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IMPACT OF EXCESS ADIPOSITY ON BLOOD PRESSURE AND CARDIOVASCULAR TARGET ORGAN DAMAGE

Olebogeng Harold Isaia Majane

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

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ABSTRACT

Epidemiological trends suggest that obesity is becoming a major public health problem. Although obesity contributes toward cardiovascular risk by promoting the development of hypertension, dyslipidaemia and diabetes mellitus (conventional risk factors), there is increasing evidence to suggest that excess adiposity may increase risk through effects on cardiovascular target organs that are independent of conventional risk factors. These obesity-induced effects may be produced by mediating damage and dysfunction of large vessels and the heart, and by promoting the development of cardiac hypertrophy. However, the independent effect of excess adiposity on large vessels has not been confirmed in all studies. Moreover, whether the impact of excess adiposity on cardiac hypertrophy or cardiac damage and dysfunction is dependent on an interaction with blood pressure (BP) is uncertain. In the present thesis I addressed these questions.

Before evaluating these questions I first identified the preferred clinical index of adiposity when predicting BP. In this regard, some, but not all studies support the notion that indexes of central adiposity (waist circumference or waist-to-hip ratio) are the preferred predictors of conventional BP over indexes of general (body mass index) or subcutaneous (skin-fold thickness) adiposity. Moreover, to my knowledge no study has been conducted in a large study sample to evaluate whether indexes of central adiposity are the preferred predictors of ambulatory BP, a measure of BP that is more closely associated with cardiovascular events than conventional BP. In the first study conducted in a relatively large, randomly selected population sample (n=300) with a high prevalence of excess adiposity (65%), I demonstrated that waist circumference is the only clinical index of adiposity that is associated with an increased conventional and ambulatory systolic and diastolic BP, independent of other indexes of adiposity.

With regards to the effects of excess adiposity on large arteries, there is inconsistency in the reports demonstrating relations between indexes of adiposity and large artery dysfunction (arterial stiffness) independent of factors such as BP, heart rate and diabetes mellitus. As convincing independent relations between clinical indexes of adiposity and arterial stiffness have been noted in older, but not in younger populations, I hypothesized that age may determine whether excess adiposity promotes increases in arterial stiffness independent of confounders. Indeed, in 508 randomly selected persons from a population sample with a high prevalence of excess adiposity (~63% overweight or obese), I was able to show that age markedly influenced the independent relationship between indexes of central adiposity and an index of large artery stiffness in women but not in men after adjusting for confounders. The adjusted effect of indexes of central obesity on arterial stiffness was ~5-fold higher in older than in younger women.

With respect to the impact of excess adiposity on cardiac growth, although severe obesity is associated with an enhanced impact of BP on left ventricular mass (LVM), there is uncertainty as to whether the same effects occur in milder forms of excess adiposity, data confounded by the high prevalence of participants receiving antihypertensive therapy in previous studies. In the present thesis I demonstrated in a randomly recruited population sample of 398 participants with a high prevalence of mild-to-moderate obesity and hypertension (~41%), but in whom antihypertensive use was limited (~17%), that adiposity is indeed associated with an enhanced impact of conventional and ambulatory BP or arterial stiffness on LVM index and wall thickness independent of additional conventional risk factors.

With regards to the impact of obesity on cardiac function, although obesity is a risk factor for heart failure independent of other conventional cardiovascular risk factors, whether this effect occurs through changes in cardiac systolic chamber function is uncertain. In the present thesis I provide the first evidence to show in an animal model of genetic

hypertension and dietary-induced obesity, that dietary-induced obesity promotes the progression from compensated cardiac hypertrophy to cardiac pump dysfunction without promoting hyperglycaemia. This effect was attributed to alterations in both intrinsic myocardial systolic dysfunction and cardiac dilatation, effects that were associated with excessive cardiomyocyte apoptosis and activation of enzymes that promote myocardial collagen degradation.

Therefore in the present thesis I provide evidence to support the notion that waist circumference should be measured when predicting BP changes, that excess adiposity does indeed decrease large vessel function independent of conventional risk factors, but that this effect is age-dependent, and that the deleterious effects of excess adiposity on cardiac hypertrophy and cardiac pump function are indeed dependent on an interaction with BP, but not other confounders.

DECLARATION

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

Olebogeng Harold Isaia Majane ……...

……..day of…… 2009.

I certify that the studies contained in this thesis have the approval of the Committee for Research in Human Subjects and the Animal Ethics Screening Committee of the University of the Witwatersrand, Johannesburg. The ethics approval numbers are M020472 (renewed as M070469) and 2006/99/03.

Olebogeng Harold Isaia Majane ……...

…….day of…… 2009.

…….	…….....
Prof. Angela Woodiwiss (supervisor 1)	Prof. Gavin Norton (supervisor 2)
Date: …….	Date: ……

……

Dr. Richard Brooksbank (supervisor 3)

Date: ……

PUBLICATIONS AND PRESENTATIONS ARISING FROM THE THESIS

Publications

Majane OHI, Woodiwiss AJ, Maseko MJ, Crowther N, Dessein P, Norton GR. Impact of age on the independent association of adiposity with pulse wave velocity in a population sample of African ancestry. Am J Hypertens 2008:21:936-942.

Majane OHI, Norton GR, Maseko MJ, Makaula S, Crowther N, Paiker J, Thijs L, Brooksbank R, Sareli P, Staessen JA, Woodiwiss AJ. The association of waist circumference with ambulatory blood pressure is independent of alternative adiposity indexes. J Hypertens 2007;25:1798-1806.

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Majane OHI, Norton GR, Maseko MJ, Makaula S, Crowther N, Paiker J, Thijs L, Brooksbank R, Sareli P, Staessen JA, Woodiwiss AJ. Preferred clinical index of adiposity when predicting obesity effects on large artery function. 34th meeting of the Physiology Society of Southern Africa (PSSA). University of KwaZulu-Natal, 2006.

Poster presentations

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Majane OHI, Woodiwiss AJ, Maseko MJ, Crowther N, Dessein P, Norton GR. Impact of age on the independent association of adiposity with pulse wave velocity in a population sample of African ancestry. 18th European Meeting on hypertension. Berlin, Germany, 2008.

Majane OHI, Vengethasamy L, duToit E, Woodiwiss AJ, Norton GR. Susceptibility of pressure overload hypertrophy to obesity-induced deleterious effects on cardiac remodeling and pump function. 15th Biennial Congress of the Southern African Hypertension Society (SAHS). Sandton Convention Centre, Johannesburg, 2007.

Majane OHI, Vengethasamy L, duToit E, Woodiwiss AJ, Norton GR. Interaction between obesity and hypertension promotes adverse cardiac chamber remodelling and pump dysfunction in rats. The Health Sciences Research Day, WITS Medical School, Parktown, 2006. (Awarded the best poster presentation in the chronic diseases & diseases of lifestyle category).

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STATEMENT OF MY CONTRIBUTION TO DATA COLLECTION AND ANALYSIS

I declare that the studies described in this thesis were designed by me in consultation with my supervisors. I collected all the data acquired in the animal studies under the supervision of my supervisors and also collected a major component of the clinical data with the assistance and supervision of the health professionals registered to practice in South Africa Dr. Carlos Libhaber, an experienced cardiologist and echocardiographer who collected the echocardiogarphic data in the APOGH study. I performed all of the data analysis for this thesis and interpreted the data.

LIST OF ABBREVIATIONS

ACE	angiotensin-converting enzyme
AGT	angiotensinogen
AI	augmentation index
AIc	central AI
AIp	peripheral AI
ARIC	atherosclerosis risk in communities
ATPase	adenosine triphosphatase
BMI	body mass index
BP	blood pressure
BP conv	conventional BP (chapter 4)
BP24	24-hour BP
BPc	conventional BP
BSA	body surface area
c	conventional (chapter 2)
Ca^{2+}	calcium ion
$CaCl_2$	calcium chloride dihydrate
CAT	computer assisted tomography
CI	confidence intervals
cm	centimetre
CNBr	cyanogen bromide
CO_2	carbon dioxide
conv	conventional (chapter 4)
DBP	diastolic blood pressure

DBPc	central DBP
DM	diabetes mellitus
DNA	nuclear deoxyribonucleic acid
E/A	early-to-late transmitral flow velocity ratio
ECG	electrocardiogram
Ees	end-systolic chamber elastance
En	end-systolic myocardial elastance
Exp	experimental
FSend	endocardial fractional shortening
g/m	gram per meter
h	hour
HbA_{1c}	glycated haemoglobin
HDL	high density lipoprotein
HPRO	hydroxyproline
HR	heart rate
HT-Trt	hypertension treatment
IVS	interventricular septal wall thickness
KCl	potassium chloride
kg	kilogram
kg/m	kg per meter
KH_2PO_4	potassium dihydrogen orthophosphate
kJ	kilojoules
LV	left ventricle
LVED	LV end diastolic
LVEDD	LV end diastolic diameter

LVEF	LV ejection fraction
LVES	LV end systolic
LVESD	LV end systolic diameter
LVH	LV hypertrophy
LVM	LV mass
LVMI	LVM indexed for height$^{2.7}$
LVMWT	LV mean wall thickness
m/sec	meters per second
MAP	mean arterial pressure
MAPc	central MAP
Menop	menopausal status
mg	milligram
$MgSO_4$	magnesium sulphate heptahydrate
MHz	megahertz
mls/day	millilitres per day
mm Hg	millimetres of mercury
mM	millimolar
mmol	millimole
mmol/l	millimoles per litre
MMP	matrix metalloproteinase
mV	millivolts
MWT	mean wall thickness
n	number (sample size)
Na^+	sodium ion
NaCl	sodium chloride

$NaHCO_3$	sodium hydrogen carbonate (sodium bicarbonate)
NIH	national institutes of health
O_2	oxygen
p value	probability value
P	pressure
PP	pulse pressure
PPc	central PP
PPp	peripheral PP
PWT	posterior wall thickness
PWV	pulse wave velocity
r	correlation coefficient
RAAS	renin-angiotensin-aldosterone system
RWT	relative wall thickness
SAS	statistical analyses software
SBP	systolic BP
SD	standard deviation
SDS	sodium dodecyl sulfate
SEM	standard error of the mean
SHR	spontaneously hypertensive rats
SK	skin-fold thickness
SNS	sympathetic nervous system
T	time
TdT	terminal deoxynucleotidyl transferase
TUNEL	terminal uridine deoxynucleotidyl transferase dUTP nick end-labeling
Unadj	unadjusted

V	volume
V_0	unstressed left ventricular volume (volume axis intercept)
V_m	left ventricular muscle volume
WC	waist circumference
WHR	waist-to-hip ratio
WKY	Wistar Kyoto
µl	microlitre
µm	micrometre
σ	systolic stress
X^2	chi-squared

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PREFACE

Epidemiological trends suggest that obesity is becoming a major public health problem in developing countries such as South Africa. The major health risk associated with obesity is through strokes, heart failure and potentially myocardial infarction effects which have largely been attributed to the impact of adipose tissue on hypertension, dyslipidaemia and diabetes mellitus. Although the latter health risks can be managed using therapeutic agents, there is increasing evidence to suggest that excessive adipose tissue may promote cardiovascular damage through an impact of factors that are not normally accounted for in risk assessment and which are not necessarily targeted by current therapeutic agents. Although the obvious solution to this conundrum is to target lifestyle or institute weight loss programmes on a large scale, weight reduction programmes seldom result in obese individuals reaching target body weights and frequently result in an inability to maintain these programmes. Consequently, clarity is required on whether obesity does indeed produce cardiovascular damage through mechanisms that are not accounted for by conventional risk assessment and to determine the mechanisms of these effects. It is only once the mechanisms are established that more appropriate therapeutic strategies can be planned. The present thesis was therefore designed to address some outstanding issues with respect to the potential mechanisms that may explain obesity-induced effects on the cardiovascular system independent of conventional cardiovascular risk factors.

In the present thesis I addressed the question of the ability of obesity to promote cardiovascular disease by assessing a number of preclinical cardiovascular target organ changes. This approach was adopted as prospective observational studies with hard end-points such as cardiovascular events or death in patients would go beyond the scope of a PhD thesis. Furthermore, many of the questions addressed in the present thesis required analysis of cardiovascular changes as continuous rather than discrete traits. In this regard, I

assessed outstanding issues with respect to excess adiposity as independent predictors of large artery dysfunction, and addressed the question of whether or not obesity promotes cardiac hypertrophy and dysfunction in-part by enhancing blood pressure effects on the heart and the potential mechanisms thereof. Moreover, in order to assess the impact of excess adiposity on target organ changes I first had to establish the most appropriate clinical index of excess adiposity that predicts blood pressure. The outcomes of the present thesis suggest that obesity does indeed produce large artery damage, but that this is only something to be concerned about in older women. Moreover, the outcomes of the present thesis indicate that the adverse effects of obesity on the heart (both hypertrophy and dysfunction) may in fact depend more on blood pressure than what was previously suggested. These data therefore provide insights into potential therapeutic approaches in patients with obesity, in that blood pressure reduction may be the key to preventing the so-called independent effects of obesity on the heart.

The present thesis is written as a series of semi-independent chapters each with its own introduction, methods, results and discussion. However, the thesis begins with a chapter that reviews the present scientific literature in the field, underscores the relevant deficiencies in the literature and ultimately leads the reader to the reasons for performing the studies described in the present thesis. Moreover, the present thesis ends with a conclusion chapter which summarises the findings of the studies and places the studies in the context of our present understanding of the field. In support of the present thesis, the data given in chapters 2 and 3 have been published in the *Journal of Hypertension* (Majane et al 2007) and the *American Journal of Hypertension* (Majane et al 2008). The data provided in other chapters is presently in-preparation for submission to international journals for consideration for publication.

CHAPTER 1

INTRODUCTION

Current Understanding and Controversies of the Impact of Excess Adiposity on Blood Pressure and Cardiovascular Organ Damage.

1.1 Introduction

The prevalence of obesity is increasing in both developed and in developing countries (Ogden et al 2007, Flegal et al 2002, Bourne et al 2002). In the United States of America in 2004, ~31% of men and ~33% of women were obese and ~3% of men and ~7% of women were considered to be extremely obese (a body mass index [BMI] > 40 kg/m^2) (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, ~29% of men and ~57% of women were obese (Bourne et al 2002). There is now increasing evidence to indicate that obesity, once established, is difficult to manage. Indeed, weight reduction programs seldom result in obese individuals reaching target body weights (Lang and Froelicher 2006, Ogden et al 2007) and frequently result in an inability to maintain these weight reduction programs (Latner et al 2002, Perri and Corsica 2002, Anderson et al 2001). The important question therefore is whether there is a cause for concern with respect to the medical implications of excess adiposity and exactly why should we be concerned.

Obesity is certainly associated with a considerable morbidity and mortality (Pi-Sunyer 1993, Bellanger and Bray 2005, McGee 2005, Giusti 2007, Allison et al 1997, Formiguera and Cantón 2004, Heitmann et al 2000, Lahmann et al 2002) and a large component of this morbidity and mortality is through an increased risk for cardiovascular events (Eckel et al 2002, Klein et al 2004a). Indeed, obesity is a risk factor for heart failure (Bahrami et al 2008, Ingelsson et al 2005, Kenchaiah et al 2002, Kenchaiah et al 2004, Poirier et al 2006, Wong and Marwick 2007, Contaldo et al 2002), myocardial infarction (Yusuf et al 2005, Steyn et al 2005) and cerebrovascular events (stroke) (Kurth et al 2002, Ahluwalia et al 2003, Suk et al 2003). This increased risk for cardiovascular events is in-part attributed to the association between obesity and classical

cardiovascular risk factors, including hypertension, diabetes mellitus, and dyslipidaemia (National Task Force on the Prevention and Treatment of Obesity, Overweight obesity and health risk 2000, Yki-Jarvinen and Koivisto 1983, Despres et al 1989, Harris et al 2000, Zhu et al 2005, Wilson et al 2002, Engeli and Sharma 2000, Grundy 2000, Eckel et al 2002, Klein et al 2004a, De Lusignan et al 2006).

With respect to the impact of excess adiposity on blood pressure (BP), this effect may be mediated largely through an impact of visceral, rather than general fat (Kanai et al 1990, Zhu et al 2005). This has important implications as measures of central (waist circumference and waist-to-hip ratio) as opposed to general (BMI) body fat are more likely to predict the effects of excess adiposity on BP and hence a portion of cardiovascular risk. Nonetheless, as shall be further discussed, these data are not entirely supported by all studies. Moreover, as shall also be highlighted, studies conducted comparing the impact of indexes of adiposity on 24-hour ambulatory BP profiles, a measure of BP that predicts cardiovascular events beyond conventional BP measurements (see later discussion), have been limited in a number of respects. Consequently, in the first part of my thesis I explored whether any one clinical index of central as opposed to general or subcutaneous (skin-fold thickness) fat predicts 24-hour ambulatory BP beyond the others. Therefore, in the first part of the present chapter I will first highlight the evidence to suggest that visceral fat may contribute toward increases in BP and underscore the controversies in this regard.

Although as previously indicated, obesity increases the risk for cardiovascular events in-part through an impact on classical cardiovascular risk factors, there is now increasing evidence to suggest that the adverse effects of obesity on the cardiovascular system are still present even after adjustments for these risk factors. Indeed, obesity is a risk factor for the development of heart failure (Kenchaiah et al 2002, Kenchaiah et al 2004, Bahrami et al 2008, Ingelsson et al 2005), myocardial infarction (Yusuf et al 2005,

Steyn et al 2005), and stroke (Kurth et al 2002, Suk et al 2003) after adjustments for age, the presence of hypertension, diabetes mellitus and dyslipidaemia. The relationship between measures of adiposity and cardiovascular outcomes independent of classical cardiovascular risk factors has engendered the notion that obesity could promote damage to key cardiovascular organs that is not necessarily mediated through an elevated BP, poor blood glucose control or abnormal lipid profiles. The natural extension to this argument is that managing hypertension, diabetes mellitus and dyslipidaemias in obesity may be insufficient to prevent obesity-induced increases in cardiovascular risk. However, there are a number of outstanding questions in this regard and these relate to the independent actions of obesity on cardiovascular structure and function. Indeed, the major focus of my thesis was to attempt to clarify outstanding issues regarding the impact of excess adiposity on cardiovascular target organs either independent of conventional cardiovascular risk factors, including BP, or mediated through interactions with BP. In the process of addressing these questions, as indicated in the aforementioned discussion I nevertheless had to first identify the most appropriate adiposity index associated with BP changes. After having completed this aspect of the thesis which is described in chapter 2 and published in the *Journal of Hypertension* (Majane et al 2007), with respect to subsequent chapters in my thesis, I performed a series of studies that add to our insights into the role of excess adiposity as a potential independent predictor of cardiovascular target organ changes. Consequently, in the introductory chapter of the present thesis I will also discuss the evidence to indicate that cardiac and vascular target organ changes are independent predictors of cardiovascular events and subsequently highlight the evidence to suggest that excess adiposity is an independent determinant of these cardiovascular changes, and the potential mechanisms thereof. In this regard, I will highlight questions that have not been adequately addressed and which were subsequently explored in the present thesis.

Before addressing the aforementioned points, it is worth first briefly reviewing the changes that occur as a consequence of excessive adipose tissue that may promote either increases in BP or cardiovascular target organ changes independent of BP or through interactions with BP. The next section is therefore intended to underscore all of the potential mechanisms through which an excess adiposity may promote many of the adverse cardiovascular effects that will subsequently be discussed.

1.1.1 Insulin resistance in obesity

Insulin resistance may occur as a consequence of excess adiposity (Kahn and Flier 2000). This change is thought not only to lead to diabetes mellitus, but also to adverse cardiovascular changes independent of diabetes mellitus. At a cellular level, insulin binds to its receptor on the surface of target cells, thereby causing tyrosine autophosphorylation and consequent intracellular signaling. These events culminate in cellular responses, such as the translocation of glucose transporters to the cell surface to allow glucose uptake for use or glycogen storage. In obesity, however, insulin signaling may be defective. Insulin-stimulated protein kinase activity of the insulin receptor, which mediates tyrosine autophosphorylation, is reduced in obese as compared to non-obese people, and it is further reduced in patients with obesity-induced type II diabetes mellitus (Caro et al 1989). However, euglycaemia is maintained in the initial stages of insulin-resistance through compensatory hyperinsulinaemia. Indeed, there is considerable evidence to suggest that a large proportion of obese individuals are insulin-resistant, but that compensatory hyperinsulinaemia maintains fasting blood glucose concentrations (Reaven 2003, Keller 2006). Thus, insulin resistance goes undetected in these individuals.

Insulin resistance will undoubtedly promote adverse cardiovascular effects once type II diabetes mellitus develops and the effects of hyperglycaemia are noted (Groop 2000, Feener and King 1997). However, insulin resistance may also promote adverse cardiovascular changes prior to the development of diabetes mellitus and poor blood glucose control through a number of mechanisms. In this regard, hyperinsulinaemia, as a consequence of insulin insensitivity associated with obesity, could promote obesity-induced increases in sympathetic nervous system (SNS) activity (Esler et al 2001, Corry and Tuck 1999, Landsberg et al 1986, Alonso-Galicia et al 1996, Anderson et al 1991), which as shall be discussed below may promote adverse cardiovascular effects. Further, hyperinsulinaemia in insulin resistance may induce hypertrophy of the vascular wall through an increased synthesis of collagen and proliferation of vascular smooth muscle cells (de Fronzo and Ferrannini 1991, Avena et al 1998, King and Wakasaki 1999, Ruiz-Torres et al 1998), induce increases in arterial stiffness (van Popele et al 2000) and promote endothelial dysfunction (Arcaro et al 2002), a change that is considered to precede large vessel pathology.

1.1.2 Activation of the sympathetic nervous system in obesity.

There is considerable evidence to suggest that the SNS is activated in obesity (Haynes et al 1997 a and b, Haynes et al 1998, Casto et al 1998, Satoh et al 1999, Tentolouris et al 2006, Yang and Barouch 2007, Mark et al 1999, Mark et al 2004, Masuo et al 2000). A number of mechanisms could explain this effect, including increased circulating concentrations of leptin, an adipokine released from fat tissue in excess in obesity (Eikelis et al 2003, Hall et al 2000, Haynes et al 1997 a and b) and as discussed above, through hyperinsulinaemia (Esler et al 2001, Corry and Tuck 1999, Landsberg et al 1986, Alonso-Galicia et al 1996, Anderson et al 1991) following the

development of insulin resistance. With respect to leptin effects, upon leptin release by adipocytes, it reaches its receptors in the brain via facilitated passage across the blood-brain barrier. In addition to reducing appetite and controlling weight gain, leptin activates the SNS. The mechanisms of leptin-induced SNS activation in obesity are still unclear but this may be via stimulation of neurons within the ventromedial hypothalamus (Satoh et al 1999). With regards to hyperinsulinaemia promoting SNS activity, because insulin-mediated glucose uptake in central hypothalamic neurons regulates SNS activity in response to dietary intake, hyperinsulinemia is also thought to provoke SNS stimulation (Landsberg 1999). However, there is controversy as to whether increased leptin or insulin concentrations in obesity can explain early SNS activation in persons with an excess adiposity as indeed, some studies do not support these hypotheses (Masuo et al 2000, Hall 1995, Hildebrandt et al 1999, Feskens et al 1995).

It is well accepted that excessive SNS activity may promote increases in BP through a number of mechanisms. Indeed, SNS activation may induce adrenergic-induced vasoconstriction (α-adrenergic receptor effects) which increases peripheral resistance. However, as shall be underscored in later discussion, obesity may promote decreases rather than increases in vascular resistance in most vascular beds, the consequence being an attenuation rather than an increment in total peripheral resistance. However, SNS activation increases heart rate and cardiac contractility (β-adrenergic-induced effects) which enhances cardiac output and hence BP. Further, SNS activation induces renal fluid retention (Izzo and Taylor 1999, Sinski et al 2006, Watson et al 2006), which also increases cardiac output and hence BP. However, the adverse cardiovascular effects of SNS activation may also occur through actions that may be unrelated to BP changes. In this regard, left ventricular hypertrophy (LVH) is associated with excessive myocardial sympathetic effects (Schlaich et al 2003) and blockade of β-adrenoreceptors may reduce LVH through effects that cannot be attributed solely to an

impact on BP (Roland et al 1997). Moreover, activation of the SNS can promote the transition from compensated LVH to heart failure through mechanisms that appear to be unrelated to BP changes (Badenhorst et al 2003a, Veliotes et al 2005).

1.1.3 Activation of the renin-angiotensin-aldosterone system in obesity

Another potential mechanism by which obesity may promote adverse cardiovascular changes is through activation of the renin-angiotensin-aldosterone system (RAAS). In this regard there is considerable evidence to suggest that the RAAS is excessively stimulated in obesity. Indeed, angiotensinogen (AGT), a protein synthesized principally in the liver and transformed by renin to ultimately produce angiotensin II and aldosterone, is also synthesized by adipocytes (Serazin-Leroy et al 2000, Cassis et al 1988, Campbell et al 1987) and circulating AGT concentrations increase in obesity (Cooper et al 1998). Angiotensin-converting enzyme (ACE), an enzyme largely derived from endothelial cells in the lung parenchyma, and which is responsible for converting inactive angiotensin I to angiotensin II, is also synthesized by adipocytes (Jonsson et al 1994) and ACE activity increases in obesity (Cooper et al 1998) and decreases with weight loss (Harp et al 2002). Moreover, adipocytes are capable of producing a lipid substance that is either converted to aldosterone by adrenal cortical cells or acts as an adrenal secretagogue and promotes aldosterone release (Ehrhart-Bornstein et al 2003).

The importance of the RAAS in promoting adverse cardiovascular effects has been reviewed on a number of occasions (Packer 1992, Mantero and Lucarelli 2000, Unger 2002, Nadar 2006, Farmer and Torre-Amione 2001). There is no question as to the importance of the RAAS in inducing increases in BP through renal salt and volume retention and vasoconstriction. Importantly, however, either angiotensin II or aldosterone

may promote cardiovascular fibrosis through direct rather than through haemodynamic changes, thus inducing large vessel stiffness (Rocha et al 2002, Mahmud and Feely 2004, Duprez 2006) or diastolic dysfunction in the heart (Norton et al 1997). Further, either angiotensin II or aldosterone can promote cardiomyocyte and vascular smooth muscle cell hypertrophy or hyperplasia independent of haemodynamic changes (Tanaka et al 2005). Moreover, angiotensin II and aldosterone can promote adverse vascular remodeling through inflammatory changes (Rocha et al 2002, Duprez 2006), rather than through the aforementioned well-recognized haemodynamic effects. Consequently, RAAS activation, such as occurs in obesity, could promote cardiovascular damage independent of measurable haemodynamic changes.

1.1.4 Haemodynamic changes in obesity

What are the potential haemodynamic changes associated with excess adiposity that may promote target organ damage through mechanisms that are not necessarily mediated by an impact on BP that are measured through conventional methods? Because of the high metabolic activity of excessive fat, obesity may result in a decrease rather than an increase in total peripheral resistance and an increase in venous return with an enhanced stroke volume and cardiac output (Messerli et al 1982, Messerli et al 1983, Carabello and Gittens 1987). Further, as SNS activity may be enhanced in obesity (see above discussion) heart rate and cardiac contractility may also contribute towards an increased cardiac output, and as SNS-induced renal fluid retention may occur in excess adiposity (Mark et al 1999), this may increase blood volume and hence further enhance stroke volume and cardiac output.

As a consequence of the high cardiac output, systolic BP increases, but this increase in BP may be partly offset by a reduced vascular resistance. Nevertheless,

irrespective of the effect of obesity on systolic BP, the increased blood volume and blood flow may induce an increase in vascular shear stress and promote vascular damage independent of BP. Moreover, an increased venous return may increase cardiac preload and subsequently induce myocardial damage. Thus, obesity may further haemodynamic-induced damage to the heart and vessels, through mechanisms that may not necessarily be mediated by an increased BP detected from brachial artery or other peripheral artery BP measurements.

