THE IMPACT OF LIFESTYLE CHANGES ON BLOOD PRESSURE AND THE HEART

Aletta Maria Esterhuyse Millen

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DECLARATION

I, Aletta Millen, declare that this thesis is my own, unaided work except where otherwise specified. It is being submitted for the degree of Doctor of Philosophy in the School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg. The work contained in this thesis has not been submitted for any degree or examination in this or any other University.

..... signed on the .....day of..... 2013.

I certify that the studies contained in this thesis have been approved by the Committee for Research in Human Subjects of the University of the Witwatersrand, Johannesburg. The ethics approval numbers are M10624 and M02-04-72 renewed as M07-04-69 and M1204108.

..... signed on the .....day of..... 2013.

Prof Angela Woodiwiss (supervisor 1)	Dr Ingrid Avidon (supervisor 2)
.....	.....
Date:	Date: .....

ABSTRACT

There is a marked increase in the prevalence of obesity worldwide. In this regard, obesity is associated with a considerable morbidity and mortality. A large component of this morbidity and mortality is through an increased risk for cardiovascular events, which is in-part attributed to the association between obesity and hypertension or cardiac dysfunction. In the present thesis I conducted a series of studies designed to advance our understanding of the role of an obesity-associated sedentary lifestyle or insulin resistance in promoting the development of hypertension or left ventricular (LV) diastolic dysfunction.

Despite the particular importance of salt-sensitivity in contributing toward increases in BP in groups of African descent, the role of obesity or insulin resistance in mediating this effect in this ethnic group is uncertain. In addition, whether this effect translates into changes in central aortic BP is unknown. I therefore aimed to determine whether obesity or insulin resistance (homeostasis model assessment of insulin resistance [HOMA-IR]) is independently associated with salt intake (24-hour urinary Na^+/K^+) blood pressure (BP) relationships in 331 participants from a community sample of African ancestry not receiving treatment for hypertension and whether this association occurs in both brachial and central aortic BP. Although log HOMA-IR was not independently associated with BP, with adjustments including diabetes mellitus and the individual terms, an interaction between log HOMA-IR and urinary Na^+/K^+ was associated with 24-hour, day systolic ($p<0.05$) and 24-hour, day and night diastolic ($p<0.002$ to $p<0.001$) BP. Neither aortic augmentation pressure, aortic augmentation index, central aortic pulse pressure not aortic pulse wave velocity were independently associated with an interaction between log HOMA-IR and urinary Na^+/K^+ The multivariate adjusted relationship between urinary Na^+/K^+ and night diastolic BP increased across tertiles of HOMA-IR (Tertile 1: β-coefficient=-0.79±0.47; Tertile 2: β-coefficient=0.65±0.35; Tertile 3: β-coefficient=1.03±0.46, $p<0.05$ tertiles 3 and 2 vs 1). In conclusion, insulin resistance is independently associated with the relationship between salt intake, as indexed by urinary Na^+/K^+, and ambulatory BP in groups of African descent. Thus,

in persons of African ancestry, salt-sensitivity may depend in-part on the presence of insulin resistance. These effects of insulin resistance cannot be accounted for by actions on central aortic haemodynamics.

In overweight or obese individuals, whether the beneficial effects of exercise training on BP can be explained by decreases in aortic systolic pressure augmentation is uncertain. Therefore I aimed to determine the impact of 6 weeks of exercise training (≥3 days/week, on a stationary bike and/or treadmill) either preceded (n=19) or followed by (n=16) a 6 week control period of no exercise, on aortic augmentation pressure and index and central aortic and brachial BP in 35 sedentary or recreationally active young-to-middle-aged overweight (40%) or obese (60%) individuals. Aortic augmentation pressure (AP), aortic and peripheral augmentation indices (AIx), central aortic BP (SphygmoCor) and brachial BP were determined before and after exercise training and a control period. Peak oxygen consumption (cardiorespiratory fitness) increased ($p=0.0001$) from 27.0±5.1 to 28.8±5.8ml.kg^{-1}.min^{-1} after 6 weeks of exercise. Exercise training decreased brachial systolic BP and diastolic BP from 142±8/94±8 mm Hg to 134±11/86±11 mm Hg ($p<0.005$/$p<0.005$); whereas no changes were observed after the control period. Neither AP (baseline: 9.2±4.2 mm Hg; after 6 weeks training: 8.7±6.1 mm Hg), aortic AIx (Baseline: 24.6±11.0%; after 6 weeks training: 22.7±11.1%), nor peripheral AIx (Baseline: 81.4±16.7%; after 6 weeks training: 76.4±16.5%) were modified by exercise training. Although aortic systolic BP decreased after exercise training (132±8 mm Hg to 124±12 mm Hg, $p<0.002$), these changes were accounted for by decreases in MAP. In conclusion, overweight or obese individuals, although short-term aerobic exercise training which improved cardiorespiratory fitness, may decrease aortic and brachial BP, these effects are not attributed to alterations in aortic systolic pressure augmentation. Considering the effects of obesity on the vasculature the repercussions on the function of the heart itself should also be considered.

In this regard whether the relationship between obesity and abnormalities in LV diastolic function can be accounted for by insulin resistance independent of adiposity indexes, or LV mass or remodelling is uncertain. In 361 participants (24% overweight, 38%

obese) from a South African community sample of black African descent not receiving treatment for hypertension, I evaluated LV dimensions and diastolic function (E/A) with echocardiography, HOMA-IR, and nurse-derived conventional BP. Log HOMA-IR was associated with E/A independent of waist circumference and additional confounders ($p<0.005$). The independent impact of log HOMA-IR on E/A (β-coefficient=-0.11±0.04, $p<0.005$) was similar to the effects of conventional DBP (β-coefficient=-0.12±0.04, $p<0.01$), but less than that of age (β-coefficient=-0.52±0.05, $p<0.0001$). Although, log HOMA-IR was independently related to relative wall thickness (partial $r=0.16$, $p<0.005$), the multivariate adjusted relationship between log HOMA-IR and E/A was not altered by further adjustments for either relative wall thickness (β-coefficient=-0.10±0.04, $p<0.01$) or LV mass index (β-coefficient=-0.11±0.04. $p<0.01$). In conclusion, insulin resistance is associated with a decreased LV diastolic function independent of adiposity indices and LV mass or remodelling. Hence insulin resistance may be an important pathophysiological mechanism responsible for the abnormalities in LV diastolic function.

Although exercise training has consistently been shown to be unable to improve obesity-associated decreases in LV diastolic function as assessed using change function measurements, the effects of exercise training on LV diastolic myocardial function as assessed using Tissue Doppler Imaging (TDI) are uncertain. Hence, in 32 overweight (n=11) or obese (n=21), sedentary or recreationally active men and women (30–57 years), I aimed to determine the impact of 6 weeks of exercise training either preceded (n=16) or followed by (n=16) a 6 week control period on TDI-derived parameters of LV diastolic function (e', e'/a', E/e'). Cardiorespiratory fitness (peak oxygen consumption, VO_{2peak}), LV diastolic function (E/A, TDI e', e'/a' and E/e') (echocardiography) and body weight were determined at baseline and after the control and exercise training periods. Baseline measures of diastolic function were comparable with those noted in overweight and obese participants from a community sample (n=245) and 56% (n=18) had baseline e' values (early diastolic abnormalities) that were below the lower 95% confidence intervals of a lean and healthy cohort (n=60) of the community sample. Exercise training increased peak oxygen consumption from 27.4±4.9 to

29.4±5.8 $ml.kg^{-1}.min^{-1}$ (p=0.0001); but had no effect on body mass index (p=0.99). No changes in TDI indices of LV diastolic function were observed after exercise training in all participants (e': p=0.74, a': p=0.98, e'/a': p=0.85; E/e': p=0.26), in participants with abnormal e' values (n=18)(e': p=0.99, a': p=0.96, e'/a': p=0.91; E/e': p=0.97) or in obese participants only (n=21)(e': p=0.67, a': p=1.00, e'/a': p=0.78; E/e': p=0.11. Thus, exercise training alone, despite producing an improved cardiorespiratory fitness is unable to improve obesity-associated decreases in LV diastolic myocardial function

In conclusion, the results of my thesis suggest the following: In groups of African ancestry, obesity effects on salt sensitive hypertension or abnormalities of LV diastolic function may in-part be accounted for by insulin resistance. In addition, short-term, regular exercise in overweight and obese individuals without weight loss, but with an increased cardiorespiratory fitness, is unable to influence those large vessel characteristics that contribute to BP or LV diastolic function. Thus, studies that assess the impact of approaches that increase insulin sensitivity on BP-responses to salt intake and LV diastolic function in obese individuals are warranted, but short-term exercise training alone may not achieve this goal.

PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS THESIS

Publications

Millen AME, Norton GR Majane OHI, Maseko MJ, Brooksbank R, Michel FS, Snyman T, Sareli P, Woodiwiss AJ. Insulin resistance and the relationship between urinary salt excretion and ambulatory blood pressure in a community of African ancestry. *Am J Hypertens* 2012, accepted for publication.

Millen AME, Norton GR, Avidon I, Woodiwiss AJ. Effects of short-term exercise training on aortic systolic pressure augmentation in overweight or obese individuals. *Eur J Appl Physiol* (under review).

Millen AME, Norton GR, Avidon I, Woodiwiss AJ. Effects of short-term exercise-training on tissue Doppler indices of left ventricular diastolic function in overweight and obese individuals *J Sports Sci* (under review).

Oral presentations

Millen AME, Avidon I, Norton GR, Woodiwiss AJ. Moderate and high intensity acute exercise decreases central pressures in people with mild to moderate hypertension. South African Sports Medicine Association Congress, Johannesburg, 2011. (First prize)

Millen AME, Avidon I, Norton GR, Woodiwiss AJ. Acute exercise decreases central pressures in people with mild to moderate hypertension. 39th Annual Congress of Physiology Society of Southern Africa, Cape Town, 2011.

Poster presentations

Millen AME, Avidon I, Norton GR, Woodiwiss AJ. Effects of short-term exercise training on aortic stiffness and central blood pressure in young-to-middle aged pre- or grade I

hypertensives. The University of the Witwatersrand Cross-Faculty Symposium, Johannesburg, 2012. (First prize)

Millen AME, Avidon I, Norton GR, Woodiwiss AJ. Effects of short-term exercise training on aortic stiffness and central blood pressure in young-to-middle aged pre- or grade I hypertensives. The High Blood Pressure Research Meeting. Washington DC, USA, 2012.

Millen AME, Avidon I, Norton GR, Woodiwiss AJ. The effects of moderate and high intensity exercise training on central and peripheral blood pressure in mild-to-moderate hypertensives. The Southern African Hypertension Society Congress. Cape Town, 2012.

STATEMENT OF CONTRIBUTION TO DATA COLLECTION AND ANALYSIS

I declare that I designed and collected all data for the intervention studies as presented in Chapters 3 and 5. The cross-sectional study was designed in conjunction with Professors AJ Woodiwiss and GR Norton. I was part of a team of researchers (including Prof AJ Woodiwiss, Dr Carlos Libhaber (cardiologist), Ms Nomonde Molebatsi and Nkele Maseko) responsible for collecting data in a large cross sectional study. I was largely responsible for the collection of the main outcome variables reported in Chapters 2 and Chapter 4. I performed all data analysis with the help of Prof AJ Woodiwiss. I wrote the manuscripts for the papers emanating from this thesis and they were reviewed by Professors GR Norton and AJ Woodiwiss. Chapter 4 forms part of a larger study conducted and will be published in conjunction with Dr Carlos Libhaber et al.

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LIST OF ABBREVIATIONS AND ACRONYMS

A	late diastolic filling
a’	late diastolic flow velocity
ACEI	angiotensin converting enzyme inhibitor
Aer	aerobic exercise
AIx	central (aortic) augmentation index
ANOVA	analysis of variance
AP	augmentation pressure
Appl tonom	applanation tonometry
art	artery
BF	body fat
bpm	beats per minute
BMI	body mass index
BP	blood pressure
CAD	coronary artery disease
CF PWV	carotid-femoral PWV
CI	confidence intervals
cm	centimetre
$cm.s^{-1}$	cm per second
CO	cardiac output
Cont	control
CRP	C-reactive protein
CV	cardiovascular
c_PWV	central PWV
D	diet
DBP	diastolic blood pressure
DM	diabetes mellitus
DT	deceleration time

dur	duration
e’	early diastolic flow velocity
E	early diastolic filling
E/A	ratio of early-to-late diastolic filling
e’/a’	ratio of early-to-late mitral annulus flow velocity
E/e’	diastolic filling pressure
ECG	electrocardiogram
EF	ejection fraction
F	female(s)
fem art	femoral artery
FMD	flow mediated dilation
FSmid	midwall fractional shortening
GFR	glomerular filtration rate
$g.m^{-2}$	grams per square meter
GTF	generalized transfer function
HbA1c	glycated haemoglobin
HOMA-IR	homeostasis model of insulin resistance
HR	heart rate
HR_{max}	maximum heart rate
HRR	heart rate reserve
HRT	hormone replacement therapy
HT	hypertensive
IDH	isolated diastolic hypertension
i.e.	namely
IGT	impaired glucose tolerance
ISH	isolated systolic hypertension
IR	insulin resistance
IVST	interventricular septal wall thickness

IVRT	isovolumic relaxation time
K^+	potassium ion
kg	kilogram(s)
$kg.m^{-2}$	kilogram per square metre
LAVI	left atrial volume index
L_T	lactate threshold
LV	left ventricle
LVED	LV end diastolic
LVEDD	LV end diastolic diameter
LVEDV	LV end diastolic volume
LVESD	LV end systolic diameter
LVESV	LV end systolic volume
LVH	LV hypertrophy
LVM	LV mass
LVMI	LVM indexed for body surface area
M	male(s)
MAP	mean arterial pressure
mEq	milli equivalent
$ml.kg^{-1}.min^{-1}$	millilitres per kilogram body weight per minute
$ml.min^{-1}$	millimetres per minute
ml	millilitres
mm Hg	millimetres of mercury
mmol	millimole
$mmol.day^{-1}$	millimoles per day
$mmol.l^{-1}$	millimole per litre
$m.s^{-1}$	meters per second
ms	milliseconds
MS	metabolic syndrome

MWT	mean wall thickness
n	number (sample size)
N	normal
Na-K-2Cl	sodium-potassium-chloride co-transporter
Na^+	sodium ion
Na^+/K^+	sodium-to-potassium ratio
N-MS	no metabolic syndrome
NT	normotensive
OB	obese
OW	overweight
QUICKI	quantitative insulin sensitivity check index
p	probability
P	prehypertensive
P1	central (aortic) forward wave pressure
P2	peak pressure of the second pressure wave of the radial pulse
PLV	peak lengthening velocity
PP	pulse pressure
PPc	central (aortic) PP
PWT	posterior wall thickness
PWV	pulse wave velocity
p_PWV	peripheral PWV
r	correlation coefficient
R	resistance exercise
RAAS	renin-angiotensin-aldosterone system
RDA	recommended daily allowance
RPE	rate of perceived exertion
RWT	relative wall thickness
s	second(s)

s’	systolic flow velocity
SAS	statistical analyses software
SBP	systolic blood pressure
SBPc	central SBP
SD	standard deviation
SEM	standard error of the mean
SF	sum of skinfolds
SV	stroke volume
TDI	tissue Doppler imaging
TPR	total peripheral resistance
Tx	treated
$\mu U.ml^{-1}$	micro units per milliliter
VO_2	oxygen consumption
VO_{2max}	maximum oxygen consumption
VO_{2peak}	peak oxygen consumption
W-HT	white coat hypertension
WC	waist circumference
WHO	World Health Organization
wk	week(s)
W_{max}	maximal workload
Wt	weight
y	year(s)

PREFACE

The prevalence of obesity is high in both economically developed and developing countries. In South Africa, at the turn of the century, about one third of men and over half of women were noted to be obese. These high prevalence rates of obesity are of great concern as obesity is a major risk factor for cardiovascular diseases. The obvious means to reduce obesity associated cardiovascular risk would be to decrease body weight; however, weight loss is difficult and reductions in body weight are seldom maintained. Hence, in order to institute effective therapeutic strategies, an understanding of the mechanisms of obesity–associated risk is required.

The present thesis was prompted by a need to address some outstanding issues regarding the impact of obesity and associated inactivity on blood pressure (BP), including central aortic BP and left ventricular (LV) diastolic function. Firstly obesity contributes to the development of hypertension and is related to central aortic BP and the determinants thereof. In order to understand the impact of obesity on peripheral and central aortic BP, it is important to understand the mechanisms involved. In this regard, it has been suggested that obesity may influence the impact of salt intake on BP. Indeed in European individuals, insulin resistance may play a role in the interaction between salt intake and BP. Despite the high prevalence of salt sensitive hypertension in persons of African ancestry the impact of obesity associated insulin resistance on the relationship between salt intake on BP has not been well studied. Hence in the present thesis I investigated the relationship between insulin resistance and the BP response to salt intake in a community sample of African Ancestry with a high prevalence of insulin resistance.

Second, obesity is associated with inactivity. Hence, one means of reducing obesity-induced increases in BP is to encourage exercise. Indeed, exercise training programmes have consistently been shown to reduce peripheral BP. Although central aortic BP predicts cardiovascular outcomes more closely than peripheral BP, the role of exercise as a central aortic BP-lowering intervention in obesity is uncertain. Exercise may reduce aortic stiffness which is one of the determinants of aortic BP; however there is limited evidence to support a

role for the beneficial effects of exercise training on aortic augmentation pressure and indices. Hence I evaluated whether exercise training-induced reductions in peripheral BP may be attributed to reductions in aortic augmentation pressures and indices in overweight and obese individuals.

Thirdly, obesity is associated with abnormalities on left ventricular diastolic function. Although it has been suggested that insulin resistance independent of the presence of diabetes mellitus may explain obesity-associated LV diastolic dysfunction, current data are controversial. Most previous studies have been conducted in select clinical samples with small sample sizes. Indeed only two large studies have been conducted. However, one of these studies failed to show a relationship between insulin resistance and LV diastolic function and in the other study the relationship was not adjusted for adiposity indices. Hence, I evaluated whether an index of insulin resistance is associated with LV diastolic function independent of adiposity indices, in a relatively large, randomly selected community sample with a high prevalence of obesity.

Lastly, bearing in mind that obesity is a strong determinant of LV diastolic dysfunction at a community level, approaches that may prevent or reverse obesity-associated LV diastolic dysfunction are required. In this regard, the role of exercise training is uncertain. The majority of studies suggest that LV diastolic function as assessed from early (E)-to-late (A) transmitral velocity measurements is unchanged after exercise training in overweight or obese individuals. However, data on the effects of exercise training of LV diastolic function assessed using tissue Doppler indices is controversial. In order to overcome the limitations and deficiencies of the previous studies (small sample sizes; men only; exercise intervention accompanied by weight loss; hypertensives receiving treatment) I assessed the impact of exercise training alone on indices of LV diastolic function, including tissue Doppler imaging, in overweight and obese individuals.

The present thesis is written as a series of semi-independent chapters, each with its own introduction, a brief summary of the methods, results and a discussion. The thesis begins with a literature review chapter that summarises the current knowledge and

incongruities in the field, which will highlight the reasons for conducting the different studies presented in this thesis. The final chapter concludes with a summary of the findings of each chapter and accentuates the relevance of the findings to the medical field. In support of the present thesis chapter 2 has been accepted for publication in the American Journal of Hypertension (Millen et al 2012). The data in the other chapters have been submitted to international journals for review.

CHAPTER 1

OBESITY EFFECTS ON BLOOD PRESSUE AND CARDIAC FUNCTION: CURRENT UNDERSTANDING AND CONTROVERSIES RELATED TO A SEDENTARY LIFESTYLE AND INSULIN RESISTANCE

1.1 Introduction

Cardiovascular disease presently accounts for the second highest cause of deaths in South Africa (Statistics South Africa 2010). In addition, there is evidence that the contribution of cardiovascular disease to deaths in South Africa may be increasing. In this regard, between 1997 and 2004, deaths from cerebrovascular disease in adults increased by 16%, deaths from hypertensive heart disease by 25% and deaths from ischaemic heart disease by 5% (Statistics South Africa 2006). These increases in cardiovascular disease in South Africa are in-line with the World Health Organisation (WHO) prediction that during the period 2006 to 2015, deaths from non-communicable diseases would increase by more than 24% in Africa. An acknowledged and major risk factor for cardiovascular disease is obesity. What is the evidence that obesity contributes to a substantial portion of the population attributable risk for cardiovascular disease?

In the United States of America (USA) in 2004, the prevalence of obesity was noted to be ~31% in men and ~33% in women (Ogden et al 2007). In South Africa, a country which from an economic perspective is considered to be a developing nation, at approximately the turn of the century, in a nationally representative population sample of 13 089 participants, ~29% of men and ~57% of women were noted to be obese (Puoane et al 2002). The high prevalence rates of obesity in both economically developed and developing countries is of grave concern. In this regard, there is substantial evidence to indicate that obesity is associated with a considerable morbidity and mortality (Allison et al 1997, Calle et al 1999, Heitmann et al 2000, Lahmann et al 2002, McGee 2005, Gu et al 2006, Jee et al 2006, Whitlock et al 2009) and a large component of this is through an increased risk for cardiovascular events (Eckel et al 2002, Klein et al 2004, Poirier et al 2006, Murphy et al 2006). This increased risk for cardiovascular events is attributed to the association between obesity and classical cardiovascular risk factors, including hypertension, diabetes mellitus, and dyslipidaemia (Eckel et al 2002, Klein et al 2004). However, obesity retains independent associations with stroke (Kurth et al 2002, Suk et al 2003), myocardial infarction (Yusuf et al

2005, Steyn et al 2005) and heart failure (Chen et al 1999, He et al 2001, Johansson et al 2001, Wilhelmsen et al 2001, Kenchaiah et al 2002, 2009, Ingelsson et al 2005a and 2005b, Nicklas et al 2006, Bahrami et al 2008, Spies et al 2009), even after adjustments for classical cardiovascular risk factors.

The obvious solution to the problem of what may currently be considered as a worldwide trend for increasing prevalence rates of obesity is to implement weight reduction programmes at a number of levels. However, there is evidence to indicate that obesity, once established, is difficult to manage. Indeed, weight reduction programmes seldom result in obese individuals reaching target body weights or the weight loss is insufficient to eradicate the damage that may be caused by obesity (Latner et al 2002, Anderson et al 2001). Moreover, the outcome of weight reduction is frequently an inability to maintain decreases in body weight (The Trials of Hypertension Prevention Collaborative Research Group 1997, Weiss et al 2007, Aucott et al 2009, Hedayati et al 2011, Jordan et al 2012). In this regard adherence to lifestyle changes decrease from as early as 6 month after the initiation of weight reduction programmes (Elmer et al 2006). Thus, in order to institute effective therapeutic strategies, a better understanding of the mechanisms of cardiovascular risk factors and damage associated with obesity is required.

In the present thesis I conducted a series of studies designed to advance our current understanding of the role of obesity-associated insulin resistance in contributing toward variations in BP, including central aortic BP, attributed to salt-intake, or the role of obesity-associated insulin resistance in contributing toward cardiac dysfunction at a community level. I also explored the possibility that in the absence of weight loss, relatively short-term exercise programmes that improve cardiorespiratory fitness, may attenuate large artery changes that play an important role in determining central aortic BP or improve obesity-associated changes in cardiac function. Consequently, in the present chapter I will argue in favour of performing these studies.

In the present chapter I will first highlight the importance of central aortic as opposed to brachial BP, the evidence to support a role for obesity and associated lack of exercise in

contributing toward large artery dysfunction and central aortic BP, and the evidence to support a role of insulin resistance in contributing toward obesity-related increases in BP in response to salt intake. Subsequently I will underscore the importance of preclinical cardiac dysfunction in predicting cardiovascular outcomes, the evidence to support a role for obesity and associated lack of exercise in contributing toward preclinical cardiac dysfunction, and the evidence to support a role of insulin resistance in contributing toward obesity-related preclinical cardiac dysfunction. In so doing in the present chapter I will highlight areas where there is a significant lack of evidence and thus where the studies conducted in the present thesis may therefore contribute toward the relevant fields of study.

1.2 Central aortic versus brachial blood pressure: A role for obesity?

Population or hospital-based studies conducted in economically developing populations in South Africa, including African populations, clearly demonstrate that in comparison to other cardiovascular risk factors, hypertension is the dominant risk factor for myocardial infarcts (Steyn et al 2005), stroke (O'Donnell et al 2010) and heart failure of non-ischaemic origins (Stewart et al 2008). Indeed, in South Africa between 2002 and 2005, 31% and 45% of deaths in men and women respectively were attributed to vascular diseases, mainly as a result of hypertension (Mayosi et al 2009). To what extent does obesity contribute toward hypertension?

1.2.1 Obesity and increases in brachial blood pressure

There is a large body of evidence supporting relationships between an excess body fat and an increased BP and that obese persons are at higher risk for developing hypertension, a change that contributes substantially to cardiovascular morbidity and mortality (Hall 1997, Must et al 1999, Uzu et al 2006, Forman et al 2009, Kotchen 2010). The importance of these relationships is illustrated by the parallel rise in prevalence rates of obesity and hypertension worldwide and the increasing prevalence of the need for anti-

hypertensive agents in the obese (Appel et al 2006, Mancia et al 2007, Jordan et al 2012). As a consequence of the evidence to support such programmes, weight loss interventions are regarded as a cornerstone of the treatment of hypertension related to obesity (Klein et al 2004, Straznicky et al 2010) and current guidelines for the management of hypertension emphasize the importance of weight loss via diet and exercise (Chobanian et al 2003, Poirier et al 2006, Mancia et al 2007). Although a review of all of the evidence to support a role for obesity as a major determinant of BP goes beyond the scope of the present thesis, it is worth considering some of the evidence from large study samples to support a role for obesity in this regard.

There is now substantial evidence from population-based studies with large study samples (n=10969-15063) in favour of obesity being a major determinant of BP and the development of hypertension (Harris et al 2000, Zhu et al 2005). Indeed, the odds of developing hypertension are ~1.7-3.4 times greater in obese individuals as compared to lean individuals (Harris et al 2000). Further, there is substantial evidence to indicate that weight reduction results in decreases in BP. Dietary interventions produce significant antihypertensive effects which are often proportional to the weight lost (The Trials of Hypertension Prevention Collaborative Research Group 1997, He et al 2000, Straznicky et al 2010) and a meta-analysis of intervention studies on the effects of weight loss on BP indicates that for every 1 kg body weight lost, systolic BP/diastolic BP decreases by 1.05/0.92 mm Hg (Neter et al 2003, Hedayati et al 2011). Moreover, in the Atherosclerosis Risk in Communities study involving 3245 participants, a ~1.5-2.0 fold chance of hypertension remission was reported to occur for every 1 kg decrease in body weight over 9 years (Juhaeri et al 2003). Weight reduction also makes a substantial contribution to BP control in persons taking antihypertensive agents (Neter et al 2003). However, none of the aforementioned studies report on the impact of obesity on central aortic BP. What is the importance of central aortic as opposed to brachial BP?

1.2.2 Central aortic versus brachial blood pressure

Although the definition of hypertension and the role of BP in cardiovascular risk prediction is still based on brachial artery BP measurements (Chobanian et al 2003, Williams et al 2004, Mancia et al 2007), brachial BP may not fully account for the adverse effects of an increased BP. In this regard, dynamic or pulsatile pressures, as indexed by pulse pressure (PP) and systolic BP are not the same at the periphery (i.e. brachial artery) as compared to centrally (aorta). Indeed, brachial pressures can be considerably higher than central aortic pressures, a finding that has been termed “PP or systolic BP amplification” (Nichols et al 2011). This difference is particularly marked in the young, where brachial systolic BP can be 12 to 20 mm Hg higher than aortic systolic BP (Mahmud and Feely 2000, Wilkinson et al 2000). With age-related changes in large vessels, this difference between central and brachial artery BP gradually decreases (Vaitkevicius et al 1993, Nichols 2005, McEniery et al 2005, 2007, Avolio et al 2009, Nichols et al 2011). Therefore, BP measured at the brachial artery may not accurately reflect central pressures until much later in life. Taking into account that BP in the aorta is more likely to reflect what the heart and cerebral vasculature are exposed to, central BP may be a better predictor of cardiovascular damage or events than brachial BP. Is there evidence to suggest that central aortic BP is more closely associated with cardiovascular damage or risk than BP measured at the brachial artery?

A number of studies have demonstrated that central aortic BP is associated with cardiovascular damage (including left ventricular hypertrophy and carotid intima-media thickness) independent of brachial BP (Roman et al 2007, 2010, Pini et al 2008, Wang et al 2009, Norton et al 2012). Moreover, central (aortic) systolic BP and/or PP are predictors of cardiovascular risk beyond peripheral (brachial) PP or systolic BP (Safar et al 2002, Chirinos et al 2005, Williams et al 2006, Roman et al 2007, Pini et al 2008, Jankowski et al 2008, Wang et al 2009). In contrast, two studies suggest that brachial BP is better or at least equivalent to aortic BP in predicting cardiovascular events (Dart et al 2006, Mitchell et al 2010). However, in one of these studies all participants were treated with either a diuretic or angiotensin-converting enzyme-inhibitor (ACEI) over the duration of the study, but in

multivariable analysis the use of antihypertensive therapy was not included as a potential confounder (Dart et al 2006). Moreover, Mitchell et al (2010) reported that when accounting for classic risk factors including conventional BP, central PP and wave reflection do not predicts cardiovascular disease risk. The difference in the predictive power between central and peripheral PP may relate to difference in technique applied among the various studies. Nevertheless as brachial BP does not necessarily reflect central aortic BP, and aortic BP may be more closely associated with cardiovascular outcomes, it is important to consider whether obesity may differentially affect central aortic as compared to brachial BP. To address this question an understanding of the mechanisms responsible for variations in central aortic as opposed to brachial BP is required.

1.2.3 Differences in the determinants of central aortic versus brachial BP

With ventricular contraction a pressure wave (incident or forward wave) is generated that travels along the arterial tree. Theoretically, part of the energy generated in large vessels by ventricular contraction is reflected back to the heart from major branches of the aorta and muscular arteries (O'Rourke et al 2002, Nichols et al 2011). The theoretically backward travelling reflected wave may return to the heart during the diastolic period of the cardiac cycle and as such, augment aortic pressures during diastole. As coronary blood flow occurs mainly during the diastolic period of the cardiac cycle, the advantage to augmenting diastolic pressures is to enhance coronary blood flow. However, if wave return is sufficiently early, it may coincide with the systolic period of the cardiac cycle, where it will meet the incident wave generated by subsequent ventricular ejection. As a result, the reflected wave may augment the incident wave and thus peak systolic pressure. That component of the aortic waveform that reflects systolic pressure augmentation is often called augmented pressure (AP). Consequently the pulsatile pressure or central systolic BP is a summation of the incident and reflected wave pressure (O'Rourke et al 2002, Nichols et al 2011, Westerhof and Westerhof 2012). Although the aforementioned has to-date been the principle mechanism proposed for systolic pressure augmentation, there is nevertheless evidence to

suggest alternative mechanisms (Reymond et al 2009, 2011). In older persons, the reflected wave often returns during systole resulting in an increased central systolic BP and PP and a decreased central diastolic BP. Hence, the early return of the reflected wave increases cardiac afterload and negatively impacts on coronary perfusion (Mitchell et al 2004). What are the factors that may influence the incident and augmented pressure waves?

Stroke volume, the elastic properties or stiffness of the aorta, and the diameter of the aorta, are believed to influence the magnitude of the incident wave (the peak of this wave may be noted at the first systolic shoulder of the aortic pulse, often termed 'P1') by altering the aortic impedance to blood flow (Nichols et al 2011). However, as a change in aortic root diameter does not contribute to hypertension progression (Ingelsson et al 2008), an increased aortic stiffness and stroke volume may be the main determinants of the amplitude of the incident wave (P1). In contrast, AP may be determined by the magnitude and speed of the pulse wave (Segers et al 2007, Nichols et al 2008, Cecelja et al 2009) (presumably of the reflected wave) and the speed of the pulse wave are increased by enhanced large artery stiffness. Thus, both P1 and AP share large artery stiffness as a common mechanism responsible for variations in their magnitude. However, in addition to arterial stiffness contributing to AP, AP is also determined by the magnitude of wave reflection, which may be affected to a large extent by characteristics of medium sized or other arteries (Cecelja et al 2009). In the context of the preceding discussion what then are the currently employed indices of large artery function that may be evaluated using non-invasive approaches?

As AP is determined in-part by alterations in aortic stiffness, the degree of amplification of central systolic BP due to early wave reflections (calculated as the central augmentation index [AIx] from AP/aortic PP) is considered to be one index of arterial stiffness (Casey et al 2008). The velocity of wave travel from the carotid to the femoral artery (carotid-femoral pulse wave velocity [PWV]) is also considered to be an index of aortic stiffness. Pulse wave velocity across other arterial beds (arm, leg or carotid artery) are often also assessed and employed as indices of large artery stiffness. Importantly, although AIx and PWV are related, they signify different aspects of arterial function (Segers et al 2007).

Indeed, as suggested in the preceding paragraph, AP is not only determined by large vessel stiffness, but is considered a composite measure, reflecting the degree and site of wave reflection and is believed to better reflect large artery changes in younger populations (Mitchell et al 2004, McEniery et al 2005, Westerhof and Westerhof 2012). In this regard, AP or AIx have been demonstrated to related to cardiovascular target organ changes or cardiovascular outcomes independent of other cardiovascular risk factors in a variety of clinical populations (Saba et al 1993, London et al 2001, Nürnberger et al 2002, Hayashi et al 2002, Weber et al 2004, 2005, Ueda et al 2004, Chirinos et al 2005, Williams et al 2006, Hashimoto et al 2007). In comparison PWV only reflects the speed of wave travel and does not account for the magnitude of wave reflection (McEniery et al 2005, Segers et al 2007, Cecelja et al 2009, Nichols et al 2011). Thus PWV mainly increases at older ages, whereas AIx mainly increases in middle age. Hence PWV is believed to be a more accurate index of arterial stiffness, at least in older individuals (McEniery et al 2005). Aortic PWV has also been demonstrated in a number of clinical or population-based studies to predict cardiovascular outcomes independent of traditional risk factors including brachial BP (Blacher et al 1999, 2003, Laurent et al 2001, Meaume et al 2001, London et al 2001, Cruickshank et al 2002, Laurent et al 2003, Hansen et al 2006, Mattace-Raso et al 2006, Mitchell et al 2010). With these mechanisms responsible for aortic BP in mind, the question thus arises as to whether obesity could differentially influence central aortic versus brachial BP.

1.2.4 Obesity effects on central aortic BP and the determinants thereof

Table 1.1 summarises the evidence to suggest that obesity modifies aspects of large artery function. In this regard, a number of studies with cross-sectional study designs suggest that obesity (Resnick et al 1997, Sutton-Tyrrell et al 2001, Mackey et al 2002, Wildman et al 2003, Danias et al 2003, Ferreira et al 2004, Snijder et al 2004, Czernichow et al 2005, Zebekakis et al 2005, Toto-Moukouo et al 1986, Majane et al 2008) or the metabolic

Table 1.1 Summary of studies that have shown the interaction between obesity and arterial stiffness.

Cross-sectional

Authors	Age	BMI	BP status	M/F	Measure of obesity	Measure of arterial stiffness	Outcome	Adjustments
Amar et al 2001	35-64	population sample		523/470	BMI, WC, WHR	CF PWV	BMI & WHR associated with PWV, but not after adjusting	SBP, CV risk factors
Czernichow et al 2005	59±5	population sample, 9% OB		n=1014	fat mass, WC, WHR	CF PWV	association between WC and PWV, but not after adjusting	age, gender, MAP, smoking, DM, height, HR, BMI
Danias et al 2003	30±8 29±5	OB N	NT/P	21/- 25/-	BMI	aortic compliance, stiffness index	↓aortic compliance and ↑ stiffness index in abdominal aorta in obese subjects	MAP
Ferreira et al 2004a	36	4.8% OB	NT	161/175	trunk peripheral fat	CF PWV, distensibility & compliance	trunk fat associated with carotid and femoral artery stiffness	gender, height, MAP, body composition, HbAlc, HR, cholesterol
Resnick et al 1997	50±3 49±5	N/OW	HT NT	17/3 8/2	BMI, abdominal subcutaneous & visceral fat	aortic distensibility	in total sample aortic distensibility related to abdominal visceral fat	
Mackey et al 2002	79±4 78±4	-	44% HT 51% HT	166/- -/190	BMI, WC, trunk fat mass	PWV	in total sample and women correlation between WC and PWV, not men	age, SBP
Majane et al 2008	41±19 42±18	population sample, prevalence of OB M=18%, F=49%		198/- -/310	WC, WHR	CF PWV, AIx	association between WC & PWV, but not AIx – only in women	adiposity, age, HTN therapy, MAP, HR height, DM
Maple-Brown et al 2005	47	DM no DM	population sample	18/25 19/35	BMI, WC, fat mass	AIx	AIx related to obesity. No difference in DM & no DM	age
Mitchell et al 2004		N	NT	188/333	weight, BMI	CF PWV, AIx	no relationship between obesity and arterial stiffness	age, sex, HR, MAP, height, weight, BMI,
Nakanishi et al 2003	35-54	25% OB	22% HT	2431/-	BMI	PWV	after adjustments no association between obesity and PWV	age, smoking, alcohol, components of metabolic syndrome
Oren et al 2003	28±1	38%OB	13% HT	240/284	BMI	CF PWV	no association after adjustments	MAP, gender, age, BMI

Ounis-Skali et al 2007	31±6 30±5	OB N	NT	-/16 -/10	BMI	CF PWV, AIx, forward wave	No difference in stiffness in lean and obese	HR, weight
Otsuka et al 2009	47±5	population sample		828/-	BMI, WC	AIx	Association between WC/BMI and AIx	age, height, HR, MAP, cholesterol, glucose
Snijder et al 2004	69±6	-	65% HT	244/240	trunk & leg fat mass	compliance CF PWV, AIx	trunk fat not associated with PWV/AIx	age, sex, height, MAP, leg lean and fat mass
Sutton-Tyrrell et al 2001	70-79	population sample		1219/1269	WC, abdominal fat	CF PWV	PWV associated only with visceral fat after adjusting	age, SBP, height, weight
Taquet et al 1993	49±3	population sample, 13% HT		-/429	BMI	CF PWV	PWV associated with BMI, but not after adjusting for SBP	SBP
Toto-Moukouo et al 1986	41 41	OB N	HT	13/14 12/13	BMI	PWV	in total sample correlation between obesity and PWV	age, sex, BP, glucose, cholesterol
Tounian et al 2001	13 12	OB N	NT	20/28 16/11	BMI, %BF, android/gynoid fat mass	arterial compliance & distensibility	↓arterial compliance in obese, android fat associated with ↓compliance	BF, BMI
Wildman et al 2003	41-70 20-40	21% OB 26% OB		125/52 85/101	weight, BMI, WC, WHR	PWV	in total sample correlation between obesity and PWV	age, SBP, race, sex
Zebekakis et al 2005	45±15 44±16	Population sample, OB M=12%, F=14%		646/- -/660	BMI	CF PWV	association between BMI & PWV only in women	age, MAP, HR, BMI, HT therapy

Longitudinal

Authors	Age	BMI	BP status	M/F	Measure of obesity	Measure of arterial stiffness	Outcome	Adjustments	follow up
Benetos et al 2002	57-64 48-54	-	HT (Tx) NT	117/70 191/105	BMI	PWV	baseline BMI not related ↑PWV	age, sex, baseline PWV	6y
Ferreira et al 2004b**	36	4.8% OB	NT	161/175	trunk fat BMI, SF	CF PWV, distensibility	childhood obesity related to adult PWV	gender, height, MAP, LBM	14y
Wildman et al 2005	20-40	-	NT	68/84	BMI	PWV	weight change showed relationship with PWV change	WC, BMI, PWV, BP	2y

Intervention										
Authors	**Age**	**BMI**	**BP status**	**M/F**	**Change in measure of obesity**	**Measure of arterial stiffness**	**Outcome**	**Adjust ments**	**Interv ention**	**BP**
Balkestein et al	39±7	OB		18/-	↓BMI	carotid distensibility &compliance	↓weight→↓PWV, but not under isobaric conditions	MAP	10 wk	↓ SBP &DBP
Barinas-Mitchell et al 2006	20-70	DM, OW/OB		n=38	↓BMI, weight	PWV	↓BMI associated with ↓PWV - not after adjusting	Baseline PWV	1y	→ BP
Dengo et al 2010	61±1	OW/ OB		16/9	↓weight, body & abdominal fat	CF PWV	↓weight, BMI& BF% correlated with ↓PWV	BP	12 wk	↓ BP →SBPc
Dengel et al 2006	55±4	OW/ OB	N/P	3/9	↓weight, %body fat	brachial distensibility &compliance	↓weight →↑ arterial compliance and distensibility		26wk	↓ SBP &DBP
Toto-Moukouo et al 1986	20-69	OB	HT	9/-	BMI	PWV	↓weight & BP→↑systemic arterial compliance		4wk	↓ SBP &DBP

BMI, body mass index; BP, blood pressure; M, males; F, females; wk, weeks; y, years; N, normal; OW, overweight; OB, obese; DM, diabetes mellitus; SF, skinfolds; WC, waist circumference; WHR, waist-to-hip ratio; BF, body fat; CV, cardiovascular; MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; SBPc, central SBP; PPc, central pulse pressure; NT, normotensive; HT, hypertensive; P, prehypertensive; HbAIc, glycated haemoglobin; CF, carotid femoral; PWV, pulse wave velocity; AIx, augmentation index.

