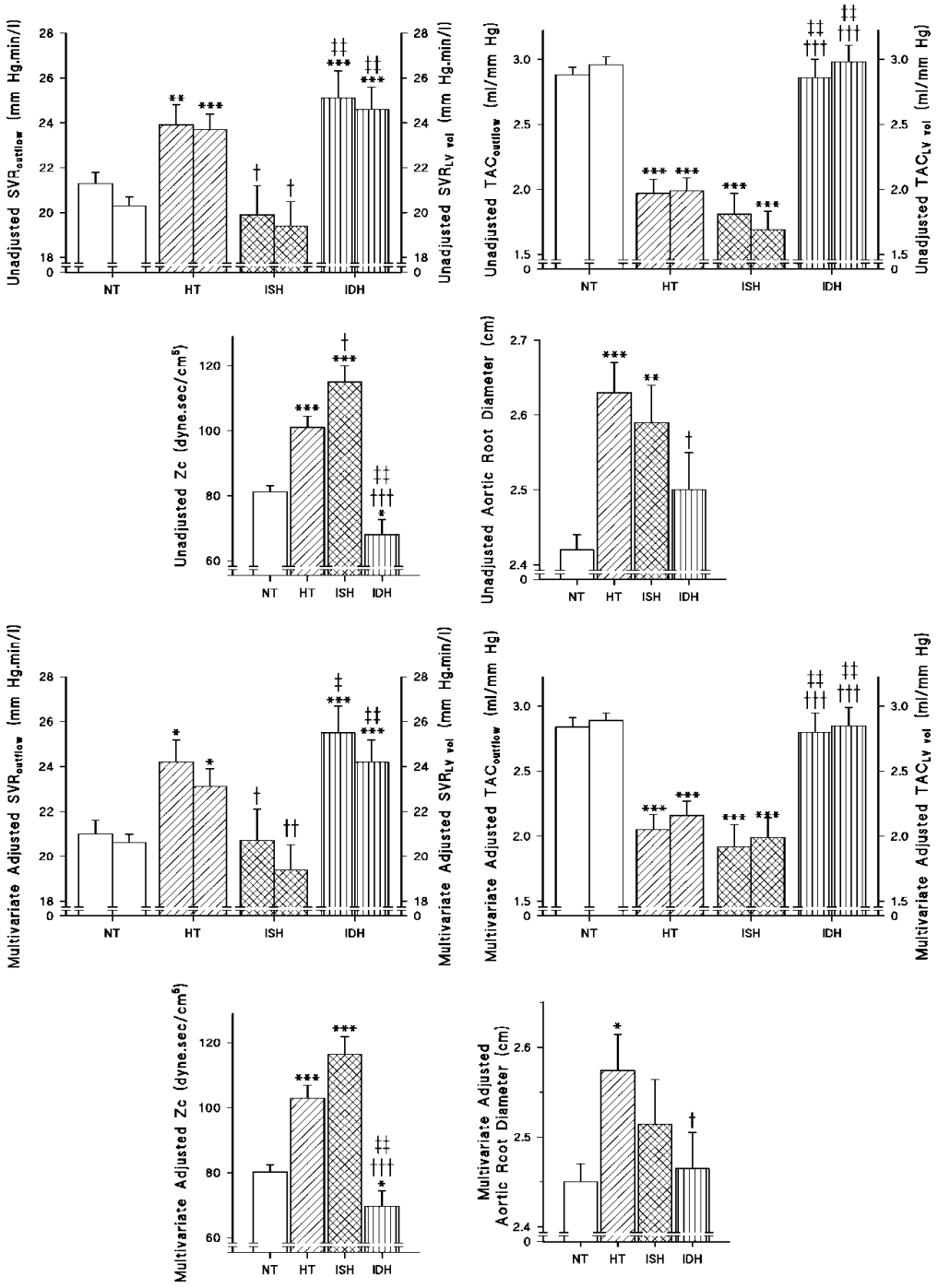


Figure 4. 3 Vascular correlates of blood pressure in untreated or inadequately controlled hypertensives (SBP≥140 mm Hg or DBP≥90 mm Hg) versus normotensives in a community sample of African ancestry. Data shown are unadjusted and multivariate adjusted mean±SEM. See Figure 4.1 for groups and adjustments, and Table 4.2 for sample sizes. SVR, systemic vascular resistance: $SVR_{outflow}$, SVR where SV and CO calculated from outflow tract measurements; $SVR_{LV\ vol}$, SVR where SV and CO calculated from LV volumes; Zc, aortic characteristic impedance; TAC,

total arterial compliance; $TAC_{outflow}$, TAC where SV calculated from outflow tract measurements; $TAC_{LV\ vol}$, TAC where SV calculated from LV volumes. *$p<0.05$, **$p<0.005$, ***$p<0.0005$ versus NT; $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.005$, $^{\dagger\dagger\dagger}p<0.0005$ versus HT; $^{\ddagger}p<0.005$, $^{\ddagger\ddagger}p<0.0005$ versus ISH.

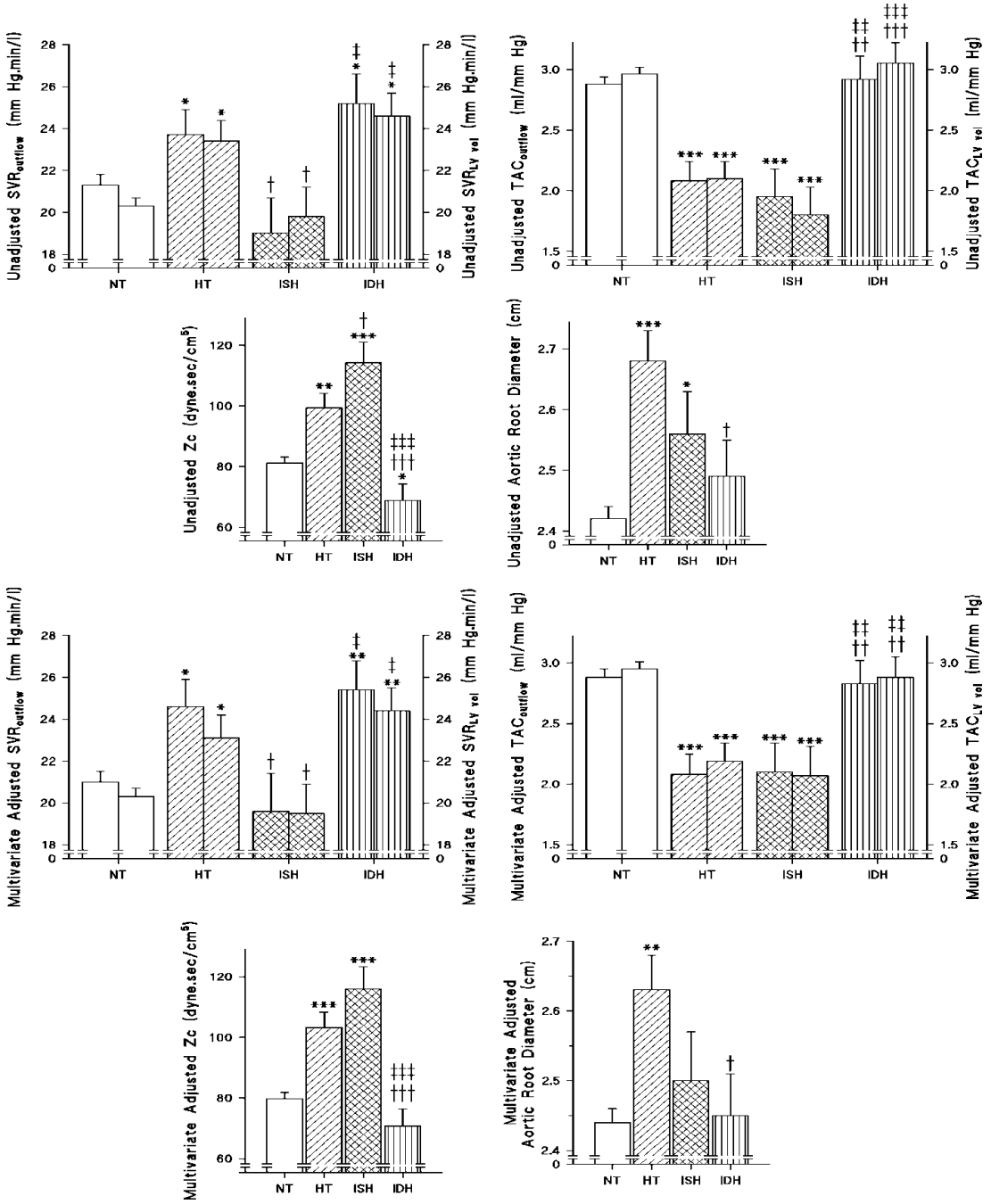


Figure 4. 4 Vascular correlates of blood pressure in untreated hypertensives (SBP≥140 mm Hg or DBP≥90 mm Hg) versus normotensives in a community sample of African ancestry. Data shown are unadjusted and multivariate adjusted mean±SEM. See Figure 4.1 for groups and adjustments, and Table 4.3 for sample sizes and characteristics. Adjustments are for age, sex, BMI, regular smoking, regular alcohol, diabetes mellitus, renal function and heart rate. See Figure 4.3 for

additional abbreviations. *p<0.05, **p<0.005, ***p<0.0005 versus NT; †p<0.05, ††p<0.005, †††p<0.0005 versus HT; ‡p<0.05, ‡‡p<0.005, ‡‡‡p<0.005 versus ISH.