An additional haemodynamic change that may also occur with an increased adiposity is augmentation of central BP produced by the early return of reflective waves in stiffer large arteries (see section on target organ changes in obesity). As will be discussed, through this change it is possible that afterload to the left ventricle will increase and subsequently induce damage to the heart through changes that are also not necessarily reflected by peripheral BP values.

1.2 Excess adiposity and blood pressure.

Based on the neurohumoral and haemodynamic consequences of excess adiposity outlined in the aforementioned discussion, it is easy to conceive of the possibility that excess adiposity also promotes cardiovascular damage because it is a major determinant of increases in BP. 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 conventional BP and the development of hypertension (Zhu et al 2005; Harris et al 2000). 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. Indeed, in a meta-analysis of a number of weight reduction studies,

with a total sample size of 4874 participants, it was estimated that a ~3.5-4.5 mm Hg decrease in BP will occur for every 5.1 kg of weight loss over 16 months (Neter et al 2003). Moreover, in the Atherosclerosis Risk in Communities (ARIC) 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).

The obvious question that arises from association studies between adiposity and BP irrespective of whether they are cross-sectional studies or weight reduction studies involving lifestyle changes, is whether fat tissue *per se* mediates the BP effects, or whether alternative dietary or exercise-related effects are the main role-players? The answer to this question lies in the impact on BP of weight reduction that is not attributed to lifestyle modifications. In this regard, in a meta-analysis conducted in a total of 1230 participants involved in a number of weight reduction studies, where weight reduction was achieved with orlistat, a pharmacological agent that specifically targets decreases in adipose tissue by decreasing fat absorption in the gastrointestinal tract, provided evidence to suggest that for each ~8% reduction in body weight a 8-9 mm Hg decrease in BP may occur (Sharma and Golay 2002). Further, in a single study conducted in 531 participants, a ~5-6% decrease in body weight mediated by orlistat resulted in a ~5-6 mm Hg reduction in BP (Broom et al 2002).

Despite the convincing evidence to indicate that excess adiposity mediates increases in BP, there is nevertheless some evidence to suggest that these effects are not persistent. Indeed, in 343 obese participants, orlistat reduced BP at one year, but despite participants largely maintaining weight reduction at two years, BP values increased to baseline values (Sjostrom et al 1998). Further, after marked weight reduction following gastric banding in obese participants, BP reduction occurred after one year, but after 8 years BP levels increased to baseline levels again (Sjostrom et al 2000). Even studies involving BP measurements that are not prone to observer errors

and take into account 24-hour profiles have produced data that suggest that the beneficial effects of weight reduction on BP are not sustained. Indeed, although ambulatory BP was reduced after two months of a low calorie diet resulting in marked weight reduction, after 14 months ambulatory BP values increased to baseline values again despite participants largely maintaining weight reduction (Laaksonen et al 2003). Thus, further work is still required to understand the apparent non-persistence of the effects of weight reduction on BP in some studies.

1.2.1 Fat distribution and blood pressure.

As indicated in the introduction to the present chapter, although the focus of my thesis was to attempt to clarify outstanding issues regarding the impact of excess adiposity on cardiovascular target organs either independent of conventional cardiovascular risk factors, including BP, or mediated through interactions with BP, in the process of addressing these questions, I first had to identify the most appropriate adiposity index associated with BP changes. In this regard, there is evidence to indicate that the distribution of adipose tissue determines the impact of excess adiposity on BP. Indeed, the ratio of visceral-to-subcutaneous fat as determined using computer assisted tomography (CAT) scans, may account for 50-60% of the variability in BP as compared to clinical indexes of adiposity which may account for less than 10% of the variability in BP (Kanai et al 1990). Further, in 10,969 nationally representative participants of the United States of America, waist circumference, an index of central adiposity, accounted for a greater proportion of the variability of BP than BMI (Zhu et al 2005), data which is supported by a number of studies assessing the impact of adiposity indexes on BP. Indeed, in this regard, present evidence suggests that waist circumference or other indexes of central fat appear to be the preferred clinical indexes when relating adiposity

to conventional BP (Okosun 1998, Williams et al 1987, Peiris et al 1989, Kanai et al 1990, Raison et al 1992, Boyko et al 1995, Lerario et al 1997, Ho et al 2001, Okosun et al 1999, Hayashi et al 2003, Ding et al 2004). Indexes of central adiposity may show either stronger relations with BP than BMI (Zhu et al 2005), or they may show relationships with BP independent of BMI (Okosun et al 1998). Importantly, subcutaneous liposuction in the abdominal region does not reduce BP in obese individuals (Klein et al 2004b) and hence, in support of the strong relationship between visceral-to-subcutaneous fat ratios and BP noted in a CAT scan study (Kanai et al 1990), it is likely that the visceral fat compartment is responsible for the impact of excess adiposity on BP.

1.2.2 Potential explanations for the relationships between indexes of visceral fat and blood pressure.

Two possibilities may explain the closer relationship between indexes of central as compared to general adiposity and BP. First, an inconsistent relationship between BMI and adiposity may occur, particularly in some populations (Luke et al 1997). Thus BMI may not be a reliable index of adipose tissue mass. Second, visceral fat mass may be more important in mediating BP changes than other fat compartments (Kanai et al 1990, Williams et al 1987, Peiris et al 1989, Kanai et al 1990, Raison et al 1992, Boyko et al 1995, Lerario et al 1997, Okosun et al 1999, Ho et al 2001, Hayashi et al 2003 and Ding et al 2004). How could visceral adipose tissue promote increases in BP beyond that produced by other fat compartments?

There are many changes that occur in people with excess adiposity that may explain BP effects and these have been discussed in sections 1.1.1-1.1.4. Importantly, however, with respect to potential mechanisms that may explain the closer relationship

between central as opposed to general adiposity with BP, one potential explanation is that visceral fat is more closely associated with SNS activity. Indeed, for the same amount of total fat or abdominal subcutaneous fat, basal muscle sympathetic activity increases in people with an increased visceral fat content (Alvarez et al 2002). Moreover, abdominal fat content is more closely associated with basal muscle sympathetic activity than is total fat content or subcutaneous abdominal fat (Alvarez et al 2002). Evidence in support of a contribution of visceral as compared to other fat compartments toward increases in SNS activity is that waist-to-thigh ratio is an independent predictor of basal muscle sympathetic activity in both males and in females (Jones et al 1996).

The closer relationship between central as opposed to general adiposity with BP, may also be explained by the propensity of visceral fat to promote adrenal steroidogenesis. Indeed, the best clinical index of excess adiposity that predicts circulating aldosterone concentrations is visceral fat measured by CAT scans and waist-to-hip ratio (Goodfriend et al 1999). This effect may be mediated by fatty acid-induced release of a secretagogue from visceral adipocytes that stimulates aldosterone production by adrenal glomerulosa cells or which is converted into aldosterone by the adrenal gland (Goodfriend et al 2004, Ehrhart-Bornstein et al 2003). As it is well recognized that aldosterone promotes increases in BP via a number of mechanisms that have been extensively reviewed (Rajagopalan and Pitt 2001, Ngarmukos and Grekin 2001, Funder 1997, Mantero and Lucarelli 2000), it is possible that visceral fat mediates increases in BP via excessive aldosterone production. Indeed, aldosterone receptor antagonism attenuates obesity-induced hypertension (de Paula et al 2004).

A further potential explanation for the closer relationship between central as opposed to general adiposity with BP, is that visceral adipocytes tend to produce more components of the RAAS than adipose tissue from other fat deposits. In this regard, a greater extent of AGT expression may occur in visceral adipocytes as compared to

adipocytes from other areas (Wajchenberg 2000). However, to my knowledge as yet there is no evidence to indicate that the activity of the circulating RAAS is more closely associated with indexes of central adiposity than BMI. Nevertheless, establishing an independent relationship between the circulating RAAS and indexes of central adiposity was a task that goes beyond the scope of the present thesis.

1.2.3 What is the outstanding evidence with respect to the role of visceral as compared to general obesity on blood pressure?

Not all studies support the notion that clinical indexes of central fat are more closely associated with BP than indexes of general adiposity. Indeed, in the ARIC study conducted in 15,063 partipants, BMI was as closely associated with BP as was waist circumference (Harris et al 2000). The ability of BMI to predict BP as closely as waist circumference was noted in two ethnic groups (Caucasians and African-Americans) and in both women and men (Harris et al 2000). Clearly, clarity on this issue is therefore still required.

One potential method of resolving the conundrum of whether indexes of central adiposity promote increases in BP beyond indexes of general adiposity is to evaluate this question using ambulatory BP monitoring. Indeed, ambulatory BP monitoring eliminates the problems associated with auscultatory BP measurement (observer error, observer bias, alerting responses, and an inability to account for BP fluctuations during the day, to name a few). Furthermore, 24-hour ambulatory BP is a better index of cardiovascular outcomes (Verdecchia et al 1994, Clement et al 2003, Staessen et al 1999 and Okhubo et al 1998) and target organ effects (Mancia and Parati 2000) than conventional BP values. Up until the time of conducting the studies reported on in the present thesis, to my knowledge studies that have compared the relative impact of

indexes of central fat and BMI on ambulatory BP have either recruited small study samples, have been non-random, have focussed on waist-to-hip ratio rather than waist circumference as the index of central fat, and have produced ambiguous results (Guagnano et al 1994, Guagnano et al 1997, Steptoe et al 1999, Lurbe et al 1998, Feldstein et al 2005). In the present thesis I therefore assessed whether any one commonly used clinical index of adiposity (waist circumference, waist-to-hip ratio, BMI and skin-fold thickness) predicts ambulatory BP independent of the others. This study was conducted in a randomly selected population sample, with a high prevalence of obesity, living in an urban developing community in South Africa. The results of this study are reported on in chapter 2 of the present thesis and have been published in the *Journal of Hypertension* (Majane et al 2007).

1.3 Target organ changes as independent predictors of cardiovascular events.

A number of cardiovascular target organ changes that are influenced by excess adiposity have now been established as independent predictors of cardiovascular events. The potential impact of adiposity on some of these target organ changes and the mechanisms thereof were studied as part of my thesis. These target organ changes that I studied include large vessel and cardiac pathology. However, before describing the role of excess adiposity as determinants of these cardiovascular target organs I will first highlight the evidence to indicate that the pre-clinical cardiac or large vessel pathology that may be determined by excess adiposity predicts cardiovascular risk independent of conventional cardiovascular risk factors. It is this evidence that has led to the recommendation in guidelines for the diagnosis and management of hypertension that measurement of some of these target organ changes are incorporated into routine clinical practice (Mancia et al 2007).

1.3.1 Abnormalities of large vessel function as independent predictors of cardiovascular outcomes.

There is now substantial evidence to indicate that increases in the stiffness of the wall of large arteries may be a risk factor for cardiovascular events independent of classical cardiovascular risk factors (Benetos et al 1997, Blacher et al 1999a, Blacher et al 1999b, de Simone et al 1999, Laurent et al 2001, London et al 2001, Guerin et al 2001, Boutouyrie et al 2002, Safar et al 2002, Blacher and Safar 2005, Dolan et al 2006, Sutton-Tyrrell et al 2005, Cruickshank et al 2002, Meaume et al 2001). Indeed, indexes of aortic stiffness are independent predictors of cardiovascular events, including stroke and myocardial infarction in patients with hypertension (Benetos et al 1997, de Simone et al 1999, Laurent et al 2001, Boutouyrie et al 2002), in patients referred to tertiary care centres for the management of hypertension (Dolan et al 2006), in patients with end stage renal disease (London et al 2001, Guerin et al 2001, Safar et al 2002), in patients with diabetes mellitus (Cruickshank et al 2002), in the elderly (Meaume et al 2001) and in well functioning older adults (Sutton-Tyrrell et al 2005). These effects of arterial stiffness on cardiovascular events are independent of conventional (de Simone et al 1999, Laurent et al 2001, Guerin et al 2001, London et al 2001, Boutouyrie et al 2002, Safar et al 2002, Sutton-Tyrrell et al 2005, Cruickshank et al 2002, Meaume et al 2001) or 24-hour ambulatory (Dolan et al 2006) BP. Increases in large artery stiffness not only predict vascular events such as stroke and myocardial infarction, but are also associated with pump dysfunction in chronic heart failure (Tartiere et al 2006) and with left ventricular diastolic dysfunction in hypertension (Palmieri et al 2003), effects that are also independent of conventional BP.

The explanations for the ability of measurements of large artery stiffness to predict cardiovascular outcomes independent of conventional cardiovascular risk factors are still uncertain. However, there are three potential theories in this regard. First, large artery stiffness may reflect the composite effects of temporal (time-dependent) changes in classical cardiovascular risk factors and as such is still dependent on classical risk factors, but obviously the sum of their detrimental effects. Second, large artery stiffness may contribute toward cardiovascular disease, in-part through the impact of early reflective waves on central aortic pressures, a pressure that is not accurately predicted by brachial artery BP measurements (Karamanoglu et al 1993, Williams et al 2006). Thus, adjustments for brachial artery BP will not eliminate relationships between large artery stiffness and cardiovascular outcomes. Third, although an increase in arterial stiffness may occur through the deleterious actions of an increased BP (Schiffrin 2004, Blacher and Safar 2005), abnormal blood glucose control (Henry et al 2003, Cruickshank et al 2002, Cameron and Cruickshank 2007, Schram et al 2004) and abnormal lipid profiles (Wilkinson et al 2002), increases in arterial stiffness may also occur through factors other than these classical cardiovascular risk factors, such as inflammatory mediators in chronic inflammatory diseases (Selzer et al 2001). Thus an increase in large artery stiffness may be a good index of the composite of classical and alternative cardiovascular changes that lead to cardiovascular morbidity and mortality. In this regard, as shall be highlighted in subsequent discussion, a potential source of stimulation of increases in large artery stiffness that may not normally be entirely accounted for by classical cardiovascular risk assessment may be from adipose tissue.

1.3.2 Cardiac hypertrophy as an independent predictor of cardiovascular outcomes.

An increased left ventricular mass (LVM) is a relatively common pre-clinical complication of poorly managed hypertension. In this regard, many studies have reported on the ability of LVM to predict cardiovascular events such as stroke, sudden cardiac death and myocardial infarction, independent of classical cardiovascular risk factors (Casale et al 1986, Levy et al 1990, Koren et al 1991, Levy et al 1994, Verdecchia et al 1996, Ghali et al 1998, Gardin et al 2001, Verdecchia et al 2001). It is also now well recognized that reductions in LVM with antihypertensive therapy are associated with a reduced risk of cardiovascular morbidity and mortality (Mathew et al 2001, Okin et al 2004, Devereux et al 2004) and that these effects are partly independent of office BP changes (Mathew et al 2001, Okin et al 2004, Devereux et al 2004). More recently, prospective evidence has also emerged to indicate that LVM is associated with a decrease in left ventricular systolic function (left ventricular ejection fraction [LVEF]) or left ventricular diastolic function independent of traditional risk factors including conventional BP (Drazner et al 2004).

The relationship between LVM and cardiovascular morbidity and mortality independent of conventional cardiovascular risk factors could be explained by three hypotheses. First, as with increases in arterial stiffness, LVM may be an index of the sum of all BP changes that have occurred over a prolonged time period. Indeed, regression of an increased LVM with antihypertensive therapy may take up to two years to achieve maximal effects (Devereux et al 2002). Second, an increased LVM (LV hypertrophy-LVH) could lead to a number of abnormalities of cardiac function and thus predispose to heart failure. These would include an impaired filling of the left ventricle through both a decreased early-diastolic relaxation and a reduced late-diastolic

compliance (Gradman and Alfayoumi 2006). This, in-turn, may increase cardiac filling pressures, augment myocardial transmural pressures during diastole and reduce coronary flow (reduced coronary reserve) (Lorell et al 1987). Left ventricular hypertrophy could also progress to an impaired left ventricular pump function through a number of mechanisms including chamber dilatation (Norton et al 2002) and a decreased myocardial contractility (Malik et al 1974). Further, the increased tissue bulk of the myocardium may, as previously suggested (Kannel et al 1970, Ghali et al 1991) increase myocardial oxygen demand - an effect that may not be accompanied by the expected increase in coronary flow because of reductions in coronary reserve. Alterations in myocardial oxygen demand-to-supply ratios in LVH could result in programmed cell death (apoptosis) (Liu et al 2000, Veliotes et al 2005) and necrosis (Tsotetsi et al 2001). It is also possible that increased filling pressures in the left ventricle and hence in the left atrium may result in atrial fibrillation and mural thrombus formation, an effect that could result in embolic phenomenon and hence strokes.

A third potential explanation for the ability of LVM to predict cardiovascular outcomes independent of conventional risk factors is that an increase in LVM may occur through factors other than classical cardiovascular risk factors. These factors may not only promote cardiomyocyte growth, but may also contribute toward alternative cardiovascular effects that ultimately lead to strokes, myocardial infarcts, sudden cardiac death and heart failure. In this regard, as shall be highlighted in subsequent discussion, a potential source of stimulation of cardiomyocyte growth that is not normally entirely accounted for by classical cardiovascular risk assessment may be from adipose tissue. Evidence to support the notion that LVM heralds the onset of alternative vascular effects is that there is an association between vascular damage in small resistance arteries and the presence of concentric LVH (Treasure et al 1993, Muiesan et al 2002).

1.3.3 Preclinical abnormalities of cardiac function as independent predictors of cardiovascular outcomes.

Left ventricular systolic or diastolic functional changes are also considered pre-clinical cardiovascular findings that may be used to predict cardiovascular outcomes. Indeed, systolic chamber dysfunction (a reduced ejection fraction) in patients without heart failure independently predicts cardiovascular mortality; and the prevention of congestive heart failure through the treatment of preclinical systolic dysfunction is therefore recommended in guidelines (Hunt et al 2001). Furthermore, a reduced left ventricular systolic myocardial function (midwall fractional shortening) despite the presence of a normal chamber function (normal ejection fraction) in patients without evidence of heart failure, predicts mortality in hypertensives independent of conventional cardiovascular risk factors and LVM (de Simone et al 1996). Moreover, a reduced early-to-late transmitral velocity ratio (E/A), an index of diastolic dysfunction, in patients with a largely normal systolic function and without heart failure, predicts cardiovascular outcomes in a general population (Bella et al 2002), and cardiovascular events in hypertensives (Schillaci et al 2002) independent of conventional cardiovascular risk factors.

The relationship between preclinical cardiac dysfunction and cardiovascular morbidity and mortality independent of conventional cardiovascular risk factors could again be explained by a number of hypotheses. First, as with other target organ changes, cardiac dysfunction may index the sum of all conventional cardiovascular effects that have occurred over prolonged time periods. Second, cardiac dysfunction could predispose to heart failure or reflect changes in the heart related to the preclinical phase of heart failure. Third, preclinical cardiac dysfunction may reflect adverse effects produced by factors other than classical cardiovascular risk factors. These factors may

not only promote cardiac dysfunction, but may also contribute toward alternative cardiovascular effects that ultimately lead to strokes and myocardial infarcts, as well as sudden cardiac death and heart failure. In this regard, as shall be highlighted in subsequent discussion, adipose tissue may promote changes that could lead to cardiac dysfunction.

1.4 Excess adiposity as an independent cause of target organ changes and the potential mechanisms thereof.

As suggested in the aforementioned discussion, excess adiposity may promote cardiovascular target organ changes by inducing hypertension, diabetes mellitus or dyslipidaemias. However, as also suggested in the previous discussion, a relationship exists between excess adiposity and target organ changes even after adjustments for conventional cardiovascular risk factors, such as hypertension, diabetes mellitus and dyslipidaemia. Nevertheless, these relationships are not entirely understood. As a clear understanding of these relationships is important to be able to design appropriate strategies to manage patients with excess adiposity, in this section of the chapter I will describe the evidence to show that a relationship may exist between excess adiposity and target organ changes even after adjustments for conventional cardiovascular risk factors and highlight those areas of understanding in this regard that are particularly lacking. I will subsequently describe how, in the present thesis, I have attempted to clarify some misunderstandings of these relationships. Importantly, these studies are either published in the *American Journal of Hypertension* (Majane et al 2008) or are presently under review for consideration of publication.

1.4.1 Excess adiposity as an independent cause of large vessel dysfunction.

A number of studies have demonstrated a relationship between excess adiposity and increases in arterial stiffness (Resnick et al 1997, Sutton-Tyrrell et al 2001, Mackey et al 2002, Wildman et al 2003, Ferreira et al 2004, Ferreira et al 2004, Snijder et al 2004) a change which may be noted even in obese children (Tounian et al 2001). Despite the consistency in these reports demonstrating relations between excess adiposity and arterial stiffness, there is nevertheless controversy as to whether this relationship is independent of haemodynamic factors such as BP and heart rate (HR), or diabetes mellitus. In this regard, although adiposity is positively correlated with pulse wave velocity (PWV), a simple and reliable index of arterial stiffness, some studies have (Sutton-Tyrrell et al 2001, Mackey et al 2002, Wildman et al 2003, Ferreira et al 2004), whilst most others have not (Ferreira et al 2004, Zebekakis et al 2005, Taquet et al 1993, Amar et al 2001, Nakanishi et al 2003, Oren et al 2003, Mitchell et al 2004, Czernichow et al 2005) been able to demonstrate strong relations between adiposity indexes and PWV independent of haemodynamic factors and diabetes mellitus.

Pre-clinical studies nevertheless suggest that body size is indeed a major determinant of PWV independent of BP, HR, and diabetes mellitus (Cosson et al 2007). In studies conducted in humans, convincing independent relations between clinical indexes of adiposity and PWV have been obtained in elderly populations (Sutton-Tyrrell et al 2001, Mackey et al 2002), whilst substantial evidence against a strong independent relationship between adiposity and PWV has been noted in samples that are predominantly young-to-middle aged (Ferreira et al 2004, Zebekakis et al 2005, Taquet et al 1993, Amar et al 2001, Nakanishi et al 2003, Oren et al 2003, Mitchell et al 2004, Czernichow et al 2005). Thus, the haemodynamic and glucose control-independent effect of adiposity on PWV may be age-dependent. Indeed, adiposity-age interactions

have previously been described (Zebekakis et al 2005) which nevertheless only achieved significance when assessing associations with measures of arterial stiffness other than PWV. However, in that study (Zebekakis et al 2005), an adiposity-age interaction was not detected when assessing associations with PWV, although the independent effect of adiposity on PWV appeared to be enhanced with increasing age (Zebekakis et al 2005). It is possible that the low mean BMI of ~25 kg/m^2 in that prior study (Zebekakis et al 2005) may have limited the capacity to detect a significant adiposity-age interaction for PWV. Further, the use of BMI as an adiposity index, rather than indexes of central adiposity, may have limited the sensitivity to detect age-adiposity interactive effects on PWV (Zebekakis et al 2005).

As a consequence of the inconsistencies in the scientific literature regarding the potential independent effect of obesity on large artery stiffness, in the present thesis, one of my aims was to clarify whether age influences the independent relationship between adiposity indexes and PWV or other indexes of large artery dysfunction in a population sample of African ancestry with a high prevalence of excess adiposity. These data are provided in chapter 3 and have been published in the *American Journal of Hypertension* (Majane et al 2008). The following discussion therefore outlines some of the potential non-classical cardiovascular risk factors that could contribute toward PWV in obesity.

1.4.1.1 Potential mechanisms of an independent relationship between adiposity and large artery stiffness.

The potential mechanism through which obesity may promote large artery dysfunction independent of conventional cardiovascular risk factors is through insulin resistance, leptin, or RAAS activation. As indicated in the aforementioned discussion insulin resistance may promote adverse effects on large arteries independent of

haemodynamic changes by inducing hypertrophy of the vascular wall through an increased synthesis of collagen and proliferation of vascular smooth muscle cells (de Fronzo and Ferrannini 1991, Avena et al 1998, King and Wakasaki 1999), changes that could subsequently increase large artery stiffness. Although, to my knowledge there are no studies that have demonstrated any relationship between insulin resistance and large artery dysfunction independent of haemodynamic factors or diabetes mellitus, this was nevertheless, not a mechanism that I could appropriately explore in the present thesis, due to the limited number of participants recruited who had an 8 hour fast at the time of blood collection. In this regard, insulin resistance is defined using fasting glucose and insulin concentrations rather than post-prandial measurements.

In contrast to the apparent lack of evidence supporting an independent role for insulin resistance as a predictor of large vessel dysfunction, with respect to leptin, there is indeed evidence in favour of an independent relationship between circulating leptin concentrations and large vessel dysfunction (Singhal et al 2002). However, the exact mechanism of this effect has not been identified. Nevertheless, as this independent relationship has previously been demonstrated, this was not a mechanism further assessed in the present thesis.

Evidence in favour of RAAS activation contributing to the deleterious effects of adiposity on large vessel function is derived from the beneficial effects of ACE inhibition on arterial stiffness (Safar et al 1997), an effect that may nevertheless have been mediated by BP changes. A BP-independent effect is nonetheless easier to understand as it is well recognized that the RAAS promotes vascular hypertrophy, hyperplasia and fibrosis (see above discussion). However, there are no convincing studies to my knowledge, conducted in large samples of humans that have assessed whether RAAS activation is related to large vessel dysfunction beyond conventional risk factors and

indexes of adiposity. This was nevertheless, a question that was beyond the scope of the present thesis.

1.4.2 Excess adiposity as an independent cause of cardiac hypertrophy.

Although there is no question that body size is the most consistent determinant of LVM, there is still no clarity as to why increases in body size produced by excess adipose tissue produces pathological effects on the heart whilst normal growth effects are physiological in nature. The strong relationship between body size and LVM is supported by a number of studies with large sample sizes (Lauer et al 1991, Lauer et al 1992, de Simone et al 1992, de Simone et al 1994, Gottdiener et al 1994, Urbina et al 1995, Gardin et al 1995, Sherif et al 2000; Lorber et al 2003, Fox et al 2004). Importantly, even in large cohorts of mild-to-moderate hypertensive patients, BMI is the strongest predictor of LVM (Lauer et al 1992, Gottdiener et al 1994). The effects of body size may be through either the impact of adiposity and muscularity, effects which are accounted for by indexing LVM for body surface area, or simply by growth effects, which are in-turn, accounted for by indexing LVM for height to appropriate allometric signals (growth signals). In support of a pathological role for obesity-induced LVH, LVM indexes that incorporate the impact of adiposity on LVM (those indexing LVM for allometric signals of height) are associated with a higher proportion of incident cardiovascular events than are LVM indexes that discount the impact of adiposity on LVM (those indexing LVM for body surface area) (de Simone et al 2005). Consequently, unlike normal growth effects on LVM which are physiological in nature, obesity-induced LVH is indeed of pathophysiological significance. However, an explanation for the pathophysiological significance of adiposity-induced LVH has not been forthcoming.

Importantly, recent evidence suggests that severe obesity (BMI>35 kg/m^2) enhances the impact of BP on LVM (Avelar et al 2007) through an as yet unidentified mechanism. These data provide one of the first potential explanations for the pathological effects of obesity on LVM as opposed to the physiological effects mediated by a normal growth. However, in milder forms of obesity, obesity may not increase the impact of BP on LVM (Fox et al 2004, Lauer et al 1992). Nevertheless, in the studies conducted in milder forms of obesity, either participants with a narrow range of BP levels were evaluated due to exclusion of treated participants (Lauer et al 1992), or analysis was conducted in a sample in whom ~50% of participants were receiving antihypertensive treatment (Fox et al 2004). The limitations of these studies in milder forms of obesity (Fox et al 2004, Lauer et al 1992), therefore prompted me, as one of the aims of the present thesis, to assess in a population sample with a high prevalence of mild-to-moderate obesity, whether adiposity is associated with an enhanced impact of BP on LVMI and wall thickness and the haemodynamic mechanisms thereof. These data are described in chapter 4.