** retrospective analysis

syndrome (Safar et al 2006), a syndrome recognised as being associated with abdominal obesity, are associated with increases in arterial stiffness, as indexed by aortic PWV or large vessel compliance estimates. Increases in indices of arterial stiffness may even occur in obese children (Tounian et al 2001). Despite the consistency of these reports, there is nevertheless controversy as to whether the relationship between obesity and aortic PWV is independent of haemodynamic factors such as BP, heart rate (HR), or diabetes mellitus. In this regard, although indices of excess adiposity are associated with aortic PWV, some studies have (Sutton-Tyrrell et al 2001, Mackey et al 2002, Wildman et al 2003), whilst most others have not (Taquet et al 1993, Amar et al 2001, Nakanishi et al 2003, Oren et al 2003, Mitchell et al 2004, Ferreira et al 2004, Zebekakis et al 2005, Czernichow et al 2005) been able to demonstrate strong relations between adiposity indices and PWV independent of haemodynamic factors and diabetes mellitus. Demonstrating relationships with aortic PWV beyond haemodynamic factors is essential as the strongest determinants of aortic PWV, other than the characteristics of the aorta itself, are distending pressures (a higher pressure will result in an increased aortic stiffness) and HR (a higher HR will result in an increased speed of wave travel). Importantly, as indicated by work from our laboratory, the inconsistencies in relationships between obesity and aortic PWV may be attributed to positive relationships noted in the elderly, but not in the young-to-middle-aged (Majane et al 2008). Indeed, aortic PWV is independently associated with an interaction between indices of an excess adiposity and age (Zebekakis et al 2005, Majane et al 2008). Nevertheless, the assessment of aortic elasticity using magnetic resonance imaging, a measure that is not dependent on acute haemodynamic changes, provides evidence that aortic stiffness is indeed increased in obesity. An important consideration of all the aforementioned studies is nevertheless the cross-sectional designs which do not allow one to draw conclusions regarding cause and effect. Is there evidence to suggest that obesity may promote large artery functional changes from study designs that allow for conclusions to be drawn regarding causality?

Data from longitudinal studies provide contrasting evidence as to whether obesity is causally related to increases in aortic stiffness. Indeed, although in one study indices of an excess adiposity were associated with increments in aortic PWV over a 2 year follow-up period (Wildman et al 2005), Benetos et al (2002) failed to show an association between baseline body mass index (BMI) and progression of aortic PWV in treated hypertensives or normotensives over a 6 year period. Nevertheless in the latter study (Benetos et al 2002), the mean BMI even in the hypertensive group was relatively low (26.6±4 $kg.m^{-2}$). Clearly, clarity on this issue should be provided by weight loss studies. Is there evidence from weight loss studies to support a role for obesity as a cause of changes in large artery function? Intervention studies have indeed provided evidence to suggest that obesity contributes toward aortic stiffness. In this regard, short term weight loss results in a reduction in indices of arterial stiffness in obesity (Dengo et al 2010, Balkestein et al 1999), type II diabetes mellitus (Barinas-Mitchell et al 2006) and in normotensives (Dengel et al 2006). Moreover, weight loss over a two year period was associated with decreases in arterial stiffness even in non-obese individuals, irrespective of changes in age or peripheral BP (Wildman et al 2005). Assuming that an excess adiposity is causally related to increases in arterial stiffness, is there evidence to indicate whether this translates into increases in central aortic BP?

As indicated in the preceding section 1.2.3, for increases in arterial stiffness to translate into increases in aortic BP, it may enhance either P1 or AP. Is there evidence to support a role for increases in either P1 or AP? In this regard, there are no studies that have reported on relationships between obesity and P1. Moreover, evidence to indicate that obesity increases AP or AIx is controversial. In this regard, indices of wave reflection or aortic pressure augmentation have been reported to both decrease (Maple-Brown et al 2005, Otsuka et al 2009) and increase (Ounis-Skali et al 2007).

Notably, none of the aforementioned studies showing an independent relationship between indices of an excess adiposity and aortic stiffness in cross-sectional studies (Sutton-Tyrrell et al 2001, Mackey et al 2002, Wildman et al 2003, Majane et al 2008) or changes in aortic stiffness in longitudinal studies (Wildman et al 2005), and studies reporting on

reflective wave changes (Maple-Brown et al 2005, Ounis-Skali et al 2007, Otsuka et al 2009) have reported on these changes translating into increments in central aortic BP. Moreover, central aortic BP was not altered following a weight loss programme despite improvements in indices of aortic stiffness (Dengo et al 2010). It is nevertheless possible that the magnitude of obesity-related effects on aortic stiffness in these studies was insufficiently large to translate into changes in AP or P1 and hence central aortic BP. The question therefore remains as to whether obesity may modify central aortic BP. Is there a potential argument to suggest that obesity may modify central aortic BP even if alterations in aortic stiffness do not translate into changes in central BP?

1.2.5 Obesity modifies the impact of salt intake on blood pressure: A potential role for effects on central aortic pressures?

A number of mechanisms may account for the aforementioned (see section 1.2.1) relationships that exist between obesity and brachial as opposed to central aortic BP. One potential mechanism that has been well described is through a modifying influence of obesity on the impact of salt intake on BP. In this regard, it is well accepted that some individuals respond to salt (Na^+) intake with a greater increase in BP than others and indeed Na^+ intake in some may produce marked increases in BP whilst in others no increase in BP or even a decrease in BP may occur. Those who develop clinically relevant increases in BP in response to Na^+ intake are considered to be 'salt-sensitive'. Is there evidence to suggest that obesity contributes toward salt-sensitivity?

In this regard, a number of studies have demonstrated that obesity or the metabolic syndrome is associated with renal tubular handling of sodium (Na^+) and an increased sensitivity of BP to Na^+ intake (Rocchini et al 1989, Hall et al 1997, Strazzullo et al 2001, 2006, Barbato et al 2004, Uzu et al 2006, Hoffmann et al 2008, Chen et al 2009). The possible mechanisms that explain this effect are currently uncertain. Nevertheless, one consideration is that obesity-associated insulin resistance (Bonadonna et al 1990, Kahn and Flier 2000) may play a role. Indeed, the concept that insulin or insulin resistance may

contribute toward obesity-associated increases in BP has been considered for a number of years (Brands and Hall 1992, Ferrannini et al 1997, Esler et al 2001). In this regard, several studies suggest that the impact of an excess adiposity on salt-sensitivity may be accounted for in-part by the relationship between insulin-resistance and salt sensitivity (Sharma et al 1991, Endre et al 1994, Shimamoto et al 1994, Zavaroni et al 1995, Bigazzi et al 1996, Galletti et al 1997, Fuenmayor et al 1998, Sechi et al 1999, Yatabe et al 2010), an association that may be explained by insulin actions on renal tubular function (Shimamoto et al 1994, Sechi et al 1999, Pearce 2001) and which could be accounted for by increases in sympathetic nervous system activity (Sechi et al 1999). Is it possible that although obesity has not been shown to be associated with central aortic BP despite relationships with indices of large vessel stiffness (see section 1.2.4), that obesity or insulin resistance may determine the impact of salt intake on central aortic BP?

Importantly, recent evidence from our laboratory shows that in a population of African ancestry, urinary salt excretion, an index of salt intake, is related to central aortic PP independent of brachial BP and that these effects of salt intake are attributed to increases in both P1 and AP, but not to aortic PWV (Redelinghuys et al 2010). It is therefore possible that in groups of African descent that obesity or the associated insulin resistance may modify the impact of salt intake on aortic BP and that these effects are largely expressed through actions which may not be associated with increases in aortic PWV. However, there is uncertainty as to the role of obesity or insulin resistance in salt sensitive hypertension in groups of African descent. What is the evidence to support a role for salt sensitivity as a major cause of hypertension in persons of African ancestry? Moreover what is the evidence to support or refute a role for obesity or insulin resistance in salt-sensitive hypertension in this ethnic group?

There is considerable evidence to support salt-sensitivity as a major pathophysiological mechanism responsible for hypertension in persons of African ancestry. This evidence is derived from a number of studies, many of which were intervention studies involving comparisons of the effect on BP of dietary Na^+ restriction or replenishment

(Weinberger et al 1986, He et al 1998, He et al 2000, Vollmer et al 2001, Wright et al 2003, Aviv et al 2004). Despite the particular importance of salt-sensitivity in contributing toward increases in BP in groups of African descent (Weinberger et al 1986, He et al 1998, He et al 2000, Vollmer et al 2001, Wright et al 2003, Aviv et al 2004), an effect that may be attributed to ethnic differences in tubular handling of Na^+ (Bochud et al 2009), neither obesity, nor insulin resistance are related to renal tubular Na^+ reabsorption in this ethnic group (Barbato et al 2004). However, apart from the latter study (Barbato et al 2004), to the best of my knowledge there are currently no other studies that have examined the role of obesity or insulin resistance as possible mechanisms involved in the pathophysiology of salt-sensitivity in persons of African ancestry. Moreover, there are no studies that have evaluated whether obesity or insulin resistance may influence the effect of salt intake on central aortic BP or the forward and reflective component pressure waveforms. Therefore, in the present thesis I evaluated whether obesity or insulin resistance is independently associated with the relationship between urinary salt excretion and either conventional, 24-hour or central aortic BP, or the component waveforms and determinants of central aortic BP in a community sample of African ancestry with a high prevalence of obesity. These data and the implications thereof are described in chapter 2 of the present thesis.

1.2.6 An obesity associated sedentary lifestyle: A potential role for exercise effects on central aortic pressures or their component waveforms?

It is well established that obesity is associated with a sedentary lifestyle. Indeed, a large study has shown that physical inactivity increases the odds of obesity in both young (25-54 years) and older (>55 years) individuals (Leroux et al 2012). It is also well recognised that a sedentary lifestyle results in a lack of physical fitness. Importantly, in comparison to other well-established cardiovascular risk factors such as smoking, physical inactivity and lack of fitness carry a higher relative risk for cardiovascular disease (Blair et al 1996, Thijssen et al 2010). In this regard, there is now considerable evidence to indicate that amongst other benefits, regular aerobic exercise is associated with reductions in brachial BP.

In-part through reductions in BP, the beneficial effects of regular exercise have been demonstrated to translate into a lower all-cause and cardiovascular mortality risk (Blair et al 1996, Hagberg 2000, Lee et al 2012, Rossi et al 2012). What is the evidence to support the view that regular exercise translates into decreases in BP? In the following I will extensively review this literature and the factors that may account for exercise training effects on BP, and subsequently I will develop the argument to support performing a study on exercise effects on central aortic BP in overweight or obese individuals. My reasons for providing a comprehensive review of the current literature available on exercise effects on brachial as opposed to central aortic BP at this point is to support my choice of exercise training programme employed in the current thesis.

1.2.6.1 *Exercise-induced effects on brachial blood pressures*

Table 1.2 summarises the results of the effects of exercise training programmes of three or more weeks duration on resting brachial BP. In most studies conducted in either younger or older hypertensives (Kiyonaga et al 1985, Somers et al 1991, Reid et al 1994, Kokkinos et al 1995, Cox et al 1996, Seals et al 1997, 2001, Moreira et al 1999, Balkestein et al 1999, Blumenthal et al 2000, Turner et al 2000, Hagberg et al 2000, Tsai et al 2002a, 2002b, 2004, Pescatello et al 2004, Pinto et al 2006, Collier et al 2008, Cornelissen et al 2009, 2010, Nualnim et al 2012) or in normotensives (Meredith et al 1991, Bursztyn et al 1993, Braith et al 1994, Cornelissen and Fagard 2005, Cornelissen et al 2009) regular physical exercise was reported to decrease resting brachial BP. These effects on BP are of clinical importance in that systematic reviews of the existing scientific literature have demonstrated decreases in resting brachial systolic BP/diastolic BP of 6.9/4.9 mm Hg after exercise training in hypertensives (Cornelissen and Fagard 2005, Fagard 2006, Fagard and Cornelissen 2007).

Despite the diversity of the different training programmes implemented in a number of studies (Marceau et al 1993, Braith et al 1994, Cox et al 1996, Moreira et al 1999, Schjerve et al 2008, Cornelissen et al 2009, 2010), systematic reviews and meta-analyses of these

Table 1.2 The effects of endurance exercise training on clinic BP.

	Characteristics				Training							
Authors	**Age (years)**	**BMI**	**BP status**	**Gender (M/F)**	**Dur (wk)**	**Mode**	**Intensity**	**Volume (timesxmin)**		**Clinic BP (SBP)/(DBP)**	**Wt**	**VO_2**
Aizawa and Petrella 2008	68±5	OB	HT(Tx)	7/2	20	Aer	70% VO_{2max}	4x	45	→(8)/→(4)	→	→
Balkestein	39±7	OB	-	18/-	10	Diet	-			↓(10)/↓(8)	↓	-
et al 1999				19/-		D+Ex	40% VO_{2max}	4x	60	↓(8)/↓(5)	↓	-
Blumenthal et al 1991	44±9	OW	HT	24/15	16	walk /jog	70% VO_{2max}	3-4x	35	→(1)/→(1)	→	↑
Blumenthal	47±1	OB	P, HT	25/19	26	cycle/walk	70-80% HRR	3-4x	55	↓(4)/↓(4)	→	↑
et al 2000	49±1	OB		n=46		diet+exercise				↓(7)/↓(6)	↓	↑
Braith et al 1994	66±5	OW	N	n=19	26	Walk	70% HRR	3x	45	↓(9)/↓(8)	→	↑
	65±4	OW	N	n=14			85% HRR	3x	35	↓(8)/↓(7)	→	↑
Bursztyn et al1993	48±7	N	HT	9/7	14	Cycle	60-70% HR_{max}	3x	60	↓(9)/↓(4)	-	↑
Cononie et al 1991	72±3	-	P	8/9	6	walk/jog	70-85% VO_{2max}	3x	35	→(4)/↓(5)	→	↑
Collier et al 2008	48±1	OW	HT	10/5	4	Walk	65%$VO2_{peak}$	3x	30	↓(5)/↓(3)	→	-
Cornelissen	59	OW	P, HT	17/19	10	Aer	33 % HRR	3x	60	↓(4)/	-	↑
et al 2010				17/19	11		66% HRR	3x	60	↓(6)/	-	↑
Cornelissen	59±1	OW	N- HT	18/21	10	walk/cycle	33% HRR	3x	60	↓(5)/→(2)	→	↑
et al 2009							66% HRR			↓(6)/↓(5)	↓	↑
Cox et al1996	42	OB	P, HT	13/-	16	Diet + Cont	18% HRR	2x	30	↓(6)/↓(2)	↓	→
		-		13/-		Diet + cycle	76% HRR	3x	30	↓(6)/↓(2)	↓	↑
		-		11/-		Cont	18% HRR	2x	30	→/→	→	→
		-		11/-		Cycle	76% HRR	3x	30	→/→	→	↑
Dengel et al1998	59±5	OW	NT, P	35/-	36	Aer	70-85%HR_{max}	3x	40	↓(6)/↓(5)	→	↑
	57±4			20/-		Diet+Aer	70-85%HR_{max}			↓(10)/↓(7)	↓	↑
Ferrier et al 2001	64±7	OW	ISH	5/5	8	Cycle	65%HRR	3x	40	→/→	→	↑
Fortmann et al1988	44±8	OW	NT	42/-	52	walk/jog	70-85%HR_{max}	3x	60	→(3)/→(2)	↓	↑
		-				Diet				→(2)/→(3)	↓	→
Gilders et al 1989	43±4	-	HT	n=7	16	Cycle	70% VO_{2max}	3x	30	→/→	→	↑
		-	NT	n=10	16					→/→	→	↑
Jessup et al 1998	69±5	-	NT, P	n=11	16	walk/stairs	50-85%HR_{max}	3x	45	→(3)/→(2)	→	↑
Ketelhut et al 1997	43±3	-	HT	10/-	68	Running	70% HR_{max}	2x	60	→(6)/↓(5)	-	-
Kiyonaga et al 1985	43±4	OW	HT	5/7	10	Cycle	Lt	3x	60	↓(14)/↓(9)	→	↑
Kokkinos et al 1995	57±10	OB	HT	18/-	16	Cycle	74 % HR_{max}	3x	45	↓(7)/↓(5)	→	↑
Marceau et al1993	43±2	N	HT	8/1	10	Cycle	50% VO_{2max}	3x	60	→(+2)/↓(3)	→	→
							70%VO_{2max}			→(2)/→(0)	↓	↑
Meredith et al 1991	36±3	N	NT	8/-	4	Cycle	60-70% W_{max}	3x	40	↓(8)/↓(5)	→	↑

Miller et al 2002	54±9	OB	HT (Tx)	n=20	9	diet+salt+ exercise	50-75% HR_{max}	3x	45	→(7)/→(6)	↓	-
Miyai et al 2002	46±2	N	NT	32/-	12	Cycle	50-60%HRR	3x	45	→(2)/→(2)	→	↑
Moreira et al 1999	52±9	OW	HT	8/6	10	Cycle	69% HR_{max}	3x	30	↓(10)/↓(7)	→	↑
	47±10			7/7			84% HRmax			↓(15)/↓(8)	→	↑
Nualnim et al 2012	58±2	OW	HT	7/17	12	Swim	60-75% HR_{max}	3-4x	45	↓(9)/→(4)	→	→
Ohkubo et al 2001	68	N	P	11/11	25	Cycle	40-60% HRR	3x	25	→(7)/→(2)	-	-
Reid et al 1994	47±4	OB	P	n=7	12	Cycle	70% VO_{2peak}	3x	30	↓(7)/↓(6)	↓	↑
	36±4	OB	P	n=6		D+cycle				↓(14)/↓(13)	↓	↑
Schjerve et al 2008		OB	-		12	walk/run	60-70% HR_{max}	3x	47	→/↓(8)	↓	↑
		OB	-				85-95% HR_{max}	3x	4x4	→/↓(6)	↓	↑
Seals et al 1997	55±1	OW	P, HT	-/9	12	Walk	62% $VO2_{max}$	3-4x	45	↓(10)/↓(7)	→	→
Seals and Reiling	61±2	OW	IDH	19/7	26	-	40-50% HRR	3-4x	40	→(4)/→(2)	→	↑
1991	63±2	OW		n=10	52	-	57% HRR	3-4x	50	→(3)/→(4)	→	↑
Somers et al 1991	35±9	-	HT	14/2	26	airforce regimen and jogging		3-4x	40	↓(10)/↓(7)	→	
Stewart et al 2005	64±6	OW	HT	21/19	26	R+aer	60-90%HR_{max}	3x	45	→(1)/↓(2)	↓	↑
Tanaka et al 2000	53±2	OW	N	20/-	13	Walk	73%HR_{max}	5x	42	→(+1)/→(+2)	→	→
Tsai et al 2002	50±9	OW	HT	7/5	12	Walk	60-70%HR_{max}	3x	30	↓(18)/↓(10)	→	↑
Tsai et al 2002	46±10	N	W-HT	12/10	12	walk/jog	60-70%HR_{max}	3x	30	↓(11)/↓(5)	→	
Tsai et al 2004	49±6	N	HT	24/28	10	walk/jog	60-70%HR_{max}	3x	30	↓(10)/↓(6)	-	↑
Van Hoof et al 1989	39±10	N	P	n=26	16	cycle/jog		3x	60	→(4)/↓(5)	↓	↑
Wijnen et al 1994	37±1	N	N, P	17/-	6	Cycle	75% VO_{2max}	3x	45	→(+3)/→(1)	→	↑
Wilmore et al 2001	35±13	OW	N	n=507	20	Cycle	55-75% VO_{2max}	3x	50	↓(1)/→(0)	-	↑
Zanettini et al 1997	49±9	N	HT	8/6	12	Aer+R	70-85% HR_{max}	3x	40	↓(15)/↓(11)	→	↑

BMI, body mass index; BP, blood pressure; M, males; F, females; Dur, duration of exercise intervention; wk, weeks; Volume, frequency of sessions per week x minutes per session; min, minutes; Wt, change in weight after training; VO_2, change in aerobic capacity after training; N, normal; OW, overweight; OB, obese; NT, normotensive; HT, hypertensive; P, prehypertensive; ISH, isolated systolic hypertension; W-HT, white coat hypertension; Tx, treated; IDH, isolated diastolic hypertension; Aer, aerobic exercise; cont, control; R, resistance exercise; $VO_{2max,}$ maximal aerobic capacity; HR_{max}, maximal heart rate; HRR, heart rate reserve; VO_{2peak}, peak aerobic capacity; L_T, lactate threshold; W_{max}, maximal workload; -, no data.

studies indicate that exercise intensity and frequency are not associated with the BP response (Halbert et al 1997, Cornelissen and Fagard 2005, Fagard 2005). Indeed, in one meta-analysis, exercise intensity and frequency of the exercise programmes explained only 4.9% and 1.1% of the variance of the response of systolic and diastolic BP respectively (Fagard 2005). Therefore, more intense exercise (> 70% VO_{2peak}) does not appear to result in additional benefits to BP as compared to moderate or low intensity exercise (40 – 70% VO_{2peak}) (Table 1.1) (Marceau et al 1993, Braith et al 1994, Cox et al 1996, Moreira et al 1999, Schjerve et al 2008, Cornelissen et al 2009, 2010).

Systematic reviews (Halbert et al 1997, Cornelissen and Fagard 2005, Fagard 2005) nevertheless were able to show that the magnitude of the BP reduction was associated with a gain in aerobic capacity. However, there are also a number of studies where an increase in aerobic capacity was not associated with a concomitant decrease in BP (Table 1.2) (Fortmann et al 1988, Gilders et al 1989, Seals and Reiling 1991, Blumenthal et al 1991, Marceau et al 1993, Wijnen et al 1994, Cox et al 1996, Jessup et al 1998, Ferrier et al 2001, Miyai et al 2002). Furthermore, in some studies a decrease in resting BP occurred after exercise training without an associated increase in aerobic capacity (Table 1.2) (Marceau et al 1993, Cox et al 1996, Seals 1997, Nualnim et al 2012). Thus, whether exercise-induced decreases in BP depend on an increased aerobic capacity is unclear.

As obesity is strongly associated with a lack of fitness and a lack of physical activity, and obesity is a strong risk factor for hypertension, the obvious question which arises is whether physical activity must be associated with weight loss to confer benefits on BP. As indicated in Table 1.2, the majority of studies that report decreases in BP after exercise training were performed in overweight or obese participants (Kiyonaga et al 1985, Fortmann et al 1988, Seals and Reiling 1991, Blumenthal et al 1991, 2000, Braith et al 1994, Reid et al 1994, Kokkinos et al 1995, Cox et al 1996, Seals et al 1997, Balkestein et al 1999, Moreira et al 1999, Tanaka et al 2000, Ferrier et al 2001, Wilmore et al 2001, Tsai et al 2002a, Miller et al 2002, Stewart et al 2005, Collier et al 2008, Aizawa and Petrella 2008, Cornelissen et al 2009, 2010, Nualnim et al 2012). However, most of these studies reported no change in body

weight after exercise training (Kiyonaga et al 1985, Gilders et al 1989, Cononie et al 1991, Meredith et al 1991, Seals and Reiling 1991, Somers et al 1991, Blumenthal et al 1991, 2000, Braith et al 1994, Wijnen et al 1994, Kokkinos et al 1995, Jessup et al 1998, Balkestein et al 1999, Moreira et al 1999, Tanaka et al 2000, Ferrier et al 2001, Wilmore et al 2001, Tsai et al 2002a, 2002b, Collier et al 2008, Nualnim et al 2012). Moreover some studies have reported no change in BP despite a decrease in body weight (Fortmann et al 1988, Marceau et al 1993, Miller et al 2002). The majority of studies that showed a decrease in body weight after exercise training combined the exercise intervention with a weight loss diet (Fortmann et al 1988, Reid et al 1994, Cox et al 1996, Dengel et al 1998, Balkestein et al 1999, Blumenthal et al 2000, Miller et al 2002). Studies comparing the impact of diet versus exercise alone or in combination reported that diet-induced weight loss resulted in similar or greater decreases in BP as compared to exercise alone (Cox et al 1996, Dengel et al 1998, Blumenthal et al 2000), or that combining exercise with diet did not have any additional BP benefits compared to diet alone (Reid et al 1994, Cox et al 1996, Dengel et al 1998, Balkestein et al 1999). In a meta-analysis, decreases in body weight could not explain the change in BP produced by exercise training (Fagard 2006). Thus, one should consider the possibility that overweight or obesity may not only be associated with increases in BP because of the deleterious effects of adiposity, but also because of a sedentary lifestyle.

1.2.6.2 *Exercise-induced effects on central aortic blood pressures, the component waveforms or the determinants thereof*

The physiological mechanisms responsible for exercise training-induced decreases in BP are still uncertain. As MAP is the product of cardiac output (CO) and total peripheral resistance (TPR), a decrease in BP may in-part be mediated by a change in one of these two variables (Pescatello et al 2004). Despite decreases in HR with exercise training (an effect that may be attributed to an enhanced vagal tone or reduced sympathetic nervous system activity), it is well-recognised that resting CO does not change significantly after exercise training. This is most likely as a result of a decreased resting HR being offset by an increase

in stroke volume (SV) after exercise training (Fagard and Cornelissen 2007). The increased SV could be attributed to an enhanced venous return, an increase in the size of the left ventricle, resulting in an increased force of cardiac contraction or a decrease in afterload to the left ventricle. Therefore, the decrease in MAP is more likely to be the result of changes in TPR (Cornelissen and Fagard 2005, Fagard 2005). A decrease in TPR could be mediated by a reduced sympathetic nervous system activity, as evidenced by decreases in circulating levels of catecholamines, an improved endothelial function, a decreased release of a number of vasoconstrictors, an increased release of vasodilators, and/or structural adaptations in the vasculature (Kiyonaga et al 1985, Kohno et al 2000, Brown et al 2002, Pescatello et al 2004, Hamer 2006, Fagard and Cornelissen 2007). With respect to the potential mechanisms responsible for decreases in BP, of importance is the possibility that regular exercise may reduce not only brachial BP, but also the aortic functional changes that account for central aortic BP. What is the evidence to suggest that aortic functional alterations may benefit from regular exercise?

There are a number of cross-sectional studies that have demonstrated that with participation in regular endurance exercise, age-related aortic or vascular stiffening is less pronounced than in sedentary counterparts (Vaitkevicius et al 1993, Kakiyama et al 1998, Tanaka et al 1998, Seals et al 1999, Tanaka et al 2000, Monahan et al 2001, Wilkinson et al 2004, Zieman et al 2005, Otsuki et al 2006a, 2006b, 2007, Kraft et al 2007, Aoyagi et al 2010). Based on this evidence, a number of intervention studies have been conducted to assess the effect of exercise training on measures of large arterial function. The results of these intervention studies are summarised in Table 1.3. It should be evident from these studies that exercise training could decrease arterial stiffness. However, it should also be obvious that these results are inconsistent. What are the possible reasons for the discrepancies between these results?

Factors that could account for the discrepancies in the effect of exercise training on large artery function include differences in the characteristics of the study populations, the

Table 1.3 The effects of aerobic exercise training on indices of arterial stiffness.

	Characteristics				Training									
Authors	**Age**	**BMI**	**BP status**	**M/F**	**Dur (wk)**	**Mode**	**Intensity**	**Volume**		**Measurement**	**Δ central stiffness**	**Δ peripheral stiffness**	**VO_2**	**Wt**	**BP**
Aizawa & Petrella 2008	68±5	OB	HT(Tx)	7/2	20	Aer	70%VO_{2max}	4x	45	Ultrasound	→ carotid distensibility	→ brachial distensibility	→	→	→
Balkestein et al 1999	39±7	OB		18/-	10	Diet	-			Appl tonom	→carotid distensibility	→brachial compliance	-	↓	↓
				19/-	10	D+Ex	40% VO_{2max}	4x	60				-	↓	↓
Baynard et al 2009	52±1	OB	N-MS	n=10	1.5	walk	70-75% VO_{2peak}	daily		Doppler	→ c_PWV	→ p_PWV	↑	→	→
	52±1	OB	MS	n=11							→ c_PWV	→ p_PWV	↑	→	→
Cameron & Dart 1994	18-32	N	NT	13/-	4	cycle	75%VO_{2peak}	3x	30	ultrasound/ appl tonom	↑ systemic art compliance		↑	-	↓
Collier et al 2008	48±1	OW	HT	10/5	4	walk	65%VO_{2peak}	3x	30	Doppler	↓ c_PWV	↓ p_PWV	-	→	↓
Currie et al 2009	25±4	N		14/-	1	cycle	65%VO_{2peak}	6x	2h	Appl tonom	↓ c_PWV	↓ p_PWV	→	→	→
Dinenno et al 2001	51±2	OW	NT	22/-	13	walk	73%HR_{max}	5x	45	Duplex ultrasound	-	↑ fem art lumen diameter	↑	→	→
Edwards et al 2004	55±8	OW	CAD	n=10	12	Aer	75-85%HRR		50	Appl tonom	↓ AIx, ↓c_PWV		-	-	→
Ferrier et al 2001	64±7	OW	ISH	5/5	8	cycle	65%HRR	3x	40	Appl tonom	→PWV		↑	→	→
Goldberg et al 2009	55±7	OB	NT-HT(Tx)	9/28	24	D+ Aer	moderate	2x	60	Appl tonom	↑ elasticity index		-	↓	↓
Guimarães et al	50±8	OW	HT(Tx)	7/9	16	walk	60%HRR	2x	40	Appl tonom (Complior)	→ CF PWV		-	→	→
2010	45±9	OW	HT(Tx)	4/12	16	walk	50/80%HRR	2x	40		↓ CF PWV		-	→	→
Hayashi et al 2005	50±3	N	NT	17/-	16	walk	75%HRR	3-4x	45	Appl tonom/ ultrasound	↓ c_PWV	→ p_PWV	↑	↓	→
Kakiyama et al 2005	21±1	N	NT	10/-	8	cycle	70%VO_{2max}	3-4x	60	Pulse wave velocimeter	↓ c_PWV	-	↑	↓	→
Laskey et al 2012	61±11		CAD	n=33	20	Aer	50-85% HR_{max}	3x	45	Appl tonom	↓ PWV → AIx		-	-	→
Liu et al 2012	51±5	OW	P	8/-	8	walk	65%VO_{2max}	4x	30	Appl tonom	→ AIx		↑	↓	↓
	55±8			-/9							↓AIx		↑	→	↓
Madden et al 2009	72±1	OB	HT	n=17	12	walk	60-75%HRR	3x	60	Appl tonom (Complior)	↓ c_PWV	↓ p_PWV	→	→	→

McNeilly et al 2012	49±9	OB	HT-IGT	6/5	12	walk	65%HR_{max}	5x	30	Pulse wave velocimeter	↓PWV		-	↓	↓
Miyaki et al 2009	50±2	OB		21/-	12	walk	RPE12-15	3x	60	Ultrasound/ appl tonom	↑ carotid compliance		↑	↓	↓
Moreau et al 2003	63±2	OW	NT-HRT	-/12	13	walk	70%HR_{max}	5x	40	Ultrasound/ appl tonom	↑ carotid compliance		↑	→	→
Mustata et al2011	64	OW	CKD	4/6	52	Aer	60%VO_{2peak}	3x	40	Appl tonom	↓AIx		↑	→	→
Nualnim et al 2012	58±2	OW	HT	7/17	12	swim	60-75% HR_{max}	3-4x	45	Ultrasound/ appl tonom	↑ carotid compliance, →AIx	→ p_PWV	→	→	↓
Rakobowchuk et al 2008	23±2	N	NT	5/5	6	cycle	65%VO_{2peak}	5x	60	Ultrasound/ appl tonom	→ carotid distensibility	↑ popliteal distensibility	↑	→	→
	24±3	N	NT	5/5	6	cycle	4x30s sprint	3x	20				↑	→	→
Seals et al 2001	62±9		HT	-/14	13		70%HR_{max}	6x	40	Ultrasound/ appl tonom	→c_PWV		→	→	↓
Stewart et al 2005	64±6	OW	HT	21/19	26	R,aer	60-90%HR_{max}	3x	45	Doppler	→c_PWV	-	↑	↓	↓
Sugawara et al 2006	58±4	N	IDH	-/8	12	cycle	40%HRR	3-5x		Ultrasound/ appl tonom	↓ carotid stiffness index		-	→	→
	59±6	N	IDH	-/9			70%HRR						-	↓	→
Tabara et al 2007	67±6	N	N/P	3/37	26	Aer	mild/mod	2x	30	Appl tonom	↓AIx		-	-	↓
Tanaka et al 2000	53±2	OW	NT	20/-	13	walk	73%HR_{max}	5x	42	Ultrasound/ appl tonom	↑ arterial compliance		→	→	→
Thijssen et al 2007	70±3	N	NT	8/-	8	cycle	65-80%HRR	3x	20	Echo Doppler	no change	↑ fem art compliance	→	→	→
Westhoff et al 2007 (abstract)	>60		HT(Tx)	n=54	12	walk	63% L_T	-		arterial compliance	→ arterial compliance		↑	-	↓
Yang et al 2011	45± 10	OW/ OB	NT-HT(Tx)	-/40	12	Aer+ R	60-75% HR_{max}	5x	45	waveform analyzer		↓ p_PWV	-	→	↓
Yokoyama et al 2004	53± 12	OW	NT, P & DM	6/17	3	Aer	50%HR_{max}	5x	40	Ultrasound	↓ carotid stiffness index	↓ femoral stiffness index	-	→	→

BMI, body mass index; BP, blood pressure; M, males; F, females; Dur, duration of exercise intervention; wk, weeks; Volume, frequency of sessions per week x minutes per session; min, minutes; Wt, change in weight after training; VO_2,change in aerobic capacity after training; NT, normotensive; HT, hypertensive; CAD, coronary artery disease; Tx, treated; ISH, isolated systolic hypertension; IDH, isolated diastolic hypertension; HRT, hormone replacement therapy; DM, diabetes mellitus; IGT, impaired glucose tolerance; N, normal; OW, overweight; OB, obese; M, males; F, females; Aer, aerobic exercise; D, diet; R, resistance exercise; HRR, heart rate reserve; L_T, lactate threshold; PWV, pulse wave velocity; CF, carotid-femoral; c_PWV, central (large artery) pulse wave velocity; p_PWV, peripheral (resistance vessel) pulse wave velocity; appl tonom, applanation tonometry; FMD, flow mediated dilation; Aix, augmentation index; fem art, femoral artery.

exercise programmes employed as well as the measurement techniques. In this regard, age has been suggested to be an important characteristic determining whether changes in indices of arterial stiffness occur. Many of the studies showing decreases in indices of aortic or large vessel stiffness after relatively short periods of exercise training (4-12 weeks) were performed in younger persons (Cameron and Dart 1994, Tanaka et al 2000, Yokoyama et al 2004, Sugawara et al 2006, Currie et al 2009, Goldberg et al 2009, Yang et al 2011, Nualnim et al 2012). However, there are also studies that show a lack of change in arterial stiffness in younger persons (Rakobowchuk et al 2008, Baynard et al 2009), which could be ascribed to small sample sizes (n=10) and a short duration of the intervention (1.5 weeks in Baynard et al 2009).

In contrast to the evidence to suggest that regular exercise may decrease indices of large artery stiffness in younger individuals, a number of studies suggest that decreases in indices of aortic and large vessel stiffness do not occur in older persons after short-term exercise training (Seals et al 2001, Ferrier et al 2001, Stewart et al 2005, Westhoff et al 2007, Aizawa and Petrella 2008) Furthermore, longer periods of exercise training (i.e. 6 months) also do not result in a change in PWV in older persons with hypertension and type 2 diabetes mellitus (Dobrosielski et al 2012). This lack of benefit in the elderly may be related to the irreversible structural effects of ageing on the vasculature rather than the effects of hypertension, as no change in PWV has been reported after moderate intensity exercise training in normotensive elderly men (Thijssen et al 2007). In this regard it is possible that after a critical age the arterial mechanical properties are resistant to modification by exercise training. However, there are also studies which show that indices of arterial stiffness improve in the elderly (Moreau et al 2003, Madden et al 2009, Laskey et al 2012).

With respect to the relationship between obesity and arterial stiffness, an important question which arises from studies conducted on the effects of exercise training on indices of arterial stiffness (Table 1.3) is whether the associations between obesity and arterial stiffness described in section 1.2.4 can be accounted for by a lack of exercise or the impact of alternative factors related to the deleterious effects of adipose tissue itself? In this regard,

one needs to consider whether exercise-related improvements in indices of arterial stiffness described in Table 1.3 are associated with reductions in body weight. What should be apparent is that in those studies conducted with a high proportion of participants who were either overweight or obese, a number of these studies demonstrated improvements in indices of large vessel stiffness despite a lack of change in body weight (Tanaka et al 2000, Moreau et al 2003, Yokayama et al 2004, Madden et al 2009, Guimarães et al 2010). Thus it would appear that in overweight and obese individuals that weight loss is not a prerequisite for producing benefits to large artery function.