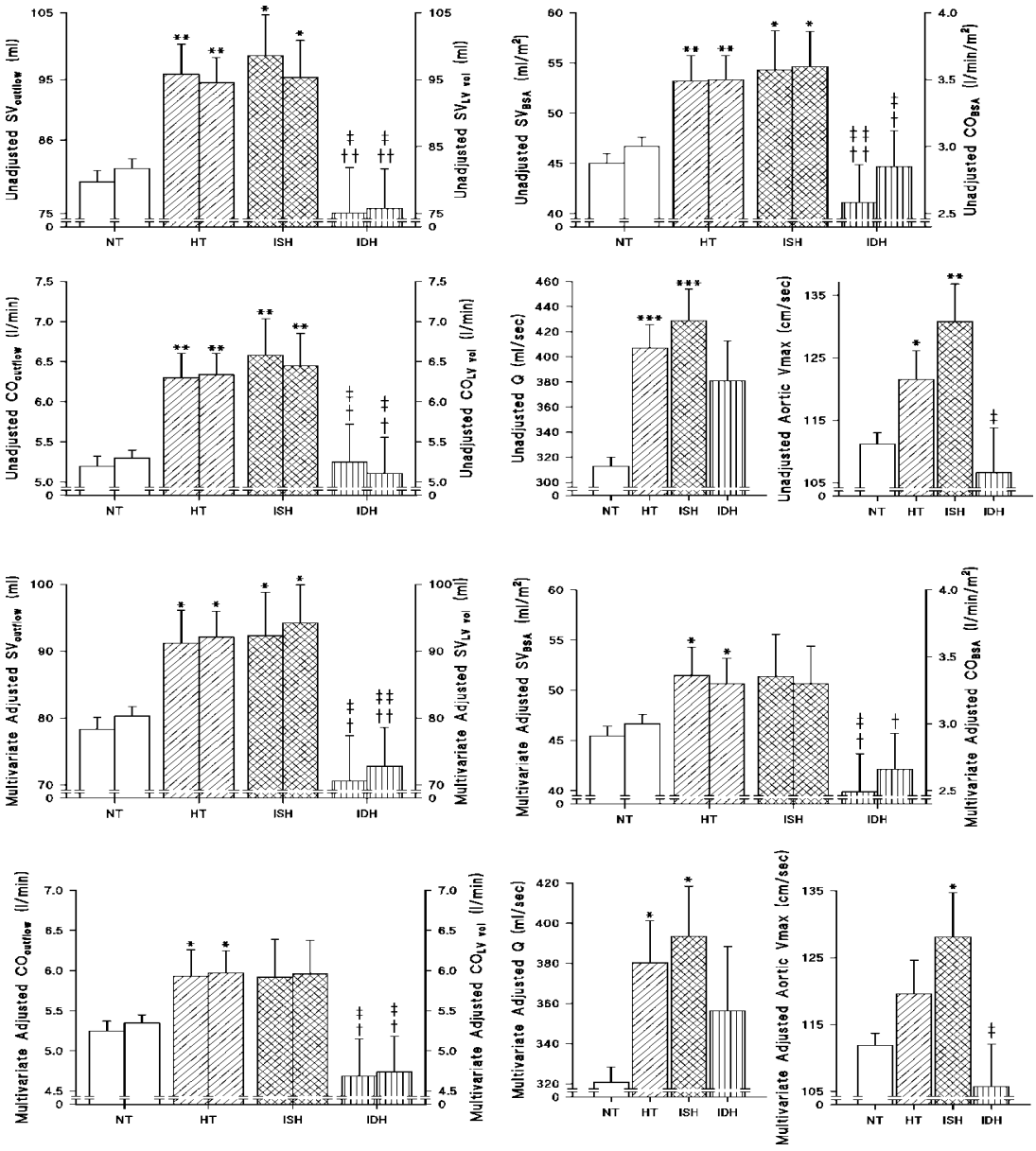


Figure 4. 5 Systemic blood flow in treated but inadequately controlled hypertensives (SBP≥140 mm Hg or DBP≥90 mm Hg) versus normotensives in a community sample of African ancestry. Data shown are unadjusted and multivariate adjusted mean±SEM. See Table 4.4 for sample sizes and characteristics. NT, normotensives; HT, treated increased systolic and diastolic blood pressure; ISH, treated isolated systolic hypertension; IDH, isolated increases in diastolic blood pressure in treated participants (isolated diastolic hypertension). Adjustments are for age, sex, BMI, regular smoking, regular alcohol, diabetes mellitus, renal function, heart rate and drug class.

See Figure 4.1 for additional abbreviations. *p<0.05, **p<0.005, ***p<0.0005 versus NT; †p<0.05, ††p<0.005 versus HT; ‡p<0.05, ‡‡p<0.005 versus ISH.

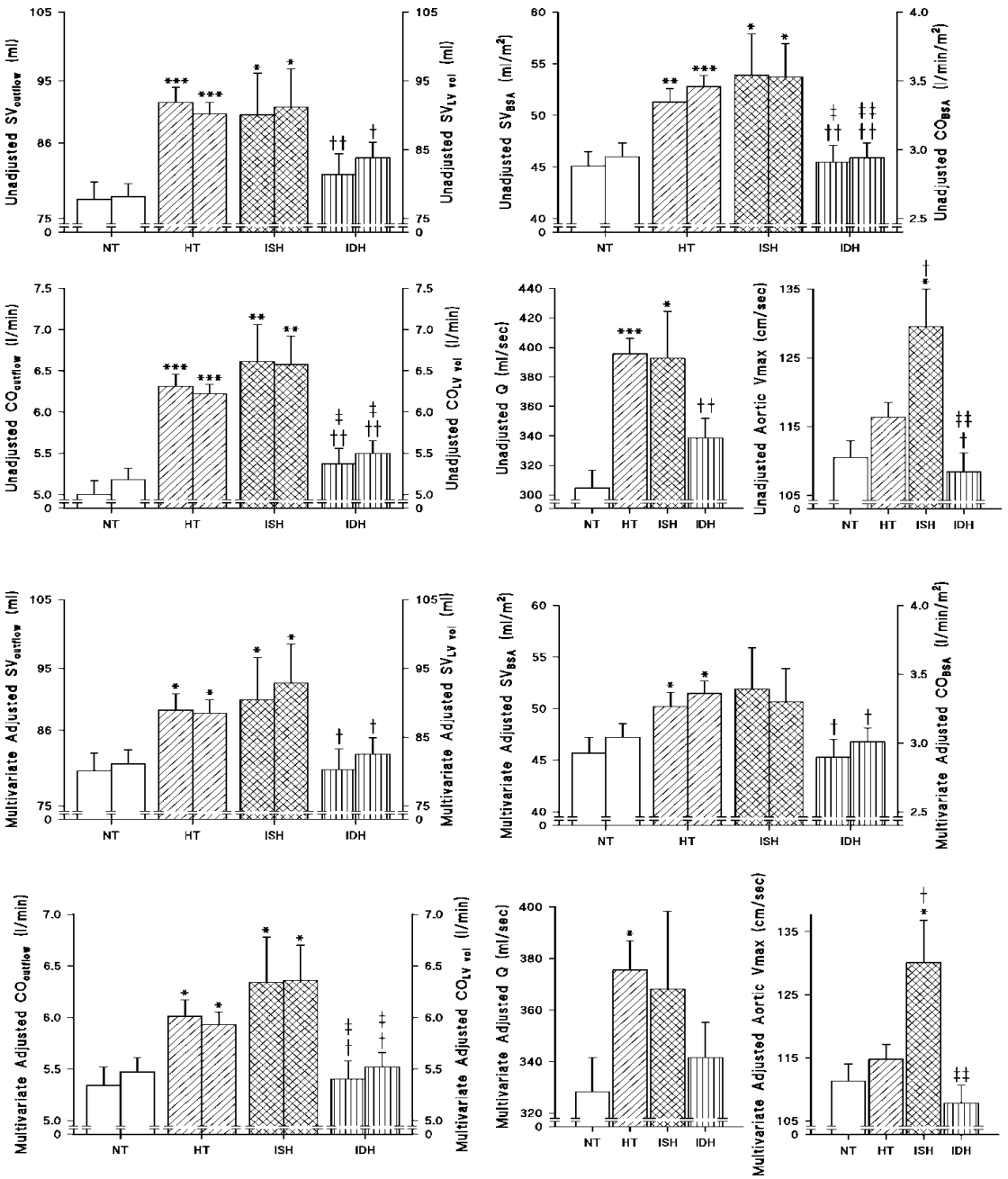


Figure 4. 6 Systemic blood flow in untreated or treated but inadequately controlled hypertensives (SBP≥130 mm Hg or DBP≥80 mm Hg) versus normotensives in a community sample of African ancestry. Data shown are unadjusted and multivariate adjusted mean±SEM. See Table 4.5 for sample sizes and characteristics. NT, normotensives; HT, untreated or inadequately controlled systolic and diastolic blood pressure; ISH, untreated or inadequately controlled isolated systolic hypertension; IDH, isolated increases in diastolic blood pressure (isolated diastolic hypertension).

Adjustments are for age, sex, BMI, regular smoking, regular alcohol, diabetes mellitus, renal function, heart rate and drug class. See Figure 4.1 for additional abbreviations. *p<0.05, **p<0.005, ***p<0.0005 versus NT; †p<0.05, ††p<0.005 versus HT; ‡p<0.05, ‡‡p<0.005 versus ISH.

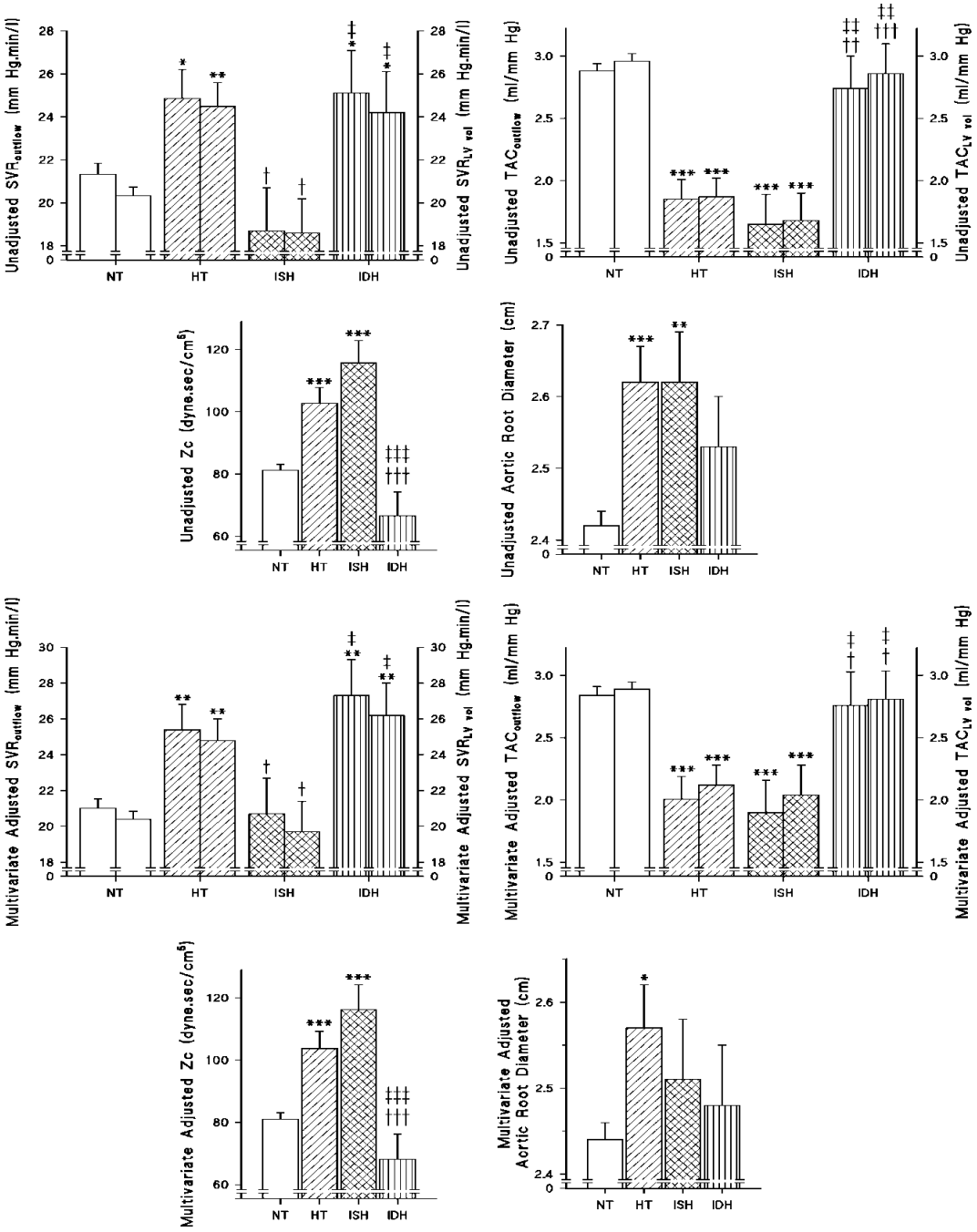


Figure 4. 7 Vascular correlates of blood pressure in treated but inadequately controlled hypertensives (SBP≥140 mm Hg or DBP≥90 mm Hg) versus normotensives in a community sample of African ancestry. Data shown are unadjusted and multivariate adjusted mean±SEM. See Table 4.4 for sample sizes and characteristics. Adjustments are for age, sex, BMI, regular smoking, regular alcohol, diabetes mellitus, renal function, heart rate and drug class. See Figure 4.3 for additional abbreviations. *p<0.05, **p<0.005, ***p<0.0005 versus NT; †p<0.05, ††p<0.005, †††p<0.0005 versus HT; ‡p<0.05, ‡‡p<0.005, ‡‡‡p<0.005 versus ISH.

as compared to normotensives when hypertension was defined according to a threshold of ≥130/80 mm Hg (Figure 4.8).