1.4.2.1 Potential mechanisms of an independent relationship between adiposity and left ventricular mass.

Haemodynamic factors that are not normally measured as part of risk assessment may mediate obesity-induced increases in LVM. In this regard, as indicated in above discussion, through the high metabolic activity of excessive fat, obesity may result in a decrease in total peripheral resistance and an increase in venous return, stroke volume and hence cardiac output (Messerli et al 1982, Messerli et al 1983, Carabello and Gittens 1987, Paulson and Tahiliani 1992). As a consequence of the high stroke volume and cardiac output, systolic BP and pulse pressure as well as cardiac

work increase and cardiac hypertrophy may be the consequence. Further, as indicated in the aforementioned discussion, obesity promotes increases in large artery stiffness, a change that will increase central artery BP, and hence further induce increases in cardiac work, and thus cardiac hypertrophy. The impact of changes in large artery function on LVM can be independent of BP. Indeed, our group has recently demonstrated a strong BP-independent relationship between PWV and LVM and wall thickness in women (Libhaber et al 2008). Thus, haemodynamic changes in obesity are likely to account for a major portion of adiposity-induced increases in LVM that are not incorporated in conventional cardiovascular risk assessment. Therefore, in the study described in chapter 4, where I assessed whether adiposity is associated with an enhanced impact of BP on LVMI and wall thickness, I not only evaluated interactions between BP and adiposity indexes as predictors of LVM, but also interactions between PWV, an index of arterial stiffness, and adiposity indexes as predictors of LVM.

With respect to a potential role of non-haemodynamic factors as mediators of obesity-induced increases in LVM, although controversial with respect to the role of angiotensin II (Reudelhuber et al 2007), there is some evidence in favor of a contribution of the SNS and RAAS to cardiac hypertrophy. Indeed, with respect to the SNS, measurements of sympathetic nerve activity to the heart indicate an increased myocardial SNS activation in hypertensive patients with LVH as compared to those without LVH at similar BP levels (Schlaich et al 2003). With regards to the RAAS, inadequate suppression of angiotensin II (Schlaich et al 1998) and angiotensin II in relation in salt intake (Schmieder et al 1996) may modulate left ventricular structure in humans, blockers of angiotensin II are the only class of antihypertensive agents that have been shown to regress LVH beyond that produced by BP effects (Devereux et al 2004), and genetic variation at the angiotensin II receptor is associated with left ventricular structure (Schmieder et al 2001). However, the bulk of evidence in genetically

modified experimental animals does not support a direct role for the RAAS in mediating cardiac hypertrophy (Reudelhuber et al 2007). Nevertheless this does not rule out a role for interactions between the RAAS and other pathological stimuli, such as obesity, in mediating LVH. Indeed, although there is no convincing evidence to suggest that SNS or RAAS activation mediates obesity-induced increases in LVM independent of haemodynamic changes, angiotensin II receptor blockade has been shown to prevent obesity-induced increases in LVM (du Toit et al 2005). Although the question of the independent role of the RAAS and SNS in mediating increases in LVM is of importance, it went beyond the scope of the present thesis.

Alternative mechanisms that may also contribute toward obesity-induced LVH independent of haemodynamic factors include either a protective effect of leptin on cardiac hypertrophy (Barouch et al 2003) or in contrast a hypertrophic action of leptin on cardiac myocytes (Rajapurohitam et al 2003). The contrasting outcomes of these studies (Barouch et al 2003, Rajapurohitam et al 2003) are nevertheless difficult to explain and hence further research is required to evaluate whether leptin either promotes or protects cardiac myocytes against hypertrophic effects.

1.4.3 Excess adiposity as an independent cause of abnormalities of cardiac function.

A number of pre-clinical studies have provided evidence to suggest that cardiomyocyte dysfunction occurs in insulin-resistant or obese states. Indeed, the function of the cardiac myocyte sarcoplasmic reticular Ca^{2+} ATPase enzyme, which is responsible for Ca^{2+} sequestration during relaxation is impaired in insulin resistant animals (Wold et al 2005). Further, decreases in myocardial adrenergic-induced contractile responsiveness may occur in dietary-induced obese animals (Carroll et al

1997), reduced cardiomyocyte contractile function may occur in dietary-induced obese animals (Relling et al 2006), and in leptin deficient animals (Dong et al 2006) and a decreased cardimoycyte contractile response to insulin and insulin-like growth factor-1 has been reported on in genetically obese rats (Ren et al 2000). Despite these data, there are no studies that have demonstrated a reduced pump function *in vivo* in euglycaemic obese animals. Indeed, when assessed *in vivo*, euglycaemic animal models of obesity have a preserved pump function (Carroll et al 2006, du Toit et al 2008).

With respect to the conventional cardiovascular risk factor-independent impact of excess adiposity on cardiac function in clinical studies, the present scientific literature is equally as unclear. In the Multiethnic Study of Atherosclerosis, although obesity was a strong predictor of the development of congestive heart failure, this relationship was abolished by the inclusion of LV systolic chamber function (LVEF) in the regression models (Bahrami et al 2008). These data suggest that obesity mediates the development of heart failure in-part through a reduction in LV pump function. However, relationships between obesity and LV pump dysfunction independent of conventional cardiovascular risk factors have been difficult to identify. In this regard although there are consistencies in the literature with respect to the impact of obesity on diastolic function, the data is discrepant with respect to systolic dysfunction. The following discussion highlights these discrepancies.

Load-independent tissue Doppler indexes of both systolic and diastolic myocardial function are reduced in overweight people even after adjustments for BP, age, gender, blood glucose control and LVM (Peterson et al 2004, Wong et al 2004). Moreover, weight loss mediated by either gastric bypass surgery (Willens et al, 2005) or lifestyle intervention (Wong et al 2006) results in improvements in myocardial diastolic function independent of conventional cardiovascular risk factors. However, the relationship between excess adiposity and systolic cardiac chamber dysfunction (a

reduced pump function) independent of conventional cardiovascular risk factors is not that clear. Indeed, previous studies imaging the heart with techniques that assess chamber rather than myocardial function have provided contradictory evidence. In this regard, the majority of studies have reported on obesity effects on diastolic dysfunction, but studies assessing the impact of obesity on systolic chamber function (pump function) independent of conventional cardiovascular risk factors have produced conflicting outcomes (Peterson et al 2004, Wong et al 2004, Pascual et al 2003, Scaglione et al 1992, de Divitiis et al 1981, Chakko 1998, Zarich et al 1991, Stoddard et al 1992, de Simone et al 1996, Mureddu et al 1996, Iacobellis et al 2002, Karason et al 1998) and any improvement in chamber systolic function with weight loss may be related to load-induced effects (Alpert et al 1995). Moreover, even with the use of load-independent tissue Doppler measures of myocardial as opposed to chamber function (Skilton et al 2007, Wong et al 2006), or with chamber function assessments (Willens et al 2005), weight loss produced by either lifestyle modification or gastric bypass does not influence left ventricular systolic function. Consequently, clarity is required on the role of obesity as a predictor of either myocardial systolic dysfunction or cardiac pump function. From a clinical perspective, a reduced cardiac pump function in otherwise well individuals is a well established risk factor for the development of heart failure (Hunt et al 2005). Consequently, there is an urgent need to assess the importance of obesity as an independent risk factor for the development of pump dysfunction.

Clearly to solve the question of whether or not obesity induces cardiac pump dysfunction through non-conventional risk effects, an alternative approach may be required. Importantly, as indicated in the aforementioned discussion, neither human studies nor animal studies of obesity alone have been able to show an impact of excess adiposity on pump function *in vivo* independent of conventional cardiovascular risk factors including glucose control. However, what has not previously been considered is

the hypothesis that an interaction between hypertension and obesity may promote the progression from compensated cardiac hypertrophy to cardiac dilatation and pump dysfunction. What is the evidence to support this hypothesis?

As shall be described in chapter 4, I was able to show that adiposity interacts with BP and arterial stiffness to promote increases in LVM and wall thickness. As LVH is a risk factor for pump dysfunction (Drazner et al 2004), it is possible that obesity may interact with hypertension to promote not only the development of LVH, but also pump dysfunction. Importantly, in studies demonstrating a conventional cardiovascular risk factor-independent relationship between obesity and myocardial systolic dysfunction, although obese participants may have been normotensive, they nevertheless still had higher BP values than lean participants (Skilton et al 2007, Peterson et al 2004, Wong et al 2004). In these studies (Skilton et al 2007, Peterson et al 2004, Wong et al 2004) no comment was made of potential interactions between adiposity indexes and BP.

In support of an hypothesis that obesity interacts with BP to promote target organ damage is recent data to show that a nonsense mutation in the leptin receptor gene in spontaneously hypertensive rats (SHR), which induces marked obesity in these animals, results in an early nephropathy despite maintaining euglycaemia (Nagase et al 2006). Further, in young adults, although obesity and hypertension separately are associated with cardiac dilatation, a combination of obesity and hypertension is clearly required to induce marked increases in cardiac cavity dimensions (Haji et al 2006). In this regard, cardiac dilatation is now a well recognized determinant of pump dysfunction in pressure overload states (Norton et al 2002). Consequently, in the present thesis I hypothesized that an interaction between hypertension and obesity may promote the progression from compensated cardiac hypertrophy to cardiac dilatation and pump dysfunction. As this is not a question that can be answered in human studies in a single centre (it would require long-term follow-up and a very large study sample), I assessed this question in an

animal model of pressure overload hypertrophy studied in our laboratory over many years (Tsotetsi et al 2001, Badenhorst et al 2003b, Veliotes et al 2005). In this regard, the SHR is an animal model of compensated cardiac hypertrophy that ultimately develops pump dysfunction after many years (Tsotetsi et al 2001) or can be induced to develop premature cardiac dilatation and pump dysfunction following SNS and RAAS activation (Badenhorst et al 2003a, Veliotes et al 2005). As excess adiposity promotes SNS and RAAS activation, as described in chapter 5 of the present thesis I therefore assessed whether dietary-induced excess adiposity in SHR can promote the progression from compensated cardiac hypertrophy to cardiac dilatation and pump dysfunction and the mechanisms thereof. Importantly, this model of dietary-induced excess adiposity when studied in isolation does not lead to cardiac pump dysfunction as assessed *in vivo* (du Toit et al 2008).

1.4.3.1 Potential mechanisms of an independent relationship between adiposity and cardiac dysfunction.

As with LVM, haemodynamic factors that are not normally measured as part of risk assessment may mediate obesity-induced decreases in cardiac systolic or diastolic function. Indeed, as previously discussed, obesity may result in a decrease in total peripheral resistance and an increase in venous return (Messerli et al 1983, Carabello and Gittens 1987). As a consequence of the high venous return, dilatation of the chambers may occur, a change that could result in a reduction in pump function (Norton et al 2002). Further, as previously discussed, obesity promotes large artery stiffness and hence increases in cardiac afterload. The impact of changes in large artery function on the heart can be independent of BP as measured using conventional techniques (Libhaber et al 2008) as brachial artery BP does not closely reflect central BP. Thus,

haemodynamic changes in obesity could still account for a major portion of adiposity-induced decreases in cardiac function that are not accounted for by conventional cardiovascular risk factors.

With respect to a potential role of non-haemodynamic factors mediating obesity-induced decreases in cardiac systolic or diastolic dysfunction, one potential mechanism is through the adverse effects of insulin on the myocardium or through myocardial insulin resistance. Indeed, fasting insulin concentrations are inversely correlated with tissue Doppler indexes of both systolic and diastolic myocardial function (Wong et al 2004). Furthermore, cardiac dysfunction may be induced by a high fat diet in association with alterations in myocardial insulin signalling in rats (Ouwens et al 2005). Second, although there is no direct evidence to support this notion, as adipose tissue promotes SNS and RAAS activation and both SNS and RAAS activation mediate cardiac dysfunction through a number of mechanisms (Veliotes et al 2005), again SNS and RAAS activation could mediate cardiac systolic and diastolic dysfunction independent of conventional cardiovascular risk factors. Indeed, an increased aldosterone production is thought to mediate, in-part, the interaction between obesity and hypertension to promote pre-clinical renal target organ damage (Nagase et al 2006). Third, a reduced cardiac efficiency and an altered myocardial substrate metabolism may accompany obesity associated with leptin deficiency or leptin resistance (Buchanan et al 2005). These changes in myocardial substrate metabolism may contribute toward an increased cardiomyocyte apoptosis in obesity models of leptin deficiency or leptin resistance (Barouch et al 2006). The cardiomyocyte apoptosis in leptin resistant or leptin deficient animals is thought to occur through a reduced cardioprotection mediated by leptin, possibly induced via a resultant increase in ectopic lipid overload in cardiac myocytes (lipoapoptosis) (Zhou et al 2000). Cardiomyocyte apoptosis is now considered to be an

important pathophysiological process in heart failure (Foo et al 2005, Wencker et al 2003).

1.5 Summary of problem statements and hypotheses

The background to the problem statements tested and the hypotheses posed in the present thesis has been thoroughly described in the aforementioned discussion. However, before describing the studies performed in the present thesis it is worth summarizing these problem statements and hypotheses. As previously indicated, the present thesis was largely designed to address outstanding issues with respect to the role of obesity in promoting target organ changes through mechanisms that cannot be attributed soley to conventional cardiovascular risk factors. In this regard, as there is still considerable uncertainty as to whether excess adiposity may promote increases in arterial stiffness independent of confounders, in the present thesis I hypothesized that age may determine whether an excess adiposity promotes increases in arterial stiffness independent of confounders. Moreover, as there is still uncertainty as to the mechanism by which excess adiposity promotes the development of LVH independent of confounders, I also evaluated whether one potential mechanism is by enhancing the effects of haemodynamic factors (BP and arterial stiffness) on LVM. Having demonstrated a marked interaction between indexes of adiposity and haemodynamic factors to promote LVH, I subsequently posed the question as to whether similar interactions may promote the transition from compensated hypertensive LVH to cardiac decompensation. As it was necessary to account for adiposity-induced effects on BP when testing the impact of excess adiposity on arterial stiffness independent of BP and when assessing interactions between adiposity and BP to promote increases in LVM, in the first part of the thesis I initially identified the most appropriate adiposity index to

employ when accounting for adiposity-induced BP effects. This was necessary as it is controversial as to whether indexes of central or general obesity are best employed when evaluating the impact of adiposity on BP. Thus, the aims of the present thesis may be summarized as follows:

1.6 Aims

a) To identify whether indexes of central adiposity are associated with both conventional and ambulatory BP independent of other indexes of adiposity. These data are presented in chapter 2 and have been published in the *Journal of Hypertension*.

b) To clarify whether age influences the independent relationship between adiposity and large artery dysfunction in a predominantly young-to-middle aged population sample with a high prevalence of excess adiposity. These data are presented in chapter 3 and have been published in the *American Journal of Hypertension*.

c) To determine whether excess adiposity enhances the impact of haemodynamic changes on LVM. These data are presented in chapter 4.

d) To determine whether obesity interacts with hypertension to promote the development of systolic cardiac dysfunction (pump dysfunction) and the mechanisms thereof. These data are presented in chapter 5.

CHAPTER 2

The Association of Waist Circumference with Ambulatory Blood Pressure is Independent of Alternative Adiposity Indexes.

Majane et al. *Journal of Hypertension*. 2007;25:1798-1806.

Abstract

Whether indexes of central adiposity, including waist circumference (WC) or waist-to-hip ratio, are associated with BP independent of other clinical indexes of adiposity is controversial. As ambulatory BP may offer more prognostic information than conventional BP, I aimed to identify whether indexes of central adiposity are associated with both conventional and ambulatory BP independent of other indexes of adiposity. The relationship between indexes of adiposity (WC, waist-to-hip ratio, body mass index (BMI), or skin-fold thickness) and ambulatory (Spacelabs model 90207) or conventional BP was determined in 300 randomly selected subjects of African descent living in an urban developing community in South Africa. Relationships were determined with multiple indexes of adiposity in the same regression model and after adjusting for age, gender, alcohol and tobacco intake, the presence or absence of diabetes mellitus or inappropriate blood glucose control (HbA1c), antihypertensive therapy and menopausal status. 65% of subjects were overweight or obese. With respect to the relationships between indexes of adiposity, BMI and WC showed the strongest correlation ($r=0.84$, $p<0.0001$). After including all indexes of adiposity and confounders in the model, WC was the only clinical index of adiposity which independently predicted 24-hour (partial $r=0.15$, $p<0.005$) and conventional (partial $r=0.14$, $p<0.005$) systolic BP and 24-hour (partial $r=0.13$, $p<0.02$) and conventional (partial $r=0.40$, $p<0.0001$) diastolic BP. After adjusting for other adiposity indexes and confounders, every 1 SD (15 cm) increase in WC was associated with a 4.04 mm Hg increase in 24-hour systolic BP and a 4.33 mm Hg increase in 24-hour diastolic BP. In conclusion, WC is the only clinical index of adiposity that is associated with 24-hour BP independent of other adiposity indexes in a community with a high prevalence of obesity.

2.1 Introduction

There is now substantial evidence from large-population-based studies in favour of excess adiposity being a major determinant of blood pressure (BP) and the development of hypertension (Zhu et al 2005, Okosun 1998 and Harris et al 2000). In this regard, present evidence suggests that waist circumference appears to be the preferred clinical index when relating adiposity to conventional BP (Zhu et al 2005, Okosun 1998, Harris et al 2000, Luke et al 1997, Williams et al 1987, Peiris et al 1989, Kanai et al 1990, Raison et al 1992, Boyko et al 1995, Lerario et al 1997, Ho et al 2001, Okosun et al 1999, Hayashi et al 2003 and Ding et al 2004). However, this has not, to my knowledge, been reproduced in all studies (Harris et al 2000). Moreover, studies that have compared the relative impact of indexes of central fat and body mass index (BMI) on ambulatory BP have either recruited small study samples, have been non-random, have focussed on waist-to-hip ratio rather than WC as the index of central fat, and have produced ambiguous results (Guagnano et al 1994, Guagnano et al 1997, Steptoe et al 1999, Lurbe et al 1998 and Feldstein et al 2005). As twenty four-hour (24-hour) ambulatory BP is a better index of cardiovascular outcomes (Verdecchia et al 1994, Clement et al 2003, Staessen et al 1999 and Okhubo et al 1998) and target organ effects (Mancia and Parati 2000) than conventional BP values, the relative impact of indexes of central and general adiposity on ambulatory BP requires elucidation. In the present study I therefore assessed whether any one commonly used clinical index of adiposity (waist circumference, waist-to-hip ratio, BMI and skin-fold thickness) predicts conventional and ambulatory BP independent of the others. This study was conducted in a randomly selected population sample, with a high prevalence of obesity, living in an urban developing community in South Africa.

2.1 Methods

Study participants. The study was approved by the University of the Witwatersrand Committee for Research in Human Subjects (approval number: M02-04-72) and conducted according to the principles outlined in the Declaration of Helsinki. Participants gave informed, written consent. This study is part of the ongoing African Project on Genes in Hypertension which has previously been described (Shiburi et al 2006, Maseko et al 2006). South Africans of black African ancestry from nuclear families of the predominant chiefdoms (tribes) of the area (Nguni, Sotho and Venda) with common ancestral backgrounds were randomly recruited from a metropolitan area of Johannesburg (South Western Townships-SOWETO). Nuclear families were recruited if at least one or two offspring of at least 17 years of age and one or both parents were available for examination. The analysis for the study was conducted in 2006 and was derived from the data base available at the end of 2005. Of the 496 South Africans of African ancestry randomly recruited by the end of 2005, 395 subjects had all clinical data. Of these participants 300 had more than 20 hours of ambulatory BP recordings and more than 10 and 5 readings for the computation of daytime and night-time means respectively.

Clinical, demographic and anthropometric measurements. A standardized questionnaire was administered to obtain demographic data and information on each participant's medical history, smoking habits, intake of alcohol, use of medication and menopausal status. The questionnaire was explained to families at an initial home visit and then subsequently completed in the presence of trained study assistants at an office visit where questions could be answered. At a second home visit, medications, and alcohol and tobacco in the household were compared against those reported on in the questionnaire. Menopause was confirmed with measurements of follicle stimulating hormone concentrations (Bayer, Leverkusen, Germany).

Height and weight were measured with the participants standing and wearing indoor clothes with no shoes. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Waist circumference was measured at the end of gentle expiration, at the point midway between the lowest rib and the iliac crest with the subject standing. Hip circumference was measured using a standard approach and waist-to-hip ratio calculated. Triceps and subscapular skin-fold thickness were determined using a Harpenden skin-fold callipers and the mean of these values obtained for statistical analyses. Participants were identified as being overweight if their BMI was ≥25 $kg.m^2$ and obese if their BMI was ≥30 $kg.m^2$. Standard laboratory blood tests of renal function, liver function, haematological parameters, blood glucose, lipid profiles and glycated haemoglobin (HbA_{1C}) were performed. Diabetes mellitus or inappropriate blood glucose control was defined as the use of insulin or oral hypoglycaemic agents or a percentage HbA_{1C} (Roche Diagnostics (Mannheim, Germany)>7.0% (Peters et al 1996).

Conventional blood pressure (BP) measurements and heart rate. Trained observers measured brachial artery BP using a mercury sphygmomanometer as well as heart rate (HR) from the radial artery pulse. The participants were seated and asked to rest for 5 minutes. The observers measured the participants' sitting BP and HR five consecutive times. Systolic and diastolic (phase V) BP were determined to the nearest 2 mm Hg according to the recommendations of the European Society of Hypertension (O'Brien et al 2003). Standard cuffs with an inflatable bladder with a length of 22 cm and a width of 12 cm were used unless the arm circumference exceeded 31 cm, when larger cuffs with a 31 x 15 cm bladder were employed. The five readings were averaged to obtain a single systolic, diastolic and mean arterial BP reading and HR. 0.76% of visits had fewer than the planned conventional BP recordings. The frequency of identical consecutive conventional BP recordings was 0.25%. The occurrence of conventional BP

values recorded as an odd number was 0% and of the 3936 systolic and diastolic conventional BP readings, 27.8% ended on a zero (expected =20%).

24-hour urine assessments. To determine 24-hour urine Na^+ excretion (an index of Na^+ intake), timed urine samples were obtained over a period of at least 24 hours after discarding urine excreted immediately prior to the start of the collection period. Urine Na^+ concentrations were measured and 24-hour urine Na^+ excretion rate calculated from the product of urine volume and urine Na^+ concentration. The quality of the urine samples was determined as previously described (Maseko et al 2006 and Staessen et al 1988). Briefly, I constructed 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 mls/day were also assumed to be incomplete urine collections. These approaches are standard approaches and have been published by other groups (Stolartz et al 2004, Kuznetsova et al 2004).

Ambulatory blood pressure measurements. Twenty four-hour ambulatory BP monitoring was performed using oscillometric monitors (Spacelabs, model 90207) performed on the same day as the conventional BP measurements. The size of the cuff was the same as that which was used for conventional BP measurements. The accuracy of ambulatory monitors was checked monthly against a mercury manometer. If the monitors recorded pressure values that deviated from a mercury reading by more than 4 mm Hg, monitors were recalibrated by the commercial suppliers. The monitors were programmed to measure BP at 15-minute intervals from 06:00 to 22:00 and then 30-minute intervals from 22:00 to 06:00. Subjects 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. From the subject's diary card data we determined the awake and asleep periods.

Considering the patterns of daily activities, the daytime and night-time intervals were defined as time intervals ranging from 09:00 h to 19:00 h and from 23:00 h to 05:00 h respectively. These fixed clock-time intervals (Thijs et al 1992, Fagard et al 1996, Fagard et al 1997) were defined in order to eliminate the transition periods (evening and morning) during which BP changes rapidly in most subjects. Intra-individual means of the ambulatory measurements were weighted by the time-interval between successive recordings (Thijs et al 1992, Fagard et al 1996, Fagard et al 1997). Ambulatory BP data were expressed as 24-hour, daytime and night-time average systolic and diastolic BP. The average (±SD) number of BP recordings obtained was 63.4±10.6 (range=31–80) for the 24-hour period, 29.7±6.6 (range=11–41) for the fixed clock-time day interval, and 9.5±0.9 (range=6 –10) for the fixed clock-time night interval.

Data analysis. Database management and statistical analyses were performed with SAS software, version 9.1 (The SAS Institute Inc., Cary, North Carolina, USA). Data from individual subjects were averaged and expressed as mean±SD. Proportions were compared with X^2 analysis. Linear regression analysis was used to determine relationships. Stepwise regression analysis was performed to determine independent effects of indexes of adiposity on BP. Included in the regression model were age, gender, alcohol and tobacco intake (defined as the presence or absence of daily tobacco or alcohol ingestion), postmenopausal status (confirmed with follicle stimulating hormone measurements), the presence or absence of diabetes mellitus or inappropriate blood glucose control (considered as a single covariate) and the use of antihypertensive therapy (either receiving therapy or not receiving therapy). Further adjustments of probability values were made for non-independence of family members using non-linear models (mixed procedure as described in the SAS software). To identify those indexes of adiposity associated with BP independent of other indexes of adiposity, indexes of adiposity were considered together in the regression model. In addition to including

antihypertensive therapy as a covariate in the regression analysis, sensitivity analyses were repeated in a subgroup of participants who were not receiving antihypertensive therapy (n=273).

2.2 Results

Characteristics of the participants. Table 2.1 gives the demographic and clinical characteristics of the participants. More women than men participated. Sixty five percent of subjects were overweight or obese, with 27.6% being overweight and 37.7% being obese. In the group 42% were hypertensive, 20% were receiving therapy for hypertension, and 8% had diabetes mellitus. A relatively small percentage of the group were smokers (12%), mostly due to lack of affordability and also due to the greater proportion of women (who traditionally don't smoke in this population) compared to men. 24-hour urine Na^+ excretion rates (an index of Na^+ intake) despite being high, were not associated with indexes of obesity (r values: BMI=0.05; waist circumference=0.003; skin-fold thickness=0.003; waist-to-hip ratio=0.11; $p>0.1$ for all). The mean age (41.4±17.9 years) and BMI (28.9±7.3 kg/m^2) and the percentage women (63%) in all subjects recruited was the same as that for subjects with all clinical data (Table 2.1). The demographic and clinical characteristics of the subjects with ambulatory BP measurements that met pre-specified quality control criteria were similar to those of subjects with all clinical data (Table 2.1). In addition, although they were younger (38.1±16.5 years), fewer individuals had hypertension (26%), and there were fewer postmenopausal women (29%), the other demographic and clinical characteristics in the participants not receiving antihypertensive therapy (n=237) did not differ from those of either the participants with ambulatory BP measurements or those with all clinical data (data not shown). Average conventional systolic and diastolic BP values were higher than average 24-hour, day or night-time systolic and diastolic BP values (Table 2.1).

Association between indexes of adiposity. As with the data described in chapters 2 and 3, BMI and waist circumference showed the strongest relationship in the cohort described in the present study (Table 2.2). The weakest relationship was noted between

Table 2.1. Demographic, anthropometric and clinical characteristics of study subjects.

	All subjects	Subjects with ambulatory BP
Sample number	395	300
% Female	63	63
Age (years)	41.8±18.1	43.0±17.9
Height (cm)	161±9	161±9
Weight (kg)	75±19	75±19
BMI (kg/m^2)	28.8±7.4	29.0±7.4
Waist circumference (cm)	89±15	89±15
Hip circumference (cm)	107±15	106±15
WHR	0.84±0.10	0.84±0.10
% Overweight/obese	65	66
Skin-fold thickness (cm)		
Subscapular	2.41±1.37	2.47±1.36
Triceps	1.90±1.20	2.01±1.25
Mean	2.15±1.16	2.24±1.19
Urine Na^+ excretion (mmol/day)	113.9±53.5	110.4±48.1
% smokers	12.2	12.7
% alcohol*	21.2	22.3
% with hypertension	42.0	41.7
% treated for hypertension	20.3	21.0
% with DM	8.2	8.1
HbA_{1C} (%)	6.02±1.07	6.04±1.07
n (%) postmenopausal	98 (25)	78 (27)
Clinic SBP/DBP (mm Hg)	130±23/84±12	131±23/84±12
Ambulatory BP (mm Hg)		
24 hour SBP/DBP (mm Hg)	-	119±16/73±11
Daytime SBP/DBP (mm Hg)	-	123±16/78±11
Night-time SBP/DBP (mm Hg)	-	112±18/65±12

BMI, body mass index; WHR, waist-to-hip ratio; DM, diabetes mellitus; HbA_{1C}, glycated haemoglobin; SBP, systolic BP; DBP, diastolic BP; * refers to % who consume alcohol on a daily basis.

Table 2.2. Correlation matrix between indexes of adiposity in the present study group (n=300).