What should be highlighted with respect to studies assessing the impact of exercise training programmes on large artery function is that only six of these studies reported on exercise training effects on AIx (Edwards et al 2004, Tabara et al 2007, Mustata et al 2011, Laskey et al 2012, Nualnim et al 2012, Liu et al 2012) and few have reported on whether changes in indices of arterial stiffness or aortic pressure augmentation translate into increases in central aortic BP. With respect to those studies evaluating changes in AIx, two studies failed to show an effect (Laskey et al 2012, Nualnim et al 2012), whilst one study reported decreases in women only (Liu et al 2012). In some of the studies showing exercise-induced decreases in AIx, the exercise training-induced decrease in BP was not accounted for in multivariate adjusted models (Tabara et al 2007, Liu et al 2012). This is obviously of importance for the reasons previously indicated. In this regard, indices of aortic stiffness, including AIx, are strongly dependent on distending pressures. Thus, any exercise training induced decreases in AIx may be attributed to alterations in BP. Importantly, only two studies conducted in 10 overweight individuals (Edwards et al 2004) and 9 overweight women (Liu et al 2012) have reported on a beneficial effect of exercise training on aortic augmentation index in overweight or obese individuals, whilst in another study conducted in 24 overweight individuals, no effect of exercise training was observed (Nualnim et al 2012). Moreover, exercise-induced changes in systolic pressure augmentation failed to translate into decreases in aortic BP in one study (Liu et al 2012).

The lack of evidence to support a beneficial effect of exercise training on systolic pressure augmentation and hence central aortic BP in overweight or obese individuals, prompted me in the present thesis to evaluate the impact of exercise training on aortic pressure augmentation and central aortic BP in overweight or obese individuals. The data for this study and the implications thereof are provided in chapter 3 of the present thesis.

1.3 Preclinical cardiac dysfunction: A role for obesity?

There are a number of abnormalities of cardiac function that may precede the development of heart failure. Many of these changes are thought to reflect damage to the heart as a consequence of traditional cardiovascular risk factors including hypertension and diabetes mellitus. As will subsequently be discussed, abnormalities of cardiac function not only predict the risk for heart failure, but alternative cardiovascular events. These measurements have therefore been recommended as part of global cardiovascular risk assessment in centres where sufficient resources are available. What has become increasingly obvious is that independent of brachial BP or alternative cardiovascular risk factors such as diabetes mellitus, and independent of coronary artery disease, obesity is also a cause of cardiac dysfunction, a change that may progress to heart failure, or at least herald the onset of obesity-associated cardiovascular damage. In the present section of this chapter I will therefore briefly describe some of the measures of cardiac function employed in the present thesis that have been shown to predict cardiovascular outcomes. I will then review the evidence to suggest that obesity is a major cause of cardiac dysfunction and subsequently the available evidence to suggest that insulin resistance or a sedentary lifestyle contribute toward obesity-associated cardiac dysfunction. In so doing I will provide the arguments that support the studies being conducted in the present thesis which address some of the issues related to obesity-associated cardiac dysfunction.

1.3.1 Measures of preclinical cardiac dysfunction as predictors of cardiovascular outcomes

The advent of high resolution ultrasound imaging of the heart (echocardiography), a tool available to most cardiologists, resulted in the development of a number of measures of cardiac function which have improved diagnostic precision and the ability to predict cardiovascular risk. Until relatively recently, the primary cardiac functional change that was thought to herald the onset of cardiovascular events was a reduced left ventricular ejection fraction (EF), a measure of left ventricular systolic chamber function. Indeed, there is no question that a reduced EF is an important predictor of the development of heart failure (Wang et al 2003). At a community level, asymptomatic mild left ventricular systolic dysfunction as indexed by an EF ≤50% may exist in 6.0% of individuals and moderate to severe systolic dysfunction, as indexed by an EF ≤40% in 2.0% of individuals (Redfield et al 2003). Currently however, there are a number of additional measures of cardiac function which have been demonstrated to predict cardiovascular outcomes independent of traditional risk factors and these changes are often noted well before left ventricular chamber systolic function is compromised. What are some of the better established measurements? In the following I will not discuss the more recent measures of cardiac dysfunction that may be obtained using echocardiography, such as those obtained with 'speckle Doppler' imaging ('strain' imaging and 'twist' mechanics) as these were not assessed in the present thesis.

It is acknowledged that myocardial systolic function may decrease in advance of systolic chamber function as chamber function may be maintained by concentric left ventricular remodelling. To detect abnormalities of myocardial systolic function in a concentrically remodelled heart, a number of measures of function may be employed such as midwall fractional shortening (FSmid), which essentially is the extent of systolic shortening in midwall fibres, or tissue Doppler measures of shortening within the septum or lateral wall of the left ventricle. Furthermore, there is currently extensive evidence to show that abnormalities of diastolic function of the heart are important in risk predicting. In this regard, echocardiography allows for imaging of the velocity of blood flow across the mitral valve and of myocardial tissue during the early (E or 'e) or late atrial (A or a') period of diastole

(ventricular filling). In a normal ventricle the velocity of blood flow or tissue lengthening is greatest during early diastole (E and e' are higher than A or a'). When relaxation of the ventricles is impaired because of diastolic dysfunction, the velocity of transmitral blood flow or tissue lengthening increases in late diastole as the ventricle begins to depend more on atrial contraction to fill. The consequence is that A and a' are higher than E or e' and the E/A or e'/a' ratios may be considerably reduced. Because E is dependent on ventricular relaxation as well as left atrial driving forces (pressures), while e' is dependent on relaxation alone, E/e' is considered to be an index of left atrial pressures or left ventricular filling pressures. Although a comprehensive review of all of the evidence demonstrating the ability of measures of cardiac function to predict risk go beyond the scope of the present thesis, a few important points should be made.

Measures of left ventricular dysfunction, including EF and E/A are independent predictors of fatal and non-fatal cardiovascular events in low risk (Iivanainen et al 1997, Yu et al 2007, Bernardo et al 2010) and high risk patients (Mishra et al 2011). Furthermore, FSmid predicts cardiovascular morbidity and mortality independent of left ventricular hypertrophy, BP and age (De Simone et al 1996). Both FSmid and E/A, have also been demonstrated to predict the development of heart failure in asymptomatic patients (Aurigemma et al 2001). Left ventricular (LV) diastolic dysfunction identified from combined assessments of E/A and tissue Doppler evaluation (E/e'), are associated with adverse cardiovascular outcomes and the development of heart failure (Aurigemma et al 2001, Schillaci et al 2002, Bella et al 2002, Redfield et al 2003, Ammar et al 2008). The ability of therapeutic interventions to slow down or even prevent the progression of asymptomatic left ventricular systolic dysfunction (reduced EF) (Konstam et al 1992, Doughty et al 1997), provides further evidence that a reduced EF should be sought in "at risk" populations (Murtagh et al 2012). As a consequence of the evidence to support a predictive role for measurements of subclinical cardiac dysfunction, the European Society of Cardiology guidelines (Dickstein et al 2008) and the American Heart Association guidelines (Hunt et al 2009) recommend their use in risk predicting.

1.3.2 Obesity is associated with cardiac dysfunction

With respect to the effect of obesity on cardiac systolic as opposed to diastolic function, tissue Doppler indices of systolic myocardial function have been shown to be reduced in overweight and obese people without conventional cardiovascular risk factors (Peterson et al 2004, Wong et al 2004). However, the independent relationship between an excess adiposity and LV systolic chamber function is controversial, with some studies showing a relationship between an excess adiposity and systolic function (Scaglione et al 1992, Alpert et al 1995, Karason et al 1998, Chinali et al 2006, Ammar et al 2008), whilst other earlier studies have failed to do so (de Divitiis et al 1981, Zarich et al 1991, Stoddard et al 1992, De Simone et al 1996, Mureddu et al 1996, Iacobellis et al 2002, Pascual et al 2003, Peterson et al 2004, Wong et al 2004). Moreover, more recent studies conducted in large samples have provided strong evidence that obesity is not associated with a reduced LV systolic chamber function (Powell et al 2006, Turkbey et al 2010, Bazzano et al 2011, Russo et al 2011). Furthermore, even with the use of load-independent tissue Doppler measures of myocardial as opposed to chamber function, or with chamber function assessments, weight loss produced by either lifestyle modification or gastric bypass does not influence left ventricular systolic function (Willens et al 2005, Wong et al 2006, Skilton et al 2008, Rider et al 2009). Nevertheless after gastric bypass surgery in 423 patients with severe obesity, an increase in FSmid was noted (Owan et al 2011). Although there is more evidence against rather than in favour of obesity producing decreases in LV systolic chamber function, it should be clearly evident that there is still considerable controversy as to whether obesity contributes toward decreases in LV systolic chamber or even regional myocardial function.

What is the evidence to support a role for obesity in contributing toward cardiac diastolic dysfunction? In the more recent past, several cross-sectional studies conducted in small (Peterson et al 2004, Wong et al 2004) and large (Redfield et al 2003, Fischer et al 2003, Powell et al 2006, Tsioufis et al 2008, Ammar et al 2008, Libahber et al 2009, Russo et al 2011) study groups and in both select study populations (Peterson et al 2004, Wong et al 2004, Tsioufis et al 2008) and randomly selected community samples (Redfield et al 2003,

Fischer et al 2003, Powell et al 2006, Ammar et al 2008, Libhaber et al 2009, Russo et al 2011) have suggested that independent of alternative risk factors, even modest levels of obesity contribute toward the pathogenesis of an abnormal LV diastolic function. Moreover, intervention studies suggest that weight loss induced either by gastric bypass surgery (Willens et al 2005, Leichman et al 2008, Rider et al 2009, Hsuan et al 2010, Algahim et al 2010, Owan et al 2011) or by lifestyle intervention (Wong et al 2006, Riordan et al 2008, de las Fuentes et al 2009, Kosmala et al 2009, Rider et al 2009) results in improvements in cardiac diastolic function independent of conventional cardiovascular risk factors. Importantly, one of these studies was conducted in a relatively large study sample (n=423) (Owan et al 2011). The relationship between obesity and cardiac diastolic dysfunction appears to be strongest for measures of abdominal obesity (Tsioufis et al 2008, Ammar et al 2008, Libhaber et al 2009) and importantly, is independent of not only conventional BP, but also aortic PWV and 24-hour BP as well as LV mass and the extent of concentric LV remodelling (Libhaber et al 2009).

Although some cross-sectional studies conducted in large study samples have failed to establish a contribution of obesity toward the pathogenesis of an abnormal LV diastolic function (Bella et al 2002, Chinali et al 2006, De Simone et al 2011) and some studies suggest that weight loss is not associated with improvements in LV diastolic function (Turner et al 2000, Stewart et al 2006, Leichman et al 2006, Skilton et al 2008, Cocco and Pandolfi 2011), there are explanations for these findings. In this regard, in the cross-sectional studies where relationships between obesity and LV diastolic function were not clearly demonstrated, only the effects of BMI were evaluated (Bella et al 2002, Chinali et al 2006, De Simone et al 2011). Moreover, weight loss interventions that failed to show improvements in LV diastolic function (Turner et al 2000, Stewart et al 2006, Leichman et al 2006, Skilton et al 2008, Cocco and Pandolfi 2011) were only conducted in small study samples (n=11-51), and the results are therefore subject to false negative findings. Thus, there is currently far stronger evidence in favour of obesity promoting abnormalities in LV diastolic as opposed to systolic

function. What are the potential mechanisms that may explain abnormalities in cardiac diastolic function in obesity?

1.3.3 Insulin resistance may explain obesity-associated decreases in cardiac diastolic function

Insulin resistance may occur as a consequence of an excess adiposity and this effect is more closely associated with the extent of abdominal as opposed to general obesity (Kahn and Flier 2000). Insulin resistance may induce adverse cardiac changes prior to the development of diabetes mellitus and poor blood glucose control through a number of mechanisms. In this regard, insulin resistance is not specific to skeletal muscle, but also involves myocardial muscle tissue as well (Nikolaidis et al 2004, Ouwens et al 2005, Coort et al 2007) where it down regulates glucose uptake and hence precludes the energetic advantage provided by glucose versus free fatty acid oxidation (Nikolaidis et al 2004, Ouwens et al 2005). In this regard, as ventricular relaxation (a key component of diastolic function) is an energy requiring process, it is possible that insulin resistance may promote an impaired relaxation. Moreover, insulin resistance is associated with an accumulation of intracellular triacylglycerol which promotes lipotoxicity (Coort et al 2007). As the ability of the ventricular chamber to actively relax may depend on the number of functional cardiomyocytes that exist at any one time, cell death induced by lipotoxicity may promote an impaired relaxation. Moreover, cell death could stimulate the development of replacement fibrosis and consequently a stiff myocardium. In addition, hyperinsulinaemia, that occurs as a consequence of insulin resistance, promotes obesity-induced increases in sympathetic nervous system activity (Esler et al 2001), a well-recognised cause of cardiac dysfunction mediated by a number of changes including cell death and interstitial abnormalities. What is the current evidence to suggest that insulin resistance could mediate obesity-induced effects on LV diastolic function?

Table 1.4 summarises the current evidence to suggest that insulin resistance may promote LV diastolic dysfunction. In this regard, several small studies (n=26-121) (Lind et al

Table 1.4 Summary of studies that have shown the interaction between insulin resistance and diastolic function.

Authors	Age	BMI	BP status	M/F	Measure of IR	Diastolic function	Outcome	Adjustments
Bajraktari et al 2006	52±6 55±7 55±7	N IR DM	NT, P	8/21 6/14 19/51	OGTT, HOMA-IR, QUICKI	E/A, DT	IR independent predictor of diastolic dysfunction in IR and DM	age, sex, BMI, WHR, glucose, cholesterol, PWT, EF
Dinh et al 2010	64±11	DM, IR, 88%HT		100/108	HOMA-IR, HbA1c	E/A,e', E/e'	relation between HbA1c and E/e'	CAD, HT, age, sex, EF, DM
Fox et al 2011	52±13 (18-84)	population sample		888/1511	HOMA-IR	mitral inflow velocity	no relation	SBP
Galderisi et al 1997	47±8 49±7	N IR	HT	9/- 20/-	euglycemic hyperinsulinemic clamp	E/A, IVRT	IVRT prolonged in IR, in total group glucose disposal independent predictor of IVRT	age, BMI, HR, DBP, LVMI
Hwang et al 2011	25-83	population sample		1161/398	HOMA-IR	IVRT, E/A	E/A decreased across increasing tertiles of IR, even after adjustments	age, gender, BP, fasting glucose
Kamide et al 1996	60±6	N/OW	HT	17/23	hyperinsulinemic euglycemic clamp	E/A	only age and insulin sensitivity was related to diastolic function in stepwise regression	
Lambert et al 2010	23±3 23±4	N OW/OB	NT	6/12 9/16	OGTT	diastolic tissue velocity	in total group no relation between IR and E/A	age, BMI
Lind et al 1995	52±9	OW	HT	27/25	hyperinsulinemic euglycemic clamp	E/A	only insulin sensitivity was correlated to diastolic filling after adjusting	age, sex, BMI, WHR
Leichman et al 2006	44±10	OB	NT	-/22	HOMA2	E/A, DT, IVRT, e', a'	No relationship	
Mureddu et al 1998	33 34	OW/OB N	NT/P NT/P	18/9 21/10	HOMA-IR	IVRT, E, A	no difference in diastolic function between groups after adjustments	age, gender, BP, LVM

Olsen et al 2003	66±6	N-OB	HT & LVH	73/26	hyperinsulinemic isoglycemic clamp	E/A, DT, IVRT	no relation between insulin sensitivity and diastolic function	
Sliem & Nasr 2012	48±8	pre-diabetic	NT	65/56	HOMA-IR	E/A, IVRT	insulin resistance associated with impaired LV diastolic function	
Utz et al 2011	44±10 46±9	N IR	NT	-/36 -/29	OGTT, HOMA-IR	PFR_E/PFR_A, PLV	diastolic function (LV filling and mitral velocity) impaired in IR	
Wada et al 2010	61±7 63±9	Normal IGT	N, P	22/27 14/29	fasting plasma glucose/ HbA1c/ HOMA-IR	E, A, E/A, DT	↑ E/A in IR, IR not correlated with E/A in total group	age, gender, BMI, WC, HOMA-IR,BP, LVMI, HR
Watanabe et al 1999	56±8	N/OW	HT	36/29	insulin suppression test	E/A	IR is an independent predictor for diastolic function in stepwise regression	
Wong et al 2004	46±10 43±10	N OW,OB	NT, P	16/17 53/56	fasting insulin	E/A, IVRT, e', E/e'	insulin associated with e' in univariate analysis	
Wu et al 2012	61±8	N/OW	NT-HT	18/14	HOMA-IR	E/A, DT, e'	no relation	age, gender, BMI, HT, HOMA-IR, CRP, LVMI

BMI, body mass index; BP, blood pressure; M, males; F, females; N, normal; OW, overweight; OB, obese; DM, diabetes mellitus; IGT, impaired glucose tolerance; WC, waist circumference; MAP, mean arterial pressure; BP, blood pressure; DBP, diastolic blood pressure; NT, normotensive; HT, hypertensive; P, prehypertensive; IR, insulin resistance; HOMA-IR, homeostasis model assessment of insulin resistance; HbAIc, glycated haemoglobin; OGTT, oral glucose tolerance test; QUICKI, quantitative insulin sensitivity check index; LV, left ventricle; LVM, left ventricular mass; LVMI, left ventricular mass index; PWT, posterior wall thickness; E/A, early-to-late ventricular diastolic filling; e'/a', TDI determined early to late diastolic filling velocity; E/e', filling pressure; E, early diastolic filling; e', early diastolic filling velocity at mitral annulus; IVRT, isovolumic relaxation time, PFR, peak filling rate in early and atrial filling phase; PLV, peak lengthening velocity; EF, ejection fraction; CAD, coronary artery disease; CRP, C-reactive protein.

1995, Kamide et al 1996, Galderisi et al 1997, Watanabe et al 1999, Wong et al 2004, Bajraktari et al 2006, Dinh et al 2010, Sliem and Nasr 2011, Utz et al 2011) and one large study (n=1599) (Hwang et al 2012) conducted in select samples, suggest that insulin resistance is associated with abnormalities of LV diastolic function. However, the relationships between indices of insulin resistance and LV diastolic function were not adjusted for adiposity indices in the large study (Hwang et al 2012) and in several of the small studies (Lind et al 1995, Wong et al 2004, Sliem and Nasr 2011, Utz et al 2011). In contrast, in alternative small (n=27-102) (Mureddu et al 1998, Olsen et al 2003, Leichman et al 2006, Lambert et al 2010, Wada et al 2010, Wu et al 2012) and one large (n=2399), study where the regression relations were nevertheless not provided (Fox et al 2011), indices of insulin resistance were not correlated with measures of LV diastolic function. The small sample sizes in many of these prior studies may have resulted in false positive or negative findings; a selection bias may have resulted in recruitment of participants that do not reflect the community at large; and relationships that have not been adjusted for adiposity indices are likely to reflect confounding effects of obesity *per se*. Thus, at present there is insufficient evidence to support a role for insulin resistance in accounting for obesity-associated decreases in LV diastolic function.

Therefore, to address the aforementioned concerns, in the present thesis I evaluated whether an index of insulin resistance is associated with LV diastolic function independent of adiposity indices or haemodynamic changes in a relatively large, randomly selected community-based sample with a high prevalence of obesity. The data for this study and the implications thereof are provided in chapter 4 of the present thesis.

1.3.4 A sedentary lifestyle may explain obesity-associated decreases in cardiac diastolic function

As indicated in section 1.2.6, it is well established that obesity is associated with a sedentary lifestyle and that a sedentary lifestyle results in a lack of physical fitness. In this regard, there is now evidence to indicate that amongst other benefits, regular aerobic exercise may be

associated with improvements in LV diastolic function. Table 1.5 summarises the results of studies assessing the effects of exercise training on echocardiographic assessments of cardiac function. The majority of studies suggest that in overweight or obese individuals E/A is unchanged by exercise training (Reid et al 1994, Sadaniantz et al 1996, Stewart et al 2006, Baynard et al 2008, Riordan et al 2008, Kosmala et al 2009, Eriksson et al 2010, Cocco and Pandolfi 2011, Guirado et al 2012, Schuster et al 2012), despite weight loss (Reid et al 1994, Stewart et al 2006, Riordan et al 2008, Kosmala et al 2009, Cocco and Pandolfi 2011) or evidence of improved cardiorespiratory fitness (Reid et al 1994, Sadaniantz et al 1996, Stewart et al 2006, Riordan et al 2008, Baynard et al 2008, Kosmala et al 2009, Guirado et al 2012, Schuster et al 2012) in many of these studies. In addition, there is also evidence to suggest that tissue Doppler indices of diastolic function are unaffected by exercise training programmes (Riordan et al 2008, Guirado et al 2012,) despite improvements in cardiorespiratory fitness in both of these studies and a decrease in body weight in one of these studies (Riordan et al 2008). Furthermore, in one study there was evidence of worsening diastolic function with exercise training as evidenced by a decreased E/A, and e' and an increased E/e' (Gondoni et al 2007). In contrast, some studies show an increased E (Levy et al 1993), or e' (Wong et al 2006, Kosmala et al 2009) in association with a decreased body weight and improved cardiorespiratory fitness; or an increased e' without a change in body weight, but with an improved cardiorespiratory fitness (Schuster et al 2012).

Importantly, studies performed assessing the impact of exercise training on diastolic function as determined from tissue Doppler imaging in overweight or obese individuals, either employed exercise together with dietary approaches as lifestyle interventions (Wong et al 2006, Gondoni et al 2007, Kosmala et al 2009); assessed effects in elderly (age=68±8 years) treated hypertensives (Guirado et al 2012), where the confounding effects of age and antihypertensive therapy on LV diastolic function may have limited the capacity to detect exercise-induced effects on LV diastolic function; evaluated the effects of exercise training in remarkably small study samples (n=10-13) (Riordan et al 2008, Schuster et al 2012) which

Table 1.5 A summary of the effects of exercise training on left ventricular diastolic function.

	Characteristics					Training							
Authors	**Age**	**BMI**	**BP status**	**M/F**	**Dur (wk)**	**Mode**	**Intensity**	**Volume**	**Diastolic function**	**Geometry**	**BP**	**Wt**	**VO_2**
Baynard et al 2008	52±1	OB	N-MS	18	1.5	walk	70-75%	daily	→E/A ↓IVRT		→	→	↑
	52±1	OB	MS	13			VO_{2peak}		→E/A →IVRT		↓	→	↑
Cocco and Pandolfi 2011	59±4	OW	HT (TX)	22/22	26	D+Aer	80% HR_{max}	5x2x 15	→E/A	↓LAVI	↓	↓	-
Eriksson et al 2010	47±8	OW/OB	NT, P	-/50	26	walk/ cycle	Low	daily	→E/A	→LVM	↓	→	-
Gondoni et al 2007	30±6	OB	NT	5/10	3	D+Aer	50% VO_{2peak}	5x2x 35	→E/A ↓e' ↑E/e'	→LVMI →RWT	↓	↓	
Guirado et al 2012	68±8	OB	HT (Tx)	6/9	26	Aer+R	60-75% HRR	3x 30	→E/A →e'/a' →E/e'	→LVM →PWT	↓	→	↑
Kelemen et al 1990	47±6	OB	HT (Tx)	19/-	10	Aer+R	14-16 RPE	3x 50	→diastolic function	↑LVMI →PWT	↓	→	-
Kosmala et al 2009	41±13		NT/	30/94	26	D+Aer	Moderate	4-5x 30	→E/A →E/e' ↑e'	↓ LVMI	→	↓	↑
	49±12	OB	P/HT	64/73	26	D+Aer		4-5x 30	→E/A →E/e' →E'	→LVMI	→	→	→
Levy et al 1993	28±3	-	N	11/-	26	Aer	50-85%HRR	4-5x 45	↑E	↑LVM	-	→	↑
	68±6	-	P	13/-					↑E	↑LVM	-	↓	↑
Miyai 2002	46±2	N	NT	32/-	12	cycle	50-60%HRR	3x 45	→E/A	→PWT →LVMI	→	→	↑
Reid et al 1994	47±4	OB	P	n=7	12	Cycle	70% VO_{2peak}	3x 30	→E/A	→RWT →LVMI	↓	→	↑
	36±4	OB	P	n=6		D+cycle	70% VO_{2peak}	3x 30	→E/A	→RWT →LVMI	↓	↓	↑
Riordan et al 2008	50-60	OW	N	6/7	52	Aer	70% HR_{max}	6x 60	→E/A →e'/a' ↓IVRT	→LVM	→	↓	↑
Rodrigues et al 2006	31±4	N	NT	23/-	26	Aer	60-80%VO_{2max}	3x 60	↑E/A ↑e'	↑PWT ↑LVMI	-	↓	↑
Sadaniantz et al 1996	39±7	OW	NT, P	16/-	52	Aer	60-80% HR_{max}	4x 60	→E/A	→LVMI →PWT	→	→	↑
Schuster et al 2012	52±3	OB	NT, P	10/-	8	Aer	50%VO_{2max}	3x 45	→E/A →e'/a' ↑e'	↓LVMI →RWT	↓	→	↑
Stewart et al 2006	64±6	OW	HT	25/26	26	Aer+R	60-90% HR_{max}	3x 45	→E/A, →E/e'	→LVMI →PWT	↓	↓	↑
Turner et al 2000	65±5	OW/OB	HT	9/2	30	Aer	60-80% HR_{max}	4x 50	→diastolic function	↓LVM ↓RWT	↓	↓	↑

Wong et al 2006	47±11	OB		25/23	8	D+Aer	Moderate	150 min	→E/A →E/e' ↑e'	↓LVMI	→	↓	↑
	48±10	OB	N, P	33/25		/Aer			→E/A →E/e'	↓LVMI	→	→	↑

BMI, body mass index; BP, blood pressure; M, males; F, females; Dur, duration of exercise intervention; wk, weeks; Volume, frequency of sessions per week x minutes per session; min, minutes; Wt, change in weight after training; VO_2, change in aerobic capacity after training; N, normal; OW, overweight; OB, obese; NT, normotensive; HT, hypertensive; P, prehypertensive; MS, metabolic syndrome; N-MS, non-metabolic syndrome; ISH, isolated systolic hypertension; W-HT, white coat hypertension; Tx, treated; IDH, isolated diastolic hypertension; Aer, aerobic exercise; D, diet; R, resistance exercise; $VO_{2max,}$ maximal aerobic capacity; HR_{max}, maximal heart rate; HRR, heart rate reserve; VO_{2peak}, peak aerobic capacity; RPE, rate of perceived exertion; LV, left ventricle; LVM, left ventricular mass; LVMI, left ventricular mass index; LAVI, left atrial volume index; PWT, posterior wall thickness; RWT, relative wall thickness; E/A, early-to-late ventricular diastolic filling; e'/a', TDI determined early to late diastolic filling velocity; E/e', filling pressure; E, early diastolic filling; e', early diastolic filling velocity at mitral annulus; IVRT, isovolumic relaxation time; -, no data.

may reflect false positive (Schuster et al 2012) (n=10) or negative (Riordan et al 2008, Guirado et al 2012) (n=13-15) findings; evaluated the effects of exercise training in men only (Schuster et al 2012), thus limiting the conclusions to one sex; or performed analysis after*post hoc* assignment to groups which either did or did not have weight loss (Wong et al 2006), an obviously flawed methodology. Hence, there is currently insufficient evidence to support or refute that exercise training alone may produce benefits to diastolic function as assessed using tissue Doppler imaging. As a consequence of these deficiencies in current evidence, to address the aforementioned concerns in the present thesis I assessed the impact of exercise training alone on indices of LV diastolic function, including tissue Doppler indices, in a substantially larger study sample than that previously reported on (Riordan et al 2008, Schuster et al 2012) of young-to-middle aged, overweight and obese individuals not receiving antihypertensive therapy and in participants of both sexes. The data for this study and the implications thereof are provided in chapter 5 of the present thesis.

1.4 Problem statement

In summary the present thesis was designed to address some of the outstanding issues regarding the mechanisms of obesity-associated cardiovascular risk. In this regard there is still considerable uncertainty regarding the role of an obesity-associated sedentary lifestyle or insulin resistance in promoting the development of hypertension or LV diastolic dysfunction. Presently the interaction between obesity and insulin resistance and the peripheral and central aortic BP response to salt intake in a population of African ancestry with a high prevalence of salt sensitivity is uncertain. Although exercise training decreases brachial BP in overweight and obese individuals, evidence to support a beneficial effect of exercise on systolic pressure augmentation and consequently central aortic BP is lacking. There is uncertainty whether insulin resistance can account for obesity related changes in LV diastolic function, independent of the confounding effects of coexisting obesity. Currently there is insufficient evidence to support or refute the notion that exercise training alone in

overweight and obese individuals may produce benefits to LV diastolic function as assessed using tissue Doppler imaging. The aims of the present thesis can therefore be summarised as follows:

1.5 Aims

1. To determine whether insulin resistance may in-part account for salt intake-BP relationships in a community sample of African ancestry with a high prevalence of insulin resistance, and whether this effect translated into changes in central aortic BP.
2. To evaluate the extent to which exercise training-induced decreases in BP may be attributed to modifications in aortic augmentation pressures or indexes (AIx) in overweight and obese persons.
3. To evaluate whether an index of insulin resistance is associated with LV diastolic function independent of adiposity indices in a relatively large, randomly selected community-based sample with a high prevalence of obesity.
4. To assess the impact of exercise training alone on indices of LV diastolic function, including TDI, in young-to-middle aged, overweight and obese individuals not receiving antihypertensive therapy and in participants of both sexes.

CHAPTER 2

INSULIN RESISTANCE ACCOUNTS FOR THE RELATIONSHIP BETWEEN URINARY SALT EXCRETION AND AMBULATORY BLOOD PRESSURE IN A COMMUNITY OF AFRICAN ANCESTRY

2.1 Abstract

Background. Although groups of African descent are particularly sensitive to the blood pressure effects of salt intake, the role of obesity and insulin resistance in mediating this effect and whether this effect translates into changes in central aortic BP are uncertain. I aimed to determine whether obesity or insulin resistance are independently associated with salt intake-BP relationships in a community sample of African ancestry and whether this effect occurs in both brachial and central aortic BP.

Methods. I measured 24-hour urinary Na^+/K^+, the homeostasis model assessment of insulin resistance (HOMA-IR), nurse-derived conventional, 24-hour ambulatory and central aortic (SphygmoCor) BP, aortic augmentation index (AIx) and aortic pulse wave velocity (PWV) in 331 participants from a South African community sample of black African descent not receiving treatment for hypertension.

Results. Although log HOMA-IR was not independently associated with BP, with adjustments including diabetes mellitus and the individual terms, an interaction between log HOMA-IR and urinary Na^+/K^+ was associated with 24-hour, and day systolic ($p<0.05$) and 24-hour, day and night diastolic ($p<0.002$ to $p<0.001$) BP. Neither aortic augmentation pressure, AIx, central aortic pulse pressure, nor aortic PWV were independently associated with an interaction between log HOMA-IR and urinary Na^+/K^+. The multivariate adjusted relationship between urinary Na^+/K^+ and night diastolic BP increased across tertiles of HOMA-IR (Tertile 1: β-coefficient=-0.79±0.47, Tertile 2: β-coefficient=0.65±0.35, Tertile 3: β-coefficient=1.03±0.46, $p<0.05$ tertiles 3 and 2 vs 1). The partial correlation coefficients for the relationships between urinary Na^+/K^+ and 24-hour (partial $r=0.19$, $p<0.02$), day (partial $r=0.17$, $p<0.05$), and night (partial $r=0.18$, $p<0.02$) diastolic BP in participants with log HOMA-IR≥median were greater than those for the relationships between urinary Na^+/K^+ and 24-hour (partial $r=-0.08$, $p=0.29$), day (partial $r=-0.10$, $p<0.22$), and night (partial $r=-0.06$, $p=0.40$) diastolic BP in participants with log HOMA-IR<median ($p<0.05$ for comparisons of r values).

Conclusion. Insulin resistance is independently associated with the relationship between salt intake, as indexed by urinary Na^+/K^+, and ambulatory BP, in groups of African descent. These effects of insulin resistance cannot be accounted for by actions on central aortic haemodynamics.

2.2 Introduction

There is substantial evidence to indicate that obesity is a major determinant of BP and the development of hypertension (Harris et al 2000, Zhu et al 2005). Indeed, the odds of developing hypertension are ~1.7-3.4 times greater in obese individuals as compared to lean individuals (Harris et al 2000). Moreover, meta-analysis of intervention studies indicate that for every 1 kg body weight lost, systolic BP/diastolic BP decreases by 1.05/0.92 mm Hg (Neter et al 2003, Hedayati et al 2011). The mechanisms of obesity-induced changes in BP are therefore of considerable interest. A number of studies have demonstrated that obesity or the metabolic syndrome is associated with renal tubular handling of sodium (Na^+) and an increased sensitivity of BP to Na^+ intake (Rocchini et al 1989, Hall et al 1997, Strazzullo et al 2001, 2006, Barbato et al 2004, Uzu et al 2006, Hoffmann et al 2008, Chen et al 2009). These associations may be accounted for by the relationship between insulin-resistance and salt sensitivity (Sharma et al 1991, Endre et al 1994, Shimamoto et al 1994, Zavaroni et al 1995, Bigazzi et al 1996, Galletti et al 1997, Fuenmayor et al 1998, Sechi 1999, Yatabe et al 2010) an effect that may be mediated in-part by insulin actions on renal tubular function (Shimamoto et al 1994, Sechi et al 1999, Pearce et al 2001). Despite the particular importance of salt-sensitivity in contributing toward increases in BP in groups of African descent (Weinberger et al 1986, He et al 1998, 2000, Vollmer et al 2001, Wright et al 2003, Aviv et al 2004), a finding that may be attributed to ethnic differences in tubular handling of Na^+ (Bochud et al 2009), the role of obesity, the metabolic syndrome or insulin resistance in the pathophysiology of salt-sensitivity in this ethnic group is uncertain. Indeed neither obesity, nor insulin resistance are related to renal tubular Na^+ reabsorption in this ethnic group (Barbato et al 2004).

Although obesity is associated with increases in large artery stiffness (Toto-Moukouo et al 1986, Resnick et al 1997, Sutton-Tyrrell et al 2001, Mackey et al 2002, Wildman et al 2003, Danias et al 2003, Ferreira et al 2004, Snijder et al 2004, Czernichow et al 2005, Wildman et al 2005, Zebekakis et al 2005, Majane et al 2008), and weight loss results in an

attenuation of indexes of aortic stiffness (Balkestein et al 1999, Wildman et al 2005, Barinas-Mitchell et al 2006, Dengel et al 2006, Dengo et al 2010), indices of wave reflection or aortic pressure augmentation have been shown to either decrease (Maple-Brown et al 2005, Otsuka et al 2009) or increase (Ounis-Skali et al 2007), and central aortic BP remained unaltered following a weight loss programme despite improvements in indices of aortic stiffness (Dengo et al 2010). One possibility to explain inconsistencies in the impact of obesity on large artery function and aortic BP is that obesity or the associated insulin resistance produces effects on large artery function by modifying the effect of Na^+ intake on aortic characteristics. Indeed, as recently demonstrated in a group of persons of black African ancestry, relationships between indices of Na^+ intake and aortic BP and aortic augmentation pressures or indices may exceed that noted between indices of Na^+ intake and brachial BP despite no relationship with an index of aortic stiffness (PWV) (Redelinghuys et al 2010). However, whether an interaction between obesity or insulin resistance and Na^+ intake determines central aortic BP or the wave characteristics has not been evaluated.

As the role of obesity, or insulin resistance in salt-sensitive hypertension in persons of African ancestry is uncertain, and whether this translates into an effect on central aortic BP or the aortic wave characteristics is unknown, in this study I evaluated whether obesity or insulin resistance are independently associated with the relationship between urinary salt excretion, an index of salt intake, and either conventional, 24-hour or central aortic BP or the aortic wave characteristics in a community sample of African ancestry with a high prevalence of obesity.

2.3 Methods

2.3.1 Study participants

This 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 numbers: M02-04-72 renewed as M07-04-69

and M1204108). Participants gave informed, written consent. The study design has previously been described (Woodiwiss et al 2009, Redelinghuys et al 2010, Michel et al 2012, Norton et al 2012). Nuclear families (either both parents and at least one sibling or one parent and two or more siblings) of black African descent (Nguni and Sotho chiefdoms) with siblings older than 16 years were randomly recruited from the South West Township (SOWETO) of Johannesburg, South Africa. Street names and addresses of households from formal dwellings represented in the 2001 census were obtained from the Department of Home Affairs**.** These households were allocated numbers and numbers were selected from a random number generator. People residing in informal dwellings or institutions/homes were not recruited. No subjects of mixed, Asian, or European ancestry were recruited and no Khoi-San subjects were recruited. Of the 508 participants not receiving treatment for hypertension that had 24-hour urine samples that met with pre-specified quality control criteria previously described (Redelinghuys et al 2010), 331 had 24-hour ambulatory BP measurements that met with pre-specified quality control criteria (Woodiwiss et al 2009) (longer than 20 hours and more than 10 and 5 readings for the computation of day and night means, respectively). Of these 331 participants derived from 96 families, 124 were singletons, 69 sibling pairs, and106 parent-child pairs.

2.3.2 Clinical, demographic, and anthropometric measurements

Demographic and clinical data were obtained using a standardized questionnaire as previously described (Woodiwiss et al 2009, Redelinghuys et al 2010, Michel et al 2012, Norton et al 2012). Included in the questionnaire were specific requests for date of birth, gender, previous medical history, the presence of hypertension, diabetes mellitus and kidney disease, prior and current drug therapy (analgesic use included), smoking status (including the number of cigarettes smoked in the past and at the present time), daily alcohol consumption (beer, traditional beer or other forms of alcohol and the daily quantity), and family history of hypertension and cardiovascular events. For females, menstrual history, history of pregnancies and oral contraceptive use was evaluated. In order to avoid

translational errors, the questionnaire was not translated into an African language, but study assistants familiar with all languages spoken in SOWETO and who either previously lived in SOWETO or currently reside in SOWETO assisted with the completion of each questionnaire. Nevertheless, the majority of participants were reasonably proficient in English. Only same sex assistants were used to assist each family member with the completion of the questionnaire. Assistance was only provided when requested. Study assistants first visited homes of subjects that agreed to participate in the study in order to familiarise participants with the questionnaire. The questionnaire was only completed at a subsequent clinic visit and then ambiguities checked by performing a follow-up home visit. If family members were absent at follow-up home visits, data was checked with them personally via telephonic conversations whenever possible. Ambiguities in answers to the questionnaire were detected by an independent observer prior to a second home visit. A pilot study was conducted in 20 participants to ensure that data obtained in the questionnaires were reproducible when obtained with the assistance of two separate study assistants.

Height and weight were measured with participants standing, wearing light clothing and no shoes, using standard approaches. Participants were identified as being overweight if their body mass index (BMI) was ≥25 $kg.m^{-2}$ and obese if their BMI was ≥30 $kg.m^{-2}$. Waist circumference were measured to the nearest millimetre (mm) with the subject's body in the anatomical position, at the narrowest point between the lower costal border and the top of the iliac crest, perpendicular to the long axis of the trunk.