4.3.5 MAP independent systemic blood flow and vascular mechanisms in hypertensives

With adjustments for MAP in addition to age, sex, standard risk factors for hypertension and anti-hypertensive drug class, participants with untreated or inadequately controlled Syst-diast HT and ISH but not IDH still showed marked increases in SV, CO and peak aortic Q compared to normotensives (Figure 4.9). In contrast, corrections for MAP resulted in no differences in SVR, Zc or TAC between untreated or inadequately controlled Syst-diast HT and normotensives. However, in ISH, Zc remained increased, and TAC decreased compared to normotensives (Figure 4.9).

4.3.6 Relative contribution of hemodynamic factors to hypertension

In multivariate regression models with various combinations of hemodynamic factors included in the models (in addition to age, sex, BMI, regular smoking, regular drinking, diabetes mellitus, renal function, heart rate and drug class), peak aortic Q, SV or CO contributed more or as much to the Odds of untreated or inadequately controlled Syst-diast HT as did SVR, Zc or TAC (Table 4.10 [Q and SV in all participants and in untreated participants] and Table 4.11 [CO in all participants and in untreated participants]). Similarly, in multivariate regression models, peak aortic Q, SV and CO contributed more to the Odds of untreated or inadequately controlled ISH than did SVR, but less than did TAC (Table 4.10 [Q and SV in all participants and in untreated participants] and Table 4.11 [CO in all participants and in untreated participants]); whereas SVR contributed more to the Odds of untreated or inadequately controlled IDH than did SV, CO or Q (Table 4.10 [Q and SV in all participants and in untreated participants] and Table 4.11 [CO in all participants and in untreated participants]). Moreover, in sensitivity analyses similar differences in the hemodynamic determinants of BP were noted in treated but inadequately controlled hypertensives as compared to normotensives (Table 4.12). Furthermore, similar differences in the hemodynamic determinants of BP were noted in untreated or inadequately controlled

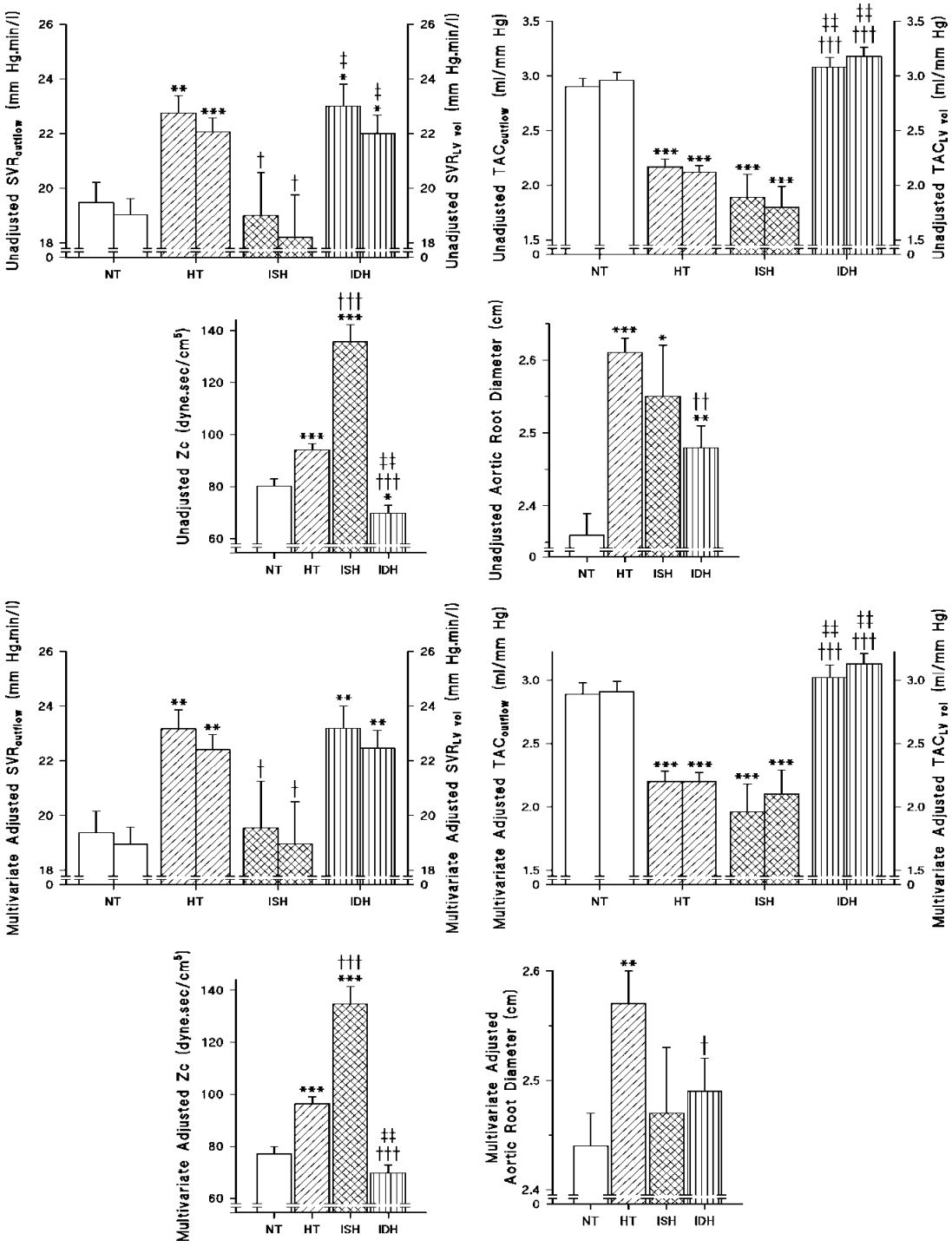


Figure 4. 8 Vascular correlates of blood pressure in untreated or treated but inadequately controlled hypertensives (SBP≥130 mm Hg or DBP≥80 mm Hg) versus normotensives in a community sample of African ancestry. Data shown are unadjusted and multivariate adjusted

mean±SEM. See Table 4.8 for sample sizes and characteristics. Adjustments are for age, sex, BMI, regular smoking, regular alcohol, diabetes mellitus, renal function, heart rate and drug class. See Figure 4.3 for additional abbreviations. *p<0.05, **p<0.005, ***p<0.0005 versus NT; †p<0.05, ††p<0.005, †††p<0.0005 versus HT; ‡p<0.05, ‡‡p<0.005, ‡‡‡p<0.005 versus ISH.

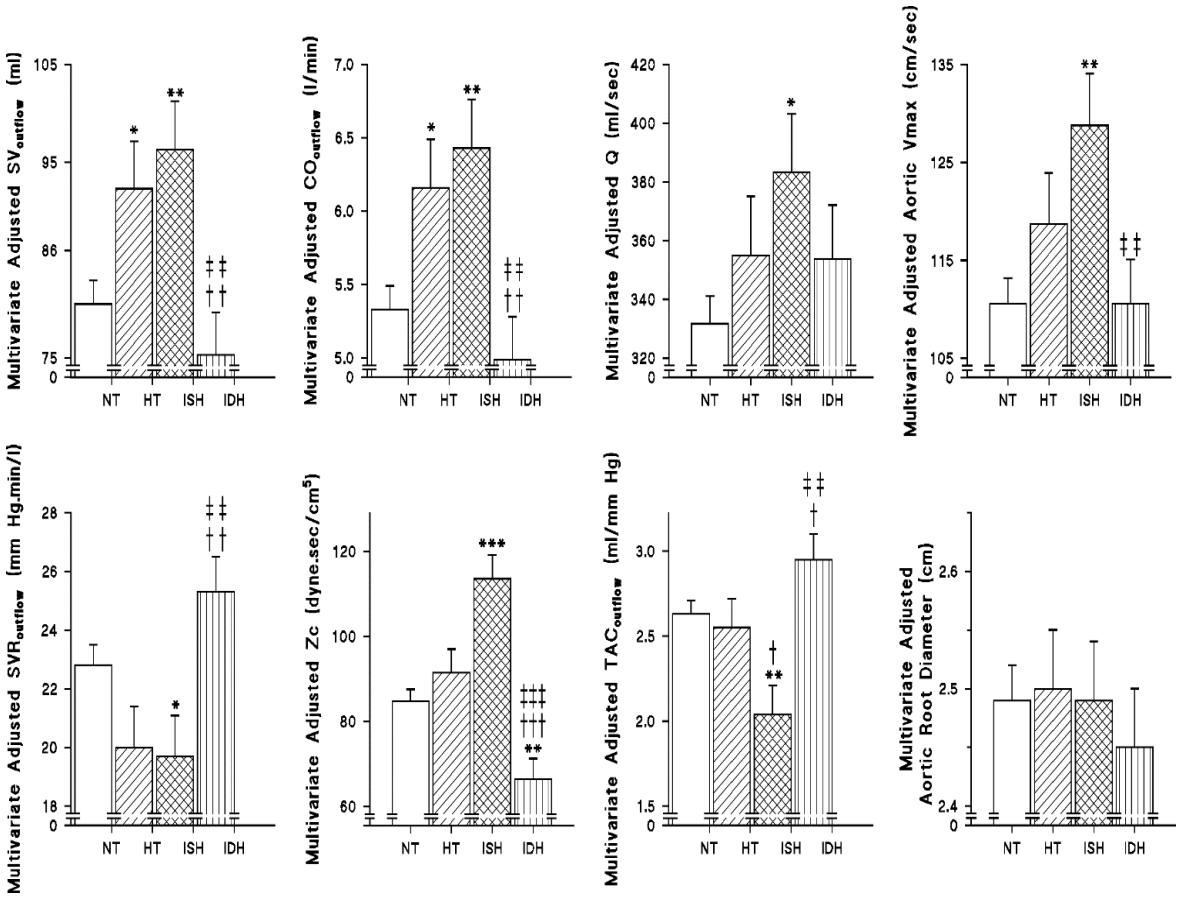


Figure 4. 9 Mean arterial pressure (MAP) independent systemic blood flow (upper panels) and vascular (lower panels) correlates of blood pressure in untreated or inadequately controlled hypertensives (SBP≥140 mm Hg or DBP≥90 mm Hg) versus normotensives in a community sample of African ancestry. Data shown are multivariate adjusted mean±SEM. Adjustments are for MAP, age, sex, BMI, regular smoking, regular alcohol, diabetes mellitus, renal function, heart rate and drug class. See Figures 4.1 and 4.3 for additional abbreviations. *p<0.05, **p<0.005, ***p<0.0005 versus NT; †p<0.05, ††p<0.005, †††p<0.0005 versus HT; ‡p<0.05, ‡‡p<0.005, ‡‡‡p<0.005 versus ISH.

Table 4. 10 Relative contribution of hemodynamic factors to untreated or treated but inadequately controlled hypertension (SBP≥140 mm Hg or DBP≥90 mm Hg).