	WC (cm)	WHR	Mean skin-fold thickness
Body mass index (kg/m^2)	0.84**	0.18*	0.66**
Waist circumference (WC) (cm)	-	0.58**	0.58**
Waist-to-hip ratio (WHR)	-	-	0.11†

WC, waist circumference, WHR, waist-to-hip ratio. Numbers are correlation coefficients (r). *p<0.01, **p<0.0001 for the relationships. †p=0.058.

waist-to-hip ratio and BMI. Furthermore, only a trend effect was noted between waist-to-hip ratio and skin-fold thickness (Table 2.2).

Unadjusted associations between indexes of adiposity and BP. Body mass index, waist circumference, waist-to-hip ratio and skin-fold thickness were all correlated with conventional systolic BP (Figure 2.1). However, BMI, waist circumference and waist-to-hip ratio, but not skin-fold thickness, were correlated with 24-hour systolic BP (Figure 2.2). Significant correlations between either BMI ($r=0.35$, $p<0.0001$), waist circumference ($r=0.41$, $p<0.0001$), waist-to-hip ratio ($r=0.27$, $p<0.0001$) or skin-fold thickness ($r=0.25$, $p<0.0001$) and conventional diastolic BP were also noted before adjustments. However, BMI ($r=0.17$, $p=0.0031$), waist circumference ($r=0.28$, $p<0.0001$), and waist-to-hip ratio ($r=0.23$, $p<0.0001$), but not skin-fold thickness ($r=0.09$, $p=0.14$) were correlated with 24-hour diastolic BP. Significant unadjusted correlations were noted between body weight and conventional BP (SBP: $r=0.29$, $p<0.0001$, DBP: $r=0.32$, $p<0.0001$), 24-hour BP (SBP: $r=0.20$, $p=0.0005$, DBP: $r=0.16$, $p=0.0052$), daytime BP (SBP: $r=0.20$, $p=0.0005$, DBP: $r=0.15$, $p=0.011$), and night-time BP (SBP: $r=0.18$, $p=0.0016$, DBP: $r=0.18$, $p=0.0019$). The unadjusted associations between indexes of adiposity and BP in the subgroups of participants who were not receiving antihypertensive therapy (data not shown) were similar to those in the group of participants with ambulatory BP.

Conventional and ambulatory BP and indexes of adiposity in separate models. When considering indexes of adiposity in separate regression models, but adjusting for other confounders, BMI, waist circumference and skin-fold thickness were independent predictors of conventional systolic and diastolic BP; whereas waist-to-hip ratio was only associated with conventional diastolic BP (Table 2.3). Waist circumference was the only index of adiposity associated with 24-hour systolic and diastolic BP after adjustments for other covariates (Table 2.3). Nevertheless, the relationship between BMI and 24-hour systolic BP was close to significant (Table 2.3). Analyses of daytime and night-time

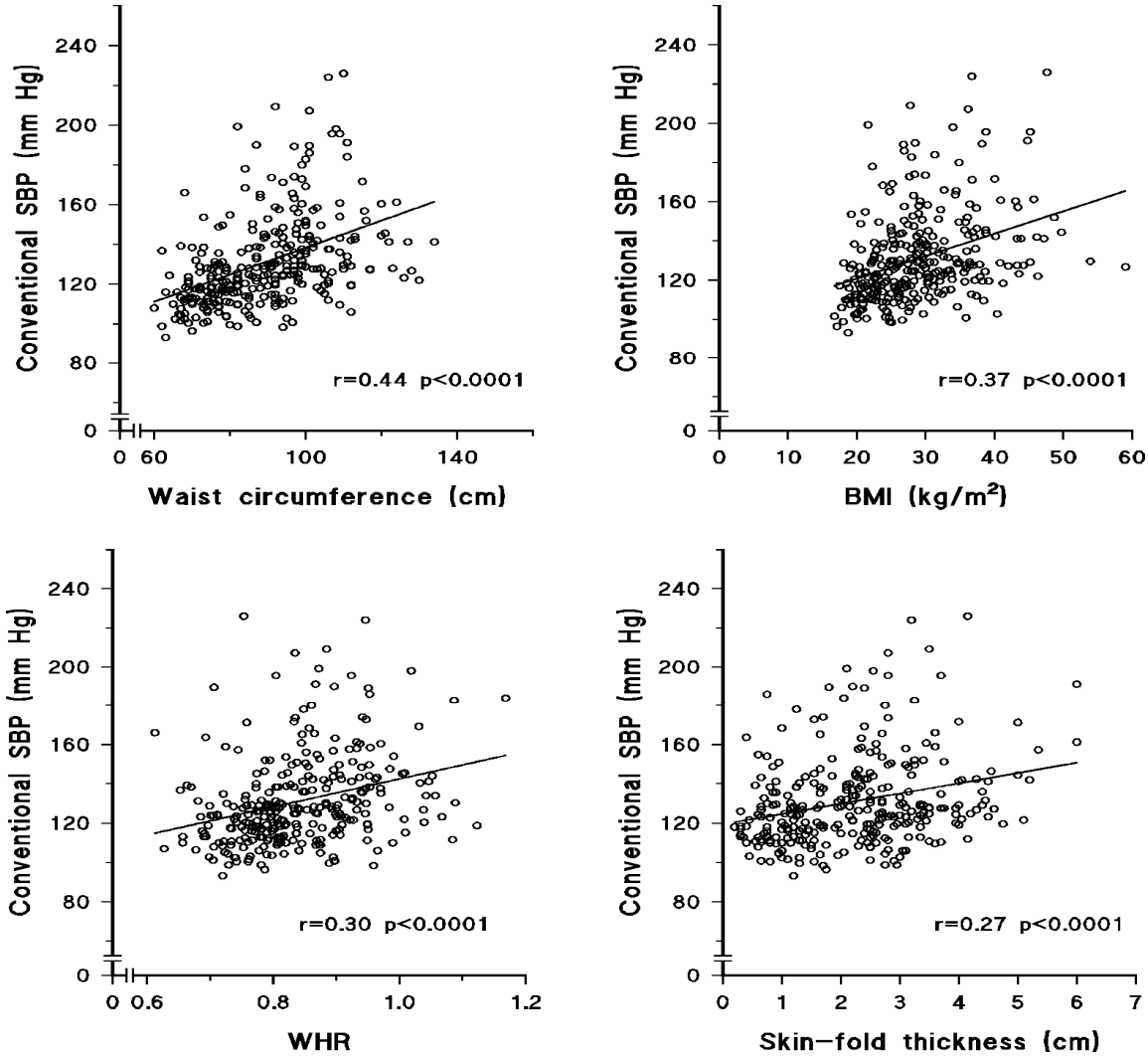


Figure 2.1. Relationships between indexes of adiposity and conventional systolic blood pressure (SBP) in study subjects (n=300). BMI, body mass index; WHR, waist-to-hip ratio.

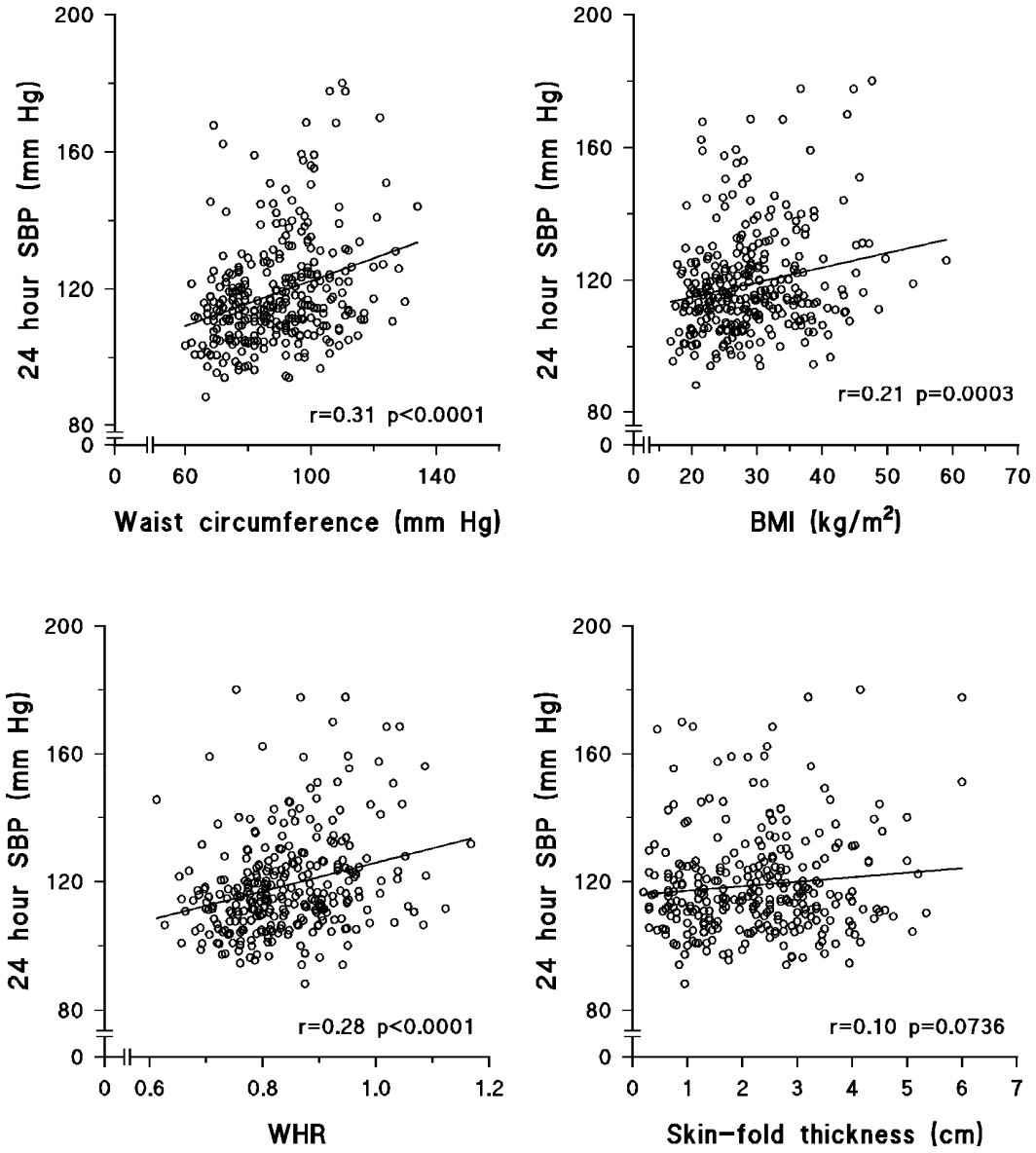


Figure 2.2. Relationships between indexes of adiposity and 24-hour ambulatory systolic blood pressure (SBP) in study subjects (n=300). BMI, body mass index; WHR, waist-to-hip ratio.

Table 2.3. Partial correlation coefficients (r) for the relationship between indexes of adiposity considered separately in the regression model and systolic (SBP) or diastolic blood pressure (DBP) in the study group (n=300).

	Partial r *	p value*	Partial r*	p value*	Partial r*	p value*	Partial r*	p value*
	Conventional SBP (mm Hg)		24 hour SBP (mm Hg)		Daytime SBP (mm Hg)		Night-time SBP (mm Hg)	
Waist circumference (cm)	0.13	**<0.005**	0.14	**0.009**	0.14	**0.009**	0.11	**0.031**
Body mass index (kg/m^2)	0.13	**<0.01**	0.10	0.054	0.09	0.098	0.09	0.088
Waist-to-hip ratio	0.05	0.255	0.08	0.107	0.10	0.055	0.08	0.151
Mean skin-fold thickness (cm)	0.12	**<0.01**	0.05	0.373	0.05	0.332	0.03	0.606
	Conventional DBP (mm Hg)		24 hour DBP (mm Hg)		Daytime DBP (mm Hg)		Night-time DBP (mm Hg)	
Waist circumference (cm)	0.41	**<0.0001**	0.12	**0.033**	0.13	**0.018**	0.11	**0.048**
Body mass index (kg/m^2)	0.21	**<0.0001**	0.06	0.231	0.06	0.314	0.06	0.237
Waist-to-hip ratio	0.12	**0.029**	0.08	0.162	0.10	0.062	0.09	0.115
Mean skin-fold thickness (cm)	0.17	**0.002**	0.02	0.675	0.03	0.637	0.03	0.584

The p values indicated in bold are significant. * from stepwise regression analysis including indexes of adiposity considered separately and age, gender, alcohol and tobacco intake, postmenopausal status (confirmed with follicle stimulating hormone measurements), the presence or absence of diabetes mellitus or inappropriate blood glucose control, and the use of antihypertensive therapy in the regression model.

systolic and diastolic BP revealed similar results, with waist circumference being the only index of adiposity associated after adjustments for other covariates (Table 2.3). Similar results were observed in the subgroup of participants not receiving antihypertensive therapy (data not shown)

Although, body weight was associated with conventional BP (systolic BP: partial r=0.10, p=0.03; diastolic BP: partial r=0.21, p<0.0001), no associations with 24-hour, daytime or night-time systolic and diastolic BP were noted after adjustments for other covariates.

Conventional and ambulatory BP and indexes of adiposity in the same model. The independent relationship between waist circumference and either conventional or 24-hour BP (Table 2.3) persisted after BMI, waist-to-hip ratio and skin-fold thickness were included in the model (Figure 2.3, left panels). In contrast, neither BMI nor skin-fold thickness were associated with clinic or 24-hour BP after adjusting for waist circumference and waist-to-hip ratio (Figure 2.3, left panels). Similarly, in the subgroup of participants not receiving antihypertensive therapy, waist circumference was independently related to either conventional or 24-hour BP after BMI, waist-to-hip ratio and skin-fold thickness were included in the model (Figure 2.3, right panels). In contrast, neither BMI, nor skin-fold thickness were significantly associated with either clinic or 24-hour BP after adjusting for waist circumference and waist-to-hip ratio (Figure 2.3, right panels). In addition, the independent relationship between waist circumference and either day or night BP persisted after BMI, waist-to-hip ratio and skin-fold thickness were included in the model (in all participants with ambulatory BP data the partial correlation coefficients for waist circumference and daytime SBP were: r=0.16, p=0.025; daytime DBP: r=0.15, p=0.009; night-time SBP: r=0.10, p=0.058; night-time DBP: r=0.12, p=0.027; in participants not receiving antihypertensive therapy, the partial correlation coefficients for waist

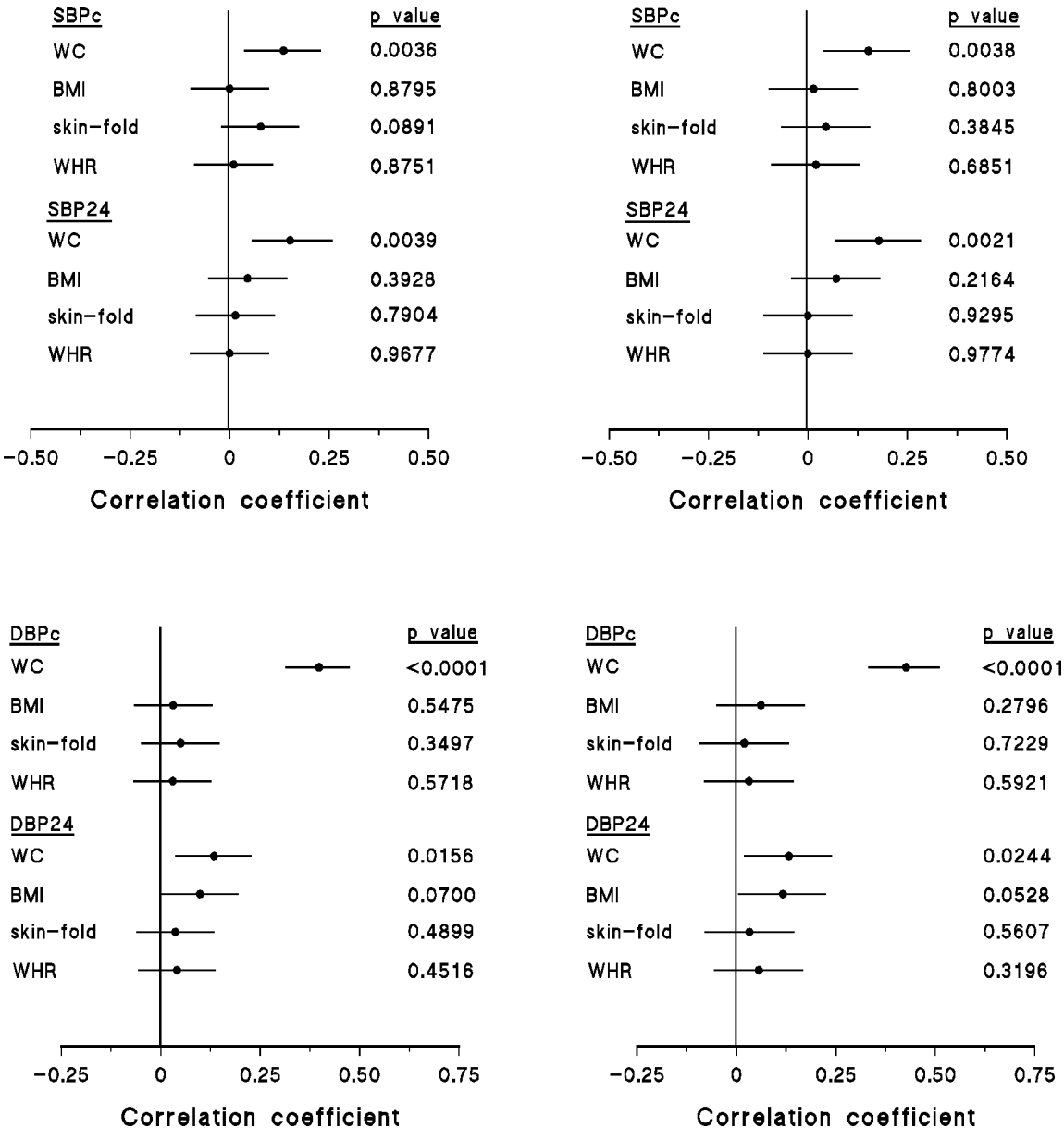


Figure 2.3. Partial correlation coefficients (r) and 95% confidence intervals for the relationship between indexes of obesity and conventional (BPc) or 24-hour (BP24) systolic and diastolic blood pressure after including all indexes of adiposity together in the regression equation. Partial correlation coefficients are after adjustments for other confounders (see Table 2.3 for additional confounders) in the study group (n=300, left panels) and in the subgroup not receiving antihypertensive therapy (untreated subjects, n=237, right panels). BMI, body mass index; skin-fold, skin-fold thickness; WC, waist circumference; WHR, waist-to-hip ratio. p values are for significant independent relationships.

circumference and daytime SBP were: r=0.18, p=0.002; daytime DBP: r=0.14, p=0.018; night-time SBP: r=0.17, p=0.005; night-time DBP: r=0.14, p=0.015).

Predicted size effects of indexes of adiposity on ambulatory BP. The size effects of waist circumference on 24-hour ambulatory systolic BP associated with a 15cm (~1 SD) increase in waist circumference were partially improved on after further adjusting for skin-fold thickness, waist-to-hip ratio and BMI (Figure 2.4A). Before adjusting for BMI, waist-to-hip ratio and skin-fold thickness, every 1 SD (15 cm) increase in waist circumference was associated with a 2.46 mm Hg increase in 24-hour systolic BP (Figure 2.4A). In comparison, after adjusting for skin-fold thickness, BMI and waist-to-hip ratio, every 1 SD (15 cm) increase in waist circumference was associated with a 4.04 mm Hg increase in 24-hour systolic BP (Figure 2.4A). In contrast, the size effects of BMI on 24-hour ambulatory systolic BP associated with a 7.4 kg/m^2 (~1 SD) increase in BMI were considerably reduced after adjusting for WC and waist-to-hip ratio (Figure 2.4B). Similarly, before adjusting for BMI, waist-to-hip ratio and skin-fold thickness, every 1 SD (15 cm) increase in waist circumference was associated with a 1.55 mm Hg increase in 24-hour diastolic BP (Figure 2.4C); whereas, after adjusting for skin-fold thickness, BMI and waist-to-hip ratio, every 1 SD (15 cm) increase in waist circumference was associated with a 4.33 mm Hg increase in 24-hour diastolic BP (Figure 2.4C). In contrast, the size effects of BMI on 24-hour ambulatory diastolic BP associated with a 7.4 kg/m^2 (~1 SD) increase in BMI were considerably reduced after adjusting for waist circumference and waist-to-hip ratio (Figure 2.4D).

The analyses in the group not receiving antihypertensive therapy were similar, with every 1 SD (14.7 cm) increase in waist circumference was associated with a 2.69 and 1.57 mm Hg increases in 24-hour systolic and diastolic BP, respectively, compared to increases of 4.74 and 4.62 mm Hg in 24-hour systolic and diastolic BP, respectively after adjusting for BMI, waist-to-hip ratio and skin-fold thickness. In contrast, the effects of BMI on 24-

hour ambulatory systolic (1.96 mm Hg) and diastolic (0.98 mm Hg) BP associated with a 6.8 kg/m^2 (~1 SD) increase in BMI were considerably reduced after adjusting for WC and waist-to-hip ratio (-2.01 and 2.42 for systolic and diastolic BP, respectively).

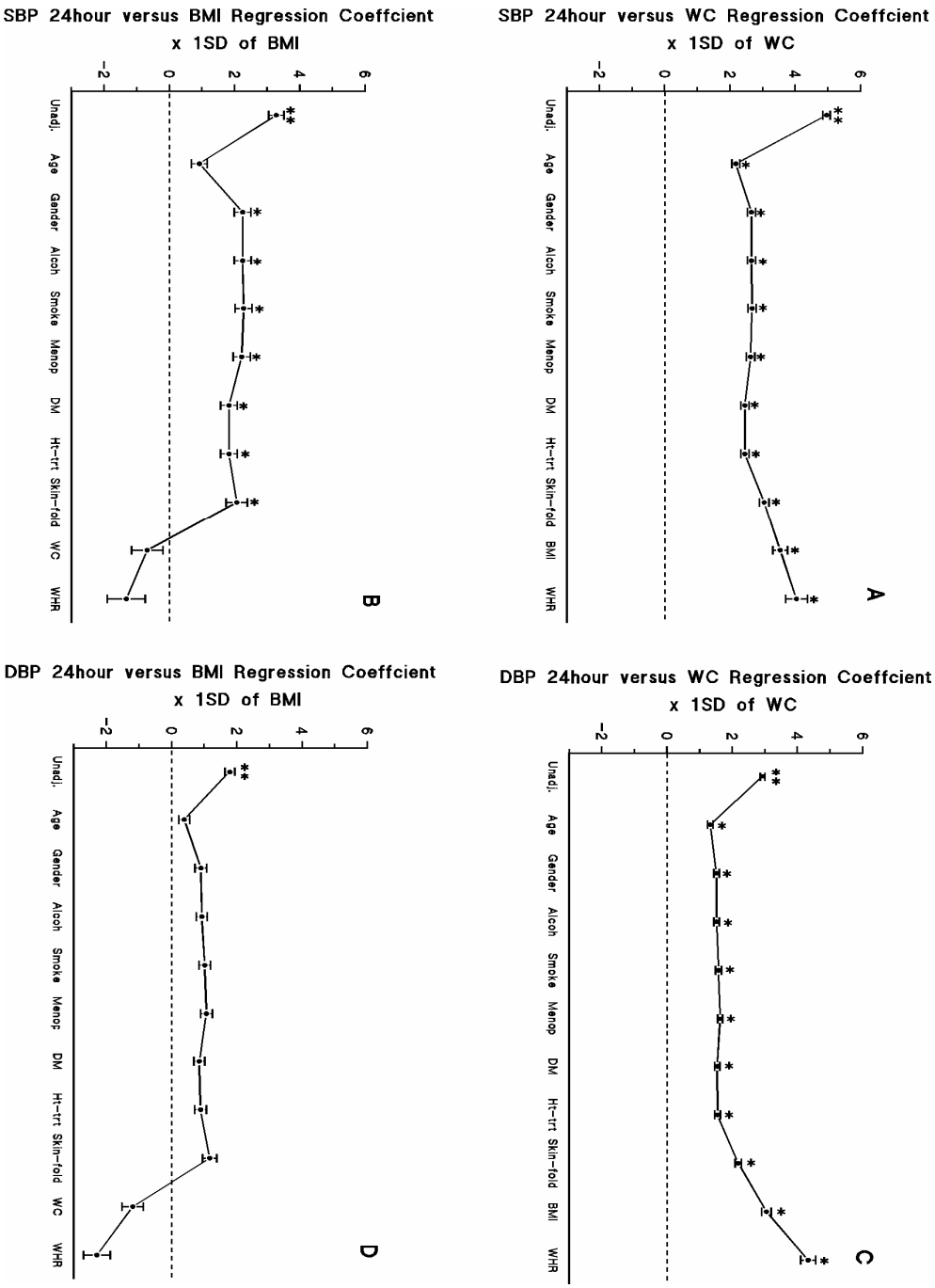


Figure 2.4. Unadjusted (unadj.) and adjusted differences in 24-hour systolic (SBP) and diastolic (DBP) blood pressure associated with one standard deviation (SD) increase in waist circumference (WC) - panels A and C, SD~15cm) or body mass index (BMI – panels B and D, SD~7 kg/m^2) in the study group (n=300). Alcoh., alcohol intake; Smoke, tobacco intake; Menop, menopausal status; DM, presence or absence of diabetes mellitus or inappropriate blood glucose control; HT-Trt, hypertension treatment; Skin-fold, skin-fold thickness; WHR, waist-to-hip ratio. *p<0.05, ** p<0.005.

2.4 Discussion

The main findings of the present study are that waist circumference is the only clinical index of adiposity that was associated with an increased ambulatory systolic and diastolic BP independent of other indexes of adiposity.

A recent large study conducted in a European population in which abdominal fat effects were not assessed, has demonstrated convincingly that BMI is associated with ambulatory BP (Kotsis et al 2005). However, the present study is the first conducted in a relatively large, randomly selected population sample that has explored whether the relationship between ambulatory BP and indexes of central adiposity (waist circumference and waist-to-hip ratio) are independent of other indexes of adiposity. In this regard, the relationship between WC and 24-hour BP was independent of BMI and skin-fold thickness, whereas the relationship between BMI and 24-hour BP was masked by adding waist circumference to the regression equation. As ambulatory BP is a better index of cardiovascular outcomes (Verdecchia et al 1994, Clement et al 2003, Staessen et al 1999 and Okhubo et al 1998) and target organ effects (Mancia et al 2000) than conventional BP values, these data support an important role for the use of waist circumference when predicting the BP that is more closely associated with cardiovascular outcomes and target organ damage than conventional BP.

Prior studies conducted in small, non-random samples have explored whether waist-to-hip ratio is associated with ambulatory BP independent of BMI (Guagnano et al 1994, Guagnano et al 1997, Steptoe et al 1999, Lurbe et al 1998 and Feldstein et al 2005). Some of these studies have suggested that waist-to-hip ratio is independently related to ambulatory BP in both adults (n=51-97) (Guagnano et al 1994, and Guagnano et al 1997) and in 140 children (Lurbe et al 1998), findings not supported by the present study. On the other hand, consistent with the present study, some previous studies have demonstrated that waist-to-hip ratio is not independently associated with ambulatory

systolic or diastolic BP in 156 school-teachers (Steptoe et al 1999) and 357 untreated hypertensives (Feldstein et al 2005). The lack of consistent relationship between waist-to-hip ratio and ambulatory BP may be explained by the poor relationship between visceral fat mass and waist-to-hip ratio (Kvist et al 1988, Seidell et al 1988, Pouliot et al 1994, Wajchenberg et al 2000 and Stewart et al 2003). In this regard, some studies have highlighted the critical role of visceral fat mass in mediating BP changes (Williams et al 1987, Peiris et al 1989, Kanai et al 1990, Raison et al 1992, Boyko et al 1995, Lerario et al 1997, Okosun et al 1999, Ho et al 2001, Hayashi et al 2003, Ding et al 2004).