2.3.3. Conventional BP

Nurse-derived conventional (brachial) BP was measured using a mercury sphygmomanometer after participants had rested in the seated position for five minutes. Five consecutive BP readings were obtained using an appropriately sized cuff, 30 to 60 seconds apart. The cuff was deflated at approximately 2 mm Hg per second and the first and fifth Korotkov phases were used to determine systolic and diastolic BP respectively. Care was taken to avoid auscultatory gaps. Standard cuffs were used with an inflatable bladder with a

length of 22 cm and a width of 12 cm except when arm circumference exceeded 31 cm, when larger cuffs with a 31 x 15 cm bladder were employed.The average of the five readings was taken as the BP. None of the visits had fewer than the planned BP recordings. The frequency of identical consecutive recordings was 0% for systolic BP and 1.2% for diastolic BP. No BP values were recorded as an odd number. Of the systolic and diastolic BP readings, 29% ended on a zero (expected =20%).

2.3.4 Ambulatory BP

Twenty four hour ambulatory BP monitoring was performed on the same day as conventional BP measurements using oscillometric monitors (SpaceLabs, model 90207) as previously described (Woodiwiss et al 2009). Monitors were programmed to measure BP at 15-minute intervals from 06:00 to 22:00 and at 30-minute intervals from 22:00 to 06:00. The calibration was checked monthly against a mercury manometer. The cuff size was the same as that used for conventional BP measurements. Participants kept a diary card for the duration of the recordings to note the time of going to bed in the evening and getting up in the morning. Diary cards were employed to identify the actual in-bed and out-of-bed periods. These periods were used to calculate the average in-bed and out-of-bed periods and thus the average transition periods during which BP changes rapidly in most participants. These average transition periods were then eliminated. The remaining periods were considered to be the night or day fixed-clock time periods. Fixed-clock time periods rather than actual in-bed and out-of-bed periods were statistically analysed to ensure that similar day and night time periods were selected for comparisons between individuals. These fixed-clock time periods were identified as ranging from 09:00 to 19:00 h and from 23:00 to 05:00 h respectively. Intra-individual means of the ambulatory measurements were weighted by the time-interval between successive recordings. The mean±SD number of BP recordings for the 24-hour period was 62.6±11.9 (range=24-81), for the day period was 29.1±7.1 (range=11-41) and for the night period was 9.4±1.0 (range=6-12).

2.3.5 Laboratory blood tests

Standard laboratory blood tests of renal function, liver function, blood glucose, lipid profiles, haematological parameters, and percentage glycated haemoglobin (HbA1c)(Roche Diagnostics, Mannheim, Germany) were performed. Diabetes mellitus (DM) or abnormal blood glucose control was defined as the use of insulin or oral hypoglycaemic agents or an HbA1c value greater than 6.5% (Bennett et al 2007). Menopause was confirmed with measurements of follicle stimulating hormone concentrations. Plasma insulin concentrations were determined from an insulin immulite, solid phase, two-site chemiluminescent immunometric assay (Diagnostic Products Corporation, Los Angeles, CA, USA) and insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) using the formula (insulin [$\mu U.ml^{-1}$] x glucose [$mmol.l^{-1}$])/22.5 (Wallace et al 2004). Estimated glomerular filtration rate (eGFR) was determined using the abbreviated Modification of Diet in Renal Disease (MDRD) study group equation: 186.3 x (serum creatinine in mg/decilitre$^{-1.154}$) x (age in years$^{-0.203}$) x 1.212 x 0.742 (if female).

2.3.6 Urinary electrolyte excretion rates

Timed urine samples were obtained over a period of at least 24-hour after discarding urine obtained immediately prior to the collection period. Urine Na^+, K^+, and creatinine concentrations were measured and 24 urine Na^+ and K^+ excretion rates calculated from the product of urine volume and urine electrolyte concentration. Creatinine clearance was determined from the product of urine volume and urine creatinine concentration/plasma creatinine concentration. The quality of urine samples was determined by constructing regression relations between 24-hour urine creatinine and body weight and 24-hour urine volume and age in gender-specific groups. Based upon the 95% confidence intervals for each group, a 24-hour urine sample was considered acceptable if 24-hour urine creatinine (mmol) was >3.5 and <35 for males and >3.5 and <30 for females. Samples with urine volumes <300 ml/day were also assumed to be incomplete urine collections and thus of insufficient quality to be included in the data analysis. As previously demonstrated, urinary

Na^+/K^+ rather than 24-hour urinary Na^+ or K^+ excretion rates are closely associated with BP (Redelinghuys et al 2010), hence in all analysis salt intake was indexed as urinary Na^+/K^+.

2.3.7 Pulse wave analysis

After participants had rested for 15 minutes in the supine position, arterial waveforms at the radial (dominant arm), carotid and femoral artery pulses were recorded by applanation tonometry, each 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) (Norton et al 2012, Shiburi et al 2006) (Figure 2.1). Recordings where the systolic or diastolic variability of consecutive waveforms exceeded 5% or the amplitude of the pulse wave signal was less than 80 mV were discarded. The pulse wave was calibrated by manual measurement (auscultation) of brachial BP taken immediately before the recordings. From a validated inbuilt transfer function an aortic waveform was generated from which central systolic, diastolic and mean arterial BP were derived (Figure 2.2). The magnitude of the augmented pressure wave was determined as the difference between central systolic BP and the inflection point at the end of the first systolic shoulder. Central PP (PPc) was calculated as the difference between central systolic BP and central diastolic BP and MAP was calculated as [central diastolic BP + 1/3(central PP)]. Central augmentation index (AIx) was determined as the augmented pressure wave/pulse pressure, expressed as a percentage.

Aortic PWV was measured from sequential waveform measurements at carotid and femoral sites as previously described (Shiburi et al 2006) (Figure 2.3). Pulse wave transit time i.e. the time it takes the pulse wave to travel from the carotid to the femoral site, was determined as the difference between the times taken to generate the femoral and carotid pulse waveforms. To assess the differences in time of the generation of the femoral and carotid pulse waveforms, a single lead electrocardiogram was performed concurrently with pulse waveform sampling. The time delay in the pulse waves between the carotid and

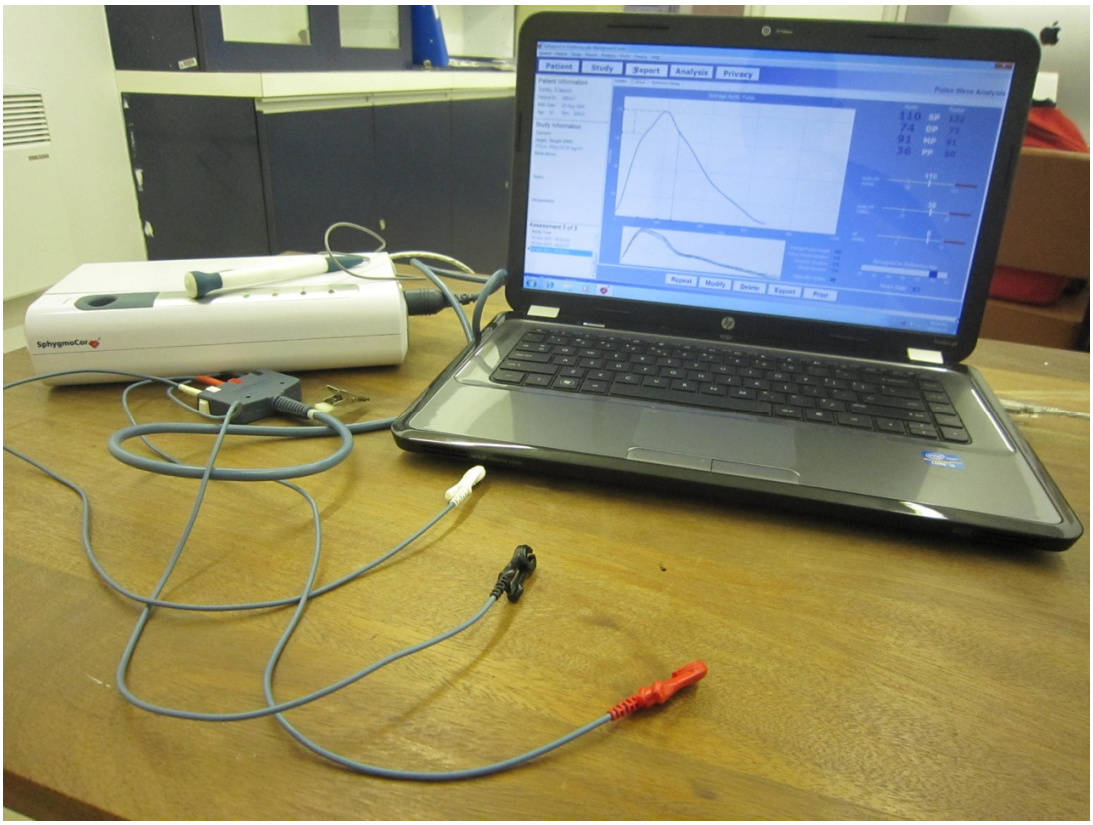


Figure 2.1 SphygmoCor device coupled to an applanation tonometer used to determine central (aortic) haemodynamics and aortic pulse wave velocity, with an image of radial artery and aortic pressure waves recorded from a participant demonstrated on the laptop (see Figure 2.2 for further details).

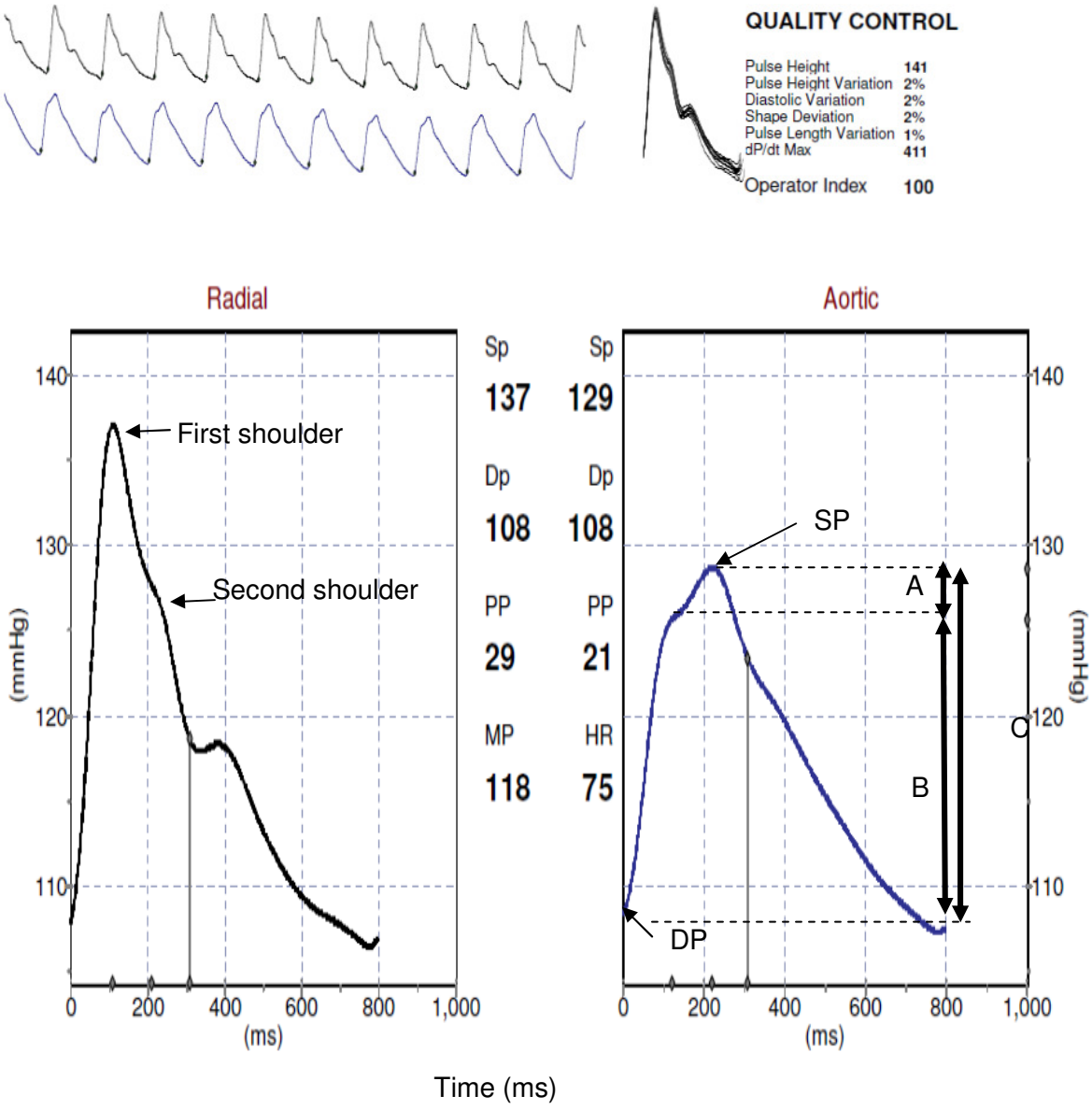


Figure 2.2 Examples of a pulse wave recording obtained to determine central haemodynamics. The figure shows the radial artery pulse wave obtained from applanation tonometry (lower left panel) and the aortic pulse wave derived from a population-based transfer function built into the software (lower right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

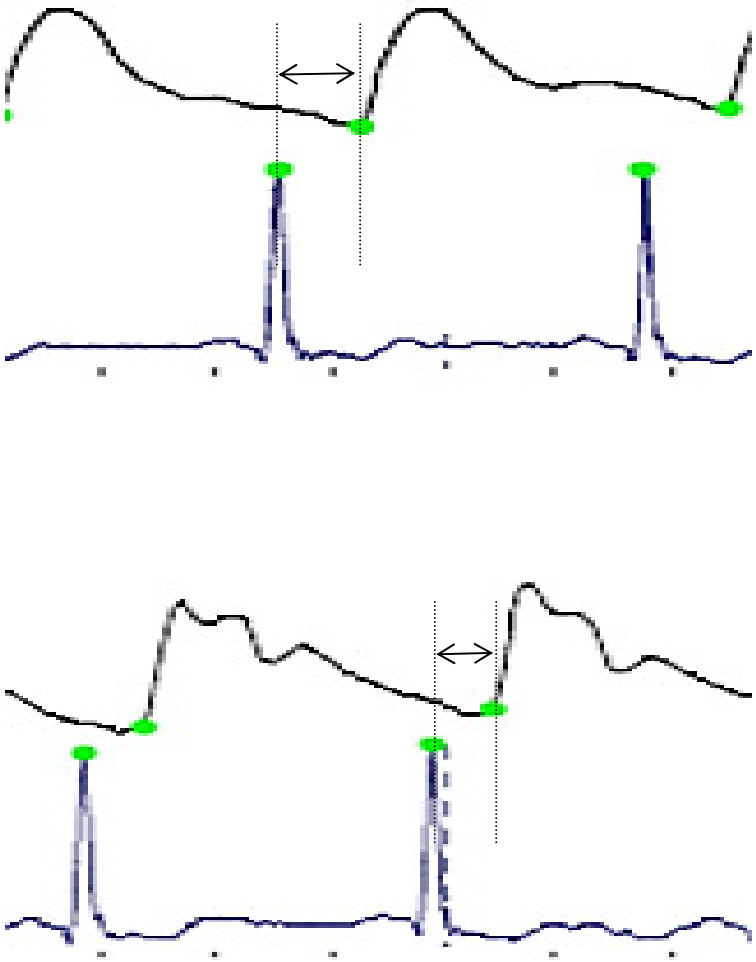


Figure 2.3 Examples of femoral and carotid artery pulse waves obtained using applanation tonometry from the same participants. Together with simultaneous electrocardiographic (ECG) recordings aortic pulse wave velocity (PWV) is calculated. The arrows indicate the time between electrical events and the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

femoral sites was determined using the R wave as a fiducial point. Pulse transit time was taken as the average of 10 consecutive beats. The distance which the pulse wave travels was determined as the difference between the distance from the femoral sampling site to the suprasternal notch, and the distance from the carotid sampling site to the suprasternal notch. Aortic PWV was calculated as distance (meters) divided by transit time (seconds).

2.3.8 Data analysis

Database management and statistical analyses were performed with SAS software, version 9.1 (SAS Institute Inc., Cary, NC, USA). Continuous data are reported as mean±SD. Unadjusted means and proportions were compared by the large-sample z-test and the χ^2-statistic, respectively. As HOMA-IR was positively skewed (skewness=3.87, kurtosis=22.54; Shapiro-Wilk's statistic=0.62, $p<0.0001$) HOMA-IR was log transformed. Log transformation of HOMA-IR resulted in an improved distribution (skewness=0.25, kurtosis=-0.77; Shapiro-Wilk's statistic=0.97). Relationships were determined from multivariate linear regression analysis with appropriate adjustors. Z-Statistics were used to compare correlation coefficients. An ANOVA with a Bonferroni or Tukey *post hoc* test was employed to compare β-coefficients where appropriate. Probability values were further adjusted for non-independence of family members using the method of maximum likelihood estimation as implemented by the mixed procedure as defined in the SAS package. Using this method the likelihood function of the model given the observed data is determined. Subsequently the probability distribution that underlies the data is identified (Probability distribution that makes observed data most likely). To ensure that the presence of diabetes mellitus or glucose lowering therapy (including insulin) did not confound the results, in secondary data analysis (sensitivity analysis) participants with diabetes mellitus or an HbA1c>6.5% were excluded. A probability value of <0.05 was considered to be significant.

2.4 Results

2.4.1 Characteristics of the participants

Table 2.1 gives the demographic and clinical characteristics of the study group. More women than men participated. In general the study group had a high BMI and waist circumference, with ~58% of participants being either overweight (~23%) or obese (~35%). Of the 2.4 % (n=8) of participants receiving glucose lowering therapy, all were receiving oral agents and 1 participant was receiving insulin in addition to oral agents. The general characteristics of untreated participants who did not have 24-hour BP values that met with pre-specified quality control were no different from the characteristics of the participants whose data are shown in Table 2.1 (see Table 2.2). The average 24-hour urinary Na^+ excretion rate was well above the recommended daily allowance (RDA) for Na^+ intake of 65 $mmol.day^{-1}$, with most of the study group (67%) 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 $mmol.day^{-1}$.

2.4.2 Relationships between indices of excess adiposity and HOMA-IR or eGFR

With age and sex adjustments, waist circumference (partial $r=0.20$, $p<0.0005$), waist-to-hip ratio (partial $r=0.21$, $p<0.0005$) and BMI (partial $r=0.11$, $p<0.05$), were related to HOMA-IR. None of the indexes of excess adiposity were associated with eGFR ($p>0.44$).

2.4.3 Independent relationships between indexes of excess adiposity, insulin resistance, or urinary indexes of salt intake or eGFR and BP

Importantly, with adjustments for age, sex, diabetes mellitus or an HbA1c>6.5%, regular alcohol intake or regular smoking, neither waist circumference, BMI, HOMA-IR, nor eGFR were correlated with urinary Na^+/K^+ ($p>0.13$). Waist circumference (Table 2.3), but neither log HOMA-IR (Table 2.3), nor eGFR (data not shown) were independently related to

Table 2.1 Characteristics of the 331 study participants.

Characteristic	mean ± standard deviation or %
Sex (% female)	58.6
Age (years)	40±17
Body mass index ($kg.m^{-2}$)	28.1±7.6
Waist circumference (cm)	87.4±15.4
Regular tobacco intake (%)	16.9
Regular alcohol intake (%)	27.8
% with diabetes mellitus or HbA1c>6.5%	7.6
% Hypertensive	25.7
Estimated GFR ($ml/min/1.73\ m^2$)	113±27 (range=66-199)
24-hour urinary Na^+ (mEq)	105.0±72.3
24-hour urinary K^+ (mEq)	29.4±22.1
24-hour urine volume (ml)	1383±739
Urinary Na^+/K^+	4.18±2.25
Urinary Na^+/creatinine	13.4±7.6
Urinary K^+/creatinine	3.54±1.70
HOMA-IR	3.37±4.60
Conventional SBP/DBP (mm Hg)	126±20/83±12
Conventional pulse pressure (mm Hg)	43±14
Pulse rate (bpm)	64±13
24hour SBP/DBP (mm Hg)	117±14/72±10
Day SBP/DBP (mm Hg)	122±13/77±10
Night SBP/DBP (mm Hg)	110±16/64±11
Aortic SBP/DBP (mm Hg)	118±21/84±12
Aortic pulse pressure (mm Hg)	35±13
Aortic augmentation pressure (mm Hg)	9.8±7.4
Aortic augmentation index (%)	25.9±13.0
Pulse wave velocity ($m.s^{-1}$)	6.39±2.56

HbA_{1C}, glycosylated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; BP, blood pressure; SBP, systolic BP; DBP, diastolic BP; bpm, beats per minute

Table 2.2 Comparison of the general characteristics of untreated study participants with and without quality 24-hour ambulatory BP values.

Characteristic	With n=331	Without n=177	p-value
Sex (% female)	58.6	65.5	=0.15
Age (years)	40±17	37±17	=0.06
Body mass index (kg/m^2)	28.1±7.6	28.4±8.3	=0.68
Waist circumference (cm)	87.4±15.4	87.3±17.3	=0.95
Regular tobacco intake (%)	16.9	21.5	=0.23
Regular alcohol intake (%)	27.8	23.2	=0.29
% overweight/obese	23.3/35.3	24.9/33.3	=0.74/=0.70
% with diabetes mellitus or HbA1c>6.1%	7.6	8.0	=0.86
% with hypertension	25.7	26.6	=0.83

HbA1c, glycosylated haemoglobin.

Table 2.3 Multivariate adjusted relationships (partial correlation coefficients, partial r) between waist circumference, an index of insulin resistance (log HOMA-IR), or urinary electrolyte excretion and blood pressure (BP) or aortic characteristics in 331 participants of African descent not receiving antihypertensive therapy

	Waist circumference vs BP		log HOMA-IR vs BP		Urinary Na^+/K^+ vs BP	
	partial r*	p value†	partial r*	p value†	partial r*	p value†
Conventional systolic BP	0.08	=0.13	0.08	=0.16	0.15	<0.005
Conventional diastolic BP	0.17	<0.005	0.002	=0.86	0.13	<0.02
24-hour systolic BP	0.19	<0.0005	-0.03	=0.73	0.13	<0.05
24-hour diastolic BP	0.08	=0.08	-0.09	=0.22	0.08	=0.27
Day systolic BP	0.19	<0.001	-0.03	=0.60	0.11	<0.05
Day diastolic BP	0.10	<0.05	-0.07	=0.29	0.06	=0.31
Night systolic BP	0.16	<0.005	-0.04	=0.55	0.12	<0.05
Night diastolic BP	0.07	=0.09	-0.06	=0.35	0.08	=0.30
Aortic pulse pressure	-0.09	=0.12	0.05	=0.36	0.21	=0.0002
Aortic augmentation pressure	-0.12	<0.05	0.005	=0.94	0.16	<0.005
Aortic augmentation index	-0.05	=0.40	-0.063	=0.69	0.06	=0.32
Aortic pulse wave velocity	-0.07	=0.27	-0.05	=0.47	0.10	=0.10

HOMA-IR, homeostasis model assessment of insulin resistance. *Adjustments are for age, sex, the presence of diabetes mellitus/ HbA1c>6.5%, regular alcohol consumption, and regular tobacco use. For aortic characteristics additional adjustments were for pulse rate and mean arterial pressure. †Probability values were further adjusted for non-independence of family members.

conventional and day diastolic BP and 24-hour, day and night systolic BP. Urinary Na^+/K^+ was independently related to conventional and ambulatory (24h, day and night) systolic BP and conventional, but not ambulatory (24h, day and night) diastolic BP (Table 2.3). Neither waist circumference, nor HOMA-IR were independently and positively related to aortic pulse pressure, augmentation pressure, AIx or PWV (Table 2.3). However, urinary Na^+/K^+ was independently associated with aortic pulse pressure and augmentation pressure, but neither AIx, nor PWV (Table 2.3).

2.4.4 Interactions between urinary Na^+/K^+ and insulin resistance or waist circumference are associated with BP

Independent of the individual terms and a number of additional confounders, interactions between log HOMA-IR and urinary Na^+/K^+ were associated with conventional diastolic and ambulatory systolic and diastolic BP (Table 2.4). Interactions between waist circumference and urinary Na^+/K^+ were also associated with conventional and day DBP (Table 2.4). With further adjustments for waist circumference, the independent relationships between the log HOMA-IR-urinary Na^+/K^+ interaction and conventional (partial $r=0.14$, $p<0.05$) and 24-hour, day and night (partial $r=0.17$ to 0.18, $p=0.002$ to $p=0.0008$) diastolic BP were retained. With further adjustments for HOMA-IR, the independent relationships between the waist circumference-urinary Na^+/K^+ interaction and BP were similarly retained for conventional systolic (partial $r=0.12$, $p<0.05$) and conventional and day diastolic (partial $r=0.12$ to 0.14, $p<0.05$ for both) BP. In sensitivity analysis conducted in participants without diabetes mellitus (or HbA1c.6.5%), interactions between log HOMA-IR and urinary Na^+/K^+ were similarly associated with conventional diastolic and ambulatory systolic and diastolic BP (Table 2.5). Independent of the individual terms and a number of additional confounders, interactions between log HOMA-IR and urinary Na^+/K^+, as well as waist circumference and urinary Na^+/K^+ were not significantly associated with central aortic pressure, augmentation pressure (AP), AIx or PWV (Table 2.6).

Table 2.4 Multivariate adjusted relationships (partial correlation coefficients, partial r) between interactions (urinary Na^+/K^+-homeostasis model assessment of insulin resistance [log HOMA-IR] interaction or urinary Na^+/K^+-waist circumference [WC] interaction) and blood pressure (BP) in 331 participants of African ancestry not receiving antihypertensive therapy.

	partial r*	confidence intervals	p value†
Urinary Na^+/K^+ x log HOMA-IR versus			
Conventional systolic BP	0.10	-0.01 to 0.21	=0.06
Conventional diastolic BP	0.13	0.03 to 0.24	<0.01
24-hour systolic BP	0.11	0.01 to 0.22	<0.05
24-hour diastolic BP	0.17	0.06 to 0.28	<0.002
Day systolic BP	0.11	0.01 to 0.22	<0.05
Day diastolic BP	0.18	0.08 to 0.29	<0.001
Night systolic BP	0.10	-0.01 to 0.21	=0.06
Night diastolic BP	0.17	0.06 to 0.27	<0.002
Urinary Na^+/K^+ x WC versus			
Conventional systolic BP	0.12	0.01 to 0.22	<0.05
Conventional diastolic BP	0.14	0.03 to 0.24	<0.02
24-hour systolic BP	0.07	-0.04 to 0.18	=0.17
24-hour diastolic BP	0.10	-0.006 to 0.21	=0.06
Day systolic BP	0.06	-0.05 to 0.17	=0.30
Day diastolic BP	0.12	0.01 to 0.23	<0.05
Night systolic BP	0.07	-0.04 to 0.18	=0.11
Night diastolic BP	0.05	-0.06 to 0.16	=0.37

*Adjustments are for the individual terms (waist circumference or log HOMA-IR and urinary Na^+/K^+), age, sex, the presence of diabetes mellitus or HbA1c>6.5%, regular alcohol consumption, and regular tobacco use. †Probability values were further adjusted for non-independence of family members.

Table 2.5 Multivariate adjusted relationships (partial correlation coefficients, partial r) between interactions (urinary Na^+/K^+-homeostasis model assessment of insulin resistance [log HOMA-IR] interaction or urinary Na^+/K^+-waist circumference [WC] interaction) and blood pressure (BP) in 306 participants of African ancestry not receiving antihypertensive therapy and without diabetes mellitus or HbA1c>6.5%.

	partial r*	confidence intervals	p value†
Urinary Na^+/K^+ x log HOMA-IR versus			
Conventional systolic BP	0.11	-0.01 to 0.22	=0.056
Conventional diastolic BP	0.14	0.03 to 0.25	<0.01
24-hour systolic BP	0.12	0.01 to 0.23	<0.05
24-hour diastolic BP	0.19	0.07 to 0.29	<0.001
Day systolic BP	0.13	0.01 to 0.24	<0.05
Day diastolic BP	0.19	0.08 to 0.30	<0.001
Night systolic BP	0.11	0.01 to 0.22	<0.05
Night diastolic BP	0.19	0.07 to 0.29	<0.001
Urinary Na^+/K^+ x WC versus			
Conventional systolic BP	0.15	0.04 to 0.26	<0.05
Conventional diastolic BP	0.15	0.04 to 0.26	<0.02
24-hour systolic BP	0.11	0.01 to 0.22	<0.05
24-hour diastolic BP	0.11	0.02 to 0.24	<0.05
Day systolic BP	0.10	-0.01 to 0.21	=0.08
Day diastolic BP	0.15	0.04 to 0.26	<0.05
Night systolic BP	0.11	-0.01 to 0.22	=0.06
Night diastolic BP	0.08	-0.03 to 0.20	=0.19

*Adjustments are for the individual terms (waist circumference or log HOMA-IR and urinary Na^+/K^+), age, sex, regular alcohol consumption, and regular tobacco use. †Probability values were further adjusted for non-independence of family members.

Table 2.6 Multivariate adjusted relationships (partial correlation coefficients, partial r) between interactions (urinary Na^+/K^+-homeostasis model assessment of insulin resistance [log HOMA-IR] interaction or urinary Na^+/K^+-waist circumference [WC] interaction) and aortic blood pressure (BP) or functional characteristics in 331 participants of African ancestry not receiving antihypertensive therapy.

	partial r*	confidence intervals	p value†
Urinary Na^+/K^+ x log HOMA-IR versus			
Aortic pulse pressure	0.02	-0.09 to 0.13	=0.66
Aortic augmentation pressure	-0.004	-0.11 to 0.11	=0.94
Aortic augmentation index	-0.03	-0.14 to 0.08	=0.59
Aortic pulse wave velocity	-0.07	-0.18 to 0.05	=0.26
Urinary Na^+/K^+ x WC versus			
Aortic pulse pressure	-0.05	-0.16 to 0.06	=0.35
Aortic augmentation pressure	-0.02	-0.13 to 0.09	=0.73
Aortic augmentation index	0.03	-0.08 to 0.14	=0.64
Aortic pulse wave velocity	-0.007	-0.12 to 0.11	=0.91

*Adjustments are for the individual terms (waist circumference or log HOMA-IR and urinary Na^+/K^+), age, sex, HR, MAP, the presence of diabetes mellitus or HbA1c>6.5%, regular alcohol consumption, and regular tobacco use. †Probability values were further adjusted for non-independence of family members.

2.4.5 Relationships between urinary Na^+/K^+ and BP in categories of HOMA-IR

In participants with a log HOMA-IR≥median for the sample, unadjusted (Table 2.7) and multivariate adjusted (Table 2.8) relationships between urinary Na^+/K^+ and conventional and ambulatory BP were noted. However, in those with a log HOMA-IR<median for the sample, no unadjusted (Table 2.7) or multivariate adjusted (Table 2.8) relationships between urinary Na^+/K^+ and conventional and ambulatory BP were noted. The Pearson's and partial r values for the urinary Na^+/K^+ and ambulatory diastolic BP relationships were greater in those with as compared to those without a log HOMA-IR≥median for the sample (Tables 2.7 and 2.8).

Markedly greater slopes (β-coefficient) of the urinary Na^+/K^+ versus ambulatory diastolic BP relationships were noted in participants with as compared to those without a log HOMA-IR≥median for the sample (Figure 2.4). The multivariate adjusted relationship between urinary Na^+/K^+ and night diastolic BP also increased across tertiles of log HOMA-IR (Tertile 1: β-coefficient= -0.79±0.47, Tertile 2: β-coefficient=0.65±0.35, Tertile 3: β-coefficient=1.03±0.46, $p<0.05$ tertiles 3 and 2 vs 1: ANOVA with a Tukey *post hoc* test). In contrast, only trends for increases in the multivariate adjusted relationships between urinary Na^+/K^+ and conventional, 24-hour, and day BP were noted across tertiles of log HOMA-IR (data not shown).

In sensitivity analysis conducted in participants without diabetes mellitus or HbA1c>6.5%, independent relationships between urinary Na^+/K^+ and conventional and ambulatory BP were also noted in participants with a log HOMA-IR≥median, but not <median for the sample (Table 2.9).

With further adjustments for waist circumference, the independent relationships between urinary Na^+/K^+ and conventional, 24-hour, day, and night systolic (partial r=0.18 to 0.25, $p<0.05$ to $p<0.005$) and diastolic (partial r=0.16 to 0.20, $p<0.05$) BP in those with a HOMA-IR≥median were retained.

Table 2.7 Unadjusted relationships (Pearson's correlation coefficients, r) between urinary electrolyte excretion rates and blood pressure (BP) in participants with a log HOMA-IR (homeostasis model of insulin resistance) above or below the median for the sample in 331 participants of African ancestry not receiving antihypertensive therapy.

	Median	log HOMA-IR≥median				log HOMA-IR<median				p value for comparison
Urinary Na^+/K^+ vs	log HOMA-IR	r	CI	p value	n	r	CI	p value	n	of r values*
Conventional systolic BP	0.577	0.17	0.02 to 0.31	<0.05	168	0.005	-0.15 to 0.16	=0.95	163	=0.13
Conventional diastolic BP	0.577	0.15	0.01 to 0.30	<0.05	168	-0.02	-0.17 to 0.13	=0.79	163	=0.11
24-hour systolic BP	0.577	0.17	0.02 to 0.32	<0.05	168	0.02	-0.13 to 0.18	=0.75	163	=0.17
24-hour diastolic BP	0.577	0.17	0.02 to 0.32	<0.05	168	-0.10	-0.25 to 0.06	=0.20	163	=0.01
Day systolic BP	0.577	0.16	0.01 to 0.30	<0.05	168	0.03	-0.13 to 0.18	=0.73	163	=0.23
Day diastolic BP	0.577	0.18	0.02 to 0.32	<0.05	168	-0.11	-0.26 to 0.05	=0.18	163	=0.01
Night systolic BP	0.577	0.16	0.01 to 0.30	<0.05	168	0.01	-0.14 to 0.17	=0.86	163	=0.20
Night diastolic BP	0.577	0.15	0.01 to 0.30	<0.05	168	-0.09	-0.24 to 0.07	=0.26	163	<0.05

*Represents a comparison of the r values between those with a HOMA-IR≥ versus < the median, as determined from Z-statistics.

Table 2.8 Multivariate adjusted relationships (partial correlation coefficients, partial r) between urinary electrolyte excretion rates and blood pressure (BP) in participants with a log HOMA-IR (homeostasis model of insulin resistance) above or below the median for the sample in 331 participants of African ancestry not receiving antihypertensive therapy.

	Median	log HOMA-IR≥median				log HOMA-IR<median				p value for comparison
Urinary Na^+/K^+ versus	log HOMA-IR	partial r	CI	p value†	n	partial r	CI	p value†	n	of r values#
Conventional systolic BP	0.577	0.25	0.10 to 0.39	<0.0005	168	0.07	-0.09 to 0.22	=0.40	163	=0.09
Conventional diastolic BP	0.577	0.20	0.05 to 0.34	<0.005	168	0.01	-0.14 to 0.17	=0.95	163	=0.08
24-hour systolic BP	0.577	0.22	0.07 to 0.36	<0.005	168	0.03	-0.12 to 0.19	=0.68	163	=0.08
24-hour diastolic BP	0.577	0.19	0.03 to 0.33	<0.02	168	-0.08	-0.23 to 0.08	=0.29	163	<0.02
Day systolic BP	0.577	0.19	0.03 to 0.33	<0.01	168	0.03	-0.13 to 0.18	=0.72	163	=0.15
Day diastolic BP	0.577	0.17	0.01 to 0.31	<0.05	168	-0.10	-0.25 to 0.06	=0.22	163	<0.02
Night systolic BP	0.577	0.23	0.07 to 0.36	<0.005	168	0.02	-0.13 to 0.18	=0.75	163	=0.05
Night diastolic BP	0.577	0.18	0.03 to 0.32	<0.02	168	-0.06	-0.21 to 0.10	=0.40	163	<0.05

*Adjustments are for age, sex, the presence of diabetes mellitus or an HbA1c>6.5%, regular alcohol consumption, and regular tobacco use. †Probability values were further adjusted for non-independence of family members. #Represents a comparison of the partial r values between those with a HOMA-IR≥ versus < the median, as determined from Z-statistics.

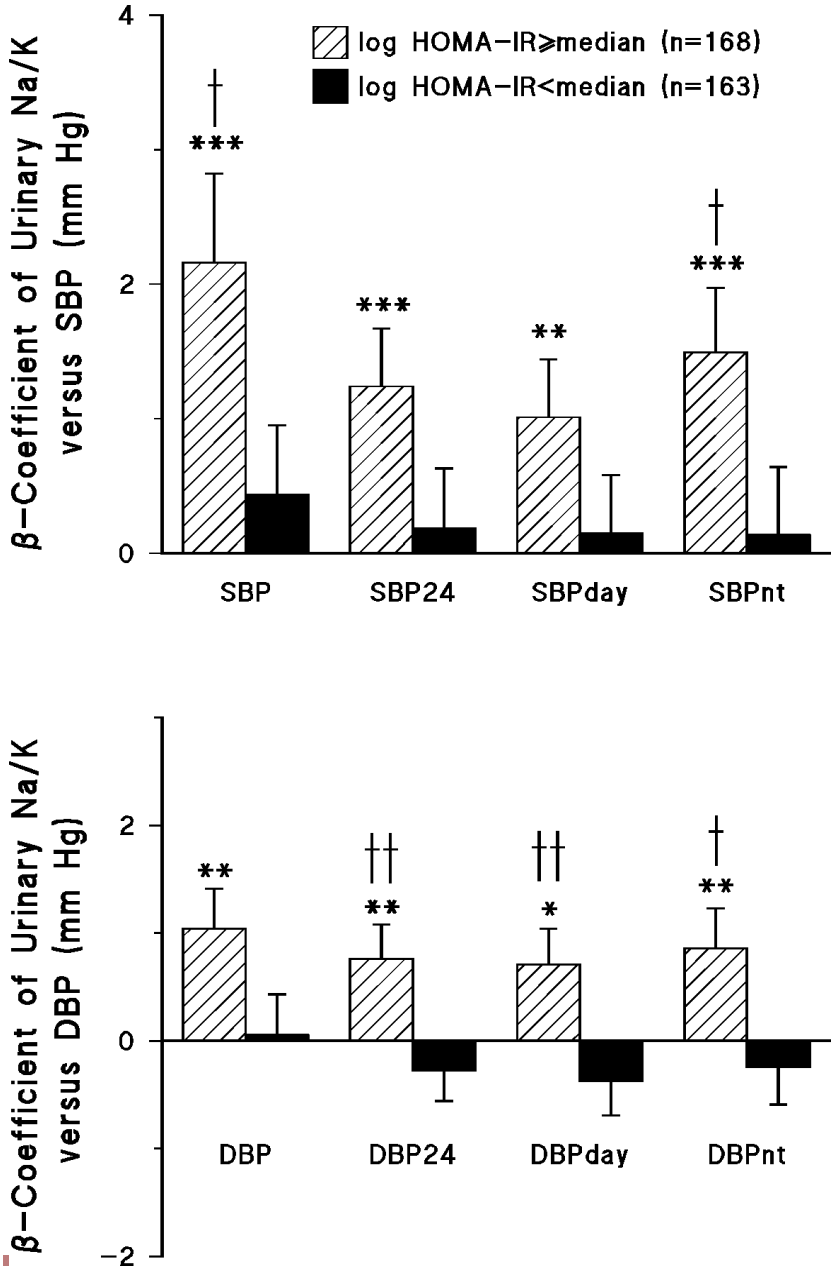


Figure 2.4 Comparison of the multivariate adjusted slopes (β-coefficients) of the urinary Na^+/K^+ (Na/K) versus conventional, 24-hour (24), day and night (nt) systolic (SBP) and diastolic (DBP) BP relations in participants with versus those without a log HOMA-IR (homeostasis model assessment of insulin resistance) ≥median for the sample (see text for values). Adjustments were for age, sex, the presence of diabetes mellitus or an HbA1c>6.5%,regular alcohol consumption, and regular tobacco use. Probability values were further adjusted for non-independence of family members. *p<0.05, **p<0.02, ***p<0.005 for relationship; †p<0.05, ††p<0.02 versus log HOMA-IR below the median as determined from an ANOVA and a Bonferroni *post hoc* test.