		Untreated or inadequately controlled hypertension					
		Syst-diast		Isolated systolic (ISH)		Isolated diastolic (IDH)	
		Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value
		All Participants					
Models							
One SD increase in							
1.	SVR	2.723 (1.239-4.844)	=0.0096	0.058 (0.004-0.561)	=0.027	5.503 (2.774-11.493)	<0.0001
	Aortic Zc	1.765 (1.188-2.654)	=0.0052	1.746 (0.809-3.733)**	=0.15	0.942 (0.603-1.441)***	=0.79
	1/TAC	1.841 (0.883-3.974)	=0.110	37.333 (7.974-270.155)***††	<0.0001	0.287 (0.126-0.609)***††	=0.0019
	SV	5.678 (3.235-9.337)*†††‡	<0.0001	5.513 (1.994-16.581)***†‡	=0.0014	1.493 (0.891-2.503)**‡‡	=0.13
2.	SVR	0.699 (0.309-1.461)	=0.37	0.019 (0.003-0.102)	<0.0001	4.729 (2.452-9.470)	<0.0001
	Aortic Zc	1.905 (1.120-3.212)*	=0.016	3.121 (1.197-7.927)***	=0.018	1.139 (0.593-2.055)**	=0.68
	1/TAC	1.713 (0.807-3.799)	=0.17	24.797 (6.149-128.915)***†	<0.0001	0.238(0.095-0.549)***††	=0.0013
	Peak aortic Q	1.675 (1.032-2.675)*	=0.032	2.761 (1.252-6.242)***‡‡	=0.012	1.324 (0.841-2.057)**‡‡‡	=0.22
		Untreated Participants					
1.	SVR	2.108 (0.966-4.414)	=0.049	0.059 (0.004-0.562)	=0.027	5.262 (2.701-10.780)	<0.0001

	Aortic Zc	1.766 (1.203-2.620)	=0.004	1.712 (0.815-3.561)**	=0.15	0.942 (0.602-1.443)***	=0.79
	1/TAC	2.311 (1.169-4.757)	=0.019	26.336 (6.528-157.475)***††	<0.0001	0.294 (0.131-0.615)***†	=0.002
	SV	5.882 (3.284-10.937)*†††‡	<0.0001	5.572 (2.002-16.870)***†	=0.0014	1.500 (0.889-2.531)**‡‡‡	=0.13
2.	SVR	0.783 (0.360-1.579)	=0.52	0.012 (0.002-0.070)	<0.0001	4.396 (2.359-8.479)	<0.0001
	Aortic Zc	1.944 (1.181-3.174)*	=0.008	1.940 (0.816-4.607)***	=0.13	1.010 (0.518-1.858)**	=0.98
	1/TAC	1.484 (0.762-3.000)	=0.25	27.898 (7.443-132.731)***††	<0.0001	0.239 (0.096-0.553)***†	=0.001
	Peak aortic Q	1.713 (1.102-2.656)*	=0.016	2.246 (1.081-4.710)***‡‡	=0.029	1.138 (0.724-1.745)***‡‡	=0.56

Syst-diast, Systolic-diastolic; SVR, systemic vascular resistance; Zc, aortic characteristic impedance; SV, stroke volume; Q, flow; TAC, total arterial compliance. All models include the hemodynamic factors listed (namely, model 1: SV, SVR, aortic Zc, 1/TAC; model 2: Q, SVR, aortic Zc, 1/TAC) as well as age, sex, BMI, regular smoking, regular alcohol, diabetes mellitus, renal function, heart rate and drug class (in all participants). *p<0.05, **p<0.005 ***p<0.0005 versus relations with SVR; †p<0.05, ††p<0.0005 versus relations with Zc; ‡p<0.05, ‡‡p<0.005, ‡‡‡p<0.0005 versus relations with 1/TAC. One SD values for Syst-diast HT, ISH and IDH respectively: SVR = 9.6, 9.2, 9.5 mm Hg/l/min; aortic Zc = 43.7, 43.4, 38.2 dynes x s/cm^5; 1/TAC = 0.26, 0.27, 0.23 mm Hg/ml; SV = 39.6, 39.2, 36.7 mls/bt; Q = 151.2, 148.5, 147.4 mls/sec.

Table 4. 11 Relative contribution of hemodynamic factors to untreated or treated but inadequately controlled hypertension (SBP≥140 mm Hg or DBP≥90 mm Hg).

		Untreated or inadequately controlled hypertension					
		Syst-diast		Isolated systolic (ISH)		Isolated diastolic (IDH)	
		Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value
Models							
One SD increase in							
		All Participants					
1.	SVR	2.333 (1.081-4.795)	=0.023	0.097 (0.011-0.651)	=0.026	4.717 (2.468-9.382)	<0.0001
	Aortic Zc	1.684 (1.140-2.519)	=0.0094	2.231 (1.077-4.587)**	=0.030	0.913 (0.584-1.398)***	=0.68
	1/TAC	1.652 (0.823-3.412)	=0.16	11.708 (3.518-49.131)***†	=0.0002	0.323 (0.147-0.662)***†	=0.0032
	CO	4.352 (2.540-7.618)†††‡	<0.0001	3.814 (1.550-10.086)**	=0.0046	1.460 (0.899-2.332)**‡‡‡	=0.11
		Untreated Participants					
1.	SVR	2.230 (1.076-4.408)	=0.023	0.125 (0.015-0.786)	=0.040	4.529 (2.410-8.846)	<0.0001
	Aortic Zc	1.661 (1.136-2.459)	=0.009	1.668 (0.862-3.264)*	=0.13	0.912 (0.582-1.401)***	=0.68
	1/TAC	1.563 (0.841-2.979)	=0.16	12.024 (3.861-47.332)***††	<0.0001	0.330 (0.152-0.667)***†	=0.003
	CO	4.336 (2.534-7.578)†††‡	<0.0001	4.041 (1.674-10.555)**	=0.003	1.467 (0.898-2.355)**‡‡	=0.11

Syst-diast, Systolic-diastolic; SVR, systemic vascular resistance; Zc, aortic characteristic impedance; TAC, total arterial compliance; CO, cardiac output. Models include the hemodynamic factors listed (namely, model 1: CO, SVR, aortic Zc, 1/TAC) as well as age, sex, BMI, regular smoking, regular alcohol, diabetes mellitus, renal function, heart rate and drug class (in all participants). *$p<0.05$, **$p<0.005$ ***$p<0.0005$ versus relations with SVR; $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.005$ versus relations with Zc; $^{\ddagger}p<0.05$, $^{\ddagger\ddagger}p<0.005$ versus relations with 1/TAC. One SD values for Syst-diast HT, ISH and IDH respectively: SVR = 11.1, 10.9, 10.9 mm Hg/l/min; aortic Zc = 43.7, 42.8, 39.4 dynes x s/cm^5; 1/TAC = 0.24, 0.25, 0.23 mm Hg/ml; CO = 2.7, 2.8, 2.6 l/min.

Table 4. 12 Relative contribution of hemodynamic factors to treated but inadequately controlled hypertension (SBP≥140 mm Hg or DBP≥90 mm Hg).

		Treated but inadequately controlled hypertension					
		Syst-diast		Isolated systolic (ISH)		Isolated diastolic (IDH)	
		Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value
Models							
One SD increase in							
1.	SVR	3.227 (1.493-6.046)	=0.003	0.024 (0.001-0.357)	=0.013	3.202 (1.440-7.643)	=0.006
	Aortic Zc	1.383 (0.911-2.119)*	=0.13	1.390 (0.694-2.949)**	=0.36	0.579 (0.317-1.020)***	=0.064
	1/TAC	1.635 (0.838-3.329)	=0.16	59.211 (9.045-680.581)***††	=0.0002	0.514 (0.212-1.143)*	=0.12
	SV	5.925 (3.200-9.675)*†††‡	<0.0001	4.370 (1.252-17.657)**†	=0.026	0.942 (0.448-1.905)*	=0.87
2.	SVR	0.815 (0.324-1.848)	=0.64	0.010 (0.001-0.030)	=0.0002	3.503 (1.536-8.721)	=0.004
	Aortic Zc	1.511 (0.875-2.609)	=0.14	1.695 (0.557-5.690)***	=0.37	0.991 (0.401-2.322)*	=0.98
	1/TAC	1.781 (0.842-4.078)	=0.15	191.708 (16.611-989.900)***†††	=0.0002	0.460 (0.147-1.233)**	=0.15
	Peak aortic Q	1.871 (1.160-3.055)*	=0.010	2.243 (1.038-5.757)***‡‡‡	=0.037	1.512 (0.925-2.833)*‡	=0.13
3.	SVR	2.636 (1.283-5.416)	=0.008	0.032 (0.001-0.494)	=0.024	2.625 (1.265-5.694)	=0.011
	Aortic Zc	1.268 (0.855-1.894)*	=0.24	1.355 (0.739-2.538)*	=0.33	0.619 (0.341-1.076)*	=0.10
	1/TAC	1.397 (0.742-2.721)	=0.31	29.119 (5.454-260.058)***††	=0.0006	0.599 (0.262-1.282)**	=0.20

CO	3.296 (1.838-6.187)††‡	=0.0001	3.338 (1.028-12.084)**‡	=0.042	1.074 (0.594-1.854)*	=0.80

Syst-diast, Systolic-diastolic; SVR, systemic vascular resistance; Zc, aortic characteristic impedance; SV, stroke volume; Q, flow; TAC, total arterial compliance; CO, cardiac output. All models include the hemodynamic factors listed (namely, model 1: SV, SVR, aortic Zc, 1/TAC; model 2: Q, SVR, aortic Zc, 1/TAC; model 3: CO, SVR, aortic Zc, 1/TAC) as well as age, sex, BMI, regular smoking, regular alcohol, diabetes mellitus, renal function, heart rate and drug class. *p<0.05, **p<0.005 ***p<0.0005 versus relations with SVR; †p<0.05, ††p<0.005 versus relations with Zc; ‡p<0.05, ‡‡p<0.005 versus relations with 1/TAC. One SD values for Syst-diast HT, ISH and IDH respectively: SVR = 11.5, 10.9, 11.4 mm Hg/l/min; aortic Zc = 41.8, 42.0, 39.9 dynes x s/cm^5; 1/TAC = 0.27, 0.25, 0.24 mm Hg/ml; SV = 38.8, 37.8, 37.6 mls/bt; Q = 158.3, 156.5, 161.5 mls/sec; CO = 2.7, 2.5, 2.6 l/min.

hypertensives as compared to normotensives when hypertension was defined according to a threshold of ≥130/80 mm Hg (Table 4.13).

4.4 Discussion

The main findings of the present study are as follows: In a large community-based sample of African ancestry living in Africa I show that an increase in systemic blood flow is strongly associated with untreated or inadequately controlled Syst-diast hypertension and ISH but not with IDH. In this regard, I show that in hypertension associated with an increased systolic and diastolic BP (Syst-diast hypertension), increases in SV, CO and peak aortic flow (Q) account for more than or as much of untreated or inadequately controlled BP as do increases in systemic vascular resistance (SVR) and characteristic impedance (Zc) and decreases in total arterial compliance (TAC). In comparison, I show that increases in SVR account more for untreated or inadequately controlled IDH than do increases in SV, CO and Q. Additionally, I show that increases in SV, CO and Q account for untreated or inadequately controlled ISH more than do increases in SVR but less than do decreases in TAC.

Although several studies have demonstrated a role for increases in Q or SV as determinants of ISH in the young and at an older age (McEniery et al., 2005; Pasierski et al., 1991), the present study is the first to show that hypertension associated with increases in both systolic and/or diastolic BP, is in part accounted for by increases in SV and Q. In this regard, previous studies conducted in alternative populations (Yano et al., 2017; Mitchell et al., 2008), suggest that when both systolic and diastolic BP are increased, SV may be unchanged (Yano et al., 2017) compared to normotensives, and Q may even be decreased (Mitchell et al., 2008), an effect attributed to increases in resistance to flow in a pulsatile system (Zc). Importantly, the contribution of increases in SV, CO or Q in the present study to the odds of untreated or inadequately controlled hypertension attributed to increases in both SBP and/or DBP, was as strong as or stronger than that noted for the contribution of increases in SVR and Zc or decreases in total arterial compliance (TAC). The present study therefore provides evidence for a fundamental hemodynamic alteration (increased systemic blood flow) that is quite distinct from other populations that contributes to untreated or inadequately controlled systolic as well as diastolic BP values in populations of African ancestry living in Africa. A relatively greater increase in systolic as compared to

Table 4. 13 Relative contribution of hemodynamic factors to untreated or treated but inadequately controlled hypertension (SBP≥130 mm Hg or DBP≥80 mm Hg).