Three possibilities may explain the independent relationship between waist circumference, but not BMI with BP in the present and in previous (Zhu et al 2005 and Okosun et al 1998) studies. First, an inconsistent relationship between BMI and adiposity may occur, particularly in populations of African descent (Luke et al 1997). Thus BMI may not be a reliable index of adipose tissue mass in some populations. Second, as visceral fat mass may be important in mediating BP changes (Williams et al 1987, Peiris et al 1989, Kanai et al 1990, Raison et al 1992, Boyko et al 1995, Lerario et al 1997, Okosun et al 1999, Ho et al 2001, Hayashi et al 2003 and Ding et al 2004), it is also possible that BMI is not that closely related to the fat compartment that contributes to BP. However, in the present study the correlation coefficient between waist circumference and BMI was remarkably strong (r=0.84). Third, the association between BMI and BP could be tempered by the presence of a genotype that moderates the relationship (Tiago et al 2003) or a genotype that increases the association between waist circumference and BP.

The lack of independent relationship between skin-fold thickness and BP in the present study may also be explained by the close relationship between visceral fat and BP (Williams et al 1987, Peiris et al 1989, Kanai et al 1990, Raison et al 1992, Boyko et al 1995, Lerario et al 1997, Okosun et al 1999, Ho et al 2001, Hayashi et al 2003 and Ding et al 2004). Skin-fold thickness is an index of subcutaneous peripheral fat and as indicated in

the present study, has a poor correlation with indexes of central fat. Interestingly, when indexes of adiposity were considered separately in the regression model, skin-fold thickness and conventional systolic and diastolic BP were correlated, whereas skin-fold thickness and ambulatory BP were not. These data would suggest that although peripheral/subcutaneous fat is associated with resting BP, it is not associated with ambulatory BP.

As compared to ambulatory BP, conventional BP is more likely to reflect an increased sympathetic activity associated with the alerting reaction (Weber et al 1994), and sympathetic overactivation has been shown to be associated with obesity (Tentolouris et al 2006). Thus, the closer relationship between skin-fold thickness and conventional BP as compared to skin-fold thickness and ambulatory BP in the present study may be explained by an increase in sympathetic activity associated with the measurement of conventional BP (alerting reaction). Similarly, the colser relationship between waist circumference asnd conventional diastolic BP as compared to waist circumference and ambulatory diastolic BP in the present study may also be explained by an increase in sympathetic activity associated with the measurement of conventional diastolic BP.

The limitations of the present study include the cross-sectional design. Moreover, more direct measures of visceral fat (such as computed tomography) were not employed. However, the present study was not designed to determine the impact of visceral fat on ambulatory BP, but rather to identify which clinical index of adiposity is independently associated with ambulatory BP. The relatively lower proportion of males as compared to females recruited in the present study also raises the question as to whether the outcomes of the present study may only apply to females. The lower sample of males prevented us from performing gender-specific analysis with confidence in the outcomes. However, it is well recognised that obesity in males is more frequently accompanied by central rather than peripheral fat accumulation. Hence, the outcomes of the present study are unlikely to

be through an effect noted only in females. As this study was performed in a population sample of African ancestry with a high prevalence of hypertension (42%) and a high proportion of participants who were overweight or obese (66%), these data may not be applicable to other populations with a different prevalence of hypertension or obesity.

In conclusion, the present study indicates that waist circumference is the only clinical index of adiposity that is associated with ambulatory systolic and diastolic BP independent of other indexes of adiposity. Therefore, the present data suggest that waist circumference is the index of adiposity to use when assessing the impact of excess adiposity on ambulatory BP, a BP measurement that is more closely associated with cardiovascular outcomes than is conventional BP.

CHAPTER 3

Impact of Age on the Independent Association of Adiposity with Pulse Wave Velocity in a Population Sample of African Ancestry.

Majane et al. *American Journal of Hypertension*. 2008;21:936-942

Abstract

There is uncertainty as to whether age influences the independent relationship between adiposity and pulse wave velocity (PWV). I therefore explored the impact of age on the independent relations between indexes of central (waist circumference [WC] and waist-to-hip ratio [WHR]), general (body mass index) or subcutaneous (skin-fold thickness) adiposity and PWV, or augmentation index (AI) in 508 randomly selected subjects greater than 16 years of age in a population sample of African ancestry with a high prevalence of excess adiposity (~63% overweight or obese). Applanation tonometry was performed at the radial, carotid and femoral arteries, from which PWV, and central (AIc) or peripheral (AIp) AI were derived. Multivariate relations were determined in sex-specific groups with confounders. ~22% of the sample were receiving antihypertensive therapy and ~8% had diabetes mellitus or an HbA1c>7.0%. In women a strong interaction between age and either WC or WHR was associated with log PWV independent of confounders and the individual terms ($r=0.68$, $p<0.0001$). This translated into a markedly greater increase in logPWV with increases in either WHR or WC in women older as compared to women younger than the median age (slopes of WHR-logPWV relations=0.37±0.13 vs -0.16±0.11, $p<0.005$; slopes of WC-logPWV relations=0.0030±0.0009 vs -0.0009±0.0007, $p<0.002$). In women older than the median age for the group, one SD increase in WC (12.8 cm) translated into a 0.69 m/sec increase in PWV as compared to a 0.16 m/sec decrease in PWV for each one SD increase in WC (13.0 cm) in women younger than the median age. No index of adiposity was independently associated with the log of AIc, or AIp. In conclusion, in women of African ancestry, age has a marked effect on the independent relationship between indexes of central adiposity and PWV, with a greater independent effect of adiposity noted in older as compared to younger women.

3.1 Introduction

As underscored in chapter 1 of the present thesis, excess adiposity contributes to adverse cardiovascular outcomes (Kurth et al 2002, Wilson et al 2002, National Task Force on the Prevention and Treatment of Obesity 2000) not only by promoting the development of conventional cardiovascular risk factors (National Task Force on the Prevention and Treatment of Obesity 2000), but also through alternative effects. One such effect may be through an increase in arterial stiffness (Sutton-Tyrrell et al 2001, Ferreira et al 2004, Mackey et al 2002, Wildman et al 2003, Czernichow et al 2005, Ferreira et al 2004, Snijder et al 2004, Zebekakis et al 2005, Cosson et al 2007, Toto-Moukouo et al 1986), a change that augments central artery systolic blood pressure (BP) through early wave reflections and hence increases cardiac and large vessel loads. Importantly, arterial stiffness determines cardiovascular outcomes independent of conventional BP (de Simone et al 1999, Laurent et al 2001, Boutouyrie et al 2002, London et al 2001, Safar et al 2002, Guerin et al 2001) as conventional BP measurements do not accurately reflect central artery BP values (Karamanoglu et al 1993, Williams et al 2006).

Despite the consistency in the reports demonstrating relations between indexes of adiposity and arterial stiffness, there is controversy as to whether this relationship is independent of factors such as BP, heart rate (HR), and diabetes mellitus. In this regard, although adiposity is associated with pulse wave velocity (PWV), a simple and reliable index of arterial stiffness that is a strong independent predictor of cardiovascular outcomes (Blacher et al 1999a, Laurent et al 2001, London et al 2001, Guerin et al 2001, Cruickshank et al 2002, Boutouyrie et al 2002, Sutton-Tyrrell et al 2005), some studies have (Sutton-Tyrrell et al 2001, Mackey et al 2002, Wildman et al 2003, Ferreira et al 2004), whilst most others have not (Ferreira et al 2004, Zebekakis et al 2005, Taquet et al 1993, Amar et al 2001, Nakanishi et al 2003, Oren et al 2003, Mitchell et al 2004, Czernichow et al 2005) been able to demonstrate strong relations between adiposity

indexes and PWV independent of haemodynamic factors and diabetes mellitus. Nevertheless, convincing independent relations between clinical indexes of adiposity and PWV have been obtained in elderly populations (Sutton-Tyrell et al 2001, Mackey et al 2002), whilst substantial evidence against a strong independent relationship between adiposity and PWV has been noted in samples that are predominantly young-to-middle aged (Ferreira et al 2004, Zebekakis et al 2005, Taquet et al 1993, Amar et al 2001, Nakanishi et al 2003, Oren et al 2003, Mitchell et al 2004, Czernichow et al 2005). Thus, the haemodynamic and glucose control-independent effect of adiposity on PWV may be age-dependent. Consequently, in the present study I sought to determine whether age influences the independent relationship between adiposity indexes and PWV or other indexes of large artery dysfunction.

3.2 Methods

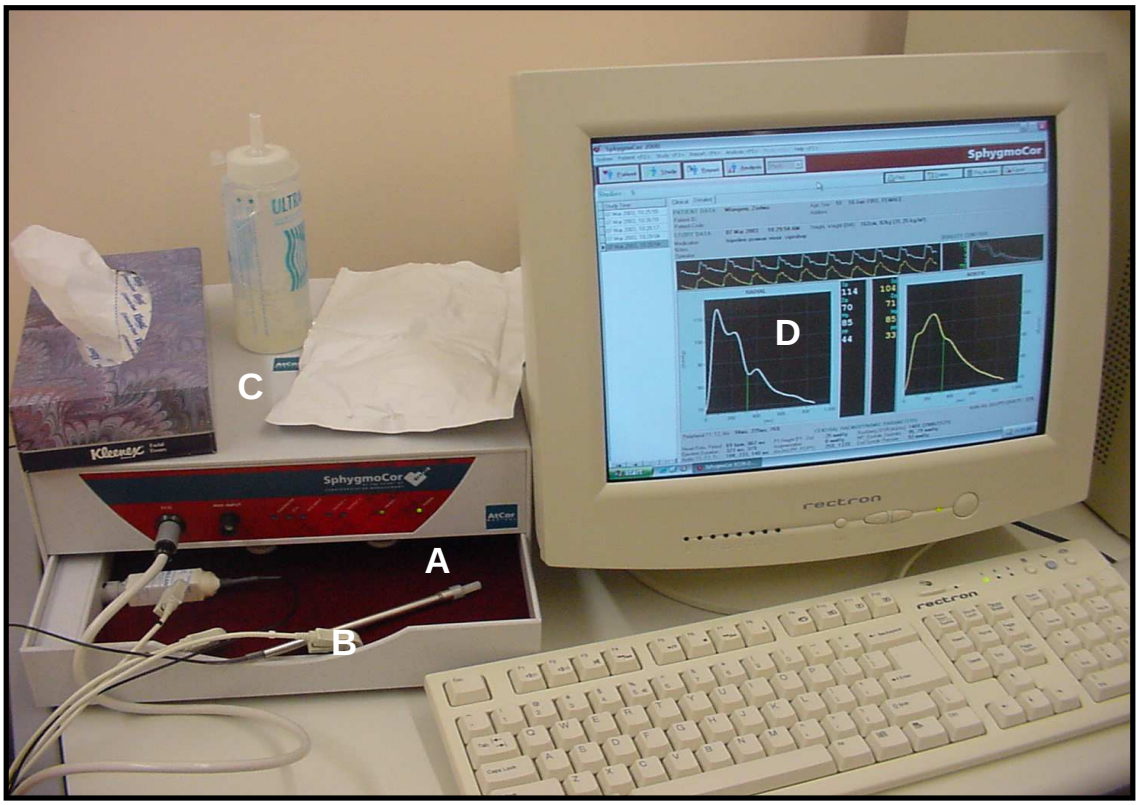
Study participants, demographic and clinical data. The study design and most of the methodology for the present study, including the questionnaire, blood measurements and BP measurements, has been described in chapter 2 (section 2.2). Importantly, the analysis for the present study was conducted in 2007 on 671 participants recruited up until November 2006. Pulse wave velocity could not be determined on 163 participants, 110 of whom were women, because they were either too obese or had a bradycardia. I discarded 73 (38 were women) because the recorded peripheral (AIp) or central (AIc) AI was of insufficient quality. Therefore, the overall number of participants statistically analyzed totalled 508 (310 women) for the carotid-femoral PWV and 598 (388 women) for AIc, AIp, and central systolic and diastolic BP and pulse pressure (PP).

With respect to conventional BP measurements, in the present study 2% of visits had fewer than the planned BP recordings. The frequency of identical consecutive recordings was 1.58% for systolic BP and 2.96% for diastolic BP. The occurrence of BP values recorded as an odd number was 0%. Of the 4988 systolic and diastolic BP

readings, 28.3 % ended on a zero (expected =20%).

Pulse wave analysis. Details of the technique employed to determine carotid-femoral PWV, AIc and AIp have previously been published (Shiburi et al 2006). All measurements were made by a single experienced observer unaware of the clinical history of the participants. Briefly, after subjects rested for 15 minutes in the supine position, the radial waveform at the dominant arm was recorded by applanation tonometry during an 8-second period using a high-fidelity SPC-301 micromanometer (Millar Instrument, Inc., Houston, Texas) interfaced with a computer employing SphygmoCor, version 6.21 software (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia) (Figure 3.1). Recordings where the systolic or diastolic variability of consecutive waveforms exceeded 5% or the amplitude of the pulse wave signal was less than 80 mV were discarded. The pulse wave was calibrated by manual measurement (auscultation) of BP immediately before the recordings. From the radial signal, SphygmoCor software calculates the aortic pulse wave by means of a validated and population-based generalized transfer function. The radial (peripheral arterial) AI (AIp) was defined as the ratio of the second to the first peak of the pressure wave expressed as a percent. The AIc was determined from the difference between the second and the first systolic peak given as a percentage of the aortic pulse pressure (Figure 3.2).

Peripheral (PPp) and central (PPc) pulse pressures (PP) were defined as the difference between systolic and diastolic BP derived from the brachial or central BP respectively. The central systolic and diastolic BP were obtained from the aortic pulse wave. Central mean arterial pressure (MAPc) was calculated from central diastolic BP + [(central systolic BP-central diastolic BP)/3]. Pulse wave velocity (PWV) was determined from carotid and femoral waveform measurements. The distance travelled by the pulse wave was measured over the body surface as the distance between the recording sites at the femoral artery to the suprasternal notch minus the distance from the



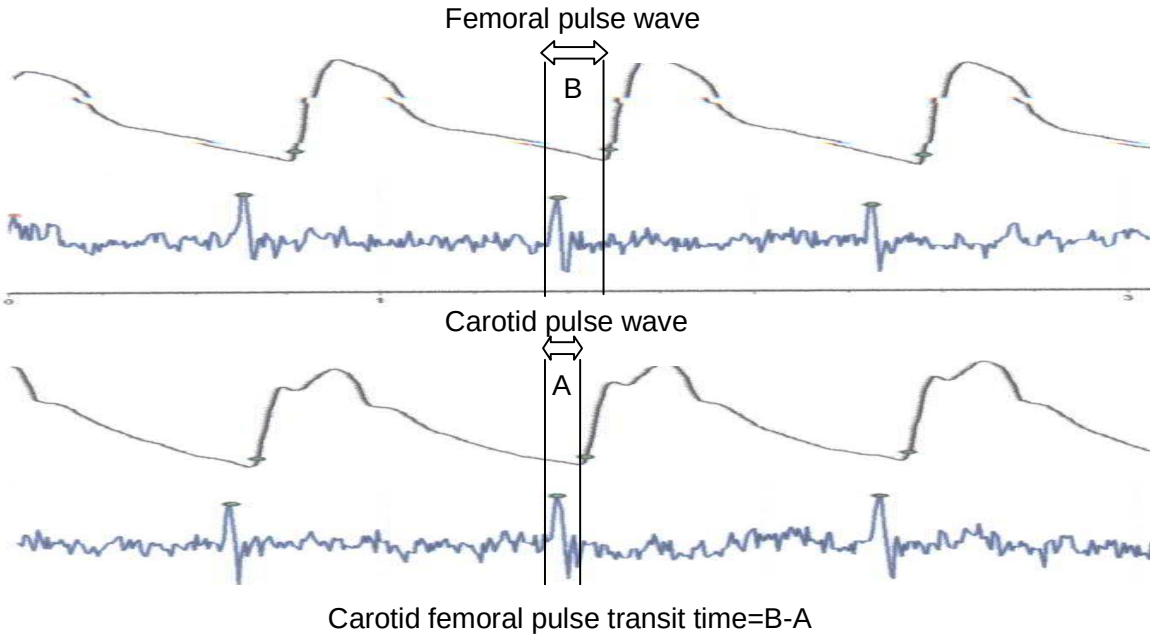
A Applanation tonometer to derive pulse waves.

B ECG electrodes to determine the timing of pulse waves.

C SphygmoCor device used to amplify pulse waves.

D Computer showing arterial pulse waves.

Figure 3.1 Hardware used to perform pulse wave analysis. A is placed over either radial, femoral or carotid arteries and via C derives pulse waves shown in D. Further analysis is determined from a simultaneous electrocardiogram (ECG) obtained from B and this analysis is explained in the text and in Figure 3.2.



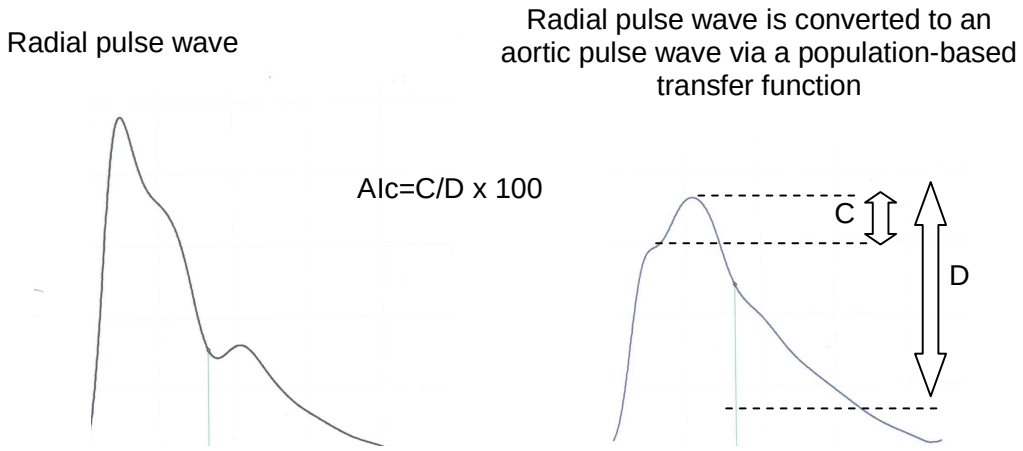


Figure 3.2. Shows the derivation of pulse transit time and central augmentation index (AIc) obtained from pulse wave analysis performed at the carotid, femoral and radial arteries.

recording site at the carotid artery to the suprasternal notch (D). The time (t) taken for the pulse wave to travel from the carotid to the femoral site (pulse transit time) was determined from the differences in the times taken to generate the femoral and carotid pulse waveforms (Figure 3.2). To assess time differences in the generation of carotid and femoral waveforms, these waveforms were matched to a simultaneous electrocardiograph recording (B in Figure 3.1). Pulse transit times were determined from an average of 10 consecutive beats. Aortic PWV was calculated as PWV = D/t.

Intra-observer variability was assessed on 25 participants on who repeat pulse wave analysis was performed within a two week period of the initial measurements. The Pearson's correlation coefficients for carotid-femoral PWV, AIc, AIp, central systolic BP and central PP were 0.86, 0.81, 0.89, 0.92 and 0.75 (all $p<0.0001$) respectively, and the variances (mean % difference ± SD) were 2.66±10.54%, 0.49±33.01%, 1.37±10.28%, 1.48±5.81% and 1.35±23.02% respectively. In addition, no significant differences between repeat measurements were evident on paired t-test analysis ($p=0.14$, $p=0.58$, $p=0.38$, $p=0.25$ and $p=0.86$ respectively).

Data analysis. Database management and statistical analyses were performed with SAS software, version 9.1 (The SAS Institute Inc., Cary, North Carolina, USA). Data from individual participants were averaged and expressed as mean±SD. The X^2-statistic was used to compare proportions. Stepwise regression analysis was performed to determine the contribution of indexes of adiposity and other factors to PWV, or AI. Again this analysis was conducted in sex-specific groups as previous studies have shown that adiposity indexes may be related to PWV and AI in a sex-specific manner (Mackey et al 2002, Zebekakis et al 2005, Protogerou et al 2007). In the present analysis, as PWV, AIc or AIp were not normally distributed, they were log-transformed. Included in the regression models were those adjustors described in chapter 2 including indexes of adiposity, age,

antihypertensive therapy, height (except when using BMI in the model), regular tobacco and alcohol use, menopausal status, and oral contraceptive use. However, in addition, to assess whether adiposity indexes are associated with indexes of large artery dysfunction independent of haemodynamic factors and glucose control, included as adjustors in the regression models were MAPc, HR, and the presence or absence of diabetes mellitus or an abnormal blood glucose control. In secondary analysis in 440 participants whom had complete lipid profiles, total/HDL cholesterol was included as an additional confounder in the model. Further adjustments of probability values were made for non-independence of family members using non-linear models (mixed procedure as described in the SAS software). To determine whether age influences the independent relationship between adiposity indexes and PWV, I tested for an interaction between age and indexes of adiposity as independent predictors of PWV in sex-specific groups. In this analysis the same aforementioned factors were included in the regression relations as well as the independent terms for age and adiposity indexes.

3.3 Results

Characteristics of the participants. Table 3.1 gives the demographic and clinical characteristics of the participants. More women than men participated. A high proportion of participants were overweight or obese, with excessive adiposity noted more frequently in women than in men and with women having greater values for all indexes of adiposity except for waist-to-hip ratio which was higher in men. Of the women 112 (~36%) were postmenopausal as confirmed with follicle stimulating hormone measurements. 7.1% of women were receiving oestrogen contraception, but none were receiving hormone replacement therapy. A greater proportion of women than men were hypertensive. On average 22% of participants were receiving antihypertensive therapy with a greater proportion of women than men receiving antihypertensive medication. On average ~8% were either receiving therapy for diabetes mellitus or had an HbA1c >7.0%, with

Table 3.1. Demographic, anthropometric and clinical characteristics of study subjects with pulse wave velocity measurements.

	Women	Men
Number	310	198
Age (years)	42.0±17.8	41.3±19.4
Height (cm)	157.2± 6.8	168.5±7.5*
Weight (kg)	74.7± 18.8	71.3±15.0*
Body mass index (kg/m^2)	30.4± 7.2	25.1±5.0*
Waist circumference (cm)	90.0± 15.4	85.4±14.0*
Hip circumference (cm)	110.6± 14.6	97.0±10.9*
Waist-to-hip ratio	0.81± 0.09	0.86±0.10*
Skin-fold thickness (cm) Subscapular	2.1± 1.1	1.2±1.0*
Triceps	2.7± 1.4	1.9±1.2*
Mean	2.4± 1.1	1.6±1.0*
% overweight/obese	24/49	30/18*
% regular tobacco use	3.2	31.9*
% regular alcohol consumption	14.2	36.4*
% postmenopausal	36	-
% oestrogen contraceptives	7.1	-
% with hypertension	33.7	20.0*
Antihypertensive medication (%)	26.8	14.0*
% diabetes mellitus or HbA1c>7.0%	8.5	7.2
HbA_{1C} (%)	6.11± 1.16	5.98±1.21
Glucose (mmol/l)	5.49± 3.24	5.37±2.66
Total cholesterol (mmol/l)	4.60± 1.15 (n=268)	4.35±1.01 (n=172)
HDL cholesterol (mmol/l)	1.47± 0.37 (n=268)	1.35±0.48 (n=172)
Total/HDL cholesterol	3.32± 1.20 (n=268)	3.52±1.27 (n=172)
Peripheral hemodynamic measurements		
SBP/DBP (mm Hg)	130± 23/84±13	132±24*/84±11
Pulse pressure (mm Hg)	46±14	49±20*
Augmentation index (%)	84.9±22.2	79.2±18.5*
Heart rate (beats/min)	66±10.9	59.5±11.2*
Central hemodynamic measurements		
SBP/DBP (mm Hg)	121± 23/85±13	123±24*/85±12
MAP (mm Hg)	97± 15.97	97±14.29
Pulse pressure (mm Hg)	37±14	38±18
Augmentation index (%)	27.8± 13.3	24.5±12.4*
Aortic PWV (m/sec)	6.76± 2.93	6.85±3.31

HbA_{1C}, glycated haemoglobin; HDL, high density lipoprotein; SBP, systolic BP; DBP, diastolic BP; MAP, mean central artery pressure, PWV, pulse wave velocity. * $p<0.05$ versus women.

marginally more women than men either receiving glucose lowering agents or with an abnormal blood glucose control. No participants were receiving statin therapy. On average a low proportion of subjects either regularly smoked or consumed alcohol, but the percentage was far greater in men than in women. Men had marginally higher mean peripheral and central systolic BP values and a higher PPp, but not PPc than women. Men had a lower pulse rate on average than women. Men had a lower peripheral and central AI than women, but PWV was similar between the two genders.

Association between indexes of obesity. As with the data shown in chapter 2, in the sample analyzed for the present study, all indexes of adiposity were strongly associated with each other in both gender groups, with the closest relationships noted between waist circumference and BMI in both women and in men (Table 3.2). However, the relationship between waist-to-hip ratio and both BMI and skin-fold thickness was markedly lower in women than in men (Table 3.2).

Relationship between adiposity and either age, haemodynamic factors or diabetes mellitus. All adiposity indexes were correlated with age (r=0.28-to-0.56, p<0.0001), MAPc (r=0.26-to-0.45, p<0.001), HR (r=0.15-to-0.21, p<0.001), and the presence of diabetes mellitus or an HbA1c >7.0% (r=0.10-to-0.25, p<0.001). Waist-to-hip ratio (r=0.18, p<0.0005) and waist circumference (r=12, p<0.02), but neither BMI, mean skin-fold thickness nor hip circumference were associated with total/HDL cholesterol.

Univariate relations between adiposity indexes and indexes of arterial stiffness or wave reflection. On univariate analysis, all indexes of adiposity were correlated with PWV (Figures 3.3 and 3.4) and with AIc (Table 3.3). In women, the relationship between BMI and PWV was similar to those between indexes of central adiposity and PWV (Figure 3.3) and AIc (Table 3.3). However, in women the relationship between skin-fold thickness and PWV tended to be lower than those for the other relationships (Figure 3.3). In men, the

Table 3.2 Correlation matrices between indexes of adiposity in gender-specific groups.

	WC (cm)	WHR	Mean skin-fold thickness
	Women (n=310)		
Body mass index (kg/m^2)	0.76**	0.09**	0.37**
Waist circumference (WC) (cm)	-	0.41**	0.28**
Waist-to-hip ratio (WHR)	-	-	0.04*
	Men (n=198)		
Body mass index (kg/m^2)	0.76**	0.30**	0.46**
Waist circumference (WC) (cm)	-	0.61**	0.36**
Waist-to-hip ratio (WHR)	-	-	0.18**

WC, waist circumference; WHR, waist-to-hip ratio. Numbers are correlation coefficients (r^2). * $p<0.001$, ** $p<0.0001$ for the relationships.