Table 2.9 Multivariate adjusted relationships (partial correlation coefficients, partial r) between urinary electrolyte excretion rates and blood pressure (BP) in participants with a log HOMA-IR (homeostasis model of insulin resistance) above or below the median for the sample in 306 participants of African ancestry not receiving antihypertensive therapy and without diabetes mellitus or an HbA1c>6.5%.

	Median	log HOMA-IR≥median				log HOMA-IR<median				p value for comparison
Urinary Na^+/K^+ vs	log HOMA-IR	partial r	CI	p value†	n	partial r	CI	p value†	n	of r values#
Conventional systolic BP	0.564	0.29	0.13 to 0.43	=0.0001	153	0.08	-0.08 to 0.24	=0.33	153	=0.06
Conventional diastolic BP	0.564	0.23	0.07 to 0.38	<0.005	153	0.02	-0.15 to 0.18	=0.93	153	=0.06
24-hour systolic BP	0.564	0.26	0.10 to 0.40	<0.001	153	0.04	-0.12 to 0.20	=0.58	153	=0.06
24-hour diastolic BP	0.564	0.23	0.07 to 0.37	<0.005	153	-0.07	-0.23 to 0.09	=0.36	153	<0.01
Day systolic BP	0.564	0.23	0.07 to 0.37	<0.005	153	0.04	-0.12 to 0.20	=0.63	153	=0.10
Day diastolic BP	0.564	0.21	0.05 to 0.35	<0.01	153	-0.10	-0.26 to 0.06	=0.23	153	<0.01
Night systolic BP	0.564	0.26	0.10 to 0.40	<0.005	153	0.03	-0.13 to 0.19	=0.65	153	<0.05
Night diastolic BP	0.564	0.22	0.06 to 0.37	<0.01	153	-0.05	-0.21 to 0.11	=0.48	153	<0.05

*Adjustments are for age, sex, regular alcohol consumption, and regular tobacco use. †Probability values were further adjusted for non-independence of family members. #Represents a comparison of the partial r values between those with a HOMA-IR≥ versus < the median, as determined from Z-statistics.

2.4.6 Relationships between urinary Na^+/K^+ and BP in categories of waist circumference

Although independent relationships between urinary Na^+/K^+ and conventional and ambulatory BP were noted in participants with a waist circumference≥median, but not <median for the sample, (Table 2.10), the slopes (β-coefficient) of the urinary Na^+/K^+ versus ambulatory diastolic BP relationships were not significantly greater in participants with as compared to those without a waist circumference≥median for the sample (Figure 2.5).With the inclusion of HOMA-IR in the regression model, the relationship between urinary Na^+/K^+ and conventional, 24-hour and night systolic (partial r=0.16-0.21, p<0.05) and conventional diastolic (partial r=0.17, p<0.05) BP in participants with a waist circumference≥median were retained. In sensitivity analysis conducted in participants without diabetes mellitus or HbA1c>6.5%, independent relationships between urinary Na^+/K^+ and conventional and ambulatory BP were also noted in participants with a waist circumference≥median, but not <median for the sample (Table 2.11)

2.4.7 Quantitative effect of insulin resistance on the relations between urinary Na^+/K^+ and BP

In participants with a log HOMA-IR equal to or greater than as compared to those with a log HOMA-IR below the median for the group, every one SD increase in urinary Na^+/K^+ was associated with an approximately 2.3, 2.4, 2.5 and 2.6 mm Hg greater positive effect on conventional, 24-hour, day and night diastolic BP respectively (Figure 2.6).

2.5 Discussion

The main findings of this study are that in a group of persons of African ancestry, despite a lack of independent relationship between log HOMA-IR and BP, interactions between log HOMA-IR and urinary Na^+/K^+ were noted to contribute to the variability of conventional and

Table 2.10 Multivariate adjusted relationships (partial correlation coefficients, partial r) between urinary electrolyte excretion rates and blood pressure (BP) in participants with a waist circumference (WC) above or below the median for the sample in 331 participants of African ancestry not receiving antihypertensive therapy.

	Median	WC≥median				WC<median				p value for comparison
Urinary Na^+/K^+ vs	WC	partial r	CI	p value	n	partial r	CI	p value	n	of r values#
Conventional systolic BP	86	0.20	0.05 to 0.35	<0.02	166	0.07	-0.09 to 0.22	=0.28	165	=0.23
Conventional diastolic BP	86	0.17	0.01 to 0.32	<0.05	166	-0.02	-0.17 to 0.14	=0.86	165	=0.09
24-hour systolic BP	86	0.16	0.01 to 0.31	<0.05	166	0.07	-0.09 to 0.22	=0.45	165	=0.41
24-hour diastolic BP	86	0.11	-0.05 to 0.26	=0.24	166	-0.02	-0.17 to 0.14	=0.82	165	=0.26
Day systolic BP	86	0.13	-0.02 to 0.28	=0.12	166	0.07	-0.08 to 0.22	=0.37	165	=0.60
Day diastolic BP	86	0.09	-0.06 to 0.24	=0.25	166	-0.05	-0.20 to 0.11	=0.60	165	=0.22
Night systolic BP	86	0.17	0.02 to 0.32	<0.05	166	0.06	-0.10 to 0.21	=0.75	165	=0.31
Night diastolic BP	86	0.08	-0.08 to 0.23	=0.35	166	0.03	-0.12 to 0.19	=0.87	165	=0.71

CI, confidence interval. *Adjustments are for age, sex, the presence of diabetes mellitus or an HbA1c>6.5%, regular alcohol consumption, and regular tobacco use. #Represents a comparison of the partial r values between those with a waist circumference≥ versus < the median, as determined from Z-statistics.

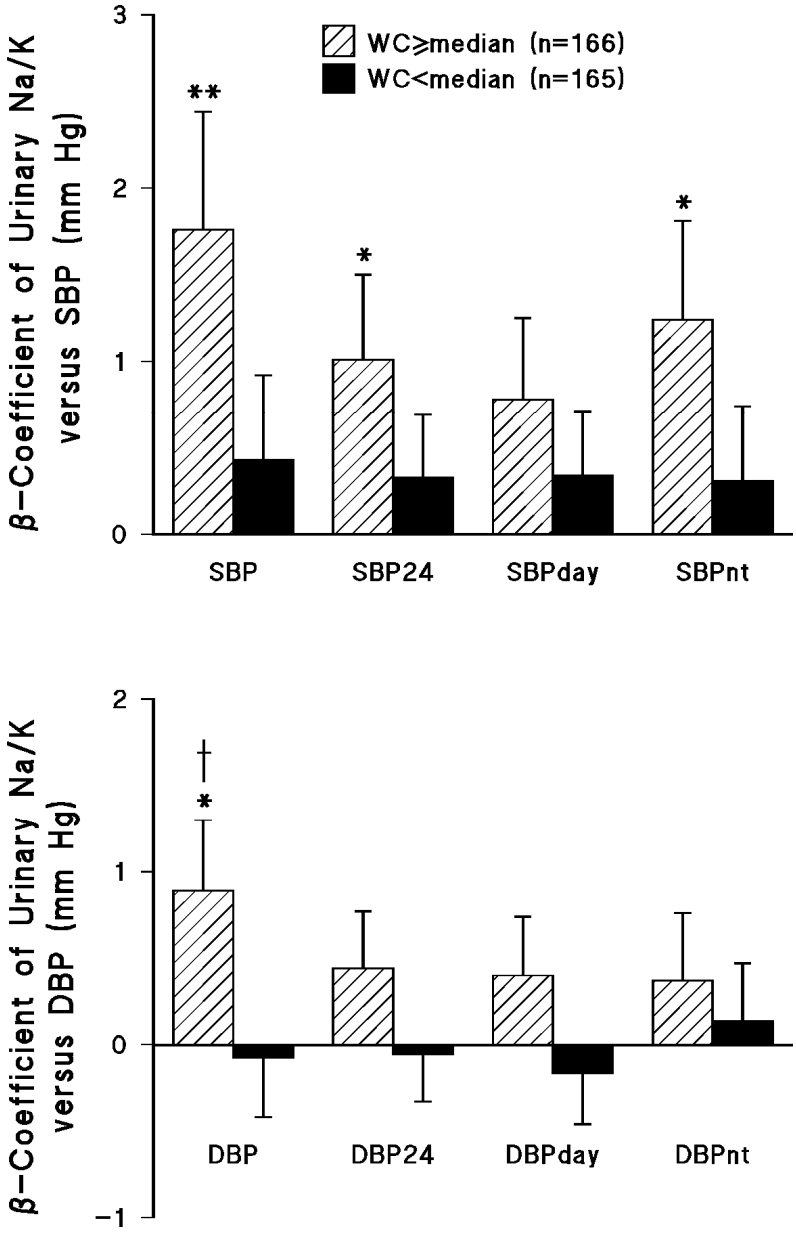


Figure 2.5 Comparison of the multivariate adjusted slopes (β-coefficients) of the urinary Na^+/K^+ (Na/K) versus conventional, 24-hour (24), day and night (nt) systolic (SBP) and diastolic (DBP) blood pressure relations in participants with versus those without a waist circumference (WC) ≥median for the sample (see text for values). Adjustments were for age, sex, the presence of diabetes mellitus or an HbA1c>6.5%, regular alcohol consumption, and regular tobacco use. Probability values were further adjusted for non-independence of family members. *p<0.05, **p<0.01 for relationship; †p=0.05 versus WC below the median as determined from an ANOVA and a Bonferroni *post hoc* test.

Table 2.11 Multivariate adjusted relationships (partial correlation coefficients, partial r) between urinary electrolyte excretion rates and blood pressure (BP) in participants with a waist circumference (WC) above or below the median for the sample in 306 participants of African ancestry not receiving antihypertensive therapy and without diabetes mellitus or an HbA1c>6.5%.

	Median	WC≥median				WC<median				p value for comparison
Urinary Na^+/K^+ vs	WC	partial r	CI	p value	n	partial r	CI	p value	n	of r values#
Conventional systolic BP	85	0.26	0.11 to 0.41	<0.005	151	0.05	-0.11 to 0.21	=0.54	155	=0.06
Conventional diastolic BP	85	0.22	0.06 to 0.37	<0.02	151	-0.03	-0.19 to 0.13	=0.71	155	<0.05
24-hour systolic BP	85	0.20	0.04 to 0.35	<0.05	151	0.07	-0.09 to 0.23	=0.38	155	=0.27
24-hour diastolic BP	85	0.15	-0.01 to 0.31	=0.09	151	-0.02	-0.18 to 0.14	=0.85	155	=0.13
Day systolic BP	85	0.17	0.01 to 0.32	<0.05	151	0.07	-0.09 to 0.23	=0.40	155	=0.38
Day diastolic BP	85	0.14	-0.02 to 0.30	=0.17	151	-0.05	-0.21 to 0.11	=0.58	155	=0.10
Night systolic BP	85	0.20	0.04 to 0.35	<0.05	151	0.06	-0.10 to 0.21	=0.49	155	=0.21
Night diastolic BP	85	0.12	-0.05 to 0.27	=0.19	151	0.04	-0.12 to 0.20	=0.65	155	=0.48

CI, confidence interval. *Adjustments are for age, sex, regular alcohol consumption, and regular tobacco use. †Probability values were further adjusted for non-independence of family members. #Represents a comparison of the partial r values between those with a waist circumference≥ versus < the median, as determined from Z-statistics.

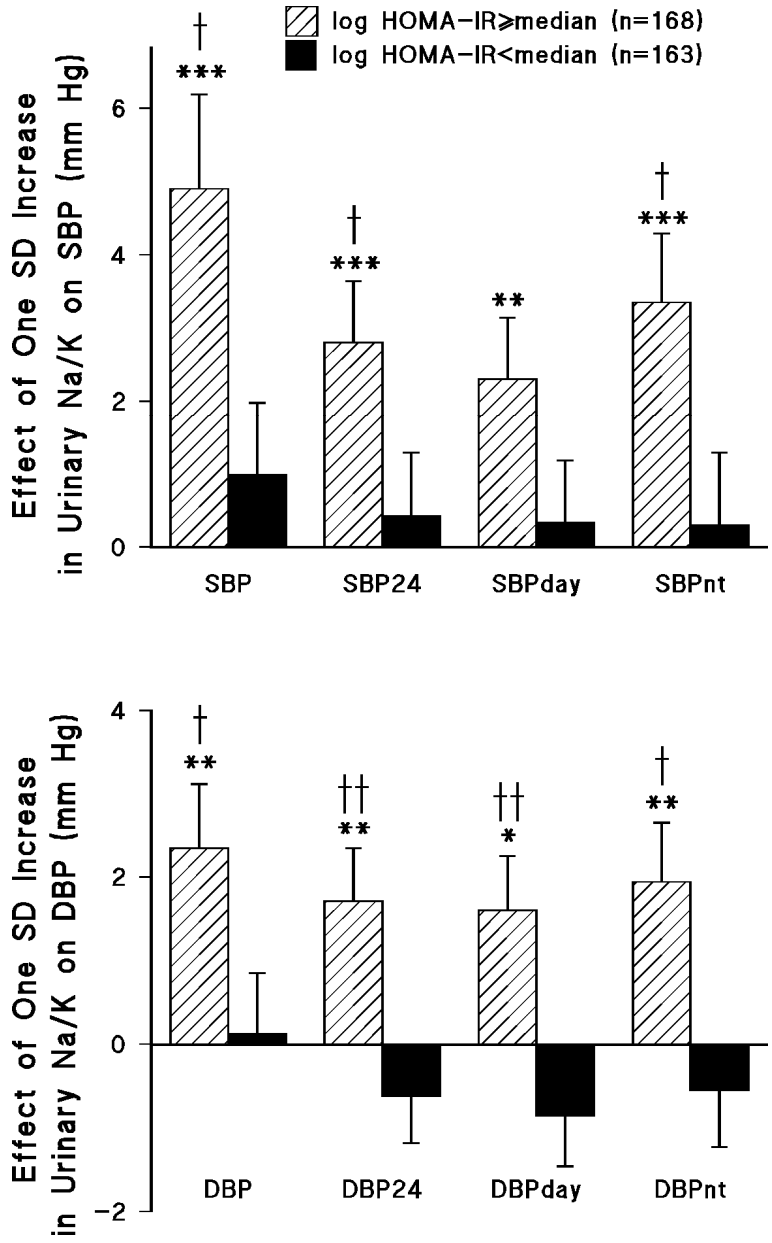


Figure 2.6 Effect of one standard deviation (SD) increase in urinary Na^+/K^+ (Na/K) and conventional, 24-hour (24), day and night (nt) systolic (SBP) and diastolic (DBP) BP (±SEM) in participants with a log HOMA-IR (homeostasis model assessment of insulin resistance) above or below the median for the sample (see text for values). Adjustments were for age, sex, the presence of diabetes mellitus or an HbA1c>6.5%, regular alcohol consumption, and regular tobacco use. Probability values were further adjusted for non-independence of family members. *p<0.05, **p<0.02, ***p<0.005 for relationship; †p<0.05, ††p<0.005 versus log HOMA-IR below the median as determined from an ANOVA and a Bonferroni *post hoc* test.

ambulatory BP with increasing multivariate adjusted slopes of urinary Na^+/K^+ versus BP noted across tertiles of log HOMA-IR. These interactions translated into relationships between urinary Na^+/K^+ and conventional or ambulatory BP in those with, but not those without a log HOMA-IR≥median for the sample and a greater relationship between urinary Na^+/K^+ with conventional, 24-hour, day and night BP in those with a log HOMA-IR above as compared to below the median for the sample. In contrast to effects on brachial BP, no interactions between log HOMA-IR and urinary Na^+/K^+ were noted to contribute to the variability of central aortic PP, augmentation pressure, AIx, or PWV.

To the best of my knowledge this study is the first to show that insulin resistance, although not independently related to BP is independently associated with salt intake (urinary Na^+/K^+)-BP relationships in a group of African descent, an ethnic group that has been well documented as having a high prevalence of salt-sensitivity (Weinberger et al 1986, He et al 1998, 2000, Vollmer et al 2001, Wright et al 2003, Aviv et al 2004). Although prior studies have demonstrated that the metabolic syndrome and insulin resistance contribute toward salt-sensitivity in-part through increases in renal tubular handling of Na^+ reabsorption (Strazzullo et al 2001, 2006, Barbato et al 2004), these findings have been noted in groups of persons of European, but not African ancestry (Strazzullo et al 2006). However, no prior studies have examined whether insulin resistance modifies BP responses to salt intake in a group of persons of African ancestry. Although I did not assess the relationship between insulin resistance and BP responses to variations in dietary salt intake, urinary Na^+/K^+ is considered to be an index of dietary salt intake (Redelinghuys et al 2010, Michel et al 2012). Thus, the present results suggest that insulin resistance in a group of persons of African ancestry may in-part account for the impact of salt intake on BP in this ethnic group.

In this study I was able to show that interactions between abdominal obesity (waist circumference) and urinary Na^+/K^+ were also associated with variations in BP and that this persisted even with further adjustments for HOMA-IR. Therefore, in this study abdominal obesity is likely to play a role in explaining urinary Na^+/K^+-BP relationships. However, this failed to translate into statistically significant differences in relationships between urinary

Na^+/K^+ and BP in those with a waist circumference above as compared to below the median for the sample. Importantly, although not supported by the present study, abdominal obesity has previously been shown to be associated with renal tubular Na^+ handling even after adjustments for insulin resistance (Strazzullo et al 2001).

Although in the present study interactions between HOMA-IR or waist circumference and urinary Na^+/K^+ were associated with variations in BP, these effects could not be explained by an impact on central aortic haemodynamics, including aortic PP, augmentation pressures, AIx or PWV. Thus, although our group have previously noted that relationships between urinary Na^+/K^+ and BP were stronger for PP (central aortic and 24-hour) than for brachial diastolic or mean arterial pressures, relationships in-part accounted for by changes in augmentation pressures and AIx (Redelinghuys et al 2010), the present results suggest that this is unlikely to be mediated by the effects of obesity or insulin resistance on aortic haemodynamics. This is in keeping with the lack of consistent relationships noted between obesity and indices of wave reflection or AIx which have been shown to either decrease (Maple-Brown et al 2005, Otsuka et al 2009) or increase (Ounis-Skali et al 2007), and the lack of effect on central aortic BP following a weight loss programme despite improvements in indices of aortic stiffness (Dengo et al 2010).

The possible clinical inferences of this study deserve consideration. If the association between insulin resistance and salt intake-BP relationships are indeed cause and effect, it is possible that as with obese adolescents of European ancestry in whom weight loss was associated an attenuated BP response to a high Na^+ intake (Rocchini et al 1989), a similar effect may occur with weight loss in persons of African ancestry. This has important implications given the relevance of salt-sensitivity to the pathogenesis of hypertension in persons of African ancestry (Weinberger et al 1986, He et al 1998, 2000, Vollmer et al 2001, Wright et al 2003, Aviv et al 2004). Studies assessing the impact of interventions that increase insulin sensitivity on BP are therefore warranted in salt-sensitive, obese individuals of African ancestry.

Assuming that the association between insulin resistance and salt intake-BP relationships are indeed cause and effect, the potential mechanisms through which insulin resistance may modify the effects of salt intake on BP in groups of African descent warrants consideration. In this regard insulin may promote renal Na^+ reabsorption through effects on the epithelial Na^+ channel (Pearce 2001). However, there is considerable controversy as to whether insulin actions alone can promote salt sensitivity (Hall 1993, 1997, 2003, Sechi 1999). Hence, further work is required to identify the mechanism that could explain the impact of insulin resistance, as determined by HOMA-IR, on salt intake-BP relationships.

The modest relationship between 24-hour urinary electrolyte excretion rates and BP in the whole group is consistent with the variable relations between urinary electrolyte excretion rates and BP in previous large studies (Intersalt Cooperative Research Group 1988, Smith et al 1988) and the lack of relationship between salt intake and BP in studies in Africa (Hoosen et al 1985, Charlton et al 2005). Suggested reasons for limited relationships include imprecision in urinary measurements because of the variability in salt intake and inaccuracies in urine collection. However, in participants with a log HOMA-IR above the median for the sample, an approximately 2.4 mm Hg greater positive effect on conventional, 24-hour, day and night diastolic BP was associated with a one standard deviation increase in urinary Na^+/K^+ as compared to that noted in participants with a log HOMA-IR< median for the sample.

In this study urinary Na^+/K^+, but not 24-hour urinary Na^+ excretion was associated with BP, data that is consistent with the stronger relations noted between urinary Na^+/K^+ and BP than between 24-hour urinary Na^+ excretion rates and BP in previous large studies (Intersalt Cooperative Research Group 1988, Smith et al 1988).This finding could be explained by a number of possibilities. A decrease in urinary K^+ excretion on a high Na^+ diet in salt-sensitive individuals (Price et al 2002) may occur as a consequence of an enhanced activity of the Na-K-2Cl co-transporter in the thick ascending limb of the renal tubule (Aviv et al 2004). Second, Na^+ sensitivity is more marked in the presence of even a modest dietary K^+ deficiency (Morris et al 1999), an effect that may be reflected in higher urinary Na^+/K^+ values.

Third, diets that are higher in Na^+ are generally lower in K^+, an effect that may also be reflected in higher urinary Na^+/K^+ values.

The limitations of this study are as follows. This study was a cross-sectional study and hence conclusions regarding cause and effect cannot be drawn. Intervention studies assessing the impact of high and low Na^+ diets in insulin resistant versus insulin sensitive persons of African descent are therefore required. Second, a high proportion of participants were women and I was not statistically powered to perform sex-specific analysis. Thus the present results may relate specifically to women. Furthermore as the estrogen/progesterone levels fluctuate at different points in the menstrual cycle in women of child-bearing potential, these may affect aspects of sodium balance and insulin sensitivity which could have confounded the results. However, due to the epidemiological nature of the study design the standardization of measures to a particular point in the menstrual cycle was not possible. Third, I did not assess the relationship between insulin resistance and proximal as compared to distal tubular Na^+ reabsorption. Further work is therefore required to address the role of insulin resistance on urinary Na^+/K^+ versus BP relations in men of African descent and the impact of insulin resistance on proximal versus distal tubular Na^+ reabsorption in this ethnic group. Fourth, I did not assess insulin resistance using the hyperinsulinaemic euglycemic glucose clamp technique, a far more accurate method of identifying insulin resistance. This approach was not possible given the epidemiological nature of the study design. However, using HOMA-IR would have underestimated the modifying effect size of insulin resistance on salt intake-BP relationships and hence would have biased against the results of the present study. Fourth, the study was conducted in a heterogeneous sample with hypertensives and participants with diabetes mellitus included in the primary analysis. However, only participants not receiving antihypertensive treatment were evaluated and the results were confirmed in sensitivity analysis conducted in those without diabetes mellitus. Fifth, the assessment of 24-hour urinary excretion rates only once is subject to inaccuracies in urine collection despite quality control measures, and does not account for daily variations in salt intake. However, the mean 24-hour urine volumes noted in the present study are higher than

those reported on in 23 of 52 sites of the Intersalt study (Intersalt Cooperative Research Group 1988). Furthermore, the electrolyte excretion rates in the present study are the same as that reported on in an alternative study conducted in the same population group and region (Hoosen et al 1985) as the present study. Sixth, I did not assess electrolyte intake from daily dietary questionnaires administered over an extended period, an approach that may have given me a temporal assessment of electrolyte intake.

In conclusion, in this study I show that in a group of African descent under usual dietary circumstances, insulin resistance is independently associated with the relationships between urinary Na^+/K^+ (an index of salt intake) and conventional or ambulatory BP. This relationship could not be accounted for by alterations in central aortic haemodynamic changes. Thus, in groups of African ancestry, salt-sensitivity may strongly depend in-part on the presence of insulin resistance. The mechanisms of this effect require elucidation and the impact of approaches that increase insulin-sensitivity require further study.

CHAPTER 3

EFFECTS OF SHORT-TERM EXERCISE TRAINING ON LARGE VESSEL AUGMENTATION INDICES AND AORTIC BLOOD PRESSURE IN OVERWEIGHT OR OBESE INDIVIDUALS

3.1 Abstract

Background. The extent to which exercise training-induced reductions in BP may be explained by decreases in aortic systolic pressure augmentation in overweight or obese individuals is uncertain. Therefore, I aimed to determine the impact of exercise training on aortic augmentation pressure and index and aortic and brachial BP in overweight or obese individuals.

Methods. Thirty-five sedentary or recreationally active men and women (30–57years) who were either overweight (40%) or obese (60%) completed 6 weeks of exercise training (≥3 days/week; stationary bike and/or treadmill) either preceded (n=19) or followed (n=16) by a 6 week control period of no exercise. Aortic augmentation pressure (AP), aortic and peripheral augmentation indices (AIx), and central aortic BP (SphygmoCor) were determined before and after exercise training and a control period.

Results. Peak oxygen consumption increased (p=0.0001) from 27.0±5.1 to 28.8±5.8 $ml.kg^{-1}.min^{-1}$after 6 weeks of exercise. Exercise decreased brachial systolic (SBP) and diastolic BP from 142±8/94±8 mm Hg to 134±11/86±11 mm Hg (p<0.005/p<0.005); whereas no changes were observed after the control period (141±11/91±9 mm Hg, p=0.81/p=0.34). Neither AP (Baseline: 9.2±4.2 mm Hg; after 6 weeks training: 8.7±6.1 mm Hg), aortic AIx (Baseline: 24.6±11.0%; after 6 weeks training: 22.7±11.1%), nor peripheral AIx (Baseline: 81.4±16.7%; after 6 weeks training: 76.4±16.5%) were modified by exercise training. Although aortic SBP decreased after exercise (132±8 mm Hg to 124±12 mm Hg, p<0.002), these changes were accounted for by decreases in MAP.

Conclusions. In overweight or obese individuals, although short-term aerobic exercise training which improved cardiorespiratory fitness, may produce marked decreases in aortic and brachial BP, these effects are not attributed to alterations in aortic systolic pressure augmentation.

3.2 Introduction

There is considerable evidence to indicate that obesity is a major determinant of BP and the development of hypertension (Harris et al 2000, Neter et al 2003, Zhu et al 2005, Hedayati et al 2011). In this regard, it is well established that obesity is associated with a sedentary lifestyle and that regular exercise decreases BP. Indeed, systematic reviews have demonstrated decreases in resting brachial systolic BP/diastolic BP of 6.9/4.9 mm Hg after exercise training (Cornelissen and Fagard 2005, Fagard 2006, Fagard and Cornelissen 2007). With the increasing evidence in various clinical or general populations that central aortic BP predicts cardiovascular outcomes more closely or independent of BP measured at the brachial artery (Safar et al 2002, Chirinos et al 2005, Williams et al 2006, Roman et al 2007, Pini et al 2008, Jankowski et al 2008, Wang et al 2009), there is now substantial interest in identifying the most effective interventions that reduce central aortic BP. Currently, the role of regular exercise as an aortic BP-lowering intervention in obesity is uncertain.

Aortic BP is determined by factors that to some extent differ from those that influence brachial BP. In this regard, aortic stiffness and pressure augmentation during the systolic period of the cardiac cycle (a possible index of wave reflection) are key role-players in determining aortic BP. A number of cross-sectional studies have demonstrated that persons who engage in regular exercise have a reduced aortic or large vessel stiffness (Vaitkevicius et al 1993, Tanaka et al 1998, Kakiyama et al 1998, Seals et al 1999, Tanaka et al 2000, Monahan et al 2001, Wilkinson et al 2004, Zieman et al 2005, Otsuki et al 2006a, 2006b, 2007, Kraft et al 2007, Aoyagi et al 2010). In addition, short-term exercise programmes may result in decreases in aortic or large vessel stiffness (Cameron and Dart 1994, Tanaka et al 2000, Moreau et al 2003, Yokoyama et al 2004, Kakiyama et al 2005, Hayashi et al 2005, Laskey et al 2005, Sugawara et al 2006, Collier et al 2008, Madden et al 2009, Goldberg et al 2009, Miyaki et al 2009, Currie et al 2009, Guimarães et al 2010, McNeilly et al 2012, Nualnim et al 2012) and many of these findings were noted in overweight or obese participants (Tanaka et al 2000, Moreau et al 2003, Yokoyama et al 2004, Collier et al 2008,

Madden et al 2009, Goldberg et al 2009, Miyaki et al 2009, Guimarães et al 2010, McNeilly et al 2012, Nualnim et al 2012). However, there is far less evidence to support a role for beneficial effects of exercise training on aortic augmentation pressures or indices. In this regard, some (Edwards et al 2004, Tabara et al 2007, Mustata et al 2011, Liu et al 2012), but not other (Laskey et al 2012, Nualnim et al 2012, Sugawara et al 2012) studies show a beneficial effect on aortic augmentation. Only two studies conducted in 10 overweight individuals (Edwards et al 2004) and 9 overweight women (Liu et al 2012) have reported on a beneficial effect of exercise training on aortic augmentation index in overweight or obese individuals, whilst in another study conducted in 24 overweight individuals, no effect of exercise training was observed (Nualnim et al 2012). Moreover, exercise-induced changes in systolic pressure augmentation failed to translate into decreases in aortic BP (Liu et al 2012). Evidence to support a beneficial effect of exercise training on systolic pressure augmentation and consequently central aortic BP in overweight or obese individuals is therefore lacking. Hence, we tested the hypothesis that exercise training-induced decreases in brachial BP may be attributed to modifications in aortic AP or AIx, in overweight and obese persons.

3.3 Methods

3.3.1 Study participants

The 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: M10624). Thirty nine sedentary or recreationally active (less than 2 hours of exercise a week) men and women, 30 – 57 years of age who were either overweight [body mass index (BMI)≥25 $kg.m^{-2}$] or obese (BMI≥30 $kg.m^{-2}$) were recruited via advertisements in the local newspaper and on a local radio station. The participants had either untreated pre- (120-139/80-89 mm Hg) or grade I (140-159/90-99 mm Hg) hypertension. Those participants who were taking anti-hypertensive medication obtained permission from their primary care physicians to discontinue their medication for 2-

weeks prior to the study and to refrain from further medication use for the duration of the study. None of the participants were taking any other form of medication. All participants were non-smokers and they were all free of any cardiovascular or metabolic diseases (confirmed by recent medical assessment by a primary care physician). None had any musculoskeletal disorders that would limit physical exercise and participants were excluded if they had a BMI greater than 35 $kg.m^{-2}$.

3.3.2 Study protocol

The study participants reported to the exercise physiology laboratory at the University of the Witwatersrand for 4 separate visits. They were asked to avoid heavy exercise for 24 hours before each visit and to fast overnight prior to the initial visit. All measurements were made in a quiet temperature-controlled room between 6:00 am and 11:00 am, after at least 15 minutes of rest and prior to exercising. The initial visit comprised of informed consent, medical history, anthropometric measurements, brachial BP measurements and pulse wave analysis. On the second visit (within 5 days of the initial visit), a maximal exercise test was performed to assess peak oxygen consumption (cardiorespiratory fitness) at baseline and a 12-lead electrocardiograph (ECG) was recorded to exclude any patients with ECG changes during exercise. The participants were then randomised to either the pre-control group (n=20) or the post-control group (n=19). The pre-control group first completed a 6 week control period of no exercise followed by 6 weeks of exercise training (≥3 days/week, on a stationary bike and/or treadmill); whereas the post-control group first completed 6 weeks of exercise training (≥3 days/week, on a stationary bike and/or treadmill) followed by a 6 week control period of no exercise. The third and fourth visits, conducted after the completion of 6 weeks of exercise training or the control period, comprised of anthropometric measurements, brachial BP measurements, pulse wave analysis and a maximal exercise test (only after 6 weeks of exercise training). The participants visited the exercise physiology laboratory once a week for supervised exercise sessions during the 6 week exercise training period.

3.3.3 Anthropometric measurements

Body weight and height and waist and hip circumference were measured using standard approaches. From height and weight measurements, body mass index (BMI) was calculated.

3.3.4 Brachial blood pressure measurements

At each visit brachial BP was measured twice in the non-dominant arm according to guidelines (see Chapter 2, page 48 for more details) and if the measures differed by more than 5 mm Hg, a third measure was taken. The average of the two or three measures was taken as the brachial BP.

3.3.5 Pulse wave analysis

Central aortic BP, aortic augmentation pressures (AP), aortic augmentation index (AIx), and carotid-femoral (aortic) pulse wave velocity (PWV) were estimated using techniques described in chapter 2, pages 51. Importantly, to determine central aortic BP (SBPc), two approaches were employed to avoid potential problems with the use of a generalised transfer function in some groups. First, the radial pressure waveform was converted into a central (aortic) waveform using a validated generalised transfer function (GTF) incorporated in SphygmoCor software. Second, central aortic SBP was also determined from the peak pressure of the second pressure wave of the radial pulse (P2) (Norton et al 2012). Mean arterial pressure was calculated as [central diastolic BP + 1/3(central PP)]. In addition to calculating AIx, peripheral augmentation index was also determined from the ratio of the second to the first peak of the peripheral (radial) pressure wave expressed as a percentage. Pulse pressure amplification was calculated as brachial PP-PPc.

3.3.6 Maximum exercise testing

Participants performed a maximal exercise test (Balke-Ware protocol) on a treadmill to exhaustion to determine peak oxygen consumption (VO_{2peak}). Throughout the maximal exercise test, breath-by-breath expired gas was sampled and analysed using the Cosmed Quark PFT ergo (Rome, Italy) metabolic system, and a 12-lead electrocardiograph recording was continuously obtained (Cosmed ECG, Rome. Italy).

3.3.7 Exercise

Participants performed a supervised period of aerobic exercise to demonstrate the level of exercise that needed to be achieved on each day of the training period. As a matter of preference and convenience the participants could either exercise at a moderate-intensity (between 60 and 75% of VO_{2peak} on a stationary bike and/or a treadmill) continuously for 50 minutes (including a 5 minute warm-up and 3 minute cool-down period at 50-60 % of VO_{2peak}), or for a shorter period (33 minutes, including a 5 minute warm-up and 3 minute cool-down period at 50-60 % of VO_{2peak}) of interrupted exercise at a higher intensity (4 x 4 minutes of exercise at 80-90% of VO_{2peak} alternating with 3 minutes of lower intensity exercise at 50-60% of VO_{2peak}, on a stationary bike and/or a treadmill). Heart rate was continuously recorded throughout the exercise session with a HR monitor (POLAR®, Polar Electro Oy, Finland). Twenty participants chose to perform continuous exercise at moderate intensity and 15 participants chose to perform interrupted exercise at higher and low intensities. Once the participants had chosen their preferred mode of exercise training, this selection was maintained throughout the duration of the exercise training period. The 6 weeks of exercise training was performed at the same intensity and for the same duration as the supervised session at least 3 days (1 supervised, 2 unsupervised) a week. All participants were asked to keep a diary of their exercise-training and all participants were given a Polar HR monitor to ensure they exercised within the prescribed exercise intensities. All staff involved in the testing and exercise sessions were qualified in CPR and basic life support (A Millen, Sr N Molebatsi), and emergency equipment was available on the premises

of the laboratory (Exercise laboratory in School of Physiology). A medical doctor (Prof G Norton) was also on site in the case of an emergency.

3.3.8 Data analysis

Descriptive statistics are reported as means and standard deviation (SD) unless otherwise specified. Data were analysed using SAS software, version 9.1 (SAS Institute, Cary, NC). The average heart rates achieved whilst performing exercise at baseline were similar between the two exercise regimes (continuous exercise, n=20: 133±7 bpm; interrupted exercise, n=15: 136±8 bpm, p=0.13). Moreover, the two exercise regimes (continuous versus interrupted) produced the same changes in VO_{2peak} [continuous exercise, n=20: 26.5±4.6 $ml.kg^{-1}.min^{-1}$ to 28.7±4.9 $ml.kg^{-1}.min^{-1}$ (8.3%, $p<0.05$); interrupted exercise, n=15: 27.0±5.5 to 29.3±6.6 $ml.kg^{-1}.min^{-1}$ (8.5%, $p<0.05$); $p>0.05$ for comparison] and hence were combined for analysis. A two-way repeated measures analysis of variance was performed to assess the impact of group (pre-control versus post-control), time (before and after exercise and control periods) and group-time interaction on brachial BP, central aortic BP, AP, aortic PWV and AIx. No group or group-time interactive effects were noted. Furthermore, the exercise-induced reductions in SBP and DBP had been washed out following the 6 week post-control period (n=16; SBP: after exercise, 136±10 mm Hg, after post-control, 148±8 mm Hg, $p<0.005$; DBP: after exercise, 89±7 mm Hg, after post-control, 97±9 mm Hg, $p<0.05$), and these values did not differ from those obtained after the pre-control period (n=19; SBP: after pre-control, 144±7 mm Hg, p=0.12 vs after post-control; DBP: after pre-control, 96±9 mm Hg, p=0.75 vs after post-control); hence justifying the combination of the pre-control and post-control periods. Tukey *post hoc* tests were performed to identify differences between specific time points. As central aortic BP, AP, aortic PWV and AIx are influenced by HR and MAP (distending pressures), adjusted means were also compared using a repeated measures ANOVA followed by Tukey *post hoc* tests. Proportions were compared using a Fisher's Exact test. To achieve statistical power at 80% with a two-sided α value of <0.05 a sample size of 11 was required, as calculated from a

mean difference and standard deviation of 5.2±4.3 % based upon significant changes in AIx previously reported (Edwards et al 2004, Liu et al 2012).

3.4 Results

3.4.1 Participant characteristics

Thirty-five of the 39 participants completed the study, 19 in the pre-control and 16 in the post-control group. Seventeen (49%) of the participants were Caucasian, 11 (31%) were black African and 7 (20%) were Indian with equivalent proportions of each in the two control groups. They all had sedentary, high stress occupations and were of moderate to high socioeconomic status, as determined via questionnaires. Baseline characteristics of participants are presented in Table 3.1. The study group had more males than females. 40% of the participants were overweight and 60% were obese, and 20% of participants were pre-hypertensives and 80% had grade 1 hypertension. There were no differences in the baseline characteristics between the two control groups. Only three participants had received antihypertensive medication prior to enrolment. One person received an angiotensin-converting enzyme inhibitor (ACEI), a second an ACEI and a diuretic (hydrochlorothiazide) and the third a calcium channel blocker. The overall adherence to the exercise training programmes was 97%.

3.4.2 Exercise training effects on participant characteristics

An exercise training effect was noted in that VO_{2peak} increased from 27.0±5.1 to 28.8±5.8 $ml.kg^{-1}.min^{-1}$ after 6 weeks of exercise (6.7%, p=0.0001) after exercise training. The time to VO_{2peak} (minutes) was similarly increased after exercise training (12.55±2.79 to 14.36±2.45, p<0.0001). Exercise training had no effect on BMI (baseline: BMI=30.90±4.12 $kg.m^{-2}$; after exercise training: 30.91±4.10 $kg.m^{-2}$, p=0.99).