	Untreated or inadequately controlled hypertension					
	Syst-diast		Isolated systolic (ISH)		Isolated diastolic (IDH)	
	Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value
Models						
One SD increase in						
1. SVR	2.735 (1.593-4.841)	=0.0004	0.119 (0.011-0.946)	=0.058	9.874(4.826-21.740)	<0.0001
Aortic Zc	1.674 (1.226-2.318)	=0.001	4.938 (2.329-11.791)**	=0.0002	1.084 (0.792-1.484)***	=0.61
1/TAC	2.143 (1.210-3.958)	=0.011	22.350 (4.846-135.286)**†	=0.0002	0.237(0.127-0.424)***†††	<0.0001
SV	4.965 (3.160-8.091)*†††‡	<0.0001	6.764 (2.208-24.020)**	=0.0014	2.218 1.382-3.624)***†††‡‡	=0.0012
2. SVR	1.158 (0.633-2.071)	=0.63	0.014 (0.001-0.129)	=0.0007	4.579(2.600-8.486)	<0.0001
Aortic Zc	2.749 (1.692-4.671)*	<0.0001	10.590 (3.649-40.427)***	<0.0001	0.979 (0.625-1.529)***	=0.93
1/TAC	1.103 (0.546-2.272)†	=0.79	20.086 (3.581-156.146)***	=0.0016	0.261(0.135-0.483)***†††	<0.0001
Peak aortic Q	2.539 (1.580-4.160)*‡	=0.0001	5.145 (2.032-14.930)***	=0.001	1.007 (0.681-1.485)***‡‡‡	=0.97
3. SVR	3.200 (1.677-6.409)	=0.0007	0.171 (0.021-1.072)	=0.074	7.395 (3.856-15.127)	<0.0001
Aortic Zc	1.826 (1.289-2.638)	=0.0009	4.441 (2.135-10.360)**	=0.0002	1.023 (0.755-1.384)***	=0.88
1/TAC	1.510 (0.796-2.949)*	=0.22	13.247 (3.172-69.785)***	=0.0009	0.304 (0.170-0.520)***†††	<0.0001

CO	5.449	(3.360-9.247)†††‡‡	<0.0001	5.435	(1.887-17.444)**	=0.0025	2.160	(1.360-3.524)**†††‡‡ =0.0016

Syst-diast, Systolic-diastolic; SVR, systemic vascular resistance; Zc, aortic characteristic impedance; SV, stroke volume; Q, flow; TAC, total arterial compliance; CO, cardiac output. All models include the hemodynamic factors listed (namely, model 1: SV, SVR, aortic Zc, 1/TAC; model 2: Q, SVR, aortic Zc, 1/TAC; model 3: CO, SVR, aortic Zc, 1/TAC) as well as age, sex, BMI, regular smoking, regular alcohol, diabetes mellitus, renal function, heart rate and drug class. *p<0.05, **p<0.005 ***p<0.0005 versus relations with SVR; †p<0.05, ††p<0.005 versus relations with Zc; ‡p<0.05, ‡‡p<0.005 versus relations with 1/TAC. One SD values for Syst-diast HT, ISH and IDH respectively: SVR = 12.1, 9.4, 11.3 mm Hg/l/min; aortic Zc = 43.0, 45.2, 35.1 dynes x s/cm^5; 1/TAC = 0.30, 0.28, 0.22 mm Hg/ml; SV = 39.5, 38.8, 37.3 mls/bt; Q = 159.0, 150.2, 146.5 mls/sec; CO = 2.7, 2.7, 2.7 l/min.

diastolic BP should therefore not be seen as indicative of a more flow-dependent form of hypertension in this ethnic group. Importantly, in contrast to alternative populations where ISH may be as frequent as or more frequent than Syst-diast hypertension (Yano et al., 2017), in the present population, Syst-diast hypertension was by far the dominant form of hypertension. Nevertheless, the present study confirms in ISH a predominant role of changes in large but not small vessels and systemic blood flow in determining variations in BP; whereas in IDH, the primary determinant of variations in BP is an increase in small vessel tone.

Although speculative, the potential mechanisms that explain the increase in systemic blood flow in hypertension associated with increases in either SBP or DBP in the present study, are worthy of consideration. In this regard, it is well recognised that groups of African ancestry develop hypertension with a high prevalence of salt-sensitivity, often quite responsive to diuretic agents (Flack et al., 2010; Luft et al., 1991; Freis et al., 1988; ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group, 2002; Aviv et al., 2004). However, recent evidence from our group (Mmopi et al., 2020) shows that previously described (Scott et al., 2011; Michel et al., 2012) relationships between SBP and urinary Na^+/K^+, a composite index of both salt intake and renal salt handling, in this ethnic group, are accounted for by an impact on Zc and not peak aortic Q. Thus, modifying salt intake may not be a solution to the flow-dependence of untreated or inadequately controlled hypertension. Nevertheless, the relationships between BP and SV, CO or Q are related to age-dependent increases in left ventricular end diastolic volumes, decreases in renin concentrations and increases in aldosterone-to-renin ratios (Woodiwiss et al., 2020). Thus, increases in SV and Q may be attributed to Frank-Starling effects mediated by a volume overload. Thus, targeting an increased plasma volume may be a therapeutic option. However, an important question raised by the present study is whether increases in SV, CO or peak aortic Q can be targeted by current approaches to the management of hypertension?

There is presently little evidence to suggest that diuretic agents employed in the management of hypertension are able to produce a sustained decrease in plasma volume and hence SV or CO and thus peak aortic Q (Rapoport et al., 2019). However, there are no studies that have specifically evaluated these effects in hypertension associated with increases in SV, CO or peak aortic Q. Importantly, the current study suggests that by targeting MAP, the vascular changes observed in systolic-diastolic hypertension can be corrected; however the high systemic blood flow is likely to remain. Further studies are therefore required to identify first the possible impact of

thiazide diuretics, thiazide-like diuretics or amiloride on SV, CO and peak aortic Q in the present or similar populations. Second, failing an ability to decrease SV with diuretic agents, additional studies should consider the possibility that in patients with heart rates at relatively high levels of normal, agents that reduce heart rate may decrease not only CO, but peak aortic Q even when SV is maintained by extending ejection duration. Whether these effects on heart rate and hence CO and peak aortic Q can be produced without markedly enhancing reflected wave pressures in central arteries (through harmonic effects on oscillating waves) (Xiao et al., 2018) does nevertheless require careful assessment.

An important finding of the present study is that while increases in Zc in participants with Syst-diast HT were associated with an enhanced aortic root diameter as compared to normotensives, the more striking increases in Zc noted in those participants with ISH, was not associated with an even greater increase in aortic root diameter as compared to those with Syst-diast hypertension. Thus, part of the greater increase in Zc in participants with ISH may be attributed not only to an increased proximal aortic stiffness, but also to an attenuated increase in aortic diameter normally associated with aging. These data are in contrast to the increased aortic root diameter that occurs with both ISH and Syst-diast hypertension in alternative populations (Yano et al., 2017), but is consistent with an attenuated increase in aortic root diameter associated with a greater age-related increase in pulse pressure and hence SBP in the Framingham Heart Study (Mitchell et al., 2003). The present data therefore support the view that aortic root diameter contributes to Zc through an impact on resistance to flow rather than through stiffness effects produced by more strain placed on collagen as opposed to elastin molecules with aortic distension.

There are several limitations to the present study that warrant consideration. First, the present study was cross-sectional in design. Hence relations may be through residual confounding. Indeed, as this was a community-based study, many participants with inadequately controlled hypertension were receiving antihypertensive agents which, may have confounded the relations noted. In this regard, the relatively low sample size of participants with untreated ISH may have limited the interpretation of data from sensitivity analysis conducted in untreated participants. Importantly however, most treated participants with inadequately controlled ISH (47.8%) or inadequately controlled Syst-diastolic hypertension (47.9%) were receiving low dose thiazide diuretics as part of their therapy (84.4% and 92.6% respectively), an approach that may have reduced volume and hence limited our ability to show an impact of SV or aortic Q. Thus, the

presence of therapy is likely to have biased against the findings of the present study. Second, in the present study, calibration of the radial waveform from brachial BP measurements ignores amplification of BP from brachial to radial arteries (Picone et al., 2015). Hence, aortic pressures and Zc may have been underestimated using the current approach.

4.4.1 Conclusions

In conclusion, in a relatively large community-based sample of African ancestry in South Africa I show that in distinct contrast to alternative populations, that age-related increases in systemic blood flow (SV, CO or peak aortic Q) are strongly associated with not only untreated or inadequately controlled ISH, but also with untreated or inadequately controlled Syst-diastolic hypertension. These data highlight the importance of systemic flow in contributing to increases in either systolic or diastolic BP in this ethnic group, and the need to identify therapeutic approaches capable of reducing systemic blood flow in a manner acceptable to patient's welfare.

CHAPTER 5

Contextual Narrative and Conclusions

5.1 Introduction

With world-wide trends for a preference of human populations to live a more urbanised and industrialised existence, a global shift in disease patterns has occurred. Whilst communicable diseases were the dominant scourge in the last century, non-communicable diseases are the major cause of death and disability in the world today (McAloon et al., 2016). Of all non-communicable diseases, the leading cause of death is cardiovascular disease (McAloon et al., 2016; Keates et al., 2017). Additionally, of all the recognised modifiable risk factors for cardiovascular disease, hypertension accounts for most population attributable risk. The impact of hypertension on cardiovascular events has been well described over more than a century of research. However, there is a distinct trend for the adverse impact of hypertension to be greater in some as opposed to other population groups depending on ancestral origins.

As reviewed in chapter 1 of the present thesis, in several studies largely conducted in the USA, ethnic differences in hypertension and the adverse effects thereof have been clearly demonstrated. In this regard, groups of African descent whose ancestors are likely to have been victims of slavery, appear to be at a higher risk than those of largely European ancestry (chapter 1). The debate as to the exact reasons for these ethnic differences continues, but a biological basis, often vigorously opposed or defended, is frequently cited and yet no distinct pathophysiological feature clearly setting hypertension apart in this as compared to alternative ethnic groups, has emerged. A major impact of hypertension on cardiovascular disease in Africa has over the past two decades nevertheless also been highlighted (reviewed in chapter 1). Despite the lack of selective pressures produced by the impact of slavery in those living in Africa, the possibility that hypertension in populations of African ancestry living in Africa may also represent distinct ethnic effects has therefore also been considered. However, there are limited studies that have been conducted in Africa and hence whether ethnic differences do indeed also occur in Africa is even more contentious. In the present thesis, I further explored the possibility that a distinct pathophysiological alteration may contribute to hypertension in Africa. In this regard I focussed my attention on the possibility that unlike most groups around the world, increases in systemic blood flow produced by excess fluid retention may contribute to hypertension in groups of African ancestry living in Africa. As highlighted in the introductory chapter, this is a particularly important question as the central role of renal mechanisms as a fundamental cause of primary hypertension

remains a source of vigorous debate almost 50 years after it was originally proposed by Arthur Guyton and colleagues in 1972 (Guyton et al., 1972). Indeed, the fundamental hemodynamic change that should characterise hypertension caused by renal mechanisms, and that is an increased systemic blood flow, has never been demonstrated in primary hypertension (Kurtz et al., 2016). Importantly, as a consequence of the work described in the present thesis, I have made major contributions to **three publications** of original work, with two published in the high impact journal ***Hypertension*** (Mmopi et al., 2020, Woodiwiss & Mmopi [equal contribution] et al., 2020) and one published in the *Journal of Hypertension* (Mmopi et al., 2021). In the present chapter, I will discuss these data from a contextual perspective, with a focus on highlighting the clinical implications of the data, including possible therapeutic implications that require urgent and extensive further work. The key findings of these 3 publications are summarised in Figure 5.1.