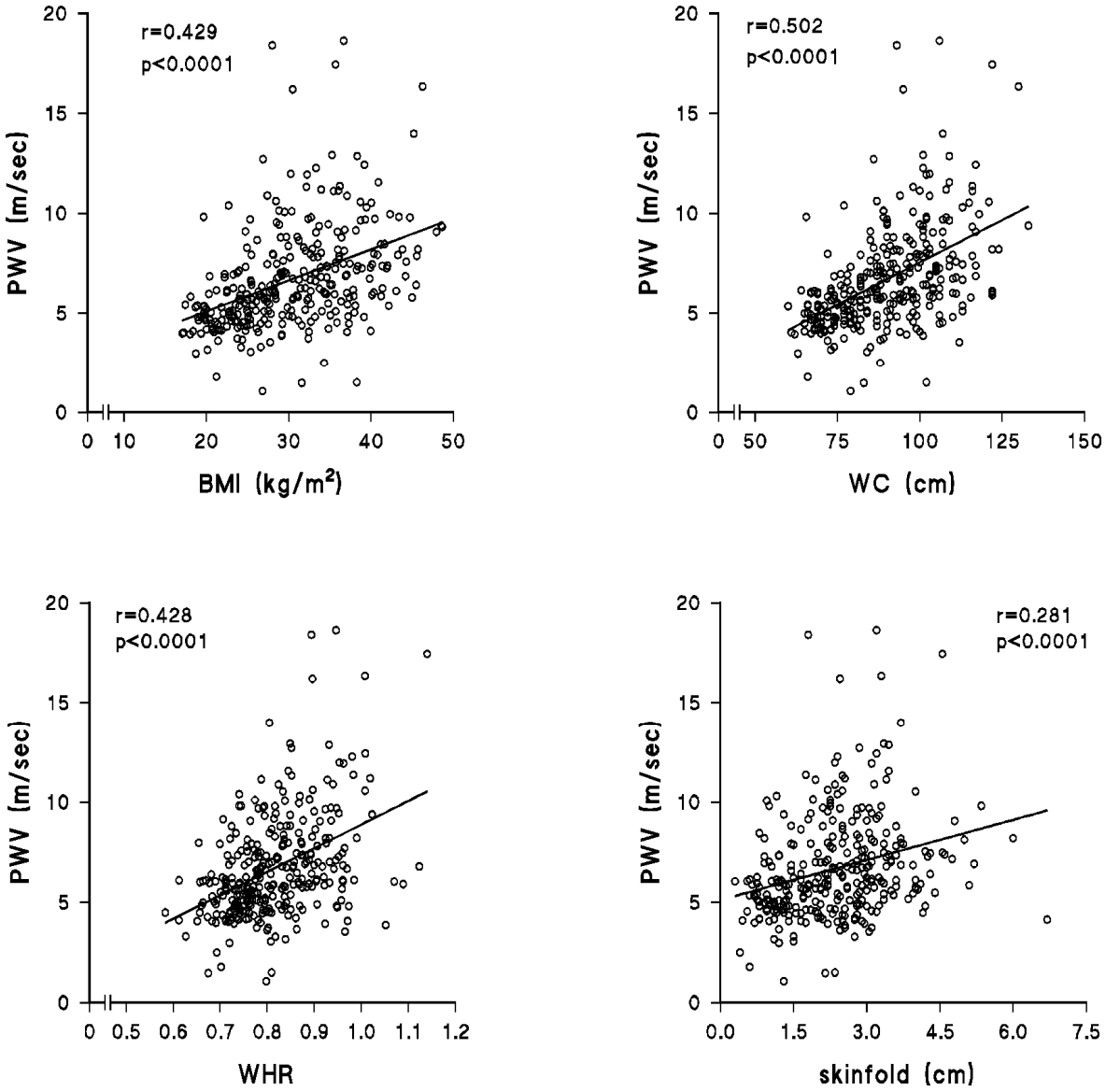


Figure 3.3. Relationship between indexes of adiposity and pulse wave velocity (PWV) in 310 women. WHR, waist-to-hip ratio; WC, waist circumference; BMI, body mass index; skinfold, skin-fold thickness.

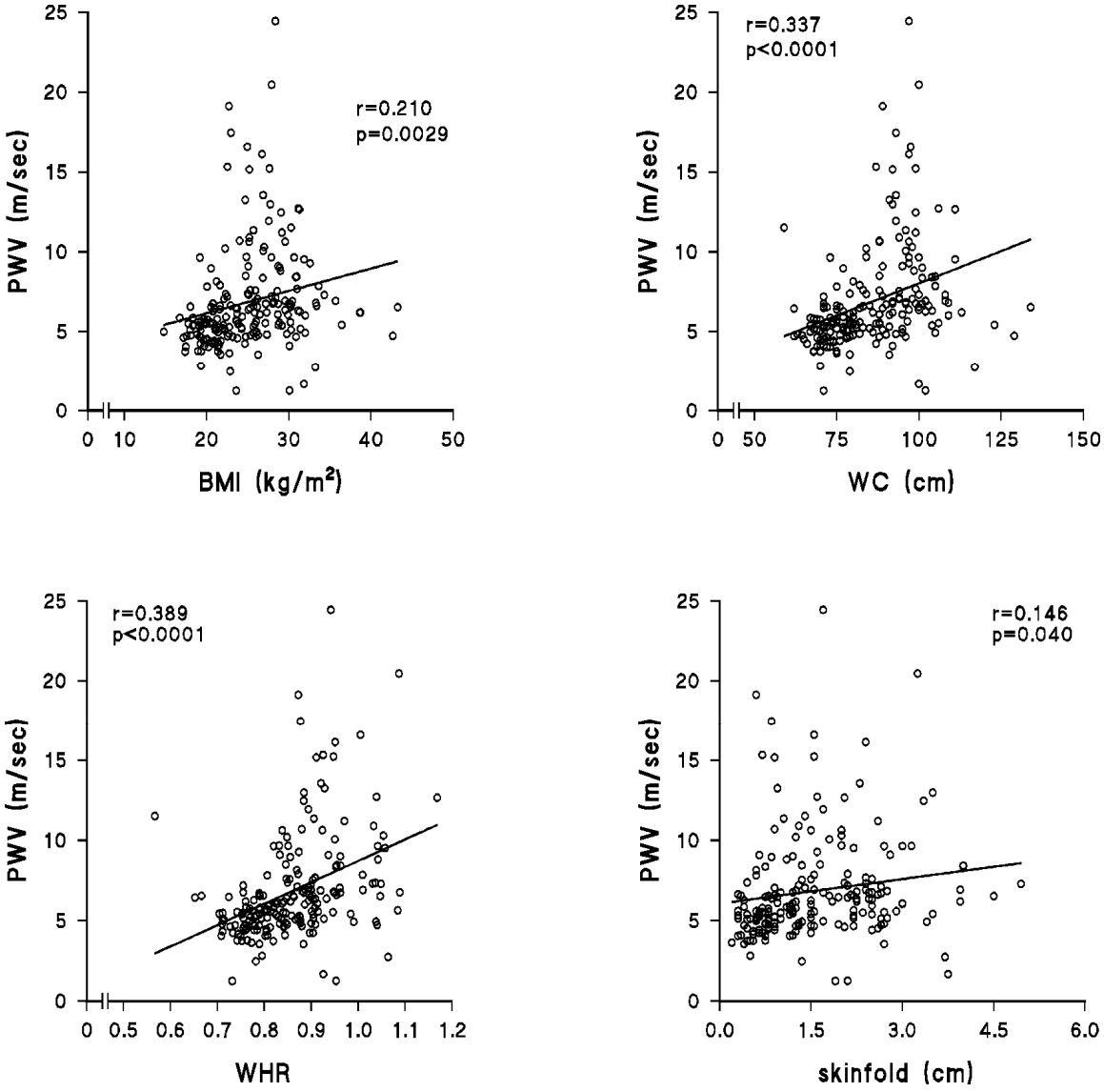


Figure 3.4 Relationship between indexes of adiposity and pulse wave velocity (PWV) in 198 men. WHR, waist-to-hip ratio; WC, waist circumference; BMI, body mass index; skinfold, skin-fold thickness.

Table 3.3 Correlation coefficients (unadjusted) for the relationships between indexes of adiposity and central augmentation index in gender-specific groups.

	Women (n=387)		Men (n=212)	
	Correlation coefficient (r)	p value	Correlation coefficient (r)	p value
Body mass index	0.25	<0.0001	0.097	=0.16
Waist circumference	0.25	<0.0001	0.24	=0.0005
Waist-to-hip ratio	0.14	=0.005	0.30	<0.0001
Skin-fold thickness	0.17	=0.0008	0.14	=0.04

relationships between BMI and PWV and between skin-fold thickness and PWV were markedly lower than those between indexes of central adiposity and PWV (Figure 3.4). Furthermore, in men, there was no significant relationship between BMI and AIc and the relationship between skin-fold thickness and AIc was lower than those relations noted between indexes of central adiposity and AIc (Table 3.3).

Factors associated with PWV, or AI. Tables 3.4 and 3.5 show the contribution of factors associated with indexes of arterial stiffness to log PWV in stepwise regression models in women and in men. Table 3.4 shows models with BMI and skin-fold thickness and Table 3.5 shows models with waist-to-hip ratio, waist circumference, and hip circumference. In both gender groups, the factor that contributed the most to the variability in log PWV was age, accounting for 39-44% of the variability in log PWV. The relationship between age and log PWV was similar irrespective of whether a linear (r=0.65), exponential (r=0.65) or power (0.64) function was used to fit the relationship. Additional factors that contributed to log PWV included MAPc, which accounted for ~5% of the variability of log PWV in women and ~2.7% of the variability in men; HR which accounted for ~4.6% of the variability in log PWV in men, but not in women; antihypertensive treatment which accounted for ~1-2% of the variability of PWV in men, but not in women, and indexes of adiposity. With respect to the contribution of indexes of adiposity to log PWV, in neither sex was BMI, skin-fold thickness (Table 3.4) or hip circumference (Table 3.5) independently associated with log PWV. In women, waist-to-hip ratio and waist circumference accounted for only ~1% of the variability of log PWV respectively (Table 3.5). Neither waist circumference, nor waist-to-hip ratio were independently associated with log PWV in men (Table 3.5). Regular alcohol intake was associated with log PWV in men, but not in women. Regular tobacco use was not independently associated with log PWV in either men or women. The presence or absence of diabetes mellitus or an

Table 3.4. The results of stepwise regression analysis showing factors associated with log pulse wave velocity in study participants. Significant associations are highlighted in bold.

	Women (n=310)		Men (n=198)	
	Partial correlation coefficient (r)*	p value*	Partial correlation coefficient (r)*	p value*
	Model with body mass index			
	(Model r^2=0.50, adjusted r^2= 0.48)		(Model r^2=0.50, adjusted r^2= 0.47)	
Body mass index	0.05	=0.26	-0.08	=0.14
Age	**0.66**	**<0.0001**	**0.63**	**<0.0001**
MAPc	**0.23**	**<0.0001**	**0.16**	**<0.01**
Heart rate	0.03	=0.45	**0.22**	**<0.001**
DM or HbA1c>7.0%	0.08	=0.08	0.02	=0.72
Antihypertensive therapy	0.000	=0.88	**0.11**	**<0.05**
Postmenopausal	0.03	=0.43	-	-
Oestrogen contraception	0.03	=0.52	-	-
Regular alcohol	0.01	=0.85	**0.11**	**<0.05**
Regular tobacco use	0.000	=0.98	-0.05	=0.39
	Model with mean skin-fold thickness			
	(Model r^2=0.50, adjusted r^2= 0.49)		(Model r^2=0.49, adjusted r^2= 0.47)	
Mean skin-folds	0.07	=0.08	-0.05	=0.36
Height	-0.08	=0.06	-0.02	=0.77
Age	**0.66**	**<0.0001**	**0.63**	**<0.0001**
MAPc	**0.23**	**<0.0001**	**0.16**	**<0.01**
Heart rate	0.08	=0.54	**0.22**	**<0.001**
DM or HbA1c>7.0%	0.08	=0.07	0.02	=0.71
Antihypertensive therapy	0.000	=0.95	**0.11**	**<0.05**
Postmenopausal	0.03	=0.39	-	-
Oestrogen contraception	0.08	=0.57	-	-
Regular alcohol	0.02	=0.71	**0.11**	**<0.05**
Regular tobacco use	0.000	=0.92	-0.04	=0.43

MAPc, mean central artery pressure; DM, treatment for diabetes mellitus. *From stepwise regression analysis with further adjustments of probability values made for non-independence of family members.

Table 3.5. The results of stepwise regression analysis showing factors associated with log pulse wave velocity in study participants. Significant associations are highlighted in bold.

	Women (n=310)		Men (n=198)	
	Partial correlation coefficient (r)*	p value*	Partial correlation coefficient (r)*	p value*
	Model with waist-to-hip ratio			
	(Model r^2=0.51, adjusted r^2= 0.49)		(Model r^2=0.49, adjusted r^2= 0.47)	
Waist-to-hip ratio	**0.10**	**<0.05**	0.01	=0.85
Height	-0.08	=0.08	-0.01	=0.78
Age	**0.66**	**<0.0001**	**0.63**	**<0.0001**
MAPc	**0.23**	**<0.0001**	**0.16**	**<0.01**
Heart rate	0.01	=0.74	**0.22**	**<0.001**
DM or HbA1c>7.0%	0.07	=0.11	0.01	=0.78
Antihypertensive therapy	0.000	=0.97	**0.11**	**<0.05**
Postmenopausal	0.04	=0.37	-	-
Oestrogen contraception	0.04	=0.34	-	-
Regular alcohol	0.02	=0.68	**0.11**	**<0.05**
Regular tobacco use	0.000	=0.97	-0.03	=0.54
	Model with waist circumference			
	(Model r^2=0.51, adjusted r^2= 0.49)		(Model r^2=0.49, adjusted r^2= 0.47)	
Waist circumference	**0.09**	**<0.05**	-0.03	=0.52
Height	-0.08	=0.06	0.000	=0.96
Age	**0.66**	**<0.0001**	**0.63**	**<0.0001**
MAPc	**0.23**	**<0.0001**	**0.16**	**<0.01**
Heart rate	0.02	=0.62	**0.22**	**<0.001**
DM or HbA1c>7.0%	**0.08**	**<0.05**	0.02	=0.74
Antihypertensive therapy	0.000	=0.88	**0.11**	**<0.05**
Postmenopausal	0.03	=0.42	-	-
Oestrogen contraception	0.04	=0.36	-	-
Regular alcohol	0.02	=0.68	**0.11**	**<0.05**
Regular tobacco use	0.000	=0.89	-0.04	=0.49
	Model with hip circumference			
	(Model r^2=0.50, adjusted r^2= 0.49)		(Model r^2=0.49, adjusted r^2= 0.47)	
Hip circumference	0.02	=0.61	-0.05	=0.36
Height	-0.08	=0.06	0.000	=0.99
Age	**0.66**	**<0.0001**	**0.63**	**<0.0001**
MAPc	**0.23**	**<0.0001**	**0.16**	**<0.01**
Heart rate	0.03	=0.53	**0.22**	**<0.001**
DM or HbA1c>7.0%	**0.08**	**<0.05**	0.02	=0.75
Antihypertensive therapy	0.000	=0.93	**0.11**	**<0.05**
Postmenopausal	0.03	=0.52	-	-
Oestrogen contraception	0.03	=0.44	-	-
Regular alcohol	0.01	=0.72	**0.11**	**<0.05**
Regular tobacco use	0.000	=0.96	-0.04	=0.49

MAPc, mean central arterial pressure; DM, treatment for diabetes mellitus. *From stepwise regression analysis with further adjustments of probability values made for non-independence of family members.

abnormal blood glucose control was only independently associated with log PWV in women in the models including either waist or hip circumference (Table 3.5).

In multivariate regression models, no single index of adiposity was independently associated with either AIc, AIp, or the log or square root of these variables in either men or women (data not shown).

In secondary analysis with total/HDL cholesterol included in the regression models, similar outcomes were noted as those in the whole group. Indeed, with respect to the impact of adiposity indexes, waist-to-hip ratio (partial $r=0.12$, $p<0.01$) and waist circumference (partial $r=0.10$, $p<0.05$) were independently associated with log PWV in women, but not in men, whilst neither BMI, skin-fold thickness nor hip circumference were independently associated with log PWV in either gender group. In these models, total/HDL cholesterol was not an independent positive predictor of log PWV in any model ($p=0.80$-0.96 in the models with waist-to-hip ratio and waist circumference in women).

Age-adiposity interactions as predictors of PWV. Although waist-to-hip ratio and waist circumference made only a minor contribution to the overall variation in PWV in women (Table 3.5), after adjustments for all potential confounders including the individual terms for age and adiposity indexes, marked interactions between age and waist-to-hip ratio ($r=0.68$, $p<0.0001$) and between age and waist circumference ($r=0.68$, $p<0.0001$) were significant independent predictors of log PWV. Secondary analysis with total/HDL cholesterol forced into the model, resulted in similar outcomes (data not shown). In contrast, in men no interactions between age and adiposity indexes were independently associated with log PWV.

The interactions between age and adiposity indexes as predictors of PWV in women, translated into a major impact of age on the effect of waist-to-hip ratio and waist circumference on PWV in women. The effect of age on the relationships between indexes of central adiposity and log PWV in women are illustrated in Figures 3.5 and 3.6 and the

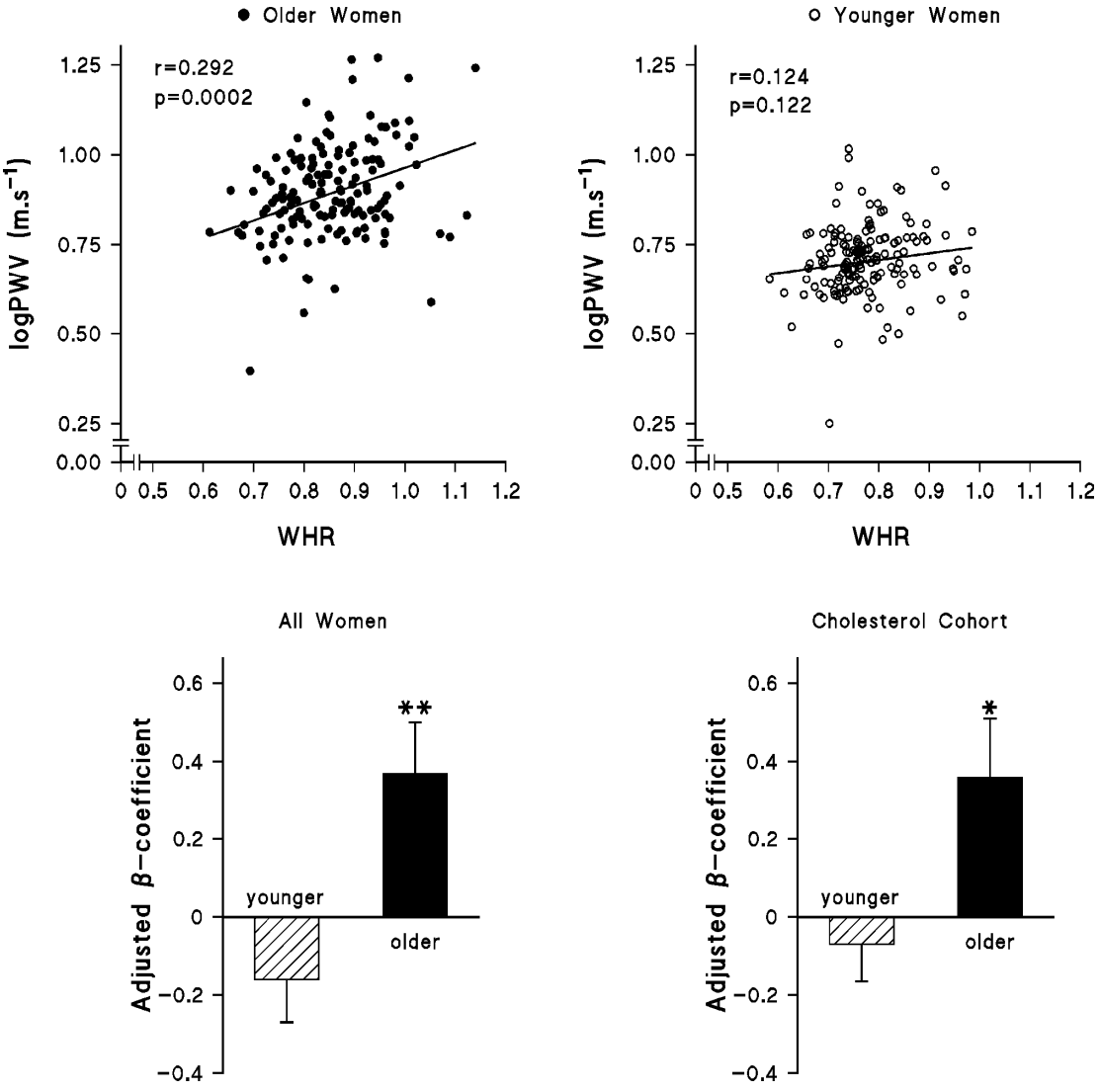


Figure 3.5. Impact of age on waist-to-hip ratio (WHR)-log pulse wave velocity (PWV) relations. Upper panel shows relations between WHR and log PWV in women older (closed dots) than the median age (41.8 years) and women younger (open dots) than the median age. The lower panels compare the multivariate adjusted slopes (β-coefficients) of the relations. The lower left panel shows slopes with adjustments for age, antihypertensive therapy, mean central artery pressure, heart rate, height, regular tobacco and alcohol use, the presence or absence of diabetes mellitus or an abnormal blood glucose control, menopausal status, and oestrogen contraception. The lower right panel shows slopes with adjustments for the same potential confounders and with total/HDL cholesterol forced into the regression models. Probability values were further adjusted for non-independence of family members. *p<0.01 ** p<0.005 versus younger women.

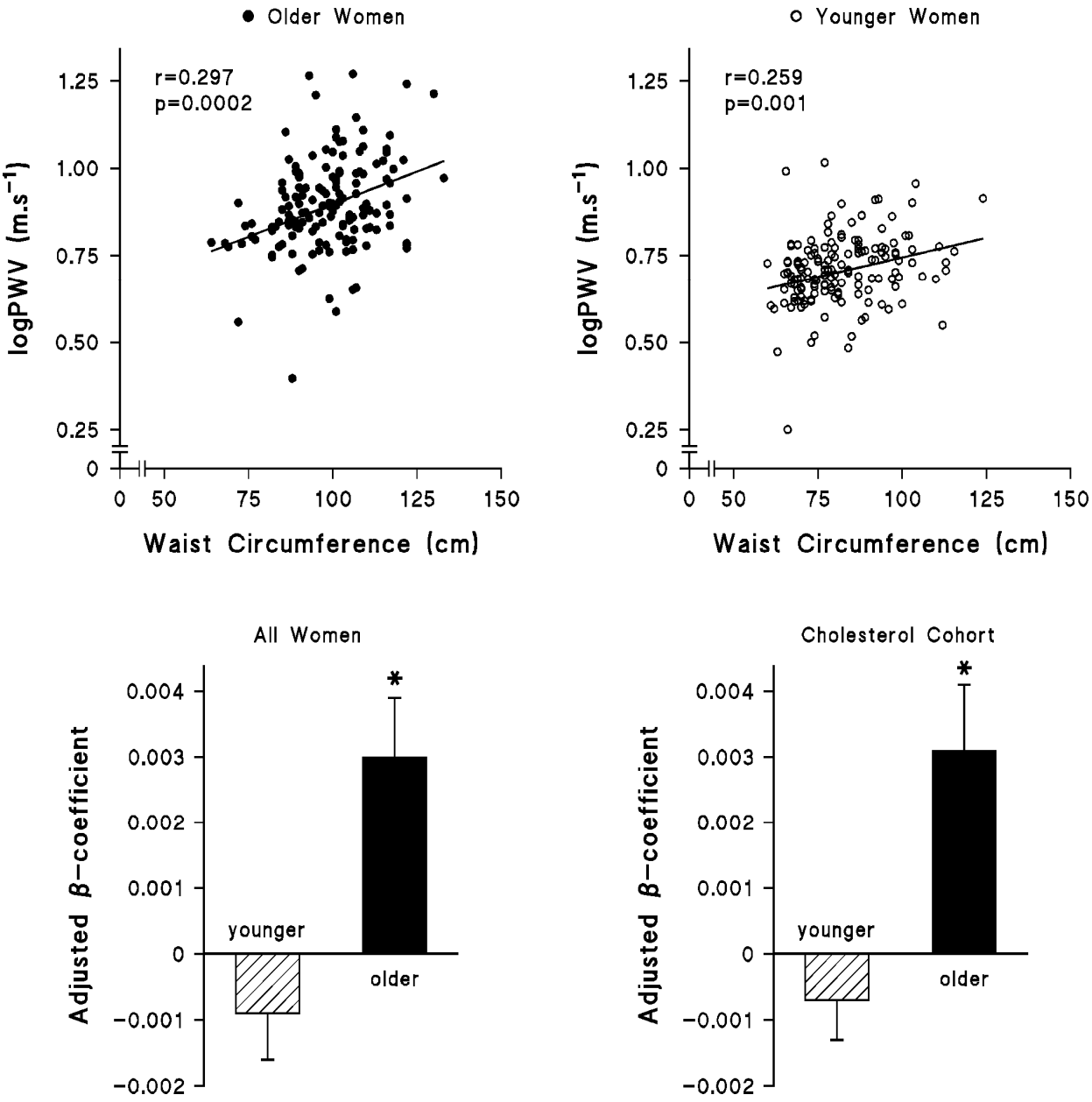


Figure 3.6. Impact of age on waist circumference (WC)-log pulse wave velocity (PWV) relations. Upper panel shows relations between WC and log PWV in women older (closed dots) than the median age (41.8 years) and women younger (open dots) than the median age. The lower panels compare the multivariate adjusted slopes (β-coefficients) of the relations. The lower left panel shows slopes with adjustments for age, antihypertensive therapy, mean central artery pressure, heart rate, height, regular tobacco and alcohol use, the presence or absence of diabetes mellitus or an abnormal blood glucose control, menopausal status, and oestrogen contraception. The lower right panel shows slopes with adjustments for the same potential confounders and with total/HDL cholesterol forced into the regression models. Probability values were further adjusted for non-independence of family members. *p<0.005 versus younger women.

factors associated with log PWV in women younger as compared to older than the median age are shown in Table 3.6. In women younger than the median age (41.8 years), no univariate relationships between either waist-to-hip ratio and log PWV (Figure 3.5, upper panel) or between waist circumference and log PWV (Figure 3.6, upper panel) were noted and no independent relationships between waist-to-hip ratio and log PWV or between waist circumference and log PWV were noted in multivariate models (Table 3.6). However, in women older than the median age, strong univariate correlations between waist-to-hip ratio and log PWV (Figure 3.5, upper panel) and between waist circumference and log PWV (Figure 3.6, upper panel) and a positive independent association between waist-to-hip ratio and log PWV and between waist circumference and log PWV was noted in a multivariate models (Table 3.6). In contrast to the ~1% contribution of either waist-to-hip ratio or waist circumference to the variation in PWV in all women (Table 3.4), waist-to-hip ratio contributed ~5.6% to the variability of log PWV and waist circumference contributed ~6% to the variability of log PWV in women older than the median age (Table 3.6), whereas waist-to-hip ratio and waist circumference made no significant positive contribution to the variation in log PWV in women younger than the median age (Table 3.6).

Figures 3.5 and 3.6, lower left panels, show the multivariate adjusted slopes (β-coefficients) of the waist-to-hip ratio-log PWV (Figure 3.5) and waist circumference-log PWV (Figure 3.6) relations in women older as compared to those younger than the median age. Age produced a marked effect on the multivariate adjusted slope of the wasit-to-hip ratio-log PWV (Figure 3.5) and the waist circumference-log PWV (Figure 3.6) relationship in women. A greater effect of either waist-to-hip ratio or waist circumference on log PWV was noted in women older as compared to those younger than the median age. Indeed, the adjusted slopes showed a positive effect of waist-to-hip ratio on log PWV in women older than the median age (β-coefficient=0.37±0.13), whilst a trend for a negative

Table 3.6. Factors associated with log pulse wave velocity in women younger and older than the median age for the study group (41.8 years). Significant associations are highlighted in bold.