Table 3.1 Baseline characteristics of participants enrolled to participate in a 6 week period of exercise-training and comparison of baseline characteristics of participants assigned to either pre-control or post-control groups.

	All	Pre-control	Post-control	p-value
n	35	19	16	
Age (years)	44.4±6.6	44.5±7.1	44.2±5.7	=0.90
Female gender (n [%])	11 (31%)	6 (32%)	5 (31%)	=0.91
Height (cm)	173.2±9.6	172.7±9.4	174.3±10.5	=0.66
Weight (kg)	93.2±17.5	91.2±16.9	97.5±18.8	=0.33
Body mass index ($kg.m^{-2}$)	30.9±4.1	30.5±4.4	31.8±3.3	=0.39
Waist circumference (cm)	99.2±13.2	98.6±14.0	100.5±11.9	=0.70
Hip circumference (cm)	109.6±7.4	108.7±7.8	111.6±6.3	=0.29
% overweight/obese	40/60	47/53	31/69	=0.49
% prehypertensive/grade I hypertension	20/80	21/79	19/81	=1.00
Peak oxygen consumption ($ml.kg^{-1}.min^{-1}$)	26.98±5.09	26.98±5.18	27.21±5.14	=0.56

3.4.3 Impact of exercise training on brachial BP and heart rate

The effects of 6 weeks of exercise training and 6 weeks of control period on brachial BP and HR are shown in Table 3.2. Six weeks of exercise training resulted in decreases in brachial systolic (5.6%), diastolic (8.5%) and mean arterial BP (7.2%); changes that were not observed after the control period (Table 3.2). Hence, exercise training decreased the proportion of participants with grade I hypertension (from 80 to 54%, $p<0.05$) and increased the proportion of participants with pre-hypertension (from 20 to 46%, $p<0.05$). Heart rate was unchanged by either exercise training or the control period (Table 3.2).

3.4.4 Impact of exercise training on systolic pressure augmentation and pulse wave velocity

Without adjustments, neither aortic AP, nor aortic and peripheral AIx were altered by 6 weeks of exercise training or the control period (Figure 3.1). A lack of effect of exercise training or the control period on aortic AP, and aortic and peripheral AIx was similarly noted even with adjustments for HR and MAP (Figure 3.1). Exercise training failed to modify aortic AP, or aortic and peripheral AIx in either males or females (Table 3.3). Without adjustments, 6 weeks of exercise training and 6 weeks of control period did not affect aortic pulse wave velocity (baseline: 4.86±0.88 $m.s^{-1}$; after control period: 5.20±0.89 $m.s^{-1}$; after 6 weeks training: 5.11±1.00 $m.s^{-1}$). Similarly, with adjustments for HR and MAP, 6 weeks of exercise training and 6 weeks of control period did not affect aortic pulse wave velocity (baseline: 4.80±0.77 $m.s^{-1}$; after control period: 5.20±0.77 $m.s^{-1}$; after 6 weeks training: 5.20±0.77 $m.s^{-1}$).

3.4.5 Impact of exercise training on central aortic BP and PP amplification

The impact of 6 weeks of exercise training or 6 weeks of control on central aortic SBP (SBPc), pulse pressure (PPc) and PP amplification from the aorta to the periphery are shown in Figure 3.2 and Table 3.2. Without adjustments for confounders, SBPc was decreased (GTF-derived=6.4%, P2-derived=6.3%) by 6 weeks of exercise training, a change which was

Table 3.2 Effect of a 6 week period of exercise training and a 6 week control period on brachial and central aortic blood pressure (BP) and pulse rate in overweight and obese individuals.

	Baseline	After Control Period	After Exercise Training
Brachial BP			
Systolic BP (mm Hg)	142±8	141±11	134±11*†
Diastolic BP (mm Hg)	94±8	91±9	86±11*†
Mean arterial BP (mm Hg)	111±7	108±9	103±10**†
Pulse pressure (mm Hg)	48±9	50±10	49±11
Heart rate (bpm)	65±9	69±10	67±11
Central aortic pulse pressure			
GTF-derived (mm Hg)	38±7	37±7	37±10
P2-derived (mm Hg)	39±8	38±8	37±11
Pulse pressure amplification (mm Hg)	11±5	13±6	12±5

GTF, generalised transfer function; P2, peak pressure at the second pressure wave of the radial pulse. *p<0.005, **p<0.0005 versus Baseline; †p<0.05 versus Control Period.

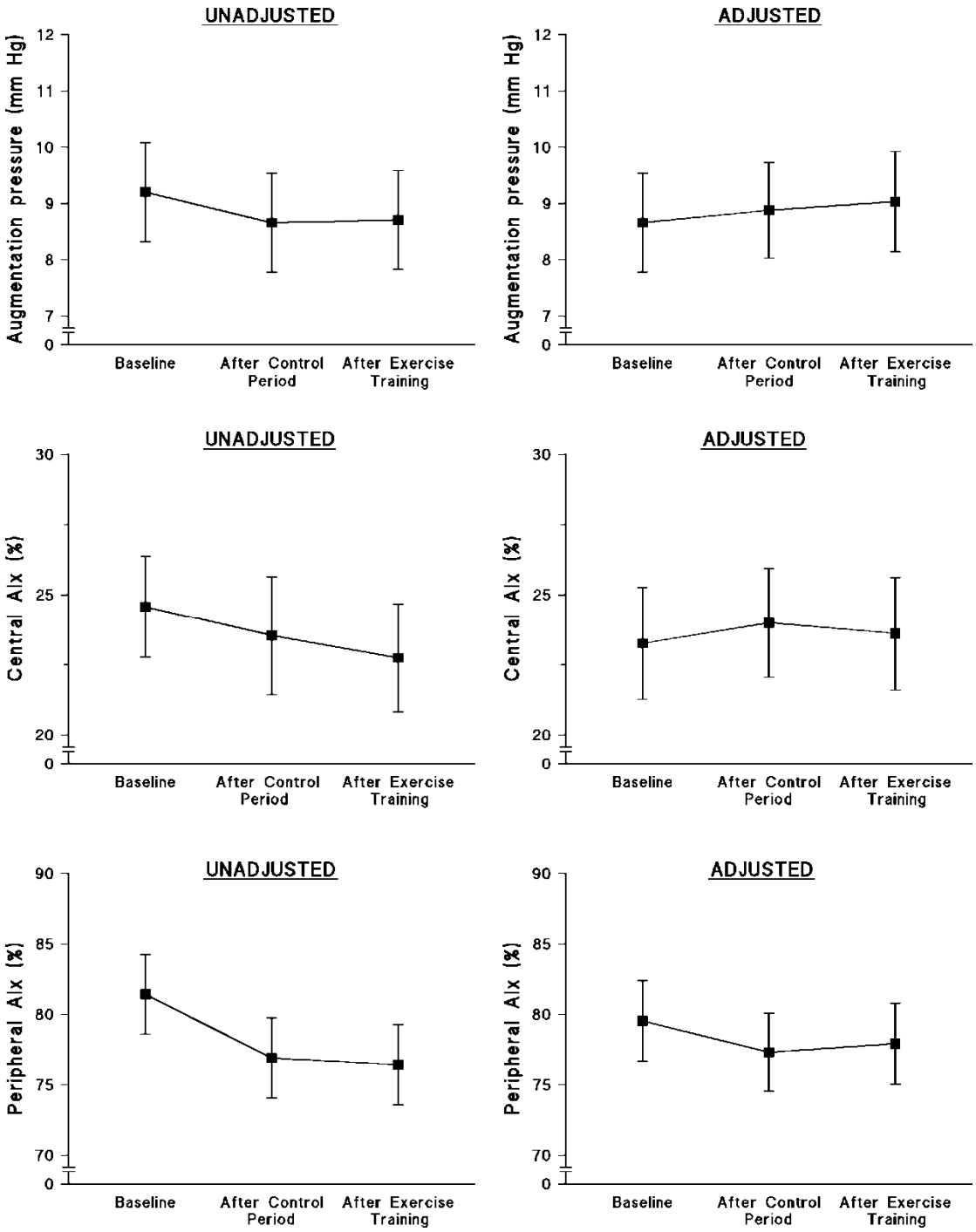


Figure 3.1 Impact of a 6 week period of exercise training and a 6 week control period on aortic augmentation pressure, and aortic or peripheral augmentation indices (AIx) before and after adjustments for HR and mean arterial pressure (distending pressures)-adjusted in overweight and obese participants. No effect of exercise training or control period was noted. Values are means and standard error of the mean.

not observed after the 6 week control period (Figure 3.2). Neither PPc, nor PP amplification from the aorta to the periphery were altered by either exercise training or the control period (Table 3.2) and with adjustments for MAP, the effects of exercise training on SBPc were eliminated (Figure 3.2).

3.4.6 Sex-specific effects of exercise training

Table 3.3 shows the effects of 6 weeks of exercise training and 6 weeks of control period on aortic augmentation pressure and index and peripheral augmentation index. Exercise training failed to modify aortic augmentation pressure and index and peripheral augmentation index in either males or females.

3.5 Discussion

The main findings of this study are as follows: In overweight and obese individuals, despite exercise training-induced effects on cardiorespiratory fitness and brachial and central aortic BP following a 6 week period of aerobic exercise, changes in BP could not be accounted for by alterations in systolic pressure augmentation as indexed by augmentation pressures and AIx.

The results of this study contribute toward our understanding of the mechanisms responsible for exercise-induced decreases in BP in overweight or obese individuals. In this regard, two previous studies conducted in 10 overweight individuals with coronary artery disease (Edwards et al 2004) and 9 overweight women (Liu et al 2012) reported on a beneficial effect of exercise training on AIx, whilst in another study conducted in 24 overweight individuals, no effect of exercise training was observed (Nualnim et al 2012). However, in that study (Nualnim et al 2012) swimming training was employed as the training method whilst in the studies demonstrating beneficial effects, running or cycling were the methods of exercise training (Edwards et al 2004, Liu et al 2012). Nevertheless, the present study conducted in a larger study sample (n=35) than previously reported on (Edwards et al

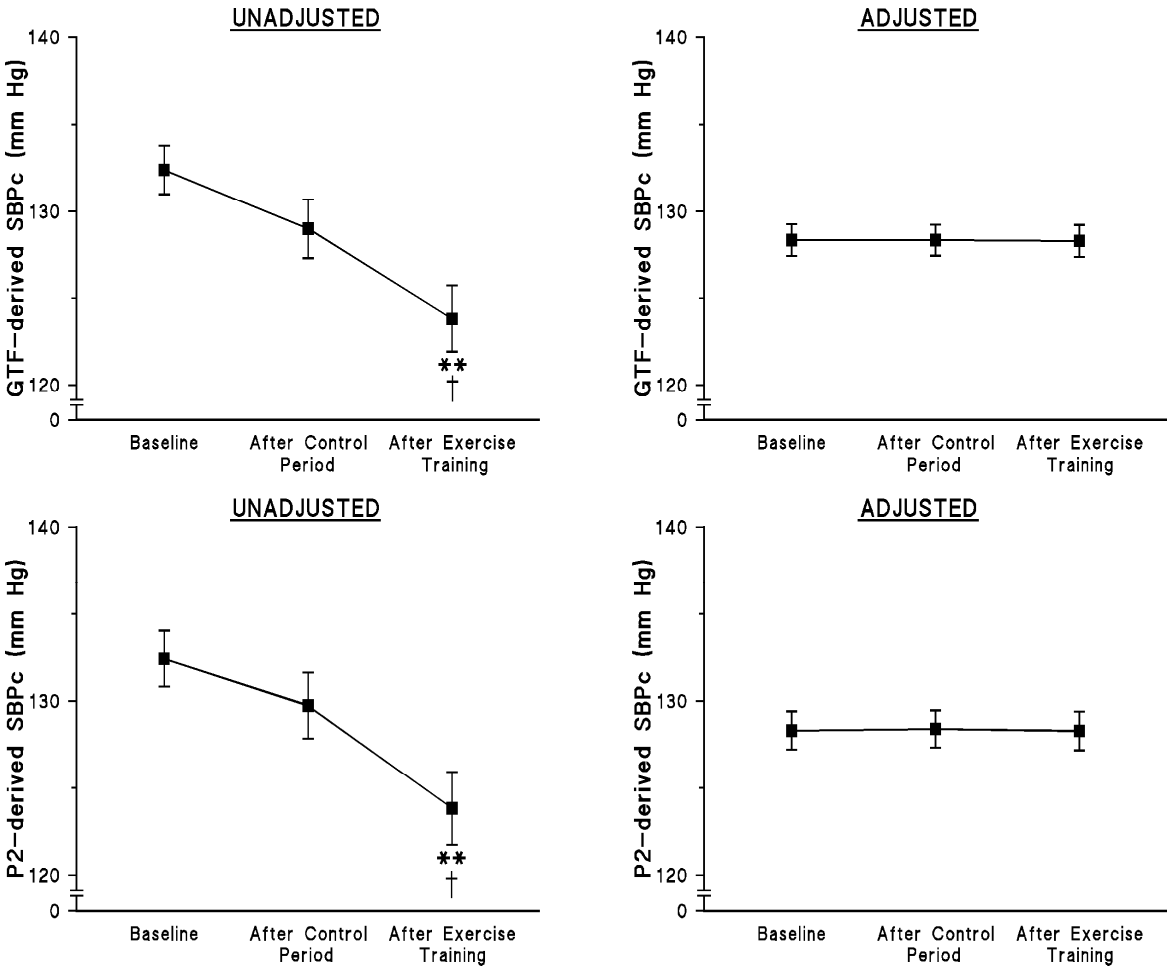


Figure 3.2 Impact of a 6 week period of exercise training and a 6 week control period on aortic systolic blood pressure (SBPc) before and after adjustments for heart rate and distending pressures (MAP) in overweight and obese participants. GTF, generalised transfer function; P2, peak pressure at the second pressure wave of the radial pulse. **p<0.005 versus Baseline; †p<0.05 versus Control Period.

Values are means and standard error of the mean.

Table 3.3 Effect of a 6 week period of exercise training and a 6 week control period on aortic augmentation pressures, and aortic and peripheral augmentation indices in overweight and obese individuals according to sex.

	Unadjusted mean±SD			Multivariate adjusted* mean±SD		
	Baseline	After Control Period	After Exercise Training	Baseline	After Control Period	After Exercise Training
			Men (n=24)			
Aortic augmentation pressure (mm Hg)	8.6±4.3	7.0±4.9	6.7±4.2	7.6±4.4	7.5±4.4	7.2±4.4
Aortic augmentation index (%)	23.2±11.0	19.4±12.3	19.2±10.5	20.8±10.3	20.3±10.3	20.7±10.3
Peripheral augmentation index (%)	77.4±12.8	71.1±13.1	71.2±13.1	73.8±11.3	71.8±10.8	74.0±11.3
			Women (n=11)			
Aortic augmentation pressure (mm Hg)	10.5±3.8	12.3±3.4	13.2±7.4	10.5±5.3	12.2±5.3	13.2±5.3
Aortic augmentation index (%)	27.6±10.8	32.6±8.6	30.6±10.0	27.7±10.0	32.5±9.6	30.5±10.0
Peripheral augmentation index (%)	90.3±21.1	89.6±18.1	87.8±18.1	90.8±19.6	89.3±18.6	87.6±19.6

* Adjusted for mean MAP and heart rate.

2004, Liu et al 2012, Nualnim et al 2012) also supports a lack of benefit of running or cycling on aortic AIx and augmentation pressures. Importantly, whilst the previous study that failed to show a beneficial effect of exercise training on aortic AIx in overweight individuals noted no improvement in cardiorespiratory fitness (Nualnim et al 2012), in the present study I demonstrated an increased VO_{2peak} and time to VO_{2peak}.

In all of the previous studies demonstrating a beneficial effect of exercise training on aortic AIx, irrespective of the underlying pathology being assessed (i.e. ageing (Tabara et al 2007) chronic kidney disease (Mustata et al 2011), coronary artery disease and overweight combined (Edwards et al 2004), or overweight alone (Liu et al 2012)), the authors did not adjust changes in AIx for alterations in MAP, despite BP decreasing in some studies (Tabara et al 2007, Liu et al 2012). In this regard, one of the strongest determinants of wave reflection and thus systolic pressure augmentation is distending pressures (Westerhof and Westerhof 2012). Thus, in at least two studies that have previously demonstrated beneficial effects of exercise training on aortic AIx, one of which was conducted in overweight individuals (Liu et al 2012), the beneficial effect may be attributed to increases in distending pressures rather than to alterations in the structural properties of the vascular wall. Moreover, in only one of the aforementioned studies that have demonstrated a beneficial effect of exercise training on aortic AIx (Edwards et al 2004, Tabara et al 2007, Mustata et al 2011, Liu et al 2012) was the central aortic BP reported on, and in this study decreases in AIx failed to translate into decreases in central aortic BP (Liu et al 2012). In the present study, exercise training failed to modify systolic pressure augmentation or AIx either before or after adjustments for MAP and hence decreases in aortic BP could not be attributed to modifications in wave reflection.

Decreases in AIx reported on in previous studies (Tabara et al 2007, Liu et al 2012) have been demonstrated to depend on the extent to which AIx is increased at baseline. In this regard, decreases in AIx in overweight women in whom baseline AIx was a mean value of 31.6±2.6% occurred with exercise training, whilst in overweight men in whom baseline AIx was noted to be a mean value of only 14.6±2.4%, AIx did not decrease with exercise training (Liu et al 2012). In this study in sex-specific analysis, I show similar values for AIx in women

after the control period (32.6±3.0%) as compared to those described by Liu et al (2012) and these values did not decrease with exercise training. Thus, it is unlikely that an inability to show exercise training effects on AIx or systolic augmentation pressures may be attributed to low baseline values.

In this study I was unable to show decreases in aortic pulse wave velocity despite increases in indices of cardiorespiratory fitness and decreases in BP after 6 weeks of exercise training in overweight and obese individuals. This is in contrast to the numerous studies that have demonstrated reductions in indices of aortic or large vessel stiffness in overweight or obese individuals after exercise training (Tanaka et al 2000, Moreau et al 2003, Yokoyama et al 2004, Collier et al 2008, Madden et al 2009, Goldberg et al 2009, Miyaki et al 2009, Guimarães et al 2010, McNeilly et al 2012, Nualnim et al 2012). Nevertheless, not all studies support the notion that exercise training modifies indices of large vessel stiffness in overweight or obese individuals (Balkestein et al 1999, Ferrier et al 2001, Stewart et al 2005, Aizawa and Petrella 2008, Baynard et al 2009). In this regard, there is presently no explanation for the discrepancies between studies. Importantly, the small study samples generally employed for exercise training studies may have resulted in false positive or negative results. Only a large study is likely to resolve the controversy of the effects of exercise training on indices of aortic stiffness.

It may be argued that as aortic PWV did not decrease with exercise training, and aortic PWV and AIx are both considered to be indices of aortic stiffness, that it is not surprising that AIx was not modified with exercise training in this study. However, aortic PWV and AIx should not be considered to be indices that reflect the same changes in large vessel function. Indeed, whilst aortic PWV is largely a stiffness index, AIx is also strongly dependent on the timing and magnitude of wave reflection which are in-turn determined by a number of factors unrelated to aortic stiffness. In this regard, whilst aortic PWV or large vessel compliance may improve with exercise training, aortic AIx may remain unchanged (Laskey et al 2012, Nualnim et al 2012). Furthermore, whilst PWV may remain unchanged after exercise training, AIx may decrease (Edwards et al 2004).

The clinical implications of this study warrant consideration. The present results suggest that the marked decreases in BP that may accompany exercise training over relatively short periods in overweight and obese individuals are unlikely to be mediated through an attenuation of systolic pressure augmentation. As augmentation indices are associated with cardiovascular events independent of conventional cardiovascular risk factors in a variety of clinical populations (Saba et al 1993, London et al 2001, Nürnberger et al 2002, Hayashi et al 2002, Weber et al 2004, Ueda et al 2004, Weber et al 2005, Chirinos et al 2005, Williams et al 2006, Hashimoto et al 2007), alternative approaches to exercise training, at least in the short-term period, may be required to modify systolic pressure augmentation. In this regard, as indices of Na^+ intake are closely associated with systolic pressure augmentation (Redelinghuys et al 2010), one possible lifestyle approach to achieving this goal is to modify Na^+ intake. Whether longer periods of exercise can reduce augmentation pressures and indices nevertheless requires further evaluation.

The limitations of this study require consideration. First, although I assessed augmentation pressures and indices using currently accepted approaches, separation of the forward and the reflected waveforms can only be accurately performed using simultaneous velocity or flow measurements in the aorta. In addition, inherent in non-invasive measurements of BP are calibration errors. In this regard calibration of the radial waveform from brachial BP measurements ignores amplification of BP from brachial to radial arteries. However, I could not accurately perform brachial artery tonometry to account for brachial-to-radial PP amplification because of obesity, and the use of wrist devices for radial BP measurement to calibrate radial pulse from radial BP measurements may carry considerable inaccuracies.

In conclusion, this study indicates that the beneficial effects of short-term exercise training on BP cannot be accounted for by an attenuation of systolic pressure augmentation in overweight and obese individuals. Whether long-term exercise training may produce benefits on aortic or peripheral systolic pressure augmentation in overweight or obesity requires further study.

CHAPTER 4

RELATIONSHIP BETWEEN INSULIN RESISTANCE AND LEFT VENTRICULAR DIASTOLIC FUNCTION INDEPENDENT OF ADIPOSITY INDICES IN A COMMUNITY SAMPLE

4.1 Abstract

Background. It is uncertain whether relationships between insulin resistance and abnormalities of left ventricular (LV) diastolic function represent false positive changes or confounding effects of coexistent obesity. I aimed to determine the relationship between insulin resistance and LV diastolic function independent of adiposity indices and alternative confounders in a community sample with a high prevalence of obesity.

Methods. I measured LV early (E)-to-atrial (A) (late) transmitral velocity with echocardiography and the homeostasis model assessment of insulin resistance (HOMA-IR), and nurse-derived conventional BP in 361 participants from a community sample of black African descent.

Results. HOMA-IR was inversely correlated with E/A ($r=-0.24$, $p<0.0001$) and in a multivariate model with adjustments for waist circumference, age, sex, conventional diastolic or systolic BP, diabetes mellitus or an HbA1c>6.1%, regular tobacco use, regular alcohol intake, pulse rate, and either left ventricular mass index (LVMI) or LV relative wall thickness in the model, the relationship between HOMA-IR and E/A persisted (partial $r= -0.14$, $p<0.01$). With HOMA-IR and waist circumference in the same multivariate regression models, HOMA-IR retained an independent relationship with E/A with a magnitude (standardised β-coefficient= -0.11 ± 0.04, $p<0.005$) that was equivalent to BP (standardised β-coefficient= -0.12 ± 0.04, $p<0.01$), whilst the relationship between waist circumference and E/A failed to achieve significance ($p=0.28$).

Conclusion. In a relatively large community-based study, relationships between insulin resistance and abnormalities of left ventricular diastolic function occur independent of coexistent obesity. Thus, insulin resistance may be an important pathophysiological mechanism responsible for abnormalities in LV diastolic function.

4.2 Introduction

A number of studies have demonstrated a relationship between the degree of adiposity and the development of heart failure independent of traditional cardiovascular risk factors and coronary artery disease (He et al 2001, Johansson et al 2001, Wilhelmsen et al 2001, Kenchaiah et al 2002, 2009, Ingelsson et al 2005a, 2005b, Nicklas et al 2006, Bahrami et al 2008, Spies et al 2009). The transition to heart failure associated with obesity may be mediated by left ventricular (LV) diastolic dysfunction. Indeed, an excess adiposity is a strong determinant of abnormalities in LV diastolic function at a community level (Redfield et al 2003, Fischer et al 2003, Powell et al 2006, Ammar et al 2008, Tsioufis et al 2008, Libhaber et al 2009, Russo et al 2011). These relationships are independent of a number of confounders including traditional risk factors, left ventricular (LV) mass index (LVMI), LV concentric remodeling (as indexed by relative wall thickness), circumferential systolic LV wall stress, 24-hour BP and aortic pulse wave velocity (Libhaber et al 2009). The possibility that independent relationships between obesity and LV diastolic function may be explained in-part by insulin resistance is nevertheless controversial.

Several studies conducted in select clinical samples and with small study sizes (Lind et al 1995, Kamide et al 1996, Galderisi et al 1997, Mureddu et al 1998, Watanabe et al 1999, Olsen et al 2003, Wong et al 2004, Leichman et al 2006, Bajraktari et al 2006, Lambert et al 2010, Wada et al 2010, Dinh et al 2010, Sliem and Nasr 2011, Wu et al 2012, Utz et al 2011) or in large study samples (Fox et al 2011, Hwang et al 2012) that have evaluated relationships between indices of insulin resistance and LV diastolic function have produced discrepant results. One large study failed to show a relationship (Fox et al 2011) and in the large study that demonstrated a relationship, the authors did not adjust for adiposity indices (Hwang et al 2012), thus raising the question of whether insulin resistance-LV diastolic function relationships are indeed independent of confounders. To address the concerns of possible false positive or negative results in small study samples and the lack of adjustment for adiposity indices in many of the small studies and the large study that demonstrated a

relationship, in this study I evaluated whether an index of insulin resistance is associated with LV diastolic function independent of adiposity indices in a relatively large, randomly selected community-based sample with a high prevalence of obesity.

4.3 Methods

4.3.1 Study participants

The study design and a description of the participants recruited has been outlined in chapter 2, page 46 of the present thesis. Of the 678 participants with echocardiographic data, both appropriate measures for the current analysis as well as fasting blood results were obtained from 361 participants.

4.3.2 Clinical, demographic and anthropometric measurements

A standardized questionnaire was administered to obtain demographic and clinical data as described in chapter 2, page 47 of the present thesis. Height, weight, waist circumference (WC), and sub-scapular and triceps skin-fold thickness (Harpenden calipers) were measured using standard approaches and participants were identified as being overweight if their body mass index (BMI) was ≥25 $kg.m^{-2}$ and obese if their BMI was ≥30 $kg.m^{-2}$. Central obesity was defined as an enlarged WC (≥88 cm in women and ≥102 cm in men). Mean skin-fold thickness was calculated as the mean of sub-scapular and triceps skin-fold thickness values.

4.3.3 Laboratory blood tests

Laboratory blood tests of renal function, liver function, haematological parameters, and percentage glycated haemoglobin (HbA1c) were performed. Diabetes mellitus or abnormal blood glucose control was defined as the use of insulin or oral hypoglycaemic agents or a glycated haemoglobin (Roche Diagnostics, Mannheim, Germany) value greater than 6.1% (Bennett et al 2007). Fasting plasma insulin concentrations were determined from

an insulin immulite, solid phase, two-site chemiluminescent immunometric assay (Diagnostic Products Corporation, Los Angeles, CA, USA) and insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) using the formula (insulin [uU/ml] x glucose [mmol/l])/22.5.

4.3.4 Conventional BP

A trained nurse-technician measured conventional (brachial) BP using a standard mercury sphygmomanometer. Details of the measurements are provided in chapter 2, page 48 of the present thesis. In the present sample the frequency of identical consecutive recordings was 0.6% for systolic BP and 1.7% for diastolic BP. No BP values were recorded as an odd number. Of the systolic and diastolic BP readings, 29.1% ended on a zero (expected =20%).

4.3.5 Echocardiography

Echocardiographic measurements were performed using previously described methods (Norton et al 2008, Libhaber et al 2008, Woodiwiss et al 2008, 2009) on an HP-5500 (Palo Alto, Ca) or a Sonosite M-Turbo ultrasound (SonoSite® Inc., Bothell, WA, USA) with the patient in the partial left decubitus position. All participants were assessed for mitral valve abnormalities as determined using 2-dimensional and color Doppler imaging. Left ventricular (LV) dimensions were determined using two-dimensional directed M-mode echocardiography in the short axis view and these recordings analysed according to the American Society of Echocardiography convention (Sahn et al 1978). During recordings, the transducer was placed perpendicular to the chest wall or pointed slightly inferiorly and laterally at the end of the long axis. M-mode images were obtained perpendicular to the posterior wall and as close to the mitral leaflet as possible without images of the mitral leaflet appearing. The interventricular septal wall thickness (IVS) at end diastole and end systole, the posterior wall thickness (PWT) at end diastole and end systole and the end diastolic and end systolic internal dimensions of the left ventricle were measured only when appropriate

visualization of both the right and the left septal surfaces occurred and where the endocardial surfaces of both the septal and posterior wall were clearly visible. Figure 4.1 shows a representative M-mode image employed to assess left ventricular mass and function.

In each participant, left ventricular diastolic function was assessed from a pulsed wave Doppler examination of the mitral inflow at rest. Pulse wave Doppler recordings of transmitral velocity were obtained with the sample volume at the tip of the mitral valve in the apical 4-chamber view. Figure 4.2 shows representative images of transmitral velocity measurements obtained during the early (E) and late (atrial-A) period of left ventricular diastolic inflow. Left ventricular diastolic function was assessed from E/A ratio.

Left ventricular end diastolic and systolic volumes were determined using the Teichholz method. (Teichholz et al 1976.) The left ventricular end systolic volume (LVESV) was calculated using the equation LVESV= [7.0/ (2.4 + LVESD)] x $(LVESD)^3$ and left ventricular end diastolic volume (LVEDV) using the equation: LVEDV= [7.0/(2.4 + LVEDD)] x $(LVEDD)^3$ (Teichholz et al1976). Left ventricular ejection fraction (EF) was calculated as [(LV end diastolic volume-LV end systolic volume)/ LV end diastolic volume] x 100. An abnormal systolic function was defined as an LV EF of ≤50%, with a moderate-to-severe reduction in LV EF defined as ≤40% (Redfield et al 2003). Left ventricular (LVM) mass was derived according to an anatomically validated formula (Devereux et al 1986) (LVM = 0.8 x [1.04 $(LVEDD + IVS + PWT)^3 - (LVEDD)^3$] + 0.6 g) and indexed to $height^{2.7}$ (LVM index, LVMI). Left ventricular relative wall thickness was calculated as (LV diastolic posterior wall thickness x2)/LV end diastolic diameter (Ganau et al 1992). Left ventricular mean wall thickness was determined from the mean of LV septal and posterior wall thickness. Left ventricular hypertrophy (LVH) was defined as a LVMI >51 $g/m^{2.7}$ for both women and men (Nunez et al 2005).

Intra-observer variability studies were conducted on 29 subjects on whom repeat echocardiographic measurements have been performed within a two week period of the initial measurements. The Pearson's correlation coefficients for LV end diastolic diameter, septal wall thickness and posterior wall thickness were 0.76, 0.94 and 0.89 (all $p<0.0001$)

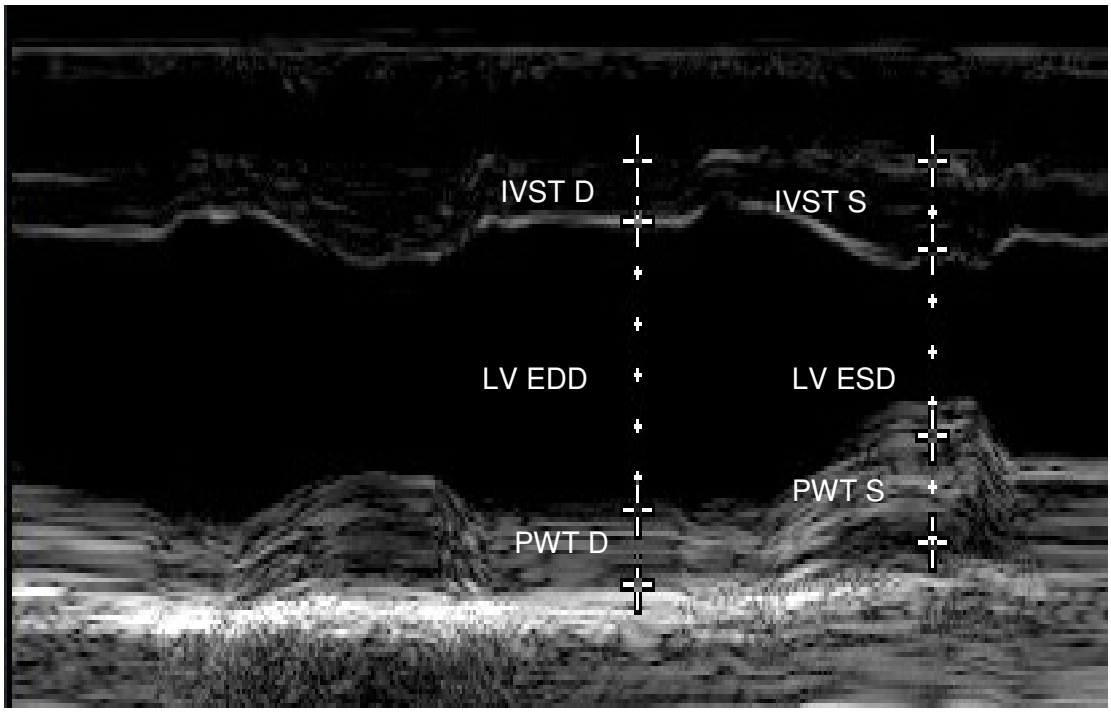


Figure 4.1 An example of a M-Mode echocardiographic image of the left ventricle obtained to assess left ventricular end systolic (LV ESD), end diastolic (LV EDD) internal diameters and the intraventricular septal wall thickness during systole (IVST S) and diastole (IVST D) and the posterior wall thickness during systole (PWT S) and diastole (PWT D). These values were used for the calculation of stroke volume, ejection fraction and LV mass (see text for further details).

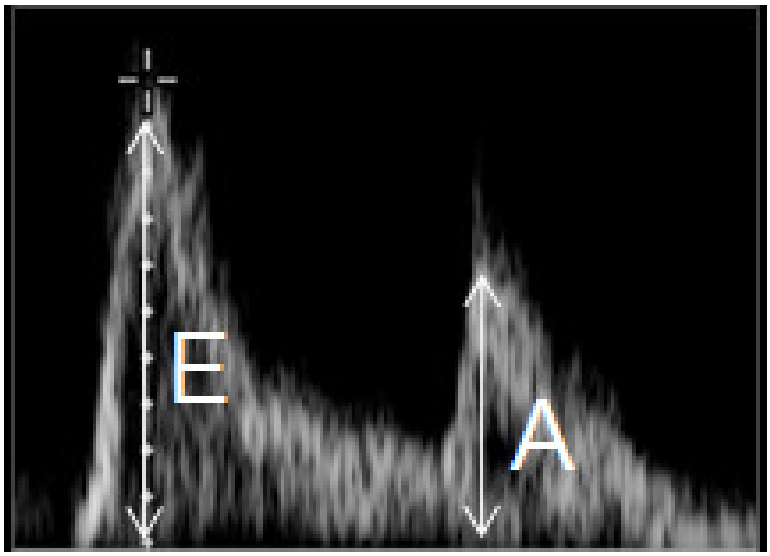


Figure 4.2 An example of a pulse waved Doppler echocardiographic image of the left ventricle obtained to assess transmitral flow patterns. E: early diastolic filling velocity, A: late diastolic filling velocity.

respectively, and the variances (mean % difference ± SD) were 0.12±5.95%, -0.77±4.47% and 0.67±5.57% respectively. In addition, no significant differences between repeat measurements were evident on paired t-test analysis (p=0.99, p=0.42 and p=0.48 respectively). The Pearson's correlation coefficients for E and A were 0.92 and 0.77 (both p<0.0001) respectively, and the variances (mean % difference ± SD) were -1.48±12.3% and -0.94±16.69% respectively. No significant differences between repeat measurements were evident on paired t-test analysis (p=0.41, p=0.94 respectively).

Inter-observer variability studies were conducted on 26 participants on whom the two echocardiographers involved in obtaining measurements performed echocardiography on the same participants whilst blinded to each other's measurements. The Pearson's correlation coefficients for LV end diastolic diameter, septal wall thickness and posterior wall thickness were 0.96, 0.84 and 0.88 (all p<0.0001) respectively, and the variances (mean % difference ± SD) were 0.49±2.71%, -1.69±7.19% and 0.23±5.89% respectively. In addition, no significant differences between measurements were evident on unpaired t-test analysis (p=0.38, p=0.26 and p=0.85 respectively). The Pearson's correlation coefficients for E and A were 0.71 and 0.86 (both p<0.0001) respectively, and the variances (mean % difference ± SD) were 4.76±16.03% and 3.58±12.13% respectively. No significant differences between repeat measurements were evident on unpaired t-test analysis (p=0.16 for both).

4.3.6 Statistical analysis

Database management and statistical analyses were performed with SAS software, version 9.1 (SAS Institute Inc., Cary, NC, USA). Descriptive data are reported as mean±SD. Unadjusted means and proportions were compared by the large-sample z-test and the χ^2-statistic, respectively. As HOMA-IR was positively skewed (skewness=3.89, kurtosis19.61; Shapiro-Wilk's statistic=0.58, p<0.0001) HOMA-IR was log transformed. Log transformation of HOMA-IR resulted in an improved distribution (skewness=0.29, kurtosis=-0.51; Shapiro-Wilk's statistic=0.98). Independent relations between HOMA-IR and E/A were determined from multivariate linear regression analysis with adjustments for waist circumference, age,

sex, conventional systolic or diastolic BP, diabetes mellitus or an HbA1c>6.1%, antihypertensive treatment, regular tobacco or alcohol intake, pulse rate and either LV mass index or relative wall thickness. Partial correlations between HOMA-IR and E/A adjusted for covariates, were determined using PROC CORR procedures defined in the SAS package. Probability values were further adjusted for non-independence of family members using the method of maximum likelihood as implemented by the PROC MIXED procedure as defined in the SAS package. The mixed model contained both fixed effects (all phenotypic data) parameters and random-effects (familial relationships) parameters. To compare the impact of the relationships between different variables and E/A, standardized β-coefficients were determined from stepwise linear regression analysis using the PROC REG procedure and these coefficients were compared using an ANOVA.

4.4 Results

4.4.1 Characteristics of the participants

Table 4.1 gives the demographic and clinical characteristics of the study group with echocardiographic data either with or without fasting blood data. The characteristics between the groups were similar. More women than men participated. In general, the study group had a high BMI and waist circumference, with 23.3% of participants being overweight and 37.7% of participants being obese. The participants also had a high prevalence of hypertension 25.2%). Of the participants 19.9% were either receiving medication for diabetes mellitus or had an impaired blood glucose control (HbA1c>6.1%), 16.3% reported regular smoking and 23.8% reported a regular intake of alcoholic beverages. The mean (±SD) HOMA-IR and log HOMA-IR values in those with fasting blood values were 3.40±4.95 and 0.60±1.09 respectively.

Table 4.1 General characteristics of study participants not receiving antihypertensive therapy with and without fasting blood data.

Fasting blood data	With	Without	p value
Number	361	161	
Women (%)	60.4	62.7	=0.63
Age (years)	38.3±16.2	36.4±15.2	=0.21
Body mass index (kg.m^{-2})	28.2±7.6	27.4±6.9	=0.25
Waist circumference (cm)	87.5±15.9	86.4±16.4	=0.47
Mean skinfold thickness (cm)	1.99±1.10	1.82±0.88	=0.08
Overweight/obese (%)	23.3/37.7	23.6/32.9	=1.00/0.72
Central obesity (%)	37.1	35.4	=0.39
Hypertension (%)	25.2	29.2	=0.77
%Diabetes mellitus or HbA1c >6.1%	19.9	12.4	=0.05
Regular smoking (%)	16.3	18.6	=0.53
Regular alcohol (%)	23.8	19.3	=0.26
Conventional systolic BP (mm Hg)	126±21	123±22	=0.14
Conventional diastolic BP (mm Hg)	83±13	82±14	=0.43

BP, blood pressure. No significant differences were noted between the groups.