5.2 Is there a case for antagonistic pleiotropy in the genesis of hypertension in Africa?

As reviewed in chapter 1 of the present thesis it has been argued that because of the terrible conditions on slave ships transporting the ancestors of African Americans and Caribbean communities to the Caribbean and America, that survival depended on a genetic predisposition to retain Na^+ and water (Wilson & Grim, 1991). Thus, African Americans presently show a propensity for this phenotype (Aviv et al., 2004), which in hot, arid conditions may enhance the chances of survival, but with aging, this phenotype translates into a reduced chance of survival because of the increased risk of hypertension (Wilson & Grim, 1991). As also described in chapter 1 of the present thesis, this is consistent with the evolutionary theory of antagonistic pleiotropy (Williams, 1957; Byars & Voskarides, 2020; Kamo et al., 2016; Mitteldorf et al., 2019). Although there is clear evidence that on average the BP response to a Na^+ load (salt-sensitivity) is greater in African Americans than in other ethnic groups (Aviv et al., 2004), this is not a phenotype that can be claimed as being distinct to African Americans. In this regard, it is well accepted that in any population a portion of the sample will show a "salt-sensitive" phenotype. Moreover, although through renal effects, which suppress renin release and a variety of alternative factors, hypertension in groups of African descent is often difficult to manage using ACEIs or ARBs, this is also not a phenotype that can be considered as distinct to African Americans (reviewed in chapter

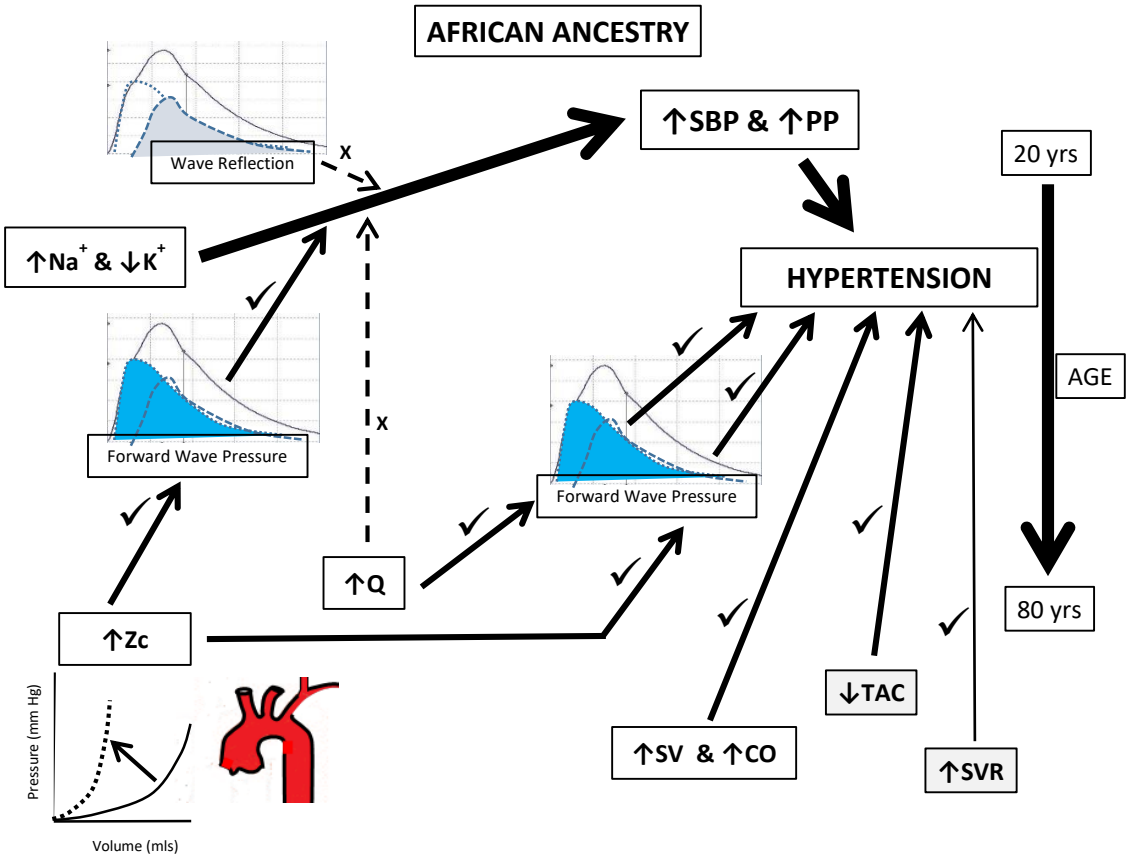


Figure 5. 1 Summary of the key findings of the 3 publications emanating from my thesis. CO, cardiac output; K^+, potassium; Na^+, sodium; PP, pulse pressure; Q, aortic blood flow; SBP, systolic blood pressure; SVR, systemic vascular resistance; TAC, total arterial compliance; Zc, aortic characteristic impedance; ✓, has an effect; **x**, has no effect; thickness of arrows indicates comparative magnitude of effect.

1). In this regard, it is well accepted that in any population a portion of sample will develop a low renin form of hypertension and a limited response to ACEIs or ARBs. Indeed, BP responses to ACEIs and ARBs are thought to be more variable within than between ethnic groups (Mokwe et al., 2004; Sehgal, 2004). Moreover, although several lines of evidence support a more important role for hypertension in African Americans as opposed to groups of African ancestry who are more recent immigrants to the USA or who are living in Africa or elsewhere (reviewed in chapter 1), there is now increasing evidence that hypertension is also a particularly important risk factor for cardiovascular disease in sub-Saharan Africa (also reviewed in chapter 1). As these populations have **not** been exposed to the same selective pressures as those who originated from victims of slavery, this postulate may not hold. However, as also reviewed in the introduction to the present thesis, many thousands of years ago there were alternative selective pressures which may have predisposed to a better survival with an enhanced ability of the kidneys to retain salt and water (Young et al., 2005). These selective pressures occurred in any group living in Africa who subsequently contributed to the gene pool of groups of African ancestry living anywhere in the world today, including groups currently living in South Africa (Patin et al., 2017). Briefly, to recapitulate, how could South Africans of African ancestry have obtained this phenotype? Southern Africans originated from West Africa via the Bantu migration which occurred thousands of years ago (Patin et al., 2017). Prior to this migration, selective pressure may have occurred in favour of survival in hot and arid environments around the equator. Thus, a genetic predisposition to retain Na^+ and water may have occurred in Africa simply through geographic location. This would also constitute a form of antagonistic pleiotropy, one which would account for salt-sensitivity and low renin hypertension in many ethnic groups as long as they originated from around the equator. Does the present thesis support a role for possible antagonistic pleiotropy in groups of African ancestry living in Southern Africa?

This question hinges on the validity of the original hypothesis of Guyton, Coleman and Granger (1972), suggesting that renal mechanisms are central to the pathogenesis of primary, rather than just secondary forms of hypertension. This hypothesis suggests that primary hypertension can only occur if through renal tubular changes, a shifted pressure- natriuresis relationship prevents the return of BP to normal levels if elevated (Guyton et al., 1972). As there is little data to support the predicted increases in systemic blood flow that should occur in primary hypertension in response to this change (Kurtz et al., 2016), this theory has more recently

encountered significant opposition (Kurtz et al., 2016). Thus, the theory of antagonistic pleiotropy can only hold if the original hypothesis of Guyton and colleagues is correct and measurable increases in systemic blood flow do indeed occur in primary hypertension in groups of African ancestry.

In chapters 3 and 4 of the present thesis, and published in part in the prestigious journal *Hypertension* (Woodiwiss, Mmopi [equal contribution] et al., 2020), I show that a major determinant of hypertension noted in a large sample of an urban developing community of African ancestry in South Africa, studied across the full adult age range, is a striking and consistent stepwise age-related increase in stroke volume (SV) and cardiac output (CO) as well as in peak aortic flow (Q). These changes contributed as much to increases in BP as did age-related increases in aortic stiffness-associated increments in characteristic impedance (Zc) and decreases in total arterial compliance (TAC) and more than increases in systemic vascular resistance (SVR) (total peripheral resistance). The age-related effects on SV and CO were identified both from left ventricular outflow tract velocity and diameter measurements and from left ventricular dimension measurements and showed remarkable consistency between these two approaches. Importantly, the age-related increases in SV and CO were strongly related to an age-associated increase in left ventricular end diastolic volume, but not to increases in left ventricular ejection fraction. These data are therefore consistent with an increased volume preload on the left ventricle resulting in an enhanced Frank-Starling effect and an increased ventricular ejection volume, rather than to an ability of the left ventricle to generate a greater force of contraction. Also importantly, the age-related increases in SV were strongly related to age-related decreases in plasma renin concentrations, thus confirming that the hypertension resulting from the increased SV and hence CO was consistent with the typical low renin state noted when renal mechanisms result in fluid overload. Although not reported on in the present thesis, more recent analyses of data obtained in this community sample show that SV, CO and Q strongly and independently associate with a reduced fractional Na^+ excretion (increased tubular Na^+ reabsorption) and to an age-related decline in glomerular filtration rate, an index of age-related loss of nephrons (Malan et al., in-preparation). In contrast, neither SVR. Zc, nor TAC are independently associated with renal function (Malan et al., in-preparation). Importantly, the relationships between systemic flow and renal function are not attributed to the impact of flow on BP (these relationships are not affected by adjustments for either MAP or P_{QxZc}) and hence caused by BP effects on renal function (Malan et al., in-

preparation). Thus, the age-related and hypertension-associated increases in systemic flow in the present study are indeed accounted for by renal effects.

As measurable increases in systemic flow were noted in primary hypertension in the present study, these data satisfy the criticisms of the original theory that renal mechanisms are central to the pathophysiology of not only secondary forms of hypertension, but also primary hypertension. In this regard, as correctly pointed out by antagonists of the theory (Kurtz et al., 2016), systemic flow, no matter how small, has to be increased for renal mechanisms to sustain primary hypertension. As increases in SV and CO are very likely to maintain a normal systemic blood flow in hot, arid environments where water loss occurs, but decrease the chances of survival with aging by increasing BP, these data are entirely consistent with the theory of antagonistic pleiotropy. However, as described in chapter 2 of the present thesis, and published in the prestigious journal *Hypertension* (Mmopi et al., 2020), these age-related increases in systemic blood flow are not accounted for by normal variations in usual salt intake. These data raise several important questions, but first, the question of how distinct these phenotypic changes are as compared to alternative ethnic groups, requires consideration.