	Older (n=155)		Younger (n=155)	
	Partial correlation coefficient (r)*	p value*	Partial correlation coefficient (r)*	p value*
		Model with waist-to-hip ratio		
	Model r^2=0.33		Model r^2=0.35	
Waist-to-hip ratio	**0.24**	**<0.01**	-0.11	=0.13
Height	-0.11	=0.13	0.000	=0.94
Age	**0.45**	**<0.0001**	0.10	=0.14
MAPc	**0.20**	**<0.01**	**0.57**	**<0.0001**
Heart rate	0.03	=0.68	0.01	=0.84
DM or HbA1c>7.0%	0.05	=0.46	-	-
Antihypertensive therapy	0.02	=0.77	0.07	=0.33
Postmenopausal	0.07	=0.36	-	-
Oestrogen contraception	0.04	=0.56	0.03	=0.65
Regular alcohol	0.08	=0.29	-0.02	=0.78
Regular tobacco use	-0.04	=0.61	0.03	=0.67
		Model with waist circumference		
	Model r^2=0.34		Model r^2=0.35	
Waist circumference	**0.24**	**<0.01**	-0.09	=0.20
Height	**-0.17**	**<0.05**	0.000	=0.94
Age	**0.45**	**<0.0001**	0.10	=0.14
MAPc	**0.18**	**<0.05**	**0.57**	**<0.0001**
Heart rate	0.03	=0.67	0.000	=0.98
DM or HbA1c>7.0%	0.10	=0.14	-	-
Antihypertensive therapy	0.000	=0.97	0.07	=0.35
Postmenopausal	0.01	=0.90	-	-
Oestrogen contraception	0.05	=0.45	0.03	=0.65
Regular alcohol	0.08	=0.24	-0.02	=0.78
Regular tobacco use	-0.02	=0.77	0.02	=0.75

MAPc, mean central arterial pressure; DM, treatment for diabetes mellitus. *From stepwise regression analysis with further adjustments of probability values made for non-independence of family members. No younger subjects had DM or an HbA1c>7.0%.

relationship was noted in women younger than the median age (β-coefficient=-0.16±0.11) (Figure 3.5). The adjusted slope of the waist-to-hip ratio-log PWV relationship in the older group was statistically greater ($p<0.005$) than that in the younger group (Figure 3.5). Moreover, the adjusted slopes showed a positive effect of waist circumference on log PWV in women older than the median age (β-coefficient=0.0030±0.0009), whilst a trend for a negative relationship was noted in women younger than the median age (β-coefficient=-0.0009±0.0007) (Figure 3.6). The slope of the waist circumference-log PWV relationship in the older group was also statistically greater ($p<0.005$) than that in the younger group (Figure 3.6).

Figures 3.5 and 3.6, lower right panels, show the outcomes of secondary analysis with total/HDL cholesterol forced into the models in participants with these data. The multivariate adjusted slopes of the waist-to-hip ratio-log PWV and waist circumference-log PWV relationships were similarly greater in older as compared to younger women (waist-to-hip ratio-log PWV: β-coefficients: older women =0.36±0.15, younger women=-0.07±0.10, $p<0.01$: waist circumference-log PWV: β-coefficients: older women =0.0031±0.0010, younger women=-0.0007±0.0006, $p<0.005$) even after adjustments for total/HDL cholesterol.

Size effects of indexes of adiposity on PWV in younger versus older women. In women older than the median age for the group a one SD increase in waist-to-hip ratio (0.096) translated into a 0.58 m/sec increase in PWV as compared to a 0.16 m/sec decrease in PWV for each one SD increase in waist-to-hip ratio (0.076) in women younger than the median age ($p<0.001$). In women older than the median age for the group a one SD increase in waist circumference (12.8 cm) translated into a 0.69 m/sec increase in PWV as compared to a 0.16 m/sec decrease in PWV for each one SD increase in waist circumference (13.0 cm) in women younger than the median age ($p<0.001$).

3.4 Discussion

The main finding of the present study is that in a predominantly young-to-middle aged population sample of African ancestry with a high prevalence of excess adiposity, age markedly influenced the independent relationship between indexes of central adiposity (waist-to-hip ratio and waist circumference) and PWV in women. In women older than the median age of the sample (~42 years), waist-to-hip ratio and waist circumference were strongly and independently associated with PWV, whereas in women younger than the median age a trend for a negative relationship was noted. Moreover, in women older than the median age of the sample, the quantitative effect of waist-to-hip ratio or waist circumference on PWV was ~5-fold greater than in women younger than the median age of the sample. Further, in older women, the contribution of waist-to-hip ratio or waist circumference to the variation in log PWV was at least as much as mean central arterial BP.

The age effect on adiposity-PWV relations noted in present study provides insight into discrepancies in studies assessing relations between adiposity and PWV. In this regard, a number of studies with study sample sizes ranging from 336-to-2431 participants have failed to show strong independent relations between adiposity and PWV in predominantly young-to-middle aged groups (Ferreira et al 2004, Zebekakis et al 2005, Taquet et al 1993, Amar et al 2001, Nakanishi et al 2003, Oren et al 2003, Mitchell et al 2004, Czernichow et al 2005). However, consistent with the impact of age on adiposity-PWV relations, marked independent relations have been reported on in 2488 participants aged 70-79 years (Sutton-Tyrrell et al 2001) and in 356 participants aged 70-90 years (Mackey et al 2002). In support of an age-dependent effect of excess adiposity on PWV noted in the present study, adiposity-age interactions have been described in another study (Zebekakis et al 2005) which nevertheless only achieved significance when assessing associations with measures of arterial stiffness other than PWV. However, in

that study (Zebekakis et al 2005), an adiposity-age interaction was not detected when assessing associations with PWV, although the independent effect of adiposity on PWV appeared to be enhanced with increasing age (Zebekakis et al 2005). It is possible that the low mean BMI of ~25 kg/m^2 in that prior study (Zebekakis et al 2005) may have limited the capacity to detect a significant adiposity-age interaction for PWV. Further, as suggested by the present study, where interactions between central adiposity and age, but not between BMI and age were noted, the use of BMI as an adiposity index may limit the sensitivity to detect age-adiposity interactive effects on PWV.

In all prior studies demonstrating either a lack of independent relationship (Ferreira et al 2004, Taquet et al 1993, Amar et al 2001, Nakanishi et al 2003, Oren et al 2003, Mitchell et al 2004) or only a weak independent relationship (Zebekakis et al 2005, Czernichow et al 2005) between adiposity indexes and PWV, the mean BMI ranged from ~23-to-26 kg/m^2, suggesting a low prevalence of excess adiposity, a characteristic of these populations that may have limited the strength of the independent relationship between adiposity and PWV. In contrast, in the present study ~63% of the sample were either overweight (~26%) or obese (~37%), and hence the outcomes are unlikely to be limited by a low prevalence of excess adiposity in the study sample.

The present study is in apparent contrast to a previous study conducted in 186 younger subjects, 21-26% of whom were obese, where adiposity indexes were strongly associated with PWV independent of age, gender and BP (Wildman et al 2003) However, in contrast to this prior study (Wildman et al 2003) where the relations between adiposity indexes and PWV were not adjusted for HR, in the present study we adjusted for HR. As indicated in the present and a prior study (Mackey et al 2002) HR is strongly associated with both adiposity indexes and PWV.

The potential mechanisms that may explain the independent relationship between adiposity and PWV in older women are numerous, have been discussed in the

introductory chapter and may be summarized as follows: First, increased insulin concentrations, without overt diabetes mellitus, may accompany obesity and insulin could promote vascular changes that result in increases in large vessel stiffness (King et al 1999). However, to my knowledge there are no studies that have demonstrated a relationship between insulin resistance and large artery dysfunction independent of haemodynamic factors or diabetes mellitus. Second, the adipokine leptin may independently promote increases in arterial stiffness (Singhal et al 2002). However, the exact mechanism of this effect has not been identified. Third, activation of the RAAS may contribute to large vessel dysfunction. Indeed, ACE inhibition reduces arterial stiffness (Safar et al 1997). However, there are no large studies that have been conducted to date that have assessed whether RAAS activation is indeed related to large vessel dysfunction beyond conventional risk factors and indexes of adiposity. Fourth, adipose tissue secretes pro-inflammatory substances and obesity is associated with increases in circulating C-reactive protein concentrations (Visser et al 1999, Berg and Scherer 2005). In this regard, C-reactive protein concentrations are associated with an increased arterial stiffness in apparently healthy individuals (Yasmin et al 2004).

It is unclear why a gender-specific independent relationship between adiposity and PWV was noted in the present study. Nevertheless, in support of the present study, in previous studies (Mackey et al 2002, Zebekakis et al 2005, Protogerou et al 2007) adiposity was independently associated with PWV in women, but not in men. It is possible that independent relationships between adiposity and PWV are sensitive to the prevalence of obesity in the population sampled. In this regard, in the present study fewer men were obese than women.

In the present study, although indexes of adiposity were independently associated with PWV, there was no independent relationship between indexes of adiposity and AI. The lack of effect of adiposity indexes on AI might relate to the dependence of AI, but not

PWV, on ventricular ejection time (Wilkinson et al 2002). Moreover, AIc, but not PWV, depends on the distance of the reflection points from the heart. It is possible that in a large population sample, subjects with a higher PWV might have reflection points further from the heart. Irrespective of the mechanism responsible for the lack of independent relationship between indexes of adiposity and AIc despite the relationship between central adiposity and PWV, our data are consistent with an alternative study similarly showing a lack of independent relationship between adiposity and AI (Vergnaud et al 2007).

The limitations of the present study are again the cross-sectional nature of the study design and the lack of more direct measures of body fat, particularly visceral or trunk fat. However, visceral (Sutton-Tyrrell et al 2001) or trunk (Ferreira et al 2004) fat is more closely associated with PWV than other fat compartments. Hence, if anything I may have underestimated the independent relationship between adiposity indexes and PWV in the present study. Further, as a considerable number of participants older than the median age for the group were receiving antihypertensive therapy, I was not statistically powered to perform sensitivity analysis in only untreated women or men. However, I did adjust for antihypertensive treatment and the inclusion of treated participants in the analysis is more likely to have biased against the outcomes by reducing the sensitivity to show an interactive effect between age and adiposity indexes.

In conclusion, in a predominantly young-to-middle-aged population sample of African ancestry with a high prevalence of excess adiposity, age modified the independent relationship between indexes of central adiposity and PWV in women. In this regard, waist-to-hip ratio and waist circumference accounted for a significant proportion of the variability of PWV in older, but not younger women independent of other confounders. Moreover, the effect of waist-to-hip ratio and waist circumference on increases in PWV was ~5-fold higher in older than in younger women after adjustments for confounders. Thus, although in younger women the impact of adiposity on PWV is likely to be mediated

principally through conventional haemodynamic factors such as BP, the effect of adiposity in older persons may be mediated by additional factors not normally included in cardiovascular risk assessment.

CHAPTER 4

Adiposity Enhances the Impact of Arterial Stiffness and Hence Blood Pressure on Left Ventricular Mass.

Abstract

Although normal growth effects on left ventricular mass (LVM) are of physiological relevance, obesity-induced LV growth may have pathophysiological significance. However, an explanation for the pathophysiological effects of adiposity-induced LV growth has not been provided. I determined, in 399 randomly recruited participants in the community (~68% with excess adiposity), whether excess adiposity enhances the impact of haemodynamic changes on LVM. I determined LVM indexed for height$^{2.7}$ (LVMI) and mean wall thickness (MWT) from echocardiography; blood pressure (BP) from conventional and 24-hour ambulatory measurements; and carotid-femoral pulse wave velocity (PWV) from applanation tonometry. After adjustments for appropriate confounders, including the individual terms, interactions between adiposity indexes (waist circumference [WC] or mean skin-fold thickness) and conventional systolic BP (SBP), 24-hour SBP, PWV, conventional pulse pressure (PP), or 24-hour PP were independently associated with LVMI and MWT ($p<0.001$). The adiposity index-haemodynamic interaction translated into a greater impact of BP on LVMI and MWT in obese as compared to lean participants. Every one SD increase in conventional SBP (~22 mm Hg) was associated with a 1.61 g/m$^{2.7}$ increase in LVMI in participants with a normal WC, in comparison to a 5.24 g/m$^{2.7}$ increase in those with an increased WC ($p<0.0001$). In conclusion, adiposity-induced increases in LVM are of pathophysiological significance in-part because they reflect an enhanced impact of arterial stiffness and hence SBP on LV growth.

4.1 Introduction

Excess adiposity predicts adverse cardiovascular outcomes independent of conventional cardiovascular risk factors (Kurth et al 2002, Wilson et al 2002, National Task Force on the Prevention and Treatment of Obesity 2000). In this regard, a potential mechanism through which obesity may mediate independent effects on cardiovascular outcomes is by promotiong left ventricular hypertrophy (LVH) (de Simone et al 1992, de Simone et al 1994, Lauer et al 1991, Lorber et al 2003, Messerli et al 1982, Messerli et al 1983, Urbina et al 1995), which is a strong independent predictor of cardiovascular events (Casale et al 1986, Devereux et al 2004, Ghali et al 1998, Koren et al 1991, Levy et al 1990, Levy et al 1994, Verdecchia et al 1996). Left ventricular mass (LVM) indexes that incorporate the impact of adiposity on LVM are associated with a higher proportion of incident cardiovascular events than are LVM indexes that discount the impact of adiposity on LVM (de Simone et al 2005). Consequently, unlike normal growth effects on LVM which are physiological in nature, obesity-induced LVH may be of pathophysiological significance. However, an explanation for the pathophysiological significance of adiposity-induced LVH has not been forthcoming.

One potential mechanism that may explain the potential pathological effects of obesity-induced increases in LVM is through an enhanced impact of blood pressure (BP) on LVM as demonstrated in severe obesity (body mass index>35 kg/m^2) (Avelar et al 2007). However, obesity may not increase the impact of BP on LVM in milder forms of obesity (Fox et al 2004, Lauer et al 1992). Nevertheless, in these studies only participants with a narrow range of BP levels (Lauer et al 1992) or where ~50% of the sample were receiving antihypertensive treatment at the time (Fox et al 2004) were studied. Therefore, in the present study I assessed in a population sample with a high prevalence of mild-to-moderate obesity (Shiburi et al 2006, chapters 2-3) whether adiposity is associated with an enhanced impact of BP or arterial stiffness on LVMI and wall thickness.

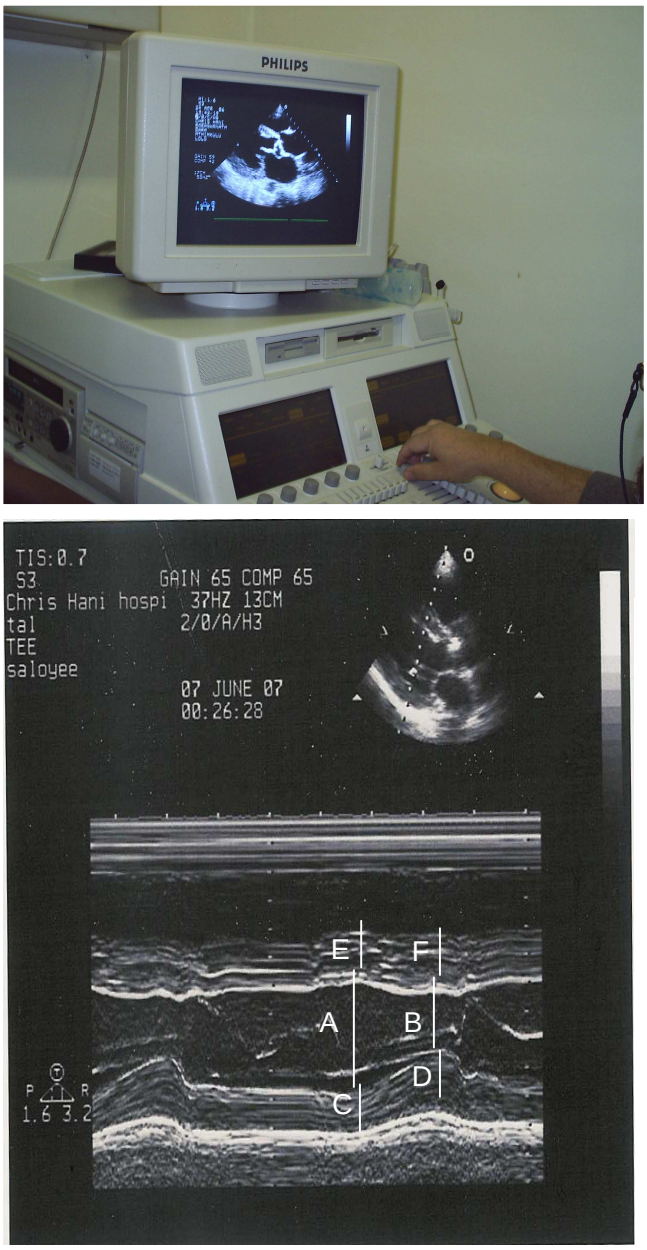
4.2 Methods

Study participants. The study design has been described in detail in chapter 2. The analysis for this component of the thesis was undertaken in early 2007 in the database available at the end of 2006. Of the 671 participants enrolled in the study up until November 2006, 418 (62%) agreed to echocardiograph assessments. Of these, 19 were discarded because of poor quality echocardiograph assessments. Thus, in total 399 subjects were assessed in this study. For statistical analysis with ambulatory BP, data from 297 participants with high quality echocardiograms who also had ambulatory BP recordings that met with pre-specified quality criteria (longer than 20 hours and more than 10 and 5 readings for the computation of daytime and night-time means, respectively) were assessed. Pulse wave velocity (PWV) could not be measured in 71 subjects because they had a bradycardia or were too obese and hence the overall number of participants statistically analyzed totaled 328 for PWV.

The techniques employed to obtain clinical, demographic and anthropometric data, conventional BP and pulse wave data have been described in chapter 2. As with other chapters, 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. In addition however, as I have demonstrated (in chapter 2) that waist circumference was the most appropriate index of adiposity for predicting BP effects, I also defined excess adiposity from waist circumference thresholds in the present study. An enlarged waist circumference was defined as $\underline{\geq}$88 cm in women and $\underline{\geq}$102 cm in men (Third report of the National Cholesterol Education Program 2002). As the present analysis was conducted in 2007, and based on a new meta-analysis published in 2007, where revised thresholds for the use of HbA1c as a diagnositic tool for diabetes mellitus have been described (Bennett et al 2007), diabetes mellitus or abnormal blood glucose control was defined as the use of insulin or oral hypoglycaemic agents or an HbA_{1c} value greater than 6.1% in the present study.

In the cohort of participants with appropriate echocardiography, the frequency of identical consecutive conventional BP recordings was 3.01%. No BP values were recorded as an odd number. Of the 3990 systolic and diastolic BP readings, 28.7% ended on a zero (expected =20%). The technique employed for 24-hour ambulatory BP monitoring has been described in chapter 2. The average (±SD) number of BP recordings obtained in the cohort with appropriate echocardiography and 24-hour BP recordings was 62.8±11.4 (range=24-81) for the 24-hour period.

Echocardiography. Echocardiographic measurements were performed using previously described methods performed by our group (Skudicky et al 2002) (see Figure 4.1 for a representative two-dimensional guided M-mode echocardiogram from which LV dimensions were determined). Briefly, two-dimensional guided M-mode echocardiography was performed using a pulse color Doppler Hewlett Packard model 5500 recorder coupled to a 2.5 MHz transducer. Data obtained in the short axis view were analyzed 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 (Sahn et al 1978). All measurements were recorded on videotape and analyzed off-line by an experienced investigator (Dr. Carlos Libhaber), who was unaware of the clinical condition of the subjects. 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 was obtained (Sahn et al 1978). Figure 4.1 shows echocardiography being performed and a representative M-mode image. Left ventricular mass was derived according to an anatomically validated formula: LVM = 0.8 x [1.04 (LVEDD + IVS +PWT) ^{3} – (LVEDD) ^{3}] + 0.6g, where LVEDD = LV internal diameter, IVS =LV septal thickness,



A-Left ventricular end diastolic (LVED) diameter

B-Left ventricular end systolic (LVES) diameter

C-LVED posterior wall thickness.

D-LVES posterior wall thickness.

E-LVED septal wall thickness.

F-LVES septal wall thickness

Figure 4.1. Upper panel illustrates the Hewlett Packard model 5500 utilised to assess left ventricular dimensions in the study sample. The lower panel shows an M-Mode image (courtesy of Dr. Carlos Libhaber).

PWT = posterior wall thickness, all measured at diastole in centimeters (Devereux et al 1986).

Intra-observer variability was assessed on 29 subjects in whom repeat echocardiographic measurements were performed within a two week period of the initial measurements. The Pearson's correlation coefficients for LV end diastolic diameter, septal wall thickness and posterior wall thickness were 0.76, 0.94 and 0.89 (all $p<0.0001$) respectively, and the variances (mean % difference ± SD) were 0.12±5.95%, -0.77±4.47% and 0.67±5.57% respectively. In addition, no significant differences between repeat measurements were evident on paired t-test analysis ($p=0.99$, $p=0.42$ and $p=0.48$ respectively).To adjust for the influence of growth on LVM without eliminating the impact of excess adiposity, LVM was indexed to height$^{2.7}$ (LVMI). Left ventricular mean wall thickness (MWT) was calculated as the mean of LV posterior and LV septal wall thickness at end diastole. Left ventricular hypertrophy was defined as an LVMI>51g/m$^{2.7}$ in both men and women (Nunez et al 2005).

*Data 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 and β coefficients (slopes) of relations as the mean±SEM. As neither waist circumference, nor the mean of triceps and sub-scapular skin-fold thickness (mean skin-fold thickness) were positively associated with height ($p>0.30$); when assessing the effect of adiposity on LVMI or MWT, waist circumference and mean skin-fold thickness were used as indexes of adiposity. Independent relationships between interactive terms and either LVMI or MWT were determined by multivariate regression analysis with adjustments for the individual components of the interactive terms (or the individual components^2) as well as age (or age^2), sex, height (for MWT only), diabetes mellitus or an HbA1c>6.1%, antihypertensive treatment, regular tobacco or alcohol intake, total/HDL cholesterol, and pulse rate. Probability values were further adjusted for non-

independence of family members in non-linear models (Mixed procedure as defined in the SAS package). To assess the extent to which adiposity enhances the impact of haemodynamic factors on LVMI or RWT, the β-coefficient (slope) of the adjusted relationship (adjusted for aforementioned confounders) between haemodynamic factors and either LVMI or MWT was compared between subjects with either an increased or a normal waist circumference. An unpaired Student's t-test was used to compare the slopes of regression relations. Sensitivity analysis was performed in subjects not receiving antihypertensive therapy.

4.3 Results

Characteristics of the participants. Table 4.1 gives the demographic and clinical characteristics, and the haemodynamic values of the study group and the group never having received antihypertensive therapy. More women than men participated. In general the group had a high BMI, with ~68% of subjects being either overweight (~26%) or obese (~43%) and ~43% having central obesity. More women than men were obese with 55% of women and 22% of men being obese and 58% of women and 15% of men having central obesity. ~41% of subjects were hypertensive, ~23% of participants had diabetes mellitus or an impaired blood glucose control. A relatively low proportion of subjects reported smoking or a regular intake of alcoholic beverages. No differences were noted in the demographic and clinical characteristics between all participants recruited and those with high quality echocardiograms (data not shown). Similarly, no differences were noted in demographic and clinical characteristics between all participants and never-treated participants.

Table 4.2 shows the left ventricular dimensions and mass of the study group and the group never having received antihypertensive therapy. Left ventricular dimensions were larger in men than in women (data not shown). However, LVMI was similar between the genders (data not shown). ~29% of men and ~27% of women had LVH.

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

	All participants	Never-treated participants
Number (%female)	399 (63.9)	309 (61.2)
Age (years)	44±18	39±17
Height (m)	161.8±8.7	162.8±8.7
Weight (kg)	76.7±18.7	75.1±18.6
Body mass index (kg/m^2)	29.4±7.4	28.5±7.2
Waist circumference (cm)	90.4±15.5	87.9±15.2
Subscapular skin-fold thickness (cm)	2.47±1.29	2.35±1.26
Triceps skin-fold thickness (cm)	1.83±1.15	1.81±1.18
Mean skin-fold thickness (cm)	2.15±1.10	2.08±1.11
% overweight/obese	25.8/42.6	24.9/38.2
% with central obesity	42.9	35.9
% with hypertension	41.0	24.3
% with DM or HbA_{1c}>6.1%	22.8	17.2
Regular smoking (%)	11.5	13.6
Regular alcohol (%)	21.8	23.6
Haemodynamics		
Conventional SBP/DBP	131±22/85±12	128±21/84±12
Conventional PP (mm Hg)	46.3±14.7	43.9±13.7
24-hour SBP/DBP (mm Hg) (n)	119±15/73±10 (n=297)	118±15/72±10 (n=231)
24-hour PP (mm Hg) (n)	45.9±8.2 (n=297)	45.5±8.0 (n=231)
Pulse wave velocity (m/sec) (n)	6.82±3.23 (n=328)	6.32±3.05 (n=254)

DM, diabetes mellitus; HbA_{1C}, glycated hemoglobin; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure.

Table 4.2. Left ventricular dimensions and mass of study participants.

	All participants	Never-treated participants
Number (%female)	399 (63.9)	309 (61.2)
LVED diastolic diameter (cm)	4.74±0.51	4.75±0.50
LVED septal thickness (cm)	1.03±0.17	1.01±0.15
LVED posterior wall thickness (cm)	0.99±0.15	0.97±0.13
LVED mean wall thickness (cm)	1.01±0.16	0.99±0.14
LV mass (g)	172±52	168±48
LV mass indexed for BSA (g/m^2)	95.4±25.3	93.7±23.1
LV mass indexed for $height^{2.7}$ ($g/m^{2.7}$)	47.1±14.3	45.2±12.6

LVED. left ventricular end diastolic; BSA, body surface area.

Relationships between indexes of adiposity and haemodynamic factors. As described in previous chapters, waist circumference was strongly and positively associated with conventional systolic BP (SBP), 24-hour SBP, conventional diastolic BP, 24-hour diastolic BP, PWV, conventional pulse pressure (PP), and 24-hour PP ($p<0.0001$ for all). In addition, waist circumference was associated with pulse rate ($p<0.002$). Likewise, mean skin-fold thickness was associated with conventional systolic and diastolic BP ($p<0.0001$ for all), 24-hour SBP ($p=0.035$), conventional PP ($p=0.002$), 24-hour PP ($p<0.03$), PWV ($p<0.0001$), but not with pulse rate ($p=0.07$). Similar univariate relations were noted in untreated subjects.

Individual factors associated with LVMI or wall thickness. With respect to adiposity indexes, waist circumference was associated with LVMI ($r=0.40$, $p<0.0001$), and MWT ($r=0.42$, $p<0.0001$); and mean skin-fold thickness was associated with LVMI ($r=0.24$, $p<0.0001$) and MWT ($r=0.16$, $p<0.002$). With respect to haemodynamic effects, the factors positively correlated with LVMI were conventional SBP ($r=0.47$) and 24-hour SBP ($r=0.35$), conventional diastolic BP ($r=0.29$) and 24-hour diastolic BP ($r=0.21$), PWV ($r=0.42$), conventional PP ($r=0.46$), and 24-hour PP ($r=0.38$) ($p<0.001$ for all). Similar relations were noted between haemodynamic factors and MWT (data not shown). Pulse rate was not associated with either LVMI or MWT. Age, hypertension treatment and DM/HbA1c>6.1% were all associated with both LVMI and MWT ($p<0.0001$ for all). Male gender was associated with MWT ($p<0.001$), but not LVMI. Neither regular tobacco, regular alcohol consumption, nor total/HDL cholesterol were associated with LVMI and MWT. Similar relationships were noted in untreated subjects.

Interactions between adiposity and haemodynamic factors as predictors of LVMI or geometry. Table 4.3 shows the multivariate adjusted association of haemodynamic factor-adiposity index interactions with either LVMI or MWT. After multivariate adjustments including the individual terms for adiposity indexes and haemodynamic factors in the

Table 4.3. Multivariate adjusted correlation coefficients (partial r) and 95% confidence intervals (CI) for independent relations between haemodynamic factor-adiposity index interactions and left ventricular mass index (LVMI) or mean wall thickness (LV MWT).