4.4.2 Left ventricular characteristics of the participants

The left ventricular characteristics of the study group are shown in Table 4.2. 19.4% of participants had LVH (LVMI>51 $g.m^{-2.7}$). None of the participants had mitral valve abnormalities. Approximately 7.2% (n=26) had mild diastolic chamber dysfunction or impaired relaxation with an E/A ≤0.75, and ~36.0% (n=130) severe diastolic chamber dysfunction with an E/A≥1.50.

4.4.3 Factors associated with log HOMA-IR

On bivariate analysis, BMI (r=0.20, p=0.0001) and waist circumference (r=0.25, p<0.0001), age (r=0.16, p<0.005), and conventional systolic BP (r=0.12, p<0.05), and diabetes mellitus or an HbA1c>6.1% (r=0.14, p<0.01) were directly correlated with HOMA-IR. However, in a multivariate regression model, only waist circumference was independently associated with HOMA-IR (p=0.005). Body mass index was not independently related to HOMA-IR (p=0.13).

4.4.4 Relationships between HOMA-IR and left ventricular mass or relative wall thickness

On both bivariate analysis (r=0.18, p<0.001) HOMA-IR was associated with LVMI. However, in multivariate regression analysis with adjustments for age, waist circumference, sex, systolic BP, regular tobacco use, regular alcohol intake, pulse rate and diabetes mellitus, there was no significant relationship between HOMA-IR and LVMI (partial r=0.10, p=0.062). However, on both bivariate (r=0.21, p<0.0001) and multivariate regression analysis (partial r=0.16, p<0.005), with adjustments for age, waist circumference, sex, systolic BP, regular tobacco use, regular alcohol intake, and diabetes mellitus, HOMA-IR was associated with LV relative wall thickness.

Table 4.2 Left ventricular parameters in study participants (n=361).

LV mass (g)	155±47
LV mass indexed for height$^{2.7}$ (g.m$^{-2.7}$)	41.6±12.7
LV hypertrophy (%)	19.4
LV relative wall thickness	0.39±0.07
LV mean wall thickness (cm)	0.92±0.17
E wave velocity (cm.s^{-1})	79.1±22.0
A wave velocity (cm.s^{-1})	62.1±18.7
E/A	1.36±0.47
LV ejection fraction (%)	66.5±7.9
% Ejection fraction<40%	n=1 (0.3%)
% Ejection fraction<50%	n=6 (1.7%)

LV, left ventricular; E, early transmitral velocity; A, late (atrial) transmitral velocity.

4.4.5 Relationships between indices of adiposity and left ventricular diastolic function

On bivariate analysis, BMI and waist circumference were strongly and inversely correlated with E/A (Figure 4.3). In a multivariate model with age, sex, systolic BP, diabetes mellitus or an HbA1c>6.1%, regular tobacco use, regular alcohol intake, and pulse rate in the model, waist circumference and BMI were independently associated with E/A (Figure 4.4). With further adjustments for LVMI or LV relative wall thickness, waist circumference and BMI retained independent relationships with E/A (Figure 4.4).

4.4.6 Relationships between HOMA-IR and left ventricular diastolic function

On bivariate analysis, HOMA-IR was strongly and inversely correlated with E/A (Figure 4.5). In a multivariate model with waist circumference, age, sex, BP, diabetes mellitus or an HbA1c>6.1%, regular tobacco use, regular alcohol intake, and pulse rate in the model, HOMA-IR was independently associated with E/A (Figure 4.6). With further adjustments for LVMI or LV relative wall thickness, HOMA-IR retained independent relationships with E/A (Figure 4.6).

4.4.7 Magnitude of the association of HOMA-IR on left ventricular diastolic function

Table 4.3 shows a comparison of the standardized β-coefficients (slopes) for the independent relations between variables associated with E/A in multivariate regression models. The relations between HOMA-IR and E/A were similar irrespective of whether LVMI was not (Table 4.3) or was (standardized β-coefficient=-0.11±0.04, $p<0.01$) included in the regression model. Moreover, the relations between HOMA-IR and E/A were similar irrespective of whether LV relative wall thickness was not (Table 4.3) or was (standardized β-coefficient=-0.10±0.04, $p<0.01$) included in the regression model. Similar outcomes were noted with systolic as opposed to diastolic BP in the regression model (HOMA-IR versus E/A adjusted for confounders and systolic BP: standardized β-coefficient=-0.11±0.04, $p=0.005$).

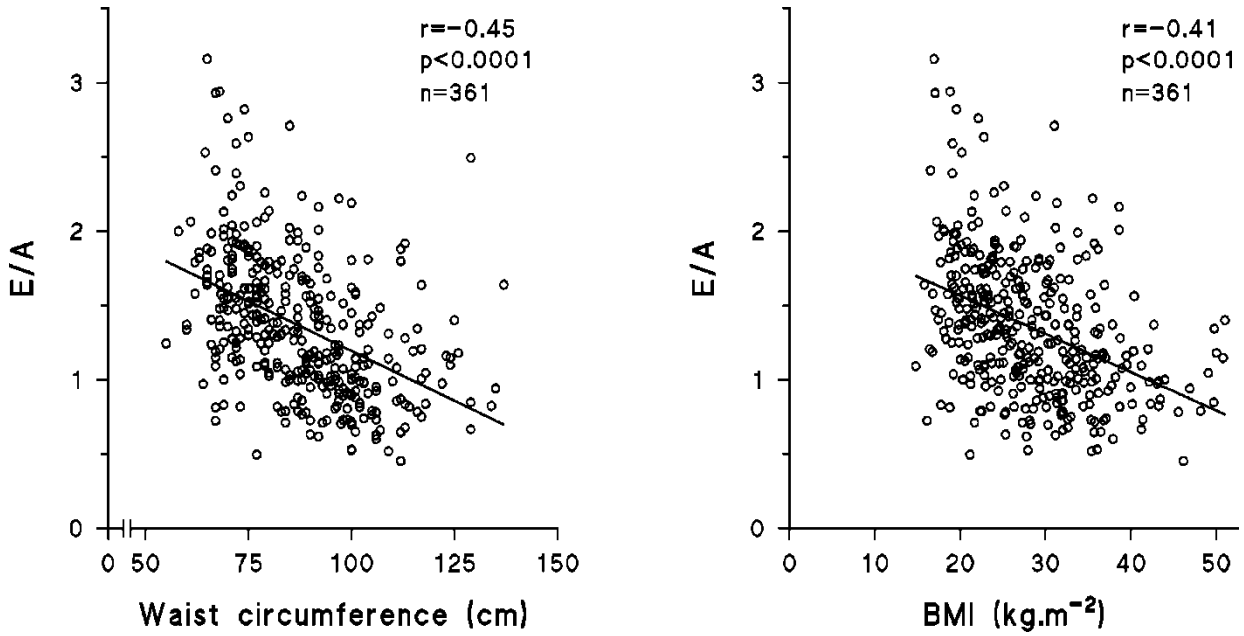


Figure 4.3 Bivariate relationship between waist circumference or body mass index and left ventricular early-to-late (atrial) transmitral velocity (E/A) in a community sample not receiving antihypertensive therapy.

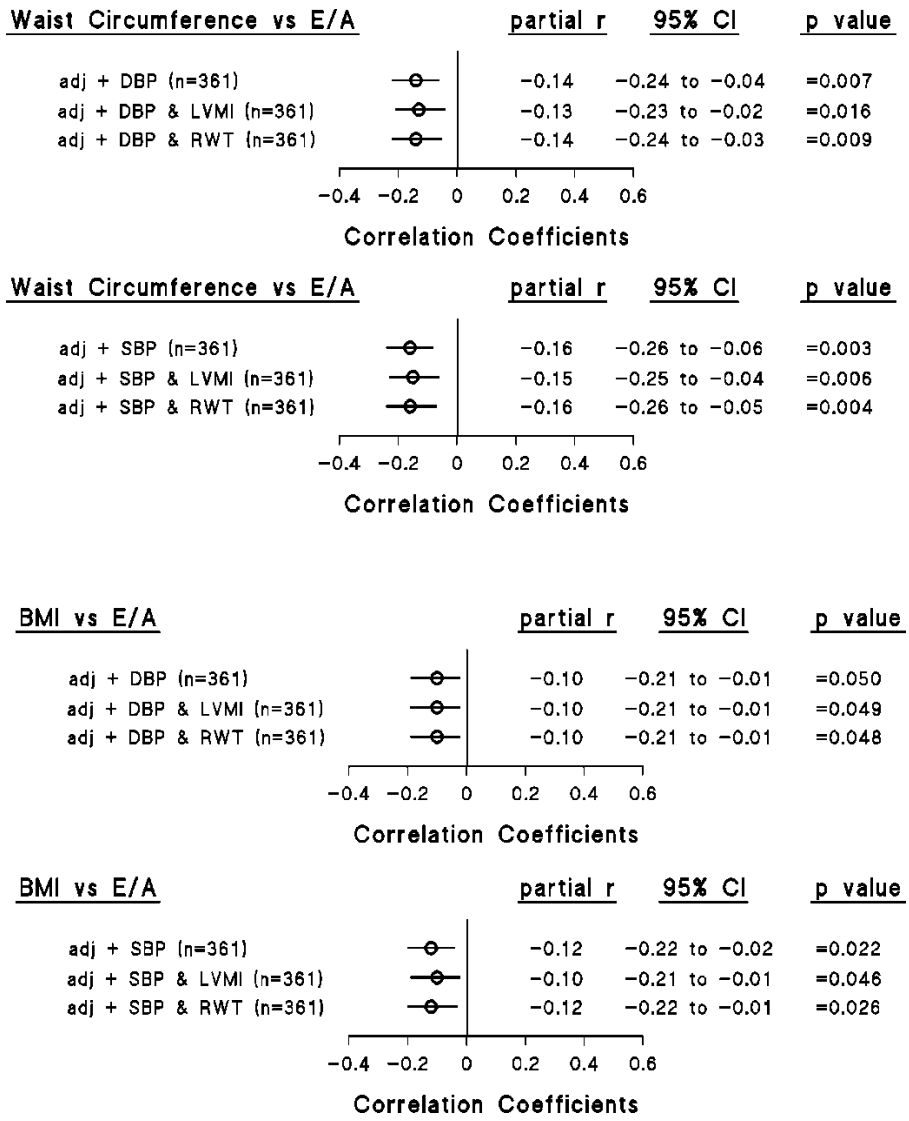


Figure 4.4 Multivariate adjusted (adj) relationships (partial correlation coefficients [partial r] and 95% confidence intervals [CI]) between waist circumference or body mass index (BMI) and left ventricular early-to-late (atrial) transmitral velocity (E/A) in a community sample not receiving antihypertensive therapy. DBP, diastolic blood pressure; SBP, systolic BP; LVMI, left ventricular mass index; RWT. Adjustments are for age, sex, conventional diastolic BP (or systolic BP as indicated), diabetes mellitus or an HbA1c>6.1%, regular tobacco use, regular alcohol intake, pulse rate, and LVMI or RWT as indicated in the models.

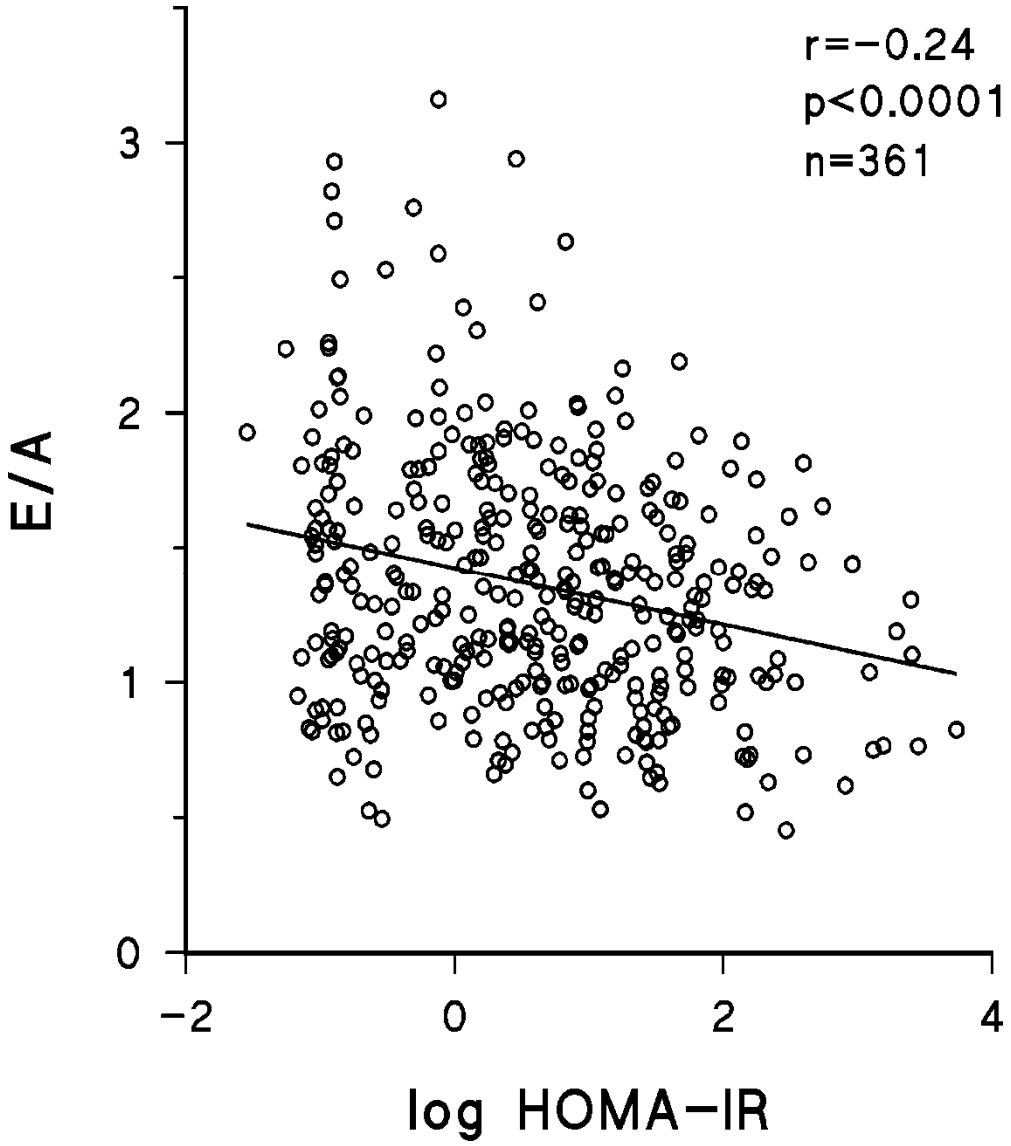
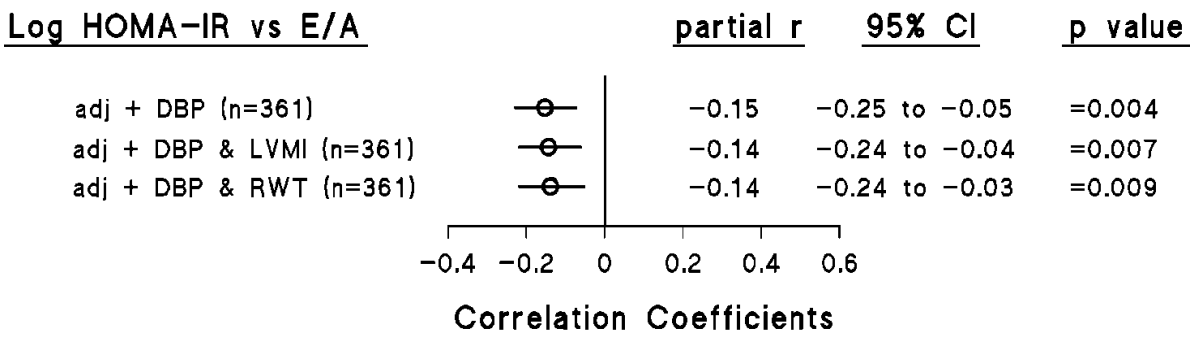


Figure 4.5 Bivariate relationship between the homeostasis model assessment of insulin resistance (HOMA-IR) and left ventricular early-to-late (atrial) transmitral velocity (E/A) in a community sample not receiving antihypertensive therapy.



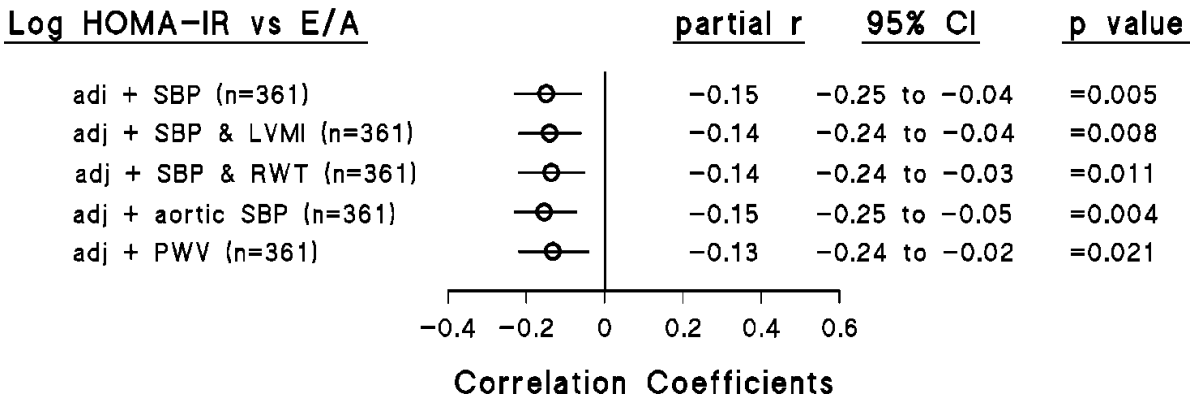


Figure 4.6 Multivariate adjusted (adj) relationships (partial correlation coefficients [partial r] and 95% confidence intervals [CI]) between the homeostasis model assessment of insulin resistance (HOMA-IR) and left ventricular early-to-late (atrial) transmitral velocity (E/A) in a community sample not receiving antihypertensive therapy. DBP, diastolic blood pressure; SBP, systolic BP; LVMI, left ventricular mass index; RWT, LV relative wall thickness. Adjustments are for waist circumference, age, sex, conventional diastolic BP (or systolic BP as indicated), diabetes mellitus or an HbA1c>6.1%, regular tobacco use, regular alcohol intake, pulse rate, and LVMI or RWT as indicated in the models.

Table 4.3 Comparison of the impact of factors associated with left ventricular E/A in a multivariate regression model in the study group (n=361).

Variable	Standardized β-coefficient±SEM	p value for relationship
Age	-0.52±0.05	**<0.0001**
Waist circumference	-0.05±0.05	=0.28
HOMA-IR	-0.11±0.04	**<0.005**
Diastolic BP	-0.12±0.04	**<0.01**
Sex	0.04±0.05	=0.37
Diabetes mellitus or glycated haemoglobin>6.1%	-0.05±0.04	=0.26
Pulse rate	-0.13±0.04	**<0.005**
Regular tobacco	0.07±0.04	=0.09
Regular alcohol	-0.008±0.04	=0.84

HOMA-IR, homeostasis model assessment of insulin resistance. Probability values were further adjusted for non-independence of family members. Significant probability values are indicated in bold. Model r^2=0.49, p<0.0001. No significant differences were noted between β-coefficients for HOMA-IR and diastolic blood pressure (p=0.86).

The magnitude of the association of HOMA-IR with E/A was comparable with the association of BP and E/A and second only to the magnitude of the association of age with E/A (comparison of the standardized β-coefficients). With both HOMA-IR and waist circumference included in a multivariate model, although the relationship between HOMA-IR and E/A persisted, the relationship between waist circumference and E/A no longer achieved significance (Table 4.3).

4.5 Discussion

The main findings of this study are that in a randomly selected community sample with a high prevalence of obesity, insulin resistance, as indexed by HOMA-IR was inversely associated with E/A independent of a number of confounders as well as waist circumference, the main adiposity index that explains variations in E/A (Libhaber et al 2009). Importantly, these independent relations were also beyond structural LV changes, including LVMI and relative wall thickness.

To the best of my knowledge this study is the first to provide evidence in a relatively large, randomly selected sample to show that insulin resistance is associated with an abnormal LV diastolic function independent of adiposity indices. In this regard, these findings are consistent with the results of several small studies (n=26-208) (Lind et al 1995, Kamide et al 1996, Galderisi et al 1997, Watanabe et al 1999, Wong et al 2004, Bajraktari et al 2006, Dinh et al 2010, Sliem and Nasr 2011, Utz et al 2011) and one large study (n=1599) (Hwang et al 2012) conducted in select samples, where the relationships were not adjusted for adiposity indexes in either the large study (Hwang et al 2012), or in several of the small studies (Lind et al 1995, Wong et al 2004, Sliem and Nasr 2011, Utz et al 2011). In contrast, in alternative small studies (n=27-102) (Mureddu et al 1998, Olsen et al 2003, Leichman et al 2006, Lambert et al 2010, Wada et al 2010, Wu et al 2012) and one large (n=2399) study where the regression relations were nevertheless not provided (Fox et al 2011), indices of insulin resistance were not correlated with measures of diastolic function. The small sample

sizes in many of these prior studies may have resulted in false positive or negative findings; a selection bias may have resulted in recruitment of participants that do not reflect the community at large; and relationships that have not been adjusted for adiposity indices are likely to reflect confounding effects of obesity *per se*.

In this study independent relationships between HOMA-IR and LVMI or relative wall thickness were noted. In this regard, LV diastolic function is also strongly determined by LVMI and relative wall thickness. However, adjustments for neither LVMI nor relative wall thickness modified the multivariate adjusted HOMA-IR-E/A relationships. These data would therefore suggest that LV structural changes are unlikely to account for relationships between HOMA-IR and abnormalities of LV diastolic function.

With respect to the clinical implications of the present findings, obesity effects on LV diastolic function (Redfield et al 2003, Fischer et al 2003, Powell et al 2006, Ammar et al 2008, Tsioufis et al 2008, Libhaber et al 2009, Russo et al 2011) may represent a progressive preclinical condition that contributes to obesity-induced heart failure (He et al 2001, Johansson et al 2001, Wilhelmsen et al 2001, Kenchaiah et al 2002, Ingelsson et al 2005, Ingelsson et al 2005, Nicklas et al 2006, Bahrami et al 2008, Kenchaiah et al 2009, Spies et al 2009). In this regard, as suggested by this study, insulin resistance may mediate this effect and hence may represent a potential target to prevent the transition to heart failure in obesity. The present findings suggest that the relationship between insulin resistance and heart failure (Ingelsson et al 2005b) may in-part be accounted for by the adverse effects of insulin resistance on LV diastolic function.

This study has several strengths and limitations. The strengths of the study include the relatively large randomly selected study sample. Thus, the chances of the independent relationship noted between HOMA-IR and E/A being a false positive finding or attributed to a selection bias, are likely to have been reduced. Second, I evaluated relations between HOMA-IR and E/A using BP and E/A as continuous rather than dichotomous traits, thus improving the chances of a positive outcome. The limitations of the study are first the cross-sectional rather than prospective nature of the study design and hence relationships between

HOMA-IR and E/A may not be causal. Second, I did not measure pulmonary venous reverse flow, mitral inflow during the peak of the valsalva manoeuvre, or perform tissue Doppler investigations and hence I may have missed participants with diastolic dysfunction. Thus, we could not assess the relationship between HOMA-IR and the presence of diastolic dysfunction reported as a discrete trait. Third, the limited proportion of male participants recruited for the study prevented sex-specific analyses.

In conclusion, the results of this study suggest that with adjustments for appropriate adiposity indexes (waist circumference) and alternative confounders, HOMA-IR contributes as much as BP to variations in LV diastolic function at a community level (not confounded by small sample size or selection bias), and that this effect is independent of LVH and geometric LV remodelling. The mechanisms of this effect require further elucidation. These data lend insights into the potential for the development of therapeutic strategies targeting insulin resistance to prevent the possible development of heart failure as a result of obesity.

CHAPTER 5

EFFECT OF SHORT TERM AEROBIC EXERCISE TRAINING ON DIASTOLIC FUNCTION IN OVERWEIGHT OR OBESE INDIVIDUALS

5.1 Abstract

Background. Overweight and obesity has consistently been associated with decreases in left ventricular (LV) diastolic function. As the effect of exercise training on LV diastolic function as assessed using tissue Doppler imaging (TDI) is uncertain, in this study I aimed to assess this question in otherwise healthy overweight and obese individuals.

Methods. Thirty-two overweight (n=14) or obese (n=18), sedentary or recreationally active men and women (30–57years), completed 6 weeks of exercise training either preceded (n=16) or followed by (n=16) a 6 week control period. Exercise capacity (peak oxygen consumption, VO_{2peak}), LV diastolic function (E/A, tissue Doppler imaging e', e'/a' and E/e') (echocardiography), and body weight were determined before and after exercise training. LV diastolic function was also determined in overweight and obese participants from a community sample (n=242) and normal values for e' determined from lower 95% confidence intervals of lean and healthy participants of the community sample (n=60).

Results. Exercise training increased VO_{2peak} from 27.4±4.9 to 29.4±5.8 $ml.kg^{-1}.min^{-1}$ (p=0.0001); but had no effect on BMI (p=0.99). Baseline measures of diastolic function were comparable with those noted in overweight and obese participants from the community sample and 56% (n=18) had baseline e' values (early diastolic abnormalities) that were below the lower 95% confidence intervals of a lean and healthy cohort of the community sample (n=60). No changes in TDI indices of LV diastolic function were observed after exercise training in all participants (e': p=0.74, a': p=0.98, e'/a': p=0.85; E/e': p=0.26), in participants with abnormal e' values (n=18)(e': p=0.99, a': p=0.96, e'/a': p=0.91; E/e': p=0.97) or in obese participants only (n=21)(e': p=0.67, a': p=1.00, e'/a': p=0.78; E/e': p=0.11).

Conclusions. Exercise training alone, despite producing an improvement in cardiorespiratory fitness is unable to improve obesity-associated decreases in TDI indices of LV diastolic function. These data suggest that a lack of exercise *per se* is unable to explain overweight and obesity-associated decreases in diastolic function.

5.2 Introduction

Obesity is associated with the development of heart failure independent of traditional cardiovascular risk factors and coronary artery disease (He et al 2001, Johansson et al 2001, Wilhelmsen et al 2001, Kenchaiah et al 2002, Ingelsson et al 2005a, 2005b, Nicklas et al 2006, Bahrami et al 2008, Kenchaiah et al 2009, Spies et al 2009). Prior to the development of heart failure obesity is a strong determinant of abnormalities in left ventricular (LV) diastolic function at a community level (Redfield et al 2003, Fischer et al 2003, Powell et al 2006, Ammar et al 2008, Tsioufis et al 2008, Libhaber et al 2009, Russo et al 2011). In order to prevent the transition of obesity associated cardiac changes to heart failure, there is therefore considerable interest in developing approaches that may prevent or reverse obesity-associated LV diastolic dysfunction. In this regard, the role of exercise training in modifying LV diastolic dysfunction is uncertain.

The majority of studies suggest that in overweight or obese individuals LV diastolic function as assessed from early (E)-to-late (atrial-A) transmitral velocity measurements are unchanged by exercise training (Reid et al 1994, Sadaniantz et al 1996, Stewart et al 2006, Baynard et al 2008, Riordan et al 2008, Kosmala et al 2009, Eriksson et al 2010, Cocco and Pandolfi 2011, Guirado et al 2012, Schuster et al 2012), despite weight loss (Reid et al 1994, Stewart et al 2006, Riordan et al 2008, Kosmala et al 2009, Cocco and Pandolfi 2011) or evidence of improved cardiorespiratory fitness (Reid et al 1994, Sadaniantz et al 1996, Stewart et al 2006, Riordan et al 2008, Baynard et al 2008, Kosmala et al 2009, Schuster et al 2012, Guirado et al 2012) in many of these studies. In addition, there is also evidence to suggest that tissue Doppler indices (TDI) of diastolic function (myocardial tissue lengthening in the early [e'] and atrial [a'] period of diastole, the e'/a' ratio, and E/e', an index of left atrial driving forces) are unaffected by exercise training programmes (Riordan et al 2008, Guirado et al 2012) despite improvements in cardiorespiratory fitness in both of these studies and a decrease in body weight in one of these studies (Riordan et al 2008). Furthermore, in one study there was evidence of worsening diastolic function with exercise training as evidenced

by a decreased E/A, and e' and an increased E/e' (Gondoni et al 2007). In contrast, some studies show an increased E (Levy et al 1993), or e' (Kosmala et al 2009) in association with a decreased body weight and improved cardiorespiratory fitness; or an increased e' without a change in body weight, but with an improved cardiorespiratory fitness (Schuster et al 2012) after exercise training. Importantly, studies performed assessing the impact of exercise training on diastolic function as determined from TDI in overweight or obese individuals, either employed exercise together with dietary approaches as lifestyle interventions (Gondoni et al 2007, Kosmala et al 2009, Wong et al 2006); assessed effects in elderly (age=68±8 years) treated hypertensives (Guirado et al 2012), where the confounding effects of age and antihypertensive therapy on LV diastolic function may have limited the capacity to detect exercise-induced effects on LV diastolic function; evaluated the effects of exercise training in remarkably small study samples (n=10-13) (Riordan et al 2008, Schuster et al 2012) which may reflect false positive (Schuster et al 2012)(n=10) or negative (Riordan et al 2008, Guirado et al 2012)(n=13-15) findings; evaluated the effects of exercise training in men only (Schuster et al 2012), thus limiting the conclusions to one sex; or performed analysis after assigning participants *post hoc* to groups where weight loss either did or did not occur (Wong et al 2006), an obviously flawed methodology. Hence, there is currently insufficient evidence to support or refute the notion that exercise training alone may produce benefits to diastolic function as assessed using TDI. As a consequence of these deficiencies in current evidence, to address the aforementioned concerns in this study I assessed the impact of exercise training alone on indices of LV diastolic function, including TDI, in a significantly larger study sample than that previously reported on (Riordan et al 2008, Schuster et al 2012) of young-to-middle aged, overweight and obese individuals not receiving antihypertensive therapy and in participants of both sexes.

5.3 Methods

5.3.1 Study participants

A description of the recruitment process and the study participants recruited is given in chapter 3, page 82 of the present thesis. Importantly, of the 39 people recruited, 35 completed the 6-week training period and 32 had high quality echocardiograms. Hence, data from 32 participants were analysed for the purposes of the current study. To ensure that tissue Doppler indices in the 32 participants recruited were representative of what would be noted in overweight and obesity, I also evaluated the relationship between overweight or obesity and tissue Doppler indices of LV diastolic function in 242 participants randomly recruited from a community sample. The recruitment procedures and method of sampling have been described in previous chapters.

5.3.2 Study protocol

A description of the study protocol is given in chapter 3, page 83 of the present thesis.

5.3.3 Anthropometric measurements

Body weight and height and waist and hip circumference were measured using standard approaches. From height and weight measurements, body mass index (BMI) was calculated.

5.3.4 Brachial blood pressure measurements

At each visit brachial BP was measured twice in the non-dominant arm according to guidelines (see Chapter 2, page 48 for more details) and if the measures differed by more than 5 mm Hg, a third measure was taken. The average of the two or three measures was taken as the brachial BP.

5.3.5 Maximal exercise testing

A description of the assessment of maximal exercise capacity is given in chapter 3, page 85 of the present thesis.

5.3.7 Exercise training

A description of the exercise training is given in chapter 3, page 85 of the present thesis.

5.3.8 Echocardiography

A description of the echocardiographic approach to the assessment of LV mass, mass index, LV relative wall thickness, LV mean wall thickness, LV internal dimensions, LV ejection fraction, and LV E/A are given in chapter 4 page 102 of the present thesis. In addition, diastolic and systolic function were also assessed using TDI. In this regard, TDI is a more sensitive indicator of the detection of diseased mitral inflow patterns compared to pulse waved Doppler imaging because it is less preload and HR dependent (Sohn et al 1997). To perform TDI, motion of the mitral annulus was recorded in the apical four-chamber view. The sample volume was positioned at the septal and lateral corners of the mitral annulus. Figure 5.1 show the peak velocities during early (e') and late (atrial) (a') diastole that were measured. Early diastolic mitral annular velocity (e') reflect the rate of myocardial relaxation. A reduction in e' is one of the earliest markers of diastolic dysfunction (Sohn et al 1997; Schillaci et al 2002, Fischer et al 2003, Willens et al 2004, Chahal et al 2010). Other measures of left ventricular function calculated from the measured variables include the E/e' ratio and the ratio of early to late mitral annular velocity (e'/a'). Because mitral annular velocity (e') remains constant and trans-mitral flow (E) increases with an increased filling pressure, E/e' ratio correlates well with left ventricular filling pressures especially in persons with an EF > 50 (George et al 2010, Kidawa et al 2005, Ommen et al 2000).

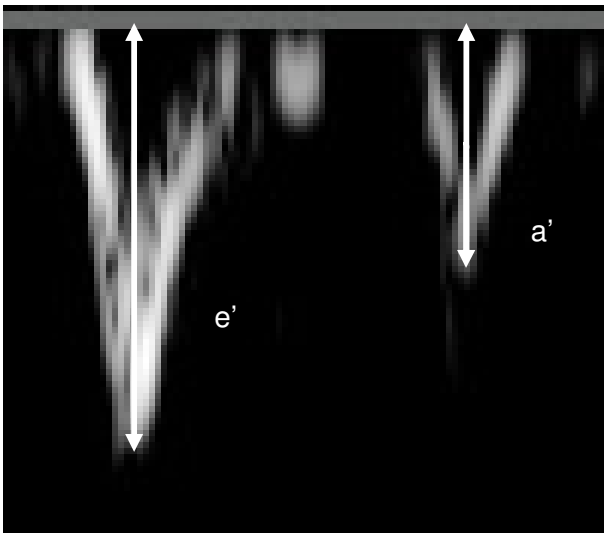


Figure 5.1 An example of a tissue Doppler image obtained from the lateral mitral annulus. e’: early diastolic mitral annulus velocity, a’: late diastolic mitral annulus velocity.

5.3.8 Data analysis

Descriptive statistics are reported as means and standard deviation (SD) unless otherwise specified. Data were analysed using SAS software, version 9.1 (SAS Institute, Cary, NC). The average heart rates achieved whilst performing exercise at baseline were similar between the two exercise regimes (continuous exercise, n=20: 133±7 bpm; interrupted exercise, n=15: 136±8 bpm, p=0.13). Moreover, the two exercise regimes (continuous versus interrupted) produced the same changes in VO_{2peak} [continuous exercise, n=20: 26.5±4.6 $ml.kg^{-1}.min^{-1}$ to 28.7±4.9 $ml.kg^{-1}.min^{-1}$ (8.3%, $p<0.05$); interrupted exercise, n=15: 27.0±5.5 to 29.3±6.6 $ml.kg^{-1}.min^{-1}$ (8.5%, $p<0.05$); $p>0.05$ for comparison] and similar lack of changes in hemodynamic, cardiac function and geometry (Table 5.3) and hence were combined for analysis. A two-way repeated measures analysis of variance was performed to assess the impact of group (pre-control versus post-control), time (before and after exercise and control periods) and group-time interaction on TDI e'/a', E/A, LVMI, RWT and EF. No group or group-time interactive effects were noted. Tukey *post hoc* tests were performed to identify differences between specific time points. Proportions were compared using a Fisher's exact test. To achieve statistical power at 80% with a two-sided α value of <0.05 a sample size of 14 in each group was required, as calculated from a mean difference and standard deviation of e' of 1.4±1.3 $cm.sec^{-1}$ based upon significant changes in TDI e' previously reported (Schuster et al 2012). To evaluate the impact of BMI on echocardiographic parameters in the community-based sample, unadjusted and multivariate adjusted values were compared between participants who were lean, overweight or obese using an ANOVA and a Tukey *post hoc* test. Multivariate adjustments were made for potential confounders associated with categories of BMI as indicated in the Tables. To ensure that the inclusion of overweight participants did not affect the results of the study, sensitivity analysis (secondary data analysis) was conducted in the exercise-trained participants in those who were obese only and in those with e' values that were below the lower 95% confidence interval of lean and healthy participants in the community-based sample.

5.4 Results

5.4.1 Characteristics of participants for exercise study

The characteristics of the 7 participants who failed to complete the exercise training programme or did not have complete data did not differ from those of the 32 participants analysed. Baseline characteristics of all 32 participants and these participants separated into pre-control (n=16) and post-control (n=16) groups are shown in Table 5.1. More males than females participated, 14 of the participants were overweight and 18 were obese. There were no differences in the baseline characteristics between the two control groups (Table 5.1).

5.4.2 General and some echocardiographic characteristics of community-based participants according to body mass index

Table 5.2 shows the general and some echocardiographic characteristics of the community-based sample grouped according to categories of BMI. Those participants with a BMI considered being in the overweight or obese range had a higher BP, more had hypertension and diabetes mellitus, and LVMI was increased in this group. Importantly, with or without adjustments for potential confounders overweight and obese individuals had a lower E/A, e' and e'/a' (Table 5.2). The mean E/A, e' and e'/a' in the participants recruited for the exercise training study (Table 5.1) were consistent with the E/A, e' and e'/a' values noted in overweight and obese individuals from the community-based study. Of the 32 participants in the exercise study 53% (n=17), 56% (n=18) and 56% (n=18) had E/A, e' and e'/a' values respectively that were below the lower 95% confidence intervals (<1.15, <10.4, <1.31) of participants in the community-based study that were lean and otherwise healthy (n=60).

Table 5.1 Baseline characteristics of all participants who completed a 6 week period of exercise training and had high quality echocardiographic data, and these participants separated into pre-control and post-control groups.

	All	Pre-control	Post-control	p-value
n	32	16	16	
Age (years)	44.6±6.1	44.9±4.5	44.2±5.7	=0.07
Female gender (n [%])	9 (35%)	4 (25%)	5 (31%)	=1.00
Height (cm)	173.6±9.9	173.3±8.2	174.3±10.5	=0.77
Weight (kg)	93.6±17.8	91.8±18.0	97.5±18.8	=0.39
Body mass index ($kg.m^{-2}$)	30.9±4.2	30.6±5.1	31.8±3.3	=0.44
Waist circumference (cm)	99.8±13.1	100.3±13.3	100.5±11.9	=0.96
Hip circumference (cm)	109.6±7.3	107.0±7.9	111.6±6.3	=0.08
% overweight/obese	34/66	38/62	31/69	=1.00
% prehypertensive/grade I hypertension	22/78	25/75	19/81	=1.00
Peak oxygen consumption ($ml.kg^{-1}.min^{-1}$)	27.4±4.9	28.1±5.5	27.2±5.1	=0.63
Systolic/Diastolic BP (mm Hg)	142±9/94±8	144±8/97±9	145±10/93±8	=0.76/0.19
Heart rate (bpm)	67.3±9.1	67.7±10.8	67.1±9.7	=0.43
E/A	1.21±0.30	1.25±0.31	1.18±0.29	=0.51
TDI e' ($cm.sec^{-1}$)	10.36±2.63	9.76±2.84	10.89±2.39	=0.23
TDI a' ($cm.sec^{-1}$)	8.45±2.51	7.90±1.71	8.93±3.01	=0.25
TDI e'/a'	1.21±0.29	1.19±0.27	1.23±0.32	=0.70
LVMI ($g.m^{-2.7}$)	30.3±6.6	29.2±7.0	31.2±6.3	=0.41
RWT	0.42±0.06	0.40±0.06	0.43±0.07	=0.18
EF (%)	61.4±9.3	61.5±8.5	61.4±10.2	=0.99

n, sample size; BP, blood pressure; E/A, early to late diastolic filling; TDI e'/a', tissue Doppler early to late diastolic filling; LVMI, left ventricular mass indexed to $height^{2.7}$; RWT, relative wall thickness; EF, ejection fraction.