5.3 How distinct is the hemodynamic basis of hypertension in Africa?

Importantly, few studies have demonstrated that the common form of hypertension that occurs in any community, that is, primary systemic hypertension, is strongly associated with an age-related increase in SV, CO or aortic Q. In this regard, in other populations only ISH has been demonstrated to be associated with an enhanced SV and hence possibly aortic Q (McEniery et al., 2005; Pasierski et al., 1991). There is some data in a small study sample to suggest that SV increases over an early adult age, but that after 50 years of age, SV begins to decrease possibly in parallel with increases in aortic stiffness and hence an increased resistance to pulsatile flow (Zc) (Alfie et al., 1999). Nevertheless, the main change noted in ISH or in any hypertensive with an increased PP has consistently been reported as an increased proximal aortic Zc produced mainly by increases in aortic stiffness (Goel et al., 2017; Segers et al., 2007; Torjesen et al., 2014; Yano et al., 2017). Moreover, most studies conducted in samples of those mainly of European descent have also demonstrated that age-related increases in BP are attributed largely to increases in proximal aortic Zc and hence to aortic stiffness (Mitchell et al., 2010*b*; Segers et al., 2007). Thus,

almost without exception in alternative ethnic groups the dominant hemodynamic changes responsible for increases in the pulsatile component of BP and hence SBP, is structural changes in the proximal aorta attributed to arteriosclerosis and hence to increases in aortic stiffness. Although consistent with these data, as demonstrated in the present thesis there is no question that increases in proximal aortic Zc and decreases in TAC are central to the development of hypertension in groups of African ancestry living in Africa (Woodiwiss, Mmopi et al., 2020; Mmopi et al., 2021, chapters 3 and 4), the impact of aortic Q on BP was as striking as Zc (Woodiwiss, Mmopi et al., 2020; Mmopi et al., 2021, chapters 3 and 4). Moreover, in the present thesis, not only do I show that increases in peak aortic Q are a strong determinant of age-related increases in PP and hence SBP (chapter 3 and Woodiwiss, Mmopi et al., 2020), but also that both ISH and systolic-diastolic hypertension relate to increases in SV and aortic Q (chapter 4). In this regard, with respect to the steady component of BP (MAP), to the best of my knowledge, in contrast to all previous studies published which describe the hemodynamic basis of hypertension, the present study is the only study to show that age-related increases in SV and CO rather than in SVR, account for age-related increases in MAP. Although as reported in chapter 4 in the present thesis, increases in SVR most certainly contributed to IDH and to systolic-diastolic hypertension, the overall effect of increases in systemic blood flow on BP irrespective of the BP that was noted to be increased (ISH, IDH, Systolic and diastolic hypertension) had an even greater impact on BP than SVR *per se*. Thus, although direct comparisons with participants from alternative ethnic groups under similar living circumstances were not possible in the studies conducted in the present thesis, the present studies suggest a quite distinct form of primary systemic hypertension in groups of African ancestry living in Africa. Despite these alterations suggesting an increased volume preload on the left ventricle, resulting in increases in systemic blood flow, one must consider whether these data are consistent with what would normally be expected with changes in renal function noted in studies conducted in largely African Americans over the past half a century. In particular, a major question which arises from the results of the present studies is what are the implications for the finding that age-related increases in systemic blood flow were not accounted for by variations in normal basal salt intake?

5.4 Implications of a lack of relationship between salt intake and systemic blood flow

The genetic variants that are likely to account for an ability to retain fluid and hence to survive better in hot and arid environments, are thought to be those that mainly influence renal (tubular reabsorption) function. These effects are thought to be best indexed by assessing the BP response to a Na^+ load (salt-sensitivity), and these renal changes are thought to translate into an impact of basal Na^+ and K^+ intake on BP over a prolonged period of time. Indeed, as reviewed in the introduction to the present thesis, there is substantial evidence that decreases in BP are produced by reductions in Na^+ intake and increases in K^+ intake over much longer periods normally evaluated when identifying the salt-sensitive phenotype (chapter 1). In this regard, our group has published several papers describing relationships between urinary indices of salt intake obtained over a 24-hour period (assuming a stable Na^+ intake and a state of equilibrium) and both office and 24-hour ambulatory SBP and PP, but not DBP (Redelinghuys et al., 2010; Scott et al., 2011; Michel et al., 2012). At first glance, this could be interpreted as an impact of excessive Na^+ intake on blood volume and hence according to conventional physiology, this would translate into an increased Frank-Starling effect and hence systemic blood flow (SV and CO) causing increases in aortic Q, PP and hence SBP, whilst the effects on MAP and hence DBP are less striking. Surprisingly however, as described in chapter 2, in the present thesis and as published in *Hypertension* (Mmopi et al., 2020), I demonstrate that the relationship between urinary indices of salt intake and PP or SBP are entirely accounted for by an enhanced proximal aortic Zc, with no relationship noted with aortic Q. Thus, variations in basal salt intake clearly cannot explain age-related increases in either SV, CO or peak aortic Q. There are several possibilities that may explain this apparent contradiction. What are these possibilities?

First, the impact of salt intake on BP was assessed in the present study without a salt challenge, but rather employing variations in usual daily salt intake normally taken by participants. In this regard, a salt challenge is usually conducted by providing diets with a Na^+ content that differs by 50-100 mmol of Na^+ per day, with pronounced BP responses only occurring when diets differ by 100 mmol per day. In this regard, the average Na^+ intake in the group studied is only approximately 100 mmol per day and hence the ability to show a salt-loading (salt-sensitive) BP response is limited. The approach employed to assess the impact of salt intake may therefore detect the long-term, cumulative effects of abnormalities in salt balance and may be too insensitive to

detect the short-term renal effects associated with salt-sensitivity. This hypothesis is supported by the relations noted with Zc, which reflect structural changes in the aorta, which could only occur over a long-term period. Are there alternative possible explanations for a lack of relationship noted between indices of salt intake and systemic flow reported on in the present thesis (Mmopi et al., 2020 and chapter 2)?

An alternative possibility is that the alterations in systemic blood flow noted in hypertensives of African descent living in Africa, although likely to be a consequence of excess fluid retention, are not caused by the same renal mechanisms as those that have been noted in African Americans (the mechanisms of antagonistic pleiotropy are therefore different). In this regard, whilst the "salt-sensitive" BP effect may be more readily identified with some renal changes, others may produce more subtle effects that take longer (years) to contribute to increases in BP. Indeed, to the best of my knowledge there are no studies that have extensively evaluated the salt-sensitive phenotype in carefully controlled conditions (metabolic units) in groups of African ancestry living in Africa. Perhaps a striking feature, which points to possible different renal mechanisms is the relationship between age and systemic blood flow noted in the present thesis, which does not support intrinsic genetic variations accounting for the impact on systemic blood flow, but rather suggests an age-related loss of nephrons with time. As previously indicated, however, more recent analysis demonstrates a combined renal effect on systemic flow and that is an effect of both an increased tubular Na^+ reabsorption and an age-related decline in nephron number as indexed by glomerular filtration rate (Malan et al., in-preparation). As with any population, glomerular filtration rate decreases dramatically with age in groups of African ancestry living in Africa (Kolkenbeck-Ruh et al., 2019). Subsequent analysis as part of future studies will therefore involve assessing the heritability and intrafamilial aggregation of flow-dependent phenotypes in the community evaluated. This analysis is presently underway, but will form part of an alternative student's PhD thesis. If heritability is obvious and strong for SV and aortic Q, then a genetic basis must be considered and the possibility of antagonistic pleiotropy does indeed exist. However, if no heritability is noted, then the possibility of antagonistic pleiotropy is unlikely. If this approach fails to show clear heritability or a lack thereof, an assessment of the salt-sensitive phenotype will be required under carefully controlled conditions (metabolic units) in groups of African ancestry living in Africa.

A third possibility that may explain the lack of relationship noted between indices of salt intake and systemic flow reported on in the present thesis (Mmopi et al., 2020 and chapter 2) is that renal mechanisms are less responsible for increases in systemic blood flow than previously thought. In this regard, the prevalence of obesity is quite remarkable in the present population, these body size changes are strongly age-dependent, and systemic flow is well-recognised as increasing with increments in body size. However, all of the relationships between either age or hypertension and systemic flow reported on in the present thesis (Woodiwiss, Mmopi et al., 2020; Mmopi et al., 2021, chapter 3 and 4) are with adjustments for body mass index and the age-related or hypertension associated increases in SV and CO are similar irrespective of whether SV and CO are expressed with or without adjustments for body surface area (BSA). Thus, neither BMI nor BSA can account for the systemic blood flow effects noted with age and hypertension.

5.5 Why does an elevated blood pressure not return systemic blood flow to normal?

As reviewed in the introduction to the present thesis (chapter 1), salt-sensitive hypertension in alternative populations is associated with an enhanced SVR and aortic stiffness, whilst SV or CO have never been demonstrated to account for an enhanced BP. This is generally explained by vascular effects such as autoregulation causing vasoconstriction or long-term vascular remodelling returning systemic flow to normal levels, but at the expense of an elevated SVR, Zc, TAC and hence BP. Clearly the same effect does not occur in groups of African ancestry living in Africa. Whether this may be accounted for by reduced autoregulatory changes at a vascular level in Africa, decreased vascular remodelling and/or because the primary abnormality that occurs in Africa produces a more pronounced alteration in the pressure natriuresis relationship and hence a greater increase in systemic flow, is unclear. The striking age-related increase in proximal aortic Zc suggests that this community undergoes similar arteriosclerotic remodelling of large arteries. However, whether remodelling of arteriolar walls is similar, is unknown. With respect to the blunted pressure-natriuresis, a striking alteration in the pressure natriuresis relationship is often noted with renal tubular damage (loss of nephrons preventing the pressure natriuretic effect occurring in the proximal tubule) and points to very different renal mechanisms explaining hypertension in Africa as opposed to in alternative populations who are reported to be salt-sensitive. These changes are more in-line with what is frequently reported to occur in the very

elderly who may have a marked age-related loss of nephrons and suggests that a similar, but premature effect could explain a significant portion of hypertension in Africa well before the age when one would normally be considered as at risk for these effects. This hypothesis is certainly worthy of further study.

5.6 Implications for the prevalence of hypertension, and control of blood pressure in Africa

The prevalence of hypertension across the adult lifespan in the community studied in the present thesis is remarkably high, approximating 40% of the sample. These data have been confirmed with ambulatory BP monitoring in several previous publications (e.g. Redelinghuys et al., 2010). As reviewed in the introductory chapter to the present thesis, these prevalence rates are similar to those noted for hypertension in adult African Americans, the ethnic group that is most often reported as having the highest prevalence of hypertension in the USA. There are several reasons for these high prevalence rates in groups of African ancestry living in Africa, but importantly, the findings that increases in SV, CO and aortic Q are equally as important determinants of hypertension in Africa as the major vascular changes that account for hypertension (Zc, TAC and SVR) has major implications for the prevalence. In this regard, whilst most communities develop hypertension only in response to vascular alterations, hypertension in Africa develops in response to a double hemodynamic burden (flow and resistance to flow). This likely compounds the impact on BP as these two factors do not summate, but rather act in synergy (pressure is determined by the product of flow and resistance to flow, with MAP=CO x SVR and PP=aortic Q x Zc). Thus, more modest alterations in systemic vascular tone (SVR) or arteriosclerotic aortic changes (proximal aortic Zc and TAC) may translate into more marked BP effects, with more persons likely to develop higher BP levels, if flow is also increased. The exact extent to which this occurs requires further analysis as it is nevertheless possible that some individuals will have an enhanced flow (CO and aortic Q) and others increases in SVR or Zc and TAC (i.e., the effects of BP are separate from each other). Furthermore, the extent to which the combined impact of flow and resistance to flow rather than either one or the other, produce end organ damage similarly requires further analysis. These analyses are currently underway but are the topic of an alternative student's PhD thesis. Assuming that flow and resistance to flow changes

occur in the same individuals, this will have obvious repercussions and may explain the high prevalence rates of hypertension and the finding that only around a half of all hypertensives receiving treatment are at target BP levels. There is therefore an urgent need to attempt to identify the pathophysiological processes causing increases in systemic blood flow and those antihypertensive agents or combinations thereof that are most likely to achieve reductions in both vascular alterations (tone) and the increases in systemic blood flow.

5.7 Implications for the management of hypertension in Africa

The implications of the findings of the studies conducted as part of the present thesis for therapy, require careful consideration. As indicated in the aforementioned section, targeting the increases in systemic blood flow that in part account for hypertension in groups of African ancestry may be an important therapeutic target. In this regard, understanding the causes of the increases in systemic blood flow is essential. As renal mechanisms are the most likely cause of increases in systemic blood flow, it may be important that effective diuretic agents are employed. However, as highlighted in chapters 3 and 4, no diuretic agent has been demonstrated to reduce either plasma volume or systemic blood flow for prolonged periods without returning these values to pre-treatment levels given a sufficiently long enough period of time to do so (Rapoport & Soleimani, 2019). However, there are no studies that have been conducted that have specifically targeted hypertensive patients with increases in plasma volume or systemic blood flow. Thus, whether diuretics are able to attenuate increases in systemic blood flow is unknown. Importantly, in the studies described in the present thesis (chapters 2-4) most treated hypertensives were receiving the thiazide diuretic agent, hydrochlorothiazide (HCTZ), and these patients still had an increased SV, CO and aortic Q values. Hence, even if HCTZ does reduce systemic blood flow it is unable to return increases in flow to levels noted in normotensive individuals. Whether this is simply a reflection of the limitations of using HCTZ at doses that are considered to have no side effects, or whether the use of thiazide-like diuretics such as chlorthalidone are more effective, is unknown. Presently, our group are analysing data where we have explored the impact of the thiazide-like diuretic, indapamide, on systemic blood flow in hypertensives of African ancestry living in Africa. The analysis of these data is nevertheless incomplete at present and will form the topic of an alternative PhD student's thesis.

As indicated in the aforementioned section, hypertension in groups of African ancestry living in Africa is more likely if both flow (SV, CO or aortic Q) and resistance to flow (SVR and Zc or TAC) increase in combination. If flow and resistance to flow changes do not occur separately, but rather act in synergy as predicted by the formulae MAP=CO x SVR and PP= Q x Zc, then just as small increases in vascular tone (SVR) or arteriosclerotic-induced increases in proximal aortic stiffness (which increases Zc) will produce dramatic increases on BP in those with an enhanced flow, so will the reverse apply. That is, small decreases in vascular tone (SVR) or proximal aortic stiffness (Zc) (which could occur through passive reductions in aortic stiffness by decreasing MAP) could produce dramatic decreases in BP in those with an increased flow. Thus, the ability to manage hypertension and the adverse effects thereof could be even more susceptible to current antihypertensive therapy as long as effective vasodilators are employed. This possibility may explain why combinations of largely vasodilators (ACEI [which do not promote a marked natriuresis] and calcium channel blocker) in groups of African descent may be equally as effective as thiazide diuretic agents employed in combination with vasodilators (calcium channel blocker and hydrochlorothiazide) (Ojji et al., 2019). However, as indicated in aforementioned discussion, these data must be taken in context as there are no data to show that thiazide diuretics effectively reduce plasma volume and hence systemic blood flow at the doses currently considered as being without side effects. Thus, further analysis of data is urgently required to determine the extent to which the impact of flow and resistance to flow on BP and end organ alterations are produced through separate effects, or through an impact of synergy between the two. This analysis will determine the extent to which targeting vascular effects alone will suffice, or whether targeting both flow and resistance is required. Nevertheless, this analysis will not identify a possibility that could occur which will limit the efficacy of employing just vasodilator therapy. What is this possibility?

Even if the use of vasodilators alone has short-term benefits, the long-term benefits on BP when renal mechanisms play a role, may be limited. In this regard, reductions in SVR and Zc in a population with a volume-dependent form of hypertension may decrease BP, but the end result will be a further reduction in pressure natriuresis and even greater increases in plasma volume and hence SV, CO and Q. This should simply return BP to hypertensive levels, a change that may result in adequate BP control with vasodilator therapy over the short-tern, but over the years as the

pressure natriuresis effect diminishes with aging, vasodilator therapy may fail to maintain BP control.

5.8 Implications of alterations in the renin-angiotensin-aldosterone system

An important observation in the present thesis is that across the adult lifespan decreases in plasma renin concentrations were largely determined by age and associated with increases in systemic blood flow (Woodiwiss, Mmopi et al., 2020, chapter 3). This has important implications as low renin hypertension is thought to occur mainly in response to salt sensitivity and often without plasma volume expansion. The present study provides clear evidence that increases in BP across the adult lifespan are indeed associated with a low renin state, but that this effect is likely to be caused by an increased systemic blood flow. This is indeed entirely in keeping with conventional physiology, which attributes a large portion of the control of renin release to the absolute amount of Na^+ presented to the macula densa. Hence, even when plasma Na^+ concentrations are normal, increases in systemic and hence renal blood flow will suppress renin release by presenting a greater amount of Na^+ to the macula densa. Thus, the age-related changes in plasma renin concentrations in the present study suggest that plasma volume expansion is indeed an important change associated with hypertension in groups of African ancestry living in Africa, but that this effect is accounted for by age-related changes.

Despite the age-related decreases in plasma renin concentrations associated with increases in systemic blood flow, in the present thesis I show that circulating aldosterone concentrations paradoxically increase in parallel with age and systemic blood flow (Woodiwiss, Mmopi et al., 2020). These changes provide one important explanation as to why suppression of renin release with increases in systemic blood flow fails to translate into a compensatory reduction in plasma volume and hence a return of blood flow to normal. The possible mechanisms of how an attenuated suppression of aldosterone occurs has been reviewed in the introductory chapter of the present thesis. This alteration cannot however be seen as the primary stimulus for increases in plasma volume and systemic blood flow in hypertension in Africa as adjustments for plasma aldosterone concentrations by no means eliminated the relationship between age and increases in SV or CO. Nevertheless, together with an altered pressure- natriuresis relationship produced by possible age-induced renal tubular damage (see above), paradoxical increments in aldosterone concentrations

with age may further attenuate the pressure-natriuresis relationship and be sufficient to explain age-related increases in plasma volume and hence systemic blood flow. Whether targeting aldosterone receptors (spironolactone or eplerenone) or ENaC (amiloride) with existing antihypertensive agents reduces systemic blood flow therefore requires consideration. This would nevertheless be a difficult approach to employ at a primary healthcare level because of the dangers of hyperkalaemia (and hyponatraemia) produced by any of these agents, and the side effects in men (breast pain and breast enlargement) of the cost-effective aldosterone receptor blocker, spironolactone.

5.9 Limitations

The limitations for the studies included in this thesis have been discussed under the relevant data chapters. However, there are some key limitations that need to be highlighted. First, the present studies were cross-sectional in design, consistent with contemporary approaches employed by several major studies around the world including the Framingham Heart Study (Mitchell et al., 2010*a*,*b*). Thus, whether relationships between salt intake or age and the hemodynamic factors demonstrated represent residual confounding is unknown. Although longitudinal studies in individual participants across the adult lifespan would be ideal, these studies would only reach fruition after the duration of at least a full adult lifespan. Although intervention studies with salt intake would also be ideal, these would have to be conducted over a significant duration to show effects on proximal aortic Zc. In this regard, comparing the effects of a high and low salt intake over a prolonged duration would not be ethical as guidelines indicate that when BP increases salt intake should be reduced. However, the large sample sizes employed in the present studies, the robust nature of the outcomes and the consistency of the data with all major confounders accounted for, nevertheless lend support to the validity of the findings. Second, the most accurate method of assessing systemic flow today is with magnetic resonance imaging (MRI). Although MRI was not employed to determine flow, age-related increases in SV were almost identical when using outflow tract evaluations and LV dimension measurements (biplane Simpson). Thus, the increases in systemic flow were confirmed using two very different measurements. Third, as highlighted in each chapter, being a community-based study, non-invasive (indirect) assessments of central arterial pressure waves were obtained to determine Zc, TAC and reflected wave pressures.

However, as also pointed out throughout the chapters these approaches have been validated on a number of occasions by various groups over several decades. Fourth, the renal mechanisms responsible for increases in systemic flow were not identified in the present thesis. In this regard, this is a topic of an alternative students PhD thesis. As indicated in previous sections however, recent analysis nevertheless demonstrates that age-related increases in SV, CO, Q and LV end diastolic volumes are indeed independently associated with both non-age-related decreases in fractional Na^+ excretion (increased tubular Na^+ reabsorption) and age-related decreases in glomerular filtration rate (an index of nephron number or nephron loss)(Malan et al., in-preparation).

5.10 Conclusions

In conclusion, in the present thesis I provide evidence published in the high impact journals *Hypertension* (2 papers) and *Journal of Hypertension* (1 paper) to support the evolutionary theory of antagonistic pleiotropy and a shifted pressure- natriuresis relationship as a cause of primary hypertension in groups of African descent living in Africa. In this regard, I show that although habitual salt intake does not cause primary hypertension through alterations in systemic blood flow, but rather through an impact on proximal aortic Zc, that age-related increases in systemic blood flow are pathognomonic of most low-renin forms of primary hypertension at a community level. Increases in systemic flow were not accounted for by decreases in resistance or impedance to flow or to an enhanced left ventricular systolic function, but rather to a volume overload causing an increased cardiac filling volume and thus a Frank-Starling effect. These effects contributed substantially to both the pulsatile and steady components of BP and hence accounted for a substantial portion of hypertension associated with increases in either systolic or diastolic BP at a community level. The age-related increases in systemic blood flow largely explained the low-renin state frequently reported to occur in hypertension in groups of African ancestry. These data therefore provide the missing evidence (increases in systemic blood flow) to substantiate the importance of a shifted pressure- natriuresis relationship as a cause of primary, rather than just secondary hypertension, proposed by Arthur Guyton and colleagues close to 50 years ago.

Key points

- In a community-based study of groups of African ancestry living in Africa (a salt-sensitive population), abnormalities in Na^+ and K^+ intake determine pulse pressure or systolic blood pressure beyond steady-state pressures mainly through a potentially irreversible impact on proximal aortic impedance than through readily modifiable increases in aortic flow (blood volume) or wave reflection.
- In a community-based study of groups of African ancestry living in Africa, systemic blood flow in addition to vascular changes that generate resistance to flow contribute significantly to age-related and hypertension associated increases in both the steady (and hence mean arterial pressure) and pulsatile (and hence systolic blood pressure, diastolic blood pressure, and pulse pressure) components of blood pressure.
- In a community-based study of groups of African ancestry living in Africa, hypertension because of increases in either systolic or diastolic blood pressure is as strongly associated with increases in systemic blood flow (stroke volume and peak aortic blood flow) as with arterial and arteriolar effects (aortic characteristic impedance, total arterial compliance, systemic vascular resistance).

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APPENDIX 1

Ethical Clearance Certificate

R14/49 Ms Keneilwe Mmopi

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170773

NAME: (Principal Investigator)	Ms Keneilwe Mmopi
DEPARTMENT:	Physiology School of Physiology Faculty of Health Sciences University of the Witwatersrand
PROJECT TITLE:	Salt Intake and Aortic Backward Wave Function in Hypertension
DATE CONSIDERED:	Adhoc
DECISION:	Approved unconditionally
CONDITIONS:	Sub-study under Primary Study (M170401)
SUPERVISOR:	Prof Gavin Norton and Prof Angela Woodiwiss
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	28/08/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004,10th floor, Senate House/3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. in this case, the study was initially review July and will therefore be due in the month of July each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date 30-08-2017

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX 2

Turn-it-in Plagiarism Report

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ORIGINALITY REPORT

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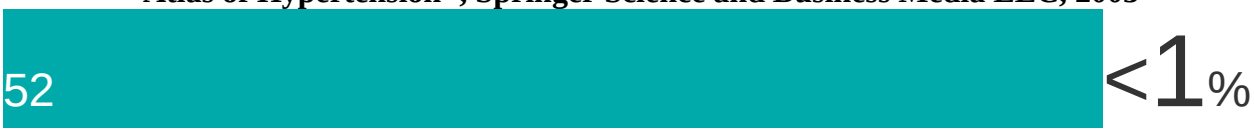
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