Table 5.2 Characteristics of participants from a community-based sample grouped according body mass index (n=245).

Body mass index ($kg.m^{-2}$)	Lean <25	Overweight ≥25 and <30	Obese ≥30
n	89	53	103
Age (years)	32.9±16.1	43.1±19.8**	49.8±14.4***†
Female gender (n [%])	42.7	60.4**	87.4**
Weight (kg)	55.9±8.7	73.3±8.9***	92.7±15.2***†††
Body mass index ($kg.m^{-2}$)	20.9±2.5	27.8±1.2***	36.5±5.8***†††
Waist circumference (cm)	73.1±8.4	89.1±8.8***	105.4±14.3***†††
% Hypertension	23.6	47.2**	62.1**
Systolic/Diastolic BP (mm Hg)	120±19/80±10	128±21/84±9	132±26/85±15***/*
Heart rate (bpm)	65.4±10.6	67.3±11.0	71.3±12.9*
LVMI ($g.m^{-2.7}$)	30.7±9.3	37.1±13.7*	43.5±15.4***†
E/A	1.63±0.58	1.45±0.55	1.14±0.39***††
Multivariate adjusted# E/A	1.45±0.46	1.46±0.41	1.30±0.46*
TDI e' ($cm.sec^{-1}$)	13.52±3.39	11.75±4.06**	9.60±2.94***††
Multivariate adjusted# e'	12.34±3.11	11.81±2.77	10.62±3.15**†
TDI a' ($cm.sec^{-1}$)	7.34±2.60	7.75±3.12	8.98±3.11**†
TDI e'/a'	2.09±0.90	1.75±0.89*	1.23±0.65***††
Multivariate adjusted# e'/a'	1.80±0.72	1.76±0.64	1.48±0.72*†

n, sample size; BP, blood pressure; E/A, early to late diastolic filling; TDI e'/a', tissue Doppler early to late diastolic filling; LVMI, left ventricular mass indexed to $height^{2.7}$. #Adjustments are for age, sex, heart rate, systolic BP, and LVMI. *p<0.05, **p<0.005, ***p<0.0001 vs lean. †p<0.05, ††p<0.005, †††p<0.0001 vs overweight.

5.4.3 Exercise training effects on body weight, blood pressure, and peak oxygen consumption

Exercise training resulted in improvements in peak oxygen consumption and decreases in systolic and diastolic BP (Table 5.4). However, no changes in body weight or BMI were observed (Table 5.4).

5.4.4 Exercise training effects on left ventricular function and geometry

No changes in left ventricular diastolic or systolic function were observed after exercise training (Table 5.5). In addition, no changes in left ventricular geometry or mass were observed after exercise training (Table 5.5). Similarly, after adjustments for baseline BP and HR, no changes in LV function or geometry were observed (data not shown). Sensitivity analysis conducted in participants with an e' less than the lower 95% confidence interval for lean and otherwise healthy participants from a community sample, or in obese participants only, similarly showed no exercise training-induced effects on LV diastolic function despite evidence of improved cardiorespiratory fitness (Table 5.6). In the group of obese participants, the mean BMI was 33.5±2.6 $kg.m^{-2}$ and this did not change with exercise training (data not shown).

5.5 Discussion

The main findings of this study are that a 6-week period of exercise training, although resulting in an improved cardiorespiratory fitness and decreasing BP, failed to translate into improvements in indices of LV diastolic function, including TDI measurements, in overweight and obese individuals, a high proportion of whom had abnormalities in LV diastolic function at baseline. Moreover, in sensitivity analysis conducted in only obese participants or in participants with an abnormal e' identified from the lower 95% confidence intervals of e' observed in lean, healthy participants from a community sample, exercise training similarly failed to modify TDI measures of diastolic function.

Table 5.3 Hemodynamic variables, and left ventricular (LV) geometry and function after exercise (Ex) training or a control (Con) period in participants separated into continuous versus interrupted exercise groups.

	Continuous Ex (n=18)			Interrupted Ex (n=14)			p-values for comparisons					
							Continuous Ex			Interrupted Ex		
	Baseline	After Con Period	After Ex Training	Baseline	After Con Period	After Ex Training	Con vs Baseline	After Ex vs Con	After Ex vs Baseline	Con vs Baseline	After Ex vs Con	After Ex vs Baseline
Systolic BP (mm Hg)	141±10	144±9	132±10	146±7	148±11	135±13	=0.64	<0.005	<0.05	=0.75	<0.01	<0.05
Diastolic BP (mm Hg)	93±7	92±9	83±14	98±9	97±11	88±9	=0.99	<0.05	<0.05	=0.97	<0.05	<0.05
Peripheral PP (mm Hg)	48±10	51±8	48±10	48±7	54±12	49±11	=0.91	=0.85	=0.98	=0.36	=0.51	=0.95
Heart rate (beats.min^{-1})	65±8	69±9	68±9	70±10	73±11	69±11	=0.45	=0.98	=0.58	=0.67	=0.57	=0.98
E (cm.sec^{-1})	97.7±35.1	93.5±29.3	103.1±32.3	103.9±29.7	86.6±27.3	106.4±33.7	=0.92	=0.65	=0.87	=0.32	=0.23	=0.98
A (cm.sec^{-1})	78.5±21.5	77.7±23.1	79.8±23.7	87.8±14.4	77.3±18.5	85.8±23.0	=0.99	=0.96	=0.98	=0.35	=0.50	=0.96
E/A	1.25±0.31	1.22±0.25	1.31±0.27	1.19±0.28	1.24±0.31	1.26±0.32	=0.92	=0.58	=0.81	=0.88	=0.98	=0.80
TDI e' (cm.sec^{-1})	10.38±2.51	11.11±2.79	9.94±2.74	10.34±2.99	10.52±2.37	11.02±2.36	=0.69	=0.39	=0.87	=0.98	=0.87	=0.78
TDI a' (cm.sec^{-1})	8.75±2.81	8.66±2.30	7.95±1.94	7.90±2.07	7.80±1.85	8.27±1.64	=0.99	=0.64	=0.58	=0.99	=0.81	=0.87
TDI e'/a'	1.28±0.41	1.33±0.34	1.29±0.38	1.34±0.37	1.38±0.30	1.36±0.26	=0.93	=0.95	=1.00	=0.94	=0.98	=0.99
TDI E/e'	10.42±5.67	8.94±3.57	11.27±5.13	10.47±3.53	9.36±3.61	9.93±3.49	=0.64	=0.33	=0.86	=0.70	=0.91	=0.92
LVEDD (cm)	4.46±0.41	4.34±0.41	4.49±0.43	4.52±0.52	4.15±0.43	4.43±0.57	=0.68	=0.52	=0.97	=0.18	=0.35	=0.91
MWT (cm)	0.95±0.09	0.91±0.07	0.89±0.10	0.84±0.07	0.84±0.05	0.85±0.07	=0.33	=0.87	=0.13	=0.98	=0.81	=0.90
RWT	0.44±0.07	0.43±0.06	0.41±0.07	0.38±0.05	0.42±0.04	0.40±0.06	=0.85	=0.75	=0.42	=0.22	=0.53	=0.81
LVM (g)	141.1±24.3	127.5±24.6	130.9±19.8	124.9±33.3	117.1±21.8	123.2±30.3	=0.19	=0.90	=0.38	=0.27	=0.34	=0.99
LVMI (g.m$^{-2.7}$)	31.9±5.8	28.9±6.0	29.7±5.9	27.7±7.3	24.7±4.5	27.0±5.4	=0.28	=0.90	=0.51	=0.21	=0.34	=0.95
EF (%)	61.5±8.6	58.5±12.8	65.5±9.9	60.7±10.5	56.9±9.9	56.3±11.6	=0.68	=0.12	=0.32	=0.65	=0.99	=0.56
FS (%)	33.2±6.6	31.5±9.7	36.3±8.4	32.8±7.4	29.9±6.8	29.9±8.6	=0.81	=0.19	=0.49	=0.61	=1.00	=0.61
mFS (%)	22.1±4.6	21.6±7.8	25.1±7.8	23.1±7.1	20.3±5.5	20.2±6.3	=0.98	=0.26	=0.36	=0.51	=1.00	=0.49
TDI s' (cm.sec^{-1})	8.09±1.48	8.87±1.92	8.51±1.56	8.30±1.25	8.57±1.75	8.67±1.33	=0.35	=0.79	=0.73	=0.88	=0.98	=0.80

Table 5.4 Anthropometry, fitness, BP and heart rate before versus after exercise training or a control period in overweight and obese participants (n=32).

				p-values for comparisons		
	Baseline	After Con Period	After Ex Training	After Con vs Baseline	After Ex vs after Con	After Ex vs Baseline
Weight (kg)	93.6±17.9	92.3±18.3	93.2±17.6	=0.97	=0.98	=0.99
BMI ($kg.m^{-2}$)	30.9±4.2	30.6±5.1	30.8±4.2	=0.98	=0.99	=0.99
VO_{2peak} ($ml.kg^{-1}.min^{-1}$)	27.4±4.9	-	29.4±5.8	-	-	=0.0001
Systolic BP (mm Hg)	142±9	145±9	135±11	=0.44	<0.0005	<0.01
Diastolic BP (mm Hg)	94±8	93±10	86±12	=0.89	<0.05	<0.01
Peripheral PP (mm Hg)	48±8	53±13	49±11	=0.24	=0.37	=0.94
Heart rate (bpm)	67±9	71±10	69±10	=0.37	=0.69	=0.86

BMI, body mass index; VO_{2peak}, Peak oxygen consumption; BP, blood pressure; PP, pulse pressure.

Table 5.5 Effect of a 6 week period of exercise training (Ex training) and a 6 week control period (Con) on left ventricular diastolic and systolic function, mass, and dimensions in overweight and obese individuals (n=32).

				p-values for comparisons		
	Baseline	After Con Period	After Ex Training	After Con vs Baseline	After Ex vs after Con	After Ex vs Baseline
E ($cm.sec^{-1}$)	98.7±33.2	89.8±28.2	104.9±32.0	=0.49	=0.13	=0.71
A ($cm.sec^{-1}$)	81.6±19.3	76.9±20.9	83.4±23.6	=0.65	=0.44	=0.94
E/A	1.21±0.30	1.22±0.26	1.28±0.28	=0.99	=0.70	=0.61
TDI e' ($cm.sec^{-1}$)	10.36±2.63	10.98±2.63	10.49±2.62	=0.62	=0.74	=0.98
TDI a' ($cm.sec^{-1}$)	8.45±2.51	8.32±2.10	8.21±1.90	=0.97	=0.98	=0.90
TDI e'/a'	1.30±0.39	1.36±0.32	1.31±0.33	=0.75	=0.85	=0.98
TDI E/e'	10.26±4.81	8.96±3.60	10.65±4.44	=0.46	=0.26	=0.93
LVEDD (cm)	4.47±0.45	4.25±0.42	4.47±0.48	=0.13	=0.13	=1.00
MWT (cm)	0.90±0.09	0.88±0.07	0.88±0.09	=0.45	=0.99	=0.39
RWT	0.42±0.06	0.43±0.05	0.41±0.07	=0.86	=0.37	=0.69
LVM (g)	133.6±28.9	120.0±24.9	128.0±24.2	=0.09	=0.42	=0.67
LVMI ($g.m^{-2.7}$)	30.3±6.6	27.1±5.9	29.0±6.2	=0.13	=0.50	=0.68
EF (%)	61.4±9.3	57.5±11.5	62.7±11.7	=0.32	=0.14	=0.88

E, early transmitral velocity; A, late (atrial) transmitral velocity; TDI e', tissue Doppler imaging of myocardial lengthening during the early filling period of diastole; TDI a', tissue Doppler imaging of myocardial lengthening during atrial contraction; LVEDD, left ventricular end diastolic diameter; MWT, mean wall thickness; RWT, relative wall thickness; LVM, left ventricular mass; LVMI, left ventricular mass indexed to $height^{2.7}$; EF, ejection fraction.

Table 5.6 Effect of a 6 week period of exercise training (Ex training) and a 6 week control period (Cont) on cardiorespiratory fitness and left ventricular diastolic function as assessed from tissue Doppler imaging in obese individuals only (n=21) or in participants with a reduced e' (e' below the lower 95% confidence interval for lean and otherwise healthy participants from a community sample) (n=18).

				p-values for comparisons		
	Baseline	After Con Period	After Ex Training	After Con vs Baseline	After Ex vs after Con	After Ex vs Baseline
			Obese participants only (n=21)			
VO_{2peak} ($ml.kg^{-1}.min^{-1}$)	26.5±4.8	-	28.3±6.0	-	-	<0.01
TDI e' ($cm.sec^{-1}$)	10.38±2.62	11.18±2.51	10.46±3.03	=0.61	=0.67	=0.99
TDI a' ($cm.sec^{-1}$)	8.91±2.84	8.68±2.12	8.64±2.10	=0.95	=1.00	=0.93
TDI e'/a'	1.26±0.30	1.33±0.30	1.25±0.25	=0.86	=0.78	=0.99
TDI E/e'	9.32±4.52	8.30±2.77	11.94±4.96	=0.72	=0.11	=0.43
			Participants with a reduced e' only (n=18)			
VO_{2peak} ($ml.kg^{-1}.min^{-1}$)	26.4±4.4	-	28.6±5.0	-	-	=0.005
TDI e' ($cm.sec^{-1}$)	9.06±1.01	10.24±2.82	10.27±2.22	=0.65	=0.99	=0.68
TDI a' ($cm.sec^{-1}$)	7.66±2.68	8.24±2.32	8.02±2.11	=0.75	=0.96	=0.89
TDI e'/a'	1.22±0.42	1.28±0.32	1.33±0.34	=0.88	=0.91	=0.64
TDI E/e'	12.73±4.75	10.04±3.98	10.39±4.74	=0.19	=0.97	=0.28

VO_{2peak}, peak oxygen consumption; TDI e', tissue Doppler imaging of myocardial lengthening during the early filling period of diastole; TDI a', tissue Doppler imaging of myocardial lengthening during atrial contraction; E, transmitral velocity during the early diastolic period.

The results of this study contribute toward our knowledge of the impact of exercise training on LV diastolic function as assessed from TDI in overweight or obese individuals. In this regard, the present results are consistent with two studies that have failed to show an improvement in TDI measures of diastolic function with exercise training in overweight and obese individuals (Riordan et al 2008, Guirado et al 2012). However, one of these studies was conducted in elderly hypertensives receiving treatment, where the confounding effects of age and treatment for hypertension cannot be excluded (Guirado et al 2012), and one study was conducted only in overweight and not obese individuals (Riordan et al 2008). In this regard, as noted in the community study sampled, LV diastolic function is considerably worse in obese as compared to overweight individuals, and in the exercise component of the present study the mean values for LV diastolic function were intermediate between overweight and obese participants. Moreover, both prior studies that failed to show an effect of exercise training on TDI indices of diastolic function in overweight or obese individuals employed small study samples (n=13-15) which raises the question of false negative results. In contrast, in the present study I employed a comparatively larger study sample (n=32).

The lack of benefit of exercise training on TDI measures of diastolic function in overweight or obese individuals in the present and prior (Guirado et al 2012, Riordan et al 2008) studies is in contrast to improvements in e' noted in three prior studies (Schuster et al 2012, Kosmala et al 2009, Wong et al 2006). However, in one study the effects were examined only in men (Schuster et al 2012), and in the other two studies the intervention involved both exercise and dietary changes (Kosmala et al 2009, Wong et al 2006), with decreases in body weight accompanying these changes. In contrast, in the present and previous studies (Guirado et al 2012, Riordan et al 2008) that failed to show an exercise training effect on e', these studies did not involve a dietary intervention and in the present and one prior study (Guirado et al 2012) no changes in body weight were noted. Moreover, in one prior study showing beneficial effects of exercise training on e', the study sample was small (n=10) (Schuster et al 2012) raising the question of a possible false positive finding, and in another study (Wong et al 2006) analysis was performed after *post hoc* assignment to

groups according to whether weight loss either did or didn't occur, an obviously flawed methodology.

It may be argued that the lack of effect of the exercise intervention on TDI indices of diastolic function in some studies (Guirado et al 2012, Riordan et al 2008), including the present study may be attributed to a limited proportion of individuals recruited with abnormal baseline LV diastolic function. In this regard the one study that has reported on improvements in TDI indices of diastolic function after exercise training alone (not combined with a dietary intervention) in obese individuals (Schuster et al 2012) had mean baseline values of e' (6.5±0.4 $cm.sec^{-1}$) that were much lower than the mean values noted in the present study (10.36±0.46 $cm.sec^{-1}$) and in prior studies (Guirado et al 2012, Riordan et al 2008)(9.10±3.60 and 10.1 $cm.sec^{-1}$ respectively) that failed to show a beneficial effect of exercise training. However, the mean baseline e' values noted in the present study were consistent with what would be noted in overweight and obese individuals in the community at large. Thus this study is representative of what one would note in overweight and obese individuals generally. Nevertheless, the improvements in e' noted by Schuster et al (2012) may represent changes noted in advanced levels of obesity. Indeed, the mean (SD) BMI in obese participants in that study (Schuster et al 2012) was 33.6±1.0 $kg.m^{-2}$. However, in this study sensitivity analysis conducted in only obese participants (with a mean BMI of 33.5±2.6 $kg.m^{-2}$) or in those with a reduced e' at baseline, similarly failed to show beneficial effects of exercise training on TDI measures of diastolic LV function including e'.

Consistent with prior studies (Reid et al 1994, Sadaniantz et al 1996, Stewart et al 2006, Baynard et al 2008, Riordan et al 2008, Kosmala et al 2009, Eriksson et al 2010, Cocco and Pandolfi 2011, Guirado et al 2012, Schuster et al 2012) in the present study I show that in overweight or obese individuals LV diastolic function as assessed from early (E)-to-late (atrial-A) transmitral velocity measurements are unchanged by exercise training. In this regard, I am unaware of any studies that have demonstrated alterations in E/A ratios with exercise training in the overweight or obese. This may suggest that exercise training is unable to produce beneficial effects on obesity-induced decreases in E/A noted at a

community level (Libhaber et al 2009, chapter 4). However, it is likely that short-term exercise training is most likely to influence more sensitive measures of diastolic function in the initial instance. In this regard, e' is one of the earliest markers of diastolic dysfunction (Schillaci et al 2002, Fischer et al 2003, Willens et al 2004, Chahal et al 2010). However, the present study suggests that in obese individuals with e' values that reflect changes in e' in obese individuals in the community at large, exercise training is unable to influence even this sensitive marker.

The limitations of this study include the selection of participants based on the presence of overweight or obesity rather than on additional criteria. As indicated in chapter 4, a possible mechanism of diastolic dysfunction is insulin resistance and hence, pre-selection of those with a raised HOMA-IR may have improved the chances of showing a beneficial effect of exercise training on diastolic dysfunction. This would nevertheless have introduced a selection bias with respect to the hypothesis tested. Second, although the aim of the study was to assess the effects of exercise training alone on TDI indices of diastolic function, an additional group with both diet and exercise designed to achieve weight loss may have caste further light on the role of exercise in obesity-associated LV diastolic dysfunction. Further studies are warranted in this regard.

In conclusion, to the best of my knowledge, this study is the first study, not markedly limited by study sample size and in otherwise healthy individuals, which shows that exercise training alone, despite producing an improvement in cardiorespiratory fitness is unable to improve obesity-associated TDI indices of LV diastolic dysfunction.

CHAPTER 6

SUMMARY AND CONCLUSION

6.1 Introduction

Cardiovascular disease is currently one of the greatest contributors to death in South Africa (Statistics South Africa 2010). One of the major risk factors contributing to cardiovascular disease is obesity. Although the association between obesity and increased risk for cardiovascular disease may be explained by the fact that obese persons are at higher risk for developing hypertension and dyslipidaemia (Hall 1997, Must et al 1999, Uzu et al 2006, Forman et al 2009, Kotchen 2010); obesity is independently associated with increased risk for cardiovascular events (Eckel et al 2002, Klein et al 2004, Murphy et al 2006, Poirier et al 2006) including heart failure (Chen et al 1999, He et al 2001, Kenchaiah et al 2002, Ingelsson et al 2005, Nicklas et al 2006, Bahrami et al 2008, Spies et al 2009), myocardial infarction (Yusuf et al 2005, Steyn et al 2005) and stroke (Kurth et al 2002, Suk et al 2003). Although the obvious solution to preventing cardiovascular disease associated with obesity is to reduce body weight it is well known that most people find it extremely difficult to decrease and maintain weight by sustaining a healthy lifestyle including weight reduction and physical activity (The Trials of Hypertension Prevention Collaborative Research Group 1997, Weiss et al 2007, Aucott et al 2009, Hedayati et al 2011, Jordan et al 2012).

Hence in order to institute effective therapeutic strategies, an understanding of the mechanism of obesity-associated cardiovascular risk is required. Therefore in the present thesis I conducted a series of studies designed to advance our understanding of the role of obesity associated insulin resistance in contributing toward alterations in brachial and central aortic BP attributed to salt-intake; and LV diastolic dysfunction at a community level. Bearing in mind the association between obesity and a sedentary lifestyle, I explored the possibility that in the absence of weight loss, short term exercise training that increases cardiorespiratory fitness may attenuate central aortic pressure augmentation and improve LV diastolic dysfunction.

Hence in the following sections, in the context of obesity I will first summarise the impact of obesity and insulin resistance on BP, including central aortic BP as well as the

impact of exercise related improvements in aerobic capacity without weight loss on large artery properties. Second I will highlight the effects of obesity and insulin resistance on LV diastolic function as well as the impact of exercise training without weight loss on obesity-related changes in LV diastolic function.

6.2 Obesity, blood pressure and arterial characteristics

There is considerable evidence in large studies that obesity contributes to the development of hypertension (The Trials of Hypertension Prevention Collaborative Research Group 1997, He et al 2000, Harris et al 2000, Neter et al 2003, Zhu et al 2005, Straznicky et al 2010, Hedayati et al 2011). Although brachial BP has been used in the prediction of cardiovascular risk over a number of decades, more recent evidence has shown that central BP may be a better predictor of cardiovascular damage or events than brachial BP (Nichols et al 2011). In this regard, although there are some opposing views (Dart et al 2006, Mitchell et al 2010), most research shows that central aortic BP, and the determinants thereof, are associated with cardiovascular damage (Roman et al 2007, 2010, Pini et al 2008, Wang et al 2009, Norton et al 2012) and are predictors of cardiovascular risk independent of brachial BP (Safar et al 2002, Chirinos et al 2005, Williams et al 2006, Roman et al 2007, Pini et al 2008, Jankowski et al 2008, Wang et al 2009). If central BP more accurately reflects cardiovascular risk, what is the role of obesity in increasing central BP?

A number of studies have shown a relationship between obesity and aortic BP and /or determinants thereof (Resnick et al 1997, Sutton-Tyrrell et al 2001, Mackey et al 2002, Wildman et al 2003, Danias et al 2003, Ferreira et al 2004, Snijder et al 2004, Toto-Moukouo et al 1986, Majane et al 2008), whilst others have shown no relationship independent of traditional risk factors and haemodynamic factors (Taquet et al 1993, Amar et al 2001, Nakanishi et al 2003, Oren et al 2003, Mitchell et al 2004, Ferreira et al 2004, Zebekakis et al 2005, Czernichow et al 2005). Furthermore most of these studies have assessed the effect

of obesity on arterial stiffness without indicating whether this translates into increases in central aortic BP.

In order to understand the impact of obesity on both peripheral and central aortic BP it is important to understand the mechanisms involved. One possible mechanism that has been suggested is the influence of obesity on the impact of salt intake on BP. A number of studies have demonstrated that obesity or the metabolic syndrome is associated with renal tubular handling of Na^+ and an increased sensitivity of BP to Na^+ intake in European individuals (Rocchini et al 1989, Hall et al 1997, Strazzullo et al 2001, 2006, Barbato et al 2004, Uzu et al 2006, Hoffmann et al 2008, Chen et al 2009) and that insulin resistance may play a role in this interaction (Sharma et al 1991, Endre et al 1994, Shimamoto et al 1994, Zavaroni et al 1995, Bigazzi et al 1996, Galletti et al 1997, Fuenmayor et al 1998, Sechi et al 1999, Yatabe et al 2010). However this relationship is uncertain in persons of African ancestry, even though it is well know that persons of African ancestry have a high prevalence of salt sensitive hypertension (Weinberger et al 1986, He et al 1998, He et al 2000, Vollmer et al 2001, Wright et al 2003, Aviv et al 2004) and that salt intake is related to central aortic PP independent of brachial BP in this population (Redelinghuys et al 2010). Therefore, in the present thesis in chapter 2, I evaluated whether obesity or insulin resistance is independently associated with the relationship between urinary salt excretion and either conventional, 24-hour or central aortic BP, or the aortic wave characteristics in a community sample of African ancestry with a high prevalence of obesity.

In 331 untreated hypertensives from a South African community sample of black African descent I demonstrated that there was no independent relationship between insulin resistance and BP, but that the interaction between insulin resistance and urinary sodium excretion contributed to the variability in BP across increasing levels of insulin resistance. However, neither aortic augmentation pressure, AIx, central aortic pulse pressure, nor aortic PWV were independently associated with an interaction between insulin resistance and salt intake. Hence in participants with greater insulin resistance there was a relationship between urinary sodium excretion as an index of salt intake and conventional and ambulatory BP but

not for central aortic BP or markers of arterial stiffness. This is in agreement with prior studies performed in participants of European ancestry showing that the metabolic syndrome and insulin resistance contribute toward salt-sensitivity (Strazzullo et al 2001, 2006, Barbato et al 2004). However, to my knowledge this is the first study to show this relationship in individuals of African ancestry, an ethic group that is believed to have a high prevalence of insulin resistance and salt sensitivity (Weinberger et al 1986, Vollmer et al 2001, He et al 1998, 2000, Wright et al 2003, Aviv et al 2004). These results suggest that although not independently associated with BP, insulin resistance may mediate an increase in conventional BP via the relationship with salt sensitivity in groups of African descent. These effects of insulin resistance cannot be accounted for by actions on central aortic haemodynamics. Hence, by improving insulin resistance in obese individuals the prevalence of salt sensitive hypertension may be reduced.

Obesity is associated with a sedentary lifestyle and both obesity and inactivity contribute toward increases in BP (Harris et al 2000, Neter et al 2003, Zhu et al 2005, Hedayati et al 2011). Indeed, systematic reviews have demonstrated decreases in brachial BP after exercise training (Cornelissen and Fagard 2005, Fagard 2006, Fagard and Cornelissen 2007). Although there is increasing evidence that central aortic BP predicts cardiovascular outcomes more closely or independent of BP measured at the brachial artery (Safar et al 2002, Chirinos et al 2005, Williams et al 2006, Roman et al 2007, Pini et al 2008, Jankowski et al 2008, Wang et al 2009), the role of exercise as an aortic BP-lowering intervention in obesity is uncertain. Although exercise may reduce artic stiffness, one of the determinants of aortic BP, there is limited evidence to support a role for the beneficial effect of exercise training on aortic augmentation pressures or indices. Two positive studies were conducted in small sample sizes (Edwards et al 2004, Liu et al 2012), whereas in another slightly larger study no effect of exercise training was observed (Nualnim et al 2012). Hence in the present thesis in chapter 3, I aimed to evaluate the extent to which exercise training-induced decreases in brachial BP may be attributed to modifications in aortic augmentation pressures or indices (AIx) in overweight or obese individuals.

This study was conducted in previously sedentary, middle-aged, overweight or obese, mild-to-moderate hypertensives. In 35 participants I demonstrated that six weeks of aerobic exercise training was successful in increasing aerobic capacity and decreasing peripheral and central systolic BP. However there were no changes in peripheral or central AIx or augmentation pressure and the changes in central systolic BP could be accounted for by decreases in MAP. Hence in overweight or obese individuals, although short-term aerobic exercise training which improved cardiorespiratory fitness, may produce marked decreases in aortic and brachial BP, these effects are not attributed to alterations in aortic systolic pressure augmentation or aortic stiffness. This is in agreement with a previous study showing no change in AIx after a period of short-term exercise training (Nualnim et al 2012). In contrast a few studies have shown decreases in aortic AIx in various populations (Edwards et al 2004, Tabara et al 2007, Mustata et al 2012, Liu et al 2012). However even though it is well known that distending pressures are one of the main determinants of systolic pressure augmentation, none of the above mentioned studies accounted for changes in MAP after exercise training. The present results suggest that the marked decreases in BP that may accompany exercise training over relatively short periods in overweight and obese individuals are unlikely to be mediated through an attenuation of systolic pressure augmentation. As augmentation indices are associated with cardiovascular events independent of conventional cardiovascular risk factors in a variety of clinical populations (Saba et al 1993, London et al 2001, Nürnberger et al 2002, Hayashi et al 2002, Weber et al 2004, Ueda et al 2004, Weber et al 2005, Chirinos et al 2005, Williams et al 2006, Hashimoto et al 2007), alternative approaches to exercise training, at least in the short-term period, may be required to modify systolic pressure augmentation. Whether long-term exercise training may produce benefits on aortic or peripheral systolic pressure augmentation in overweight or obese individuals requires further study.

6.3 Obesity and left ventricular diastolic function

A number of studies have shown a relationship between obesity and LV diastolic dysfunction (Redfield et al 2003, Fischer et al 2003, Peterson et al 2004, Wong et al 2004 Powell et al 2006, Tsioufis et al 2008, Ammar et al 2008, Libahber et al 2009, Russo et al 2011). One potential mechanism that may explain the abnormalities in cardiac function associated with obesity is insulin resistance, independent of the presence of diabetes mellitus. However current data are controversial. Although a number of studies have shown a relationship between insulin resistance and abnormal LV diastolic function (Lind et al 1995, Kamide et al 1996, Galderisi et al 1997, Watanabe et al 1999, Wong et al 2004, Bajraktari et al 2006, Dinh et al 2010, Sliem and Nasr 2011, Utz et al 2011, Hwang et al 2012), many studies have shown no relationship between insulin resistance and LV diastolic function (Mureddu et al 1998, Olsen et al 2003, Leichman et al 2006, Lambert et al 2010, Wada et al 2010, Fox et al 2011, Wu et al 2012). However most of these studies were conducted in select clinical samples and with small sample sizes and hence may have been false positive or false negative results. Only two large studies have been conducted (Fox et al 2011, Hwang et al 2012). One failed to show a relationship and in the other the relationship was not adjusted for adiposity indices. Hence in the present thesis in chapter 4, I evaluated whether an index of insulin resistance is associated with LV diastolic function independent of adiposity indices in a relatively large, randomly selected community-based sample with a high prevalence of obesity.

I demonstrated in a community sample of African ancestry (n = 361), that LV diastolic function was associated with insulin resistance and that the relationship was independent of the confounders waist circumference, age, sex, conventional diastolic or systolic BP, diabetes mellitus or an HbA1c>6.1%, regular tobacco use, regular alcohol intake and pulse rate. Furthermore I showed that even after adjusting for LVM and RWT (as markers of concentric remodeling), the relationship remained significant. Also in a multivariate regression analysis insulin resistance and BP had a similar independent relationship with

diastolic function. However, there was no significant relationship between waist circumference and diastolic function. To the best of my knowledge this study is the first to provide evidence in a relatively large, randomly selected sample that insulin resistance is associated with an abnormal LV diastolic function independent of adiposity indices and concentric remodeling. Hence, insulin resistance might explain the relationship between obesity and the development of LV diastolic dysfunction independent of traditional measures of obesity and concentric remodeling. This illustrates that insulin resistance may have a direct impact on the heart that is not mediated via traditional risk factors and presents a potential for the development of therapeutic strategies targeting insulin resistance to prevent the transition to heart failure in obesity.

As obesity is a strong determinant of abnormalities in LV diastolic function at a community level (Redfield et al 2003, Fischer et al 2003, Powell et al 2006, Amar et al 2008, Tsioufis et al 2008, Libhaber et al 2009, Russo et al 2011), there is considerable interest in developing approaches that may prevent or reverse obesity-associated LV diastolic dysfunction. Currently, however, the role of exercise training in modifying obesity-associated LV diastolic dysfunction is uncertain. Despite evidence of weight loss (Reid et al 1994, Stewart et al 2006, Riordan et al 2008, Kosmala et al 2009, Cocco and Pandolfi 2011) or an improved cardiorespiratory fitness (Reid et al 1994, Sadaniantz et al 1996, Sewart et al 2006, Riordan et al 2008, Baynard et al 2008, Kosmala et al 2009, Schuster et al 2012, Guirado et al 2012), the majority of studies suggest that in overweight or obese individuals LV diastolic function as assessed from early-to-late transmitral velocity measurements are unchanged by exercise training. In addition, there is also evidence to suggest that TDI indices of LV diastolic function are unaffected by exercise training (Riordan et al 2008, Guirado et al 2012). Nevertheless, studies assessing the impact of exercise training on diastolic function as determined from TDI in overweight or obese individuals, either employed exercise together with dietary approaches (Wong et al 2006, Gondoni et al 2007, Kosmala et al 2009); assessed effects in the elderly treated for hypertension (Guirado et al 2012); had remarkably small sample sizes (Riordan et al 2008, Schuster et al 2012); evaluated the effects in men

only (Schuster et al 2012) or performed analysis after assigning participants *post hoc* to weight loss or no weight loss groups (Wong et al 2006). Hence in chapter 5 of my thesis, I assessed the impact of exercise training alone on indices of LV diastolic function, including TDI, in a substantially larger study sample of young-to-middle aged overweight and obese individuals, not receiving antihypertensive therapy and in participants of both sexes.

In this study I demonstrated, that even though a short-term exercise training programme was sufficient to improve aerobic capacity in 32 overweight and obese individuals; no improvements in either pulsed Doppler or TDI measures of LV diastolic function were observed even though a high proportion had abnormalities in LV diastolic function at baseline. This is consistent with prior studies that have shown no changes in TDI indices of LV diastolic function with exercise training in overweight and obese individuals (Riordan et al 2008, Guirado et al 2012); however both these studies were conducted in very small sample sizes which may have confounded the results. In contrast two other studies have shown improvements in diastolic function after exercise training, however these studies employed a dietary and exercise intervention and hence the results could have been confounded by the accompanying weight loss. This implies that exercise-induced improvements in aerobic capacity are insufficient in improving obesity-associated decreases in TDI indices of LV diastolic function. Hence, it is unlikely that exercise *per se* explains overweight and obesity-associated decreases in LV diastolic function.

6.4 Strengths and limitations

Although the strengths and limitations of each of the studies have been highlighted in the respective chapters, I would like to highlight some general strengths and limitations of the thesis. Generally the strengths of these studies included the random recruitment of participants and the large sample sizes (n>300) in the cross sectional studies. Furthermore some of the aspects highlighted in the cross sectional data were investigated in intervention studies. One of the major limitations of the intervention study, however, was the short

duration of the intervention. Nevertheless studies of shorter duration (4 weeks or less) have shown significant decreases in BP, however a longer duration might have been more effective. Another potential limitation was the use of a generalized transfer function to estimate central aortic pressures. However it has been reported that central BP can be accurately estimated with this technique (Chen et al 1997).

6.5 Conclusions and future studies

In conclusion, evidence presented in the present thesis clarifies some unresolved matters regarding the mechanisms of obesity-associated cardiovascular risk. In this regard I have shown in cross sectional studies that obesity related insulin resistance affects the association between salt sensitivity and BP. Furthermore insulin resistance independently of measures of obesity affects LV diastolic function. In an exercise intervention study I have shown that an increased aerobic capacity as a result of short term aerobic exercise training without weight loss is insufficient to alter either large artery properties or LV diastolic function. The relationships between insulin resistance and BP and cardiac function were shown in cross sectional studies and hence causality cannot be inferred. Furthermore, in the exercise intervention studies we did not measure insulin resistance.

The result of these studies raise a number of possibilities for future studies, which could include the following: in order to assess whether insulin resistance indeed accounts for the BP effects of salt intake in groups of African descent, a proof-of-principle study should be performed to evaluate the impact of insulin resistance on BP responses to salt (Na^+) loading and diuretic therapy in participants of African ancestry. If in the intervention study this relationship can be confirmed, future studies should evaluate the impact of insulin-sensitising agents on changes in BP responses to a Na^+ load. To further explore the possible impact of exercise training on obesity-associated LV diastolic dysfunction, participants with insulin resistance should be recruited for an exercise training study.

Another approach to test the hypothesis that insulin resistance influences the effect of salt intake on BP and LV diastolic function independent of obesity in groups of African ancestry is to assess the impact of non-drug approaches (exercise and diet) that improve insulin resistance on the BP response to a salt load and on LV diastolic function. As these approaches (diet and exercise) could reduce BP responses to a salt load and cardiac function through mechanisms unrelated to insulin resistance, the interpretation of data obtained from such a study will nevertheless be difficult. However as lifestyle interventions are key to the management of obesity and hypertension related diseases, these studies are well worth performing. Furthermore as hypertension and obesity are major causes of cardiovascular morbidity and mortality in South Africa, identifying population-wide healthcare strategies to reduce this burden of disease is a major priority area of research. However, it will most likely require a dedicated, enthusiastic effort of combining multiple lifestyle changes as a single lifestyle change for a short duration will not have a significant impact on cardiovascular health.

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

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

CLEARANCE CERTIFICATE	**M1204108**
PROJECT	Gene Candidates as Determinants of Blood Pressure and Intermediary Phenotypes in Pathogenesis of Hypertension in Black South Africans (Previously M020472 and M070469)
INVESTIGATORS	Prof A/G Woodiwiss/Norton.
DEPARTMENT	School of Physiology
DATE CONSIDERED	Ad hoc
DECISION OF THE COMMITTEE*	Renewal Approved

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

DATE 2012/05/18 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

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

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

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

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

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Ms Aletta Esterhuyse

CLEARANCE CERTIFICATE	**M10624**
PROJECT	The Effect of Exercise Training on the Acute Hypotensive Response and Arterial Stiffness in Mildly Hypertensive Hman Subjects
INVESTIGATORS	Ms Aletta Esterhuyse.
DEPARTMENT	School of Physiology
DATE CONSIDERED	25/06/2010
DECISION OF THE COMMITTEE*	Approved unconditionally

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

DATE 02/08/2010 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

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

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UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

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

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

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

DATE 07.05.09 **CHAIRPERSON** .....

(Professors PE Cleaton-Jones, A Dhai, M Vorster, C Feldman, A Woodiwiss)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Woodiwiss A Prof

DECLARATION OF INVESTIGATOR(S)

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UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

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

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

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

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

DEPARTMENT School of Physiology, Wits Medical School

DATE CONSIDERED 02-04-26

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

DECISION OF THE COMMITTEE *

Approved unconditionally

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

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

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

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

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