	All participants			Never-treated participants		
Interactive term	partial r*	CI	p value*	partial r*	CI	p value*
	Interactions with waist circumference (WC)					
LVMI vs						
Conventional SBP X WC	0.17	0.06-0.27	<0.003	0.21	0.10-0.32	<0.0005
24-hour SBP X WC	0.15	0.03-0.27	<0.02	0.25	0.11-0.37	<0.001
Conventional PP X WC	0.19	0.08-0.28	<0.001	0.20	0.09-0.31	<0.001
24-hour PP X WC	0.14	0.02-0.26	<0.03	0.27	0.13-0.39	=0.0002
Pulse wave velocity x WC	0.17	0.05-0.28	=0.003	0.23	0.10-0.35	<0.001
LV MWT vs						
Conventional SBP X WC	0.17	0.06-0.27	<0.002	0.21	0.09-0.32	=0.0005
24-hour SBP X WC	0.14	0.02-0.26	<0.02	0.19	0.05-0.32	<0.01
Conventional PP X WC	0.18	0.08-0.28	<0.001	0.22	0.10-0.33	=0.0003
24-hour PP X WC	0.13	0.01-0.25	<0.05	0.20	0.06-0.33	=0.005
Pulse wave velocity x WC	0.17	0.05-0.28	=0.003	0.19	0.06-0.31	<0.004
	Interactions with skin-fold thickness (SK)					
LVMI vs						
Conventional SBP X SK	0.17	0.06-0.27	<0.003	0.21	0.10-0.32	<0.0005
24-hour SBP X SK	0.15	0.03-0.27	<0.02	0.25	0.11-0.37	<0.001
Conventional PP X SK	0.19	0.08-0.28	<0.001	0.20	0.09-0.31	<0.001
24-hour PP X SK	0.14	0.02-0.26	<0.03	0.27	0.13-0.39	=0.0002
Pulse wave velocity x SK	0.17	0.05-0.28	=0.003	0.23	0.10-0.35	<0.001
LV MWT vs						
Conventional SBP X SK	0.17	0.06-0.27	<0.002	0.21	0.09-0.32	=0.0005
24-hour SBP X SK	0.14	0.02-0.26	<0.02	0.19	0.05-0.32	<0.01
Conventional PP X SK	0.18	0.08-0.28	<0.001	0.22	0.10-0.33	=0.0003
24-hour PP X SK	0.13	0.01-0.25	<0.05	0.20	0.06-0.33	=0.005
Pulse wave velocity x SK	0.17	0.05-0.28	=0.003	0.19	0.06-0.31	<0.004

See Table 4.1 for abbreviations. *Multivariate adjusted correlation coefficients with adjustments for haemodynamic factors (individual terms), adiposity index (individual term), age, sex, diabetes mellitus or an HbA1c>6.1%, antihypertensive treatment (in all participants), regular tobacco or alcohol intake, total/HDL cholesterol, and pulse rate. Probability values were further adjusted for non-independence of family members.

models, statistically significant interactions between adiposity indexes and either SBP, PWV, or PP were noted as independent predictors of LVMI and MWT. However, no interactions between adiposity indexes and diastolic BP were noted as independent predictors of LVMI. Moreover, no interactions between adiposity indexes and diastolic BP were noted as independent predictors of MWT (data not shown). Adjusting for adiposity index^2, haemodynamic factor^2 and age^2 in the regression models did not affect the main outcomes.

Impact of an increased waist circumference on the relations between haemodynamic factors and LVMI or MWT. Figures 4.2 and 4.3 show the impact of an increased waist circumference on the univariate relations (left and middle panels) and the multivariable adjusted slopes (β-coefficients) (right panels) of the relations between haemodynamic factors and LVMI. For comparable increases in either SBP (Figure 4.2), or PWV and PP (Figure 4.3), participants with a high waist circumference had a greater increase in LVMI as compared to participants with a normal waist circumference. Indeed, the multivariate adjusted slopes of the relations between haemodynamic factors and LVMI were considerably greater in participants with an increased waist circumference as compared to participants with a normal waist circumference. Adjusting for adiposity index^2 and age^2 in the regression models did not affect the main outcomes.

Impact of an increased waist circumference on the effect of haemodynamic factors on LVMI or MWT. Figure 4.4 summarizes the impact of an increased waist circumference on the multivariate adjusted increase in LVMI and MWT for every one SD increase in either conventional or 24-hour SBP or PP, or PWV. In participants with an increased waist circumference, conventional SBP, PP, 24-hour SBP and PP and PWV were associated with a greater increase in LVMI or MWT. Importantly, every one SD increase in conventional systolic BP (~22 mm Hg) translated into a 5.24 g/m$^{2.7}$ increase in LVMI in

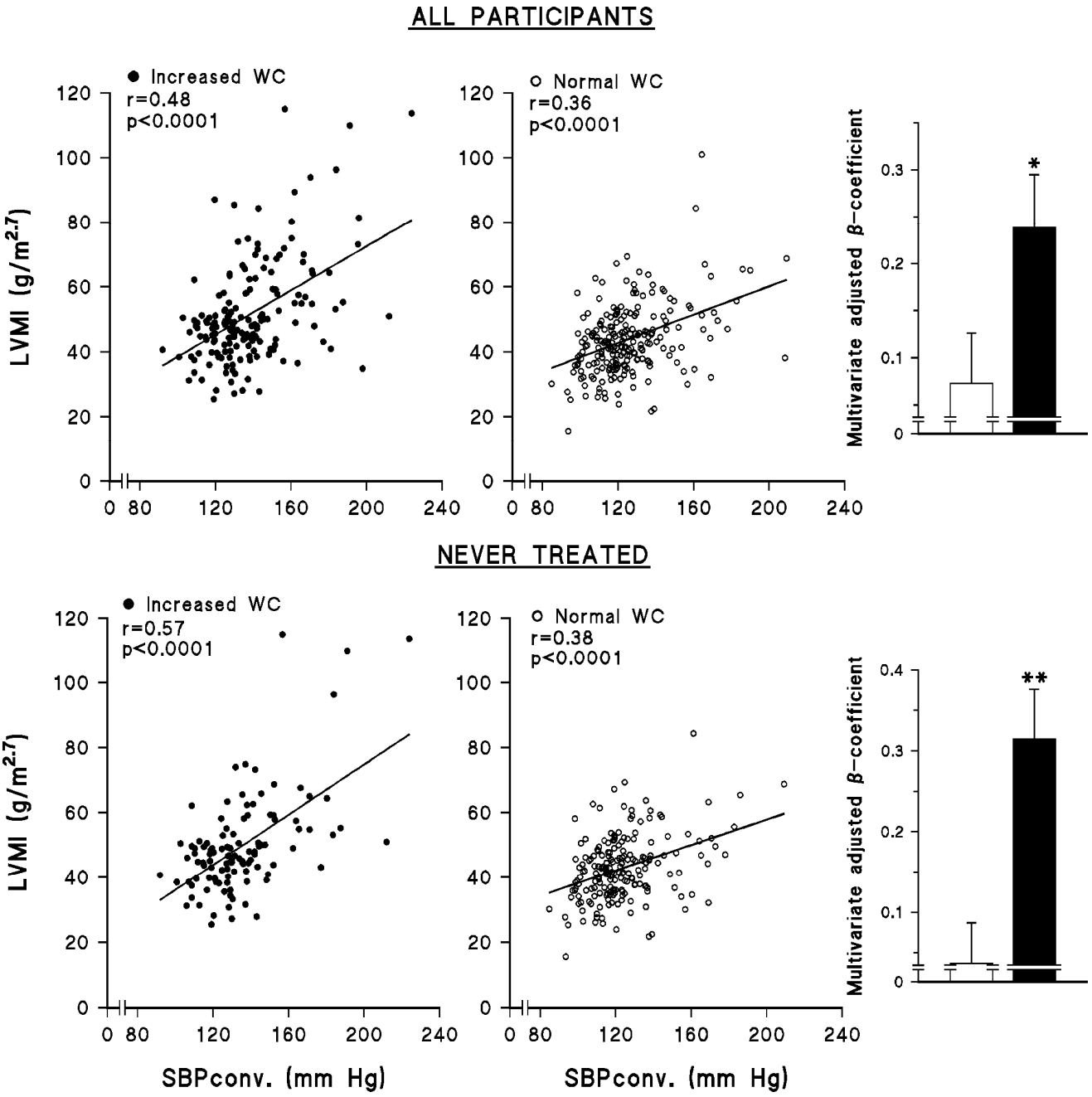


Figure 4.2. Impact of an increased waist circumference (WC) on the associations between conventional systolic blood pressure (SBPconv.) and left ventricular mass index (LVMI) in all and never-treated participants. Left and middle panels show univariate relations in participants with a normal and an increased WC and right panel shows comparisons of the multivariate adjusted slopes (β-coefficient) of relations between participants with a normal and an increased WC. Adjustments were for WC, age, sex, diabetes mellitus or an HbA1c>6.1%, antihypertensive treatment (in all participants), regular tobacco or alcohol intake, total/HDL cholesterol, and pulse rate. Probability values were further adjusted for non-independence of family members. *p<0.05, **p<0.001 compared to normal WC.

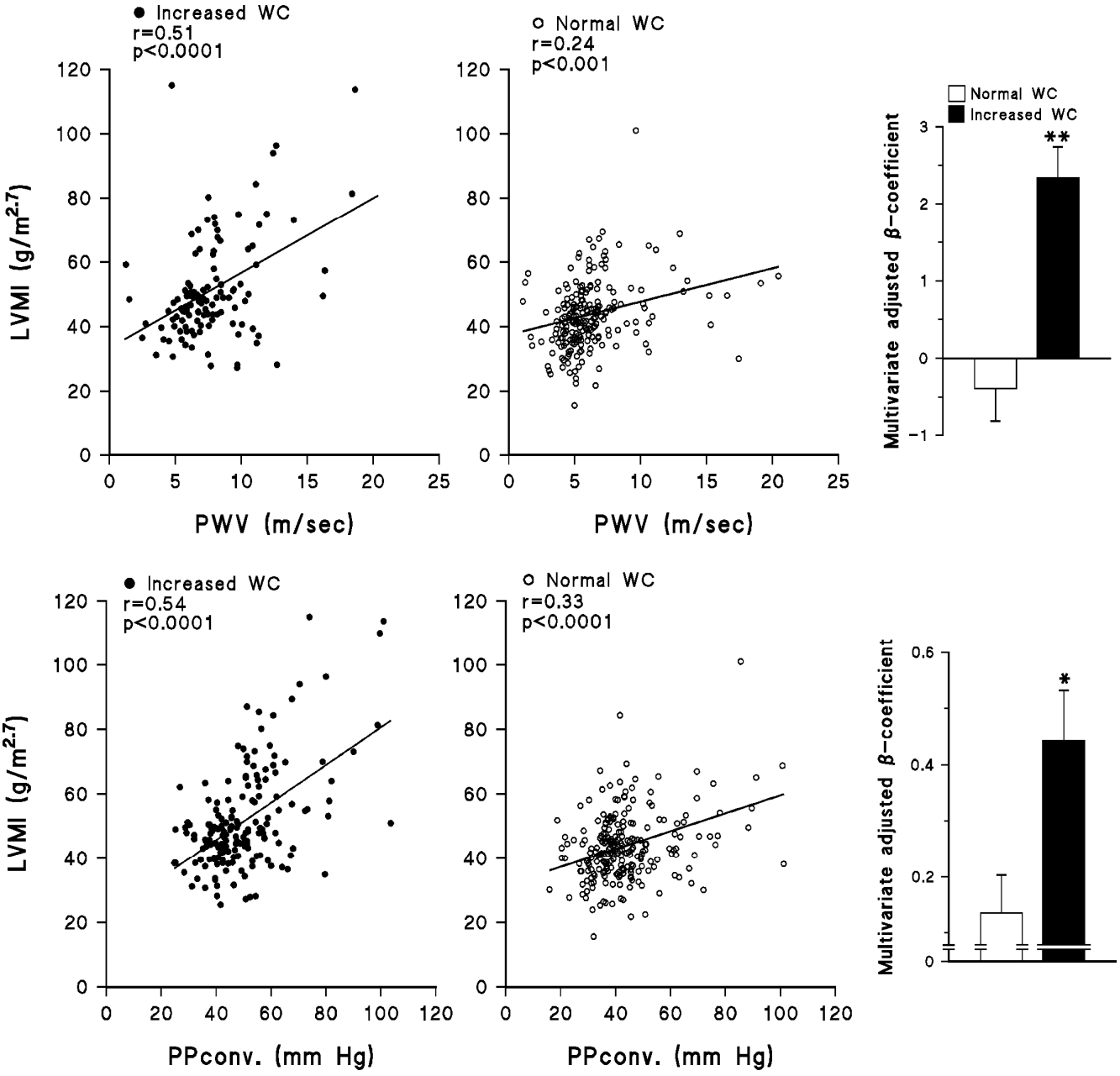


Figure 4.3. Impact of an increased waist circumference (WC) on the associations between indexes of large artery dysfunction (pulse wave velocity [PWV] and conventional pulse pressure [PPconv]) and left ventricular mass index (LVMI). Left and middle panels show univariate relations in participants with a normal and an increased WC and right panels show comparisons of the multivariate adjusted slopes (β-coefficient) of relations between participants with a normal and an increased WC. Adjustments are as in Figure 1. *p<0.01, ** p<0.001 compared to normal WC.

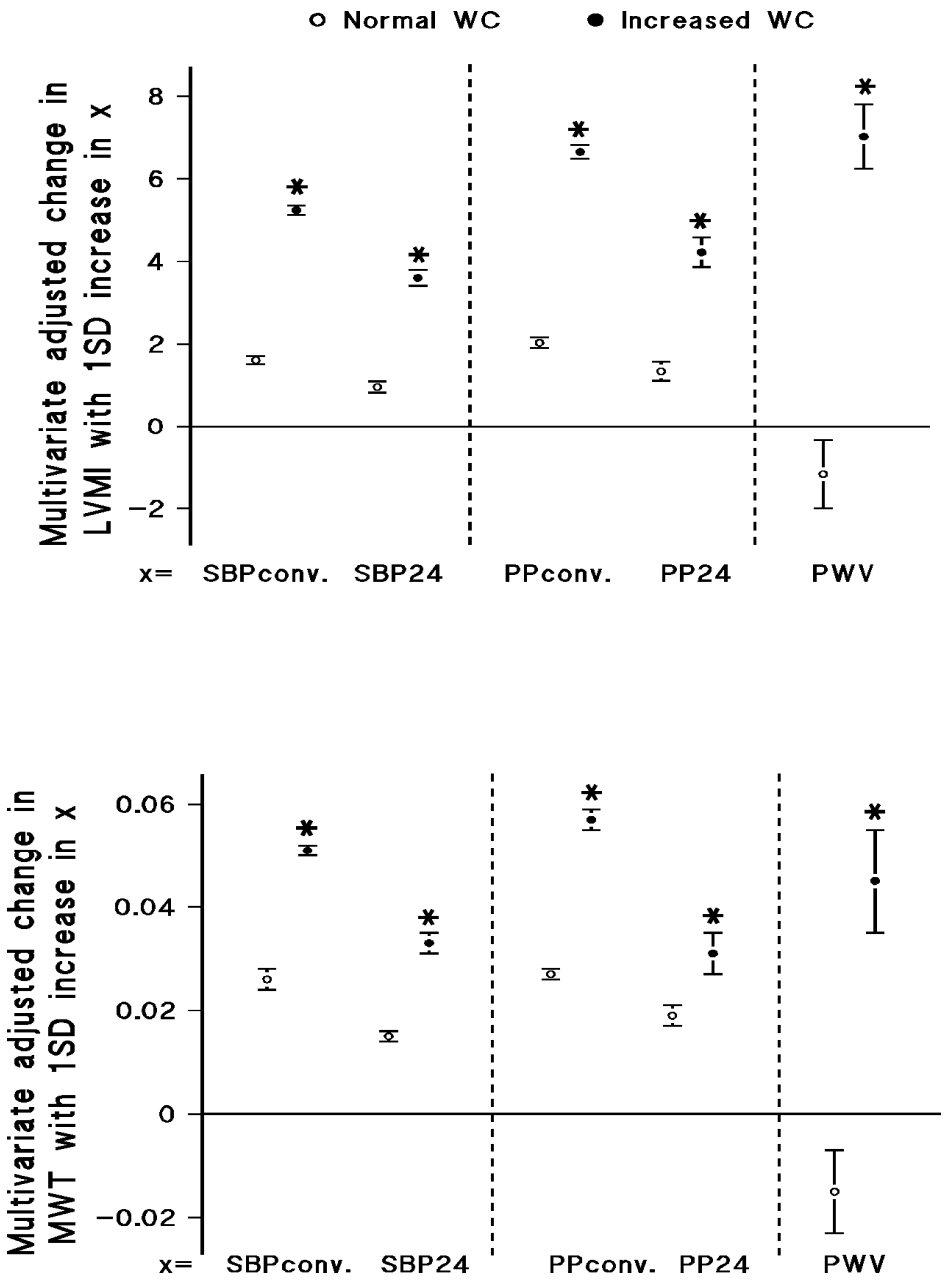


Figure 4.4. Impact of an increased waist circumference (WC) on the multivariate adjusted change in left ventricular mass index (LVMI) or mean wall thickness (MWT) (±95% confidence intervals) for every 1 SD increase in systolic blood pressure (SBP), pulse pressure (PP) or pulse wave velocity (PWV). Adjustments were for age, WC, sex, height (for mean wall thickness only), diabetes mellitus or an HbA1c>6.1%, antihypertensive treatment, regular tobacco or alcohol intake, total/HDL cholesterol, and pulse rate. Probability values were further adjusted for non-independence of family members. conv., conventional; 24, 24-hour. *p<0.0001 vs normal WC.

participants with an increased waist circumference, but only a 1.61 g/m$^{2.7}$ increase in LVMI in participants with a normal waist circumference. Thus, an increased adiposity was associated with a 3.26 times greater impact of BP on LVMI.

4.4 Discussion

The main findings of the present study are as follows. In a population sample with a high prevalence of excess adiposity (~68% overweight or obese and ~43% with central obesity) and untreated hypertension (~19%), synergy between indexes of adiposity (waist circumference and skin-fold thickness) and either conventional or 24-hour SBP, or indexes of large artery dysfunction (PWV and PP) were independently associated with LVMI and MWT after multivariate adjustments including the individual adiposity indexes and haemodynamic variables. The synergistic effects between indexes of adiposity and haemodynamic variables translated into a greater impact of these haemodynamic factors on LVMI and MWT in participants with an increased as compared to a normal waist circumference. For example, for comparable increases in SBP, participants with an excess adiposity (a high waist circumference) had a ~3-4 times greater increase in LVMI after adjustments for confounders.

The present study provides the first evidence to indicate that mild-to-moderate forms of excess adiposity not only promote the development of increases in BP as is well recognized (Majane et al 2007, chapter 2 and as summarized in chapter 1), but also enhance the impact of BP on LVMI. In this regard the present study results agree with the finding that severe obesity (BMI>35 kg/m^2) is associated with an enhanced effect of hypertension on LVM (Avelar et al 2007). However, in contrast to the present study, in a predominantly middle-aged population sample (Fox et al 2004), and in a randomly selected Framingham sample (Lauer et al 1992), interactions between discrete categories of BMI (lean, overweight and obese) and BP (normal, high-normal and hypertensive) failed to predict LVMI. In this regard the assessment of BP as a discrete trait could potentially

reduce the sensitivity of detecting an interactive effect. Furthermore, in contrast to the present study where ~17% of subjects were receiving antihypertensive therapy and ~24% were hypertensive but not receiving therapy, in one study (Fox et al 2004) ~50% of subjects (the majority of hypertensives) were receiving antihypertensive therapy, thus potentially modifying the effects of BP on LVMI. Further in the Framingham Heart Study, by excluding treated hypertensives from the analysis a very narrow range of BP values was obtained in the participants (Lauer et 1992), thus potentially limiting the sensitivity of detecting an interactive effect. Last, the use of BMI as an index of adiposity as previously studied (Lauer et al 1992, Fox et al 2004), which may not closely reflect the degree of adiposity in some populations (Luke et al 1997), could confound the interpretation of these data.

In the present study, interactions between adiposity indexes and SBP, PP and PWV were independently associated with LVMI and MWT. As SBP, PP and PWV are all affected by large artery dysfunction, these data suggest that excess adiposity not only promotes large artery dysfunction (see chapter 3), but also enhances the impact of large artery dysfunction on LV growth via systolic BP effects. This synergy between adiposity and large artery dysfunction to promote LV growth clearly represents a pathological afterload effect on the LV. This finding may therefore provide insight into why adiposity-induced increases in LVM are associated with deleterious cardiovascular outcomes independent of conventional risk factors (de Simone et al 2005), in direct contrast to normal growth-induced increases in LVM that are considered to be entirely benign.

The strengths of the present study are as follows. I analysed haemodynamic factors and indexes of adiposity as continuous and not as discrete variables in a random population sample of subjects with a high prevalence of hypertension (~41%), but in whom antihypertensive use was limited (~17%). Using this approach the interactive effects between haemodynamic factors and indexes of adiposity as predictors of LVMI were

remarkably robust even after multiple adjustments, and striking increases in the slopes of the relations in subjects with an increased as compared to a normal waist circumference were noted. Moreover, these interactive effects were confirmed on sensitivity analysis in never-treated subjects. In the present study I also examined two adiposity indexes (waist circumference and skin-fold thickness) that were unrelated to height and I did not rely on BMI, which is well recognized to be a poor index of excess adiposity in some populations (Luke et al 1997). Last, I confirmed the interactive effects with a number of measurements including pulse wave analysis, conventional SBP and PP measurements and ambulatory SBP and PP measurements. In this regard 24-hour BP is more closely associated with LVMI than conventional BP measurements (Mancia and Parati 2000).

As this was a cross-sectional study, further prospective studies are still required to determine whether adipose tissue loss or gain modifies the impact of haemodynamic changes on LVM. Further, in the present study I did not evaluate the potential cellular mechanisms of the effects of interactions between BP and body size on LVMI. In this regard, cardiomyocytes may respond to tension transduction as well as circulating factors released from adipocytes through common downstream pathways.

The potential mechanisms that may explain the interaction between BP and excess adiposity to promote increases in LVM warrant consideration. In this regard, although controversial with respect to a role for angiotensin II (Reudelhuber et al 2007), the renin-angiotensin system, which is activated in obesity (Serazin-Leroy et al 2000, Cassis et al 1988, Campbell et al 1987, Cooper et al 1998, Jonsson et al 1994, Harp et al 2002) may contribute toward obesity-induced increases in LVM. Indeed, angiotensin II receptor blockade has been shown to prevent obesity-induced increases in LVM (du Toit et al 2005). Alternatively, either a protective effect of leptin on cardiac hypertrophy (Barouch et al 2003) or in contrast, a hypertrophic action of leptin on cardiac myocytes (Rajapurohitam et al 2003) may contribute toward cardiac growth effects.

The potential clinical implications of the present study are underscored by comparing the increase in LVMI produced by an equivalent change in BP in subjects with as compared to those without an increased waist circumference. As compared to subjects with a normal waist circumference where a 22 mm Hg increase in systolic BP (~one SD) was associated with a 1.61 $g/m^{2.7}$ increase in LVMI, in subjects with an increased waist circumference, a 22 mm Hg increase in systolic BP (~one SD) was associated with a 5.24 $g/m^{2.7}$ increase in LVMI. Thus for an equivalent increase in systolic BP, participants with an increased waist circumference have a three-four times greater increase in LVMI.

In conclusion, the present study indicates that obesity-induced increases in LVM are characterized in-part by a considerably enhanced impact of arterial stiffness and hence systolic BP on LVM and wall thickness. The results of the present study therefore provide unique insights into why, as opposed to normal growth effects on LVM which have no pathological relevance, adipose tissue-induced effects on cardiac growth may be of pathophysiological relevance.

CHAPTER 5

Dietary-Induced Obesity Promotes the Progression From Compensated Cardiac Hypertrophy to Cardiac Dilatation and Pump Dysfunction in Hypertensive Rats.

Abtsract

The role of obesity in the development of cardiac pump dysfunction is uncertain. I explored whether excess adiposity can promote the transition from compensated cardiac hypertrophy to pump dysfunction in spontaneously hypertensive rats (SHR) and the mechanisms thereof. After feeding rats a diet for 4-5 months that resulted in an increased energy intake, obesity was induced in SHR and Wistar Kyoto (WKY) control rats at an age when cardiac pump function is maintained. Dietary-induced obesity was not associated with abnormal blood glucose control (HbA1c) or with increases in systolic blood pressure. However, dietary-induced obesity resulted in a reduced left ventricular (LV) endocardial fractional shortening (echocardiography) and LV end systolic elastance (isolated perfused heart studies) in SHR, but not in WKY controls. The reduced LV systolic chamber function in SHR was associated with a marked increase in LV end diastolic diameter (LVEDD) and a right shift of the LV diastolic pressure-volume relation in obese SHR, but not in obese WKY. Moreover, LV intrinsic myocardial systolic function, as determined from the slope of the linearized LV systolic stress-strain relationship, was markedly reduced in obese as compared to lean SHR, whilst intrinsic myocardial systolic function was maintained in obese as compared to lean WKY rats. Obese SHR had a greater increase in LV weight as compared to obese WKY rats and cardiomyocyte apoptosis (TUNEL) and the activity of myocardial matrix metalloproteinases (zymography) was enhanced in obese SHR, but not in obese WKY rats. As compared to lean rats, obesity in either SHR or in WKY failed to alter either total or cross-linked LV collagen concentrations. In conclusion, these data suggest that in hypertension, obesity may promote the progression from compensated LV hypertrophy to pump dysfunction independent of further BP changes or alterations in blood glucose control, changes that may be mediated through both cardiomyocyte apoptotic-induced decreases in myocardial function and activation of myocardial collagenases to promote cardiac dilatation.

5.1 Introduction

Obesity is risk factor for heart failure and this effect may be independent of other conventional cardiovascular risk factors (Bahrami et al 2008, Ingelsson et al 2005, Kenchaiah et al 2002, Kenchaiah et al 2004, Poirier et al 2006, Wong and Marwick 2007, Contaldo et al 2002). As adjustments for baseline cardiac systolic chamber function abolish the independent relationship between obesity and heart failure (Bahrami et al 2008), it is possible that heart failure in obesity is mediated by pump dysfunction. Nevertheless, whether obesity is associated with cardiac pump (chamber) dysfunction independent of conventional cardiovascular risk factors is controversial (Peterson et al 2004, Wong et al 2004, Pascual et al 2003, Scaglione et al 1992, de Divitiis et al 1981, Chakko 1998, Zarich et al 1991, Stoddard et al 1992, de Simone et al 1996, Mureddu et al 1996, Iacobellis et al 2002, Karason et al 1998, Carroll et al 2006, du Toit et al 2008). Although sensitive myocardial tissue Doppler techniques indicate that obesity is associated with myocardial systolic abnormalities independent of BP, age, gender, blood glucose control and left ventricular mass (LVM) (Peterson et al 2004, Wong et al 2004); weight loss produced either by lifestyle modification or gastric bypass does not influence myocardial systolic function of the LV (Skilton et al 2007, Wong et al 2006). Moreover, although animal models of obesity without diabetes mellitus are associated with a reduction in isolated cardiomyocyte contractile function (Carroll et al 1997, Relling et al 2006, Dong et al 2006, Ren et al 2000), euglycaemic animal models of obesity have a preserved pump function (Carroll et al 2006, du Toit et al 2008). As a reduced cardiac pump function is a well recognized feature of the development of heart failure (Hunt et al 2005), clarity on the role of obesity in the development of pump dysfunction independent of conventional risk factors is required.

In studies demonstrating a relationship between obesity and myocardial systolic dysfunction, BP was greater in obese as compared to lean participants (Skilton et al 2007,

Peterson et al 2004, Wong et al 2004). It is therefore possible that obesity may promote the development of myocardial systolic and hence pump dysfunction through an interaction with BP effects. As obesity may enhance the impact of BP on LV hypertrophy (LVH) (see preceding chapter, Avelar et al 2007) and an increased LV mass is a risk factor for the development of pump dysfunction (Drazner et al 2004), I therefore hypothesized that one potential mechanism that may explain the relationship between obesity and the development of heart failure (Kenchaiah et al 2002) is that obesity may enhance the detrimental effects of blood pressure (BP) on the heart. To test this hypothesis I assessed whether dietary-induced obesity promotes the development of LV systolic chamber and myocardial dysfunction in spontaneously hypertensive rats (SHR) as compared to normotensive Wistar Kyoto control (WKY) rats and the mechanisms thereof.

5.2 Methods

This study was conducted in accordance with the Principles of Laboratory Animal Care of the National Society for Medical Research and the Guide for the Care and use of Laboratory Animals of the National Academy of Sciences (NIH publication no 80-23, revised 1985) and was approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (Approval number: 2006/99/3). Eight month old SHR (an age when compensatory LVH occurs)(Tsotetsi et al 2001) and age-matched WKY rats were assigned to receive an obesity-inducing or control diet for 5 months (du Toit et al 2008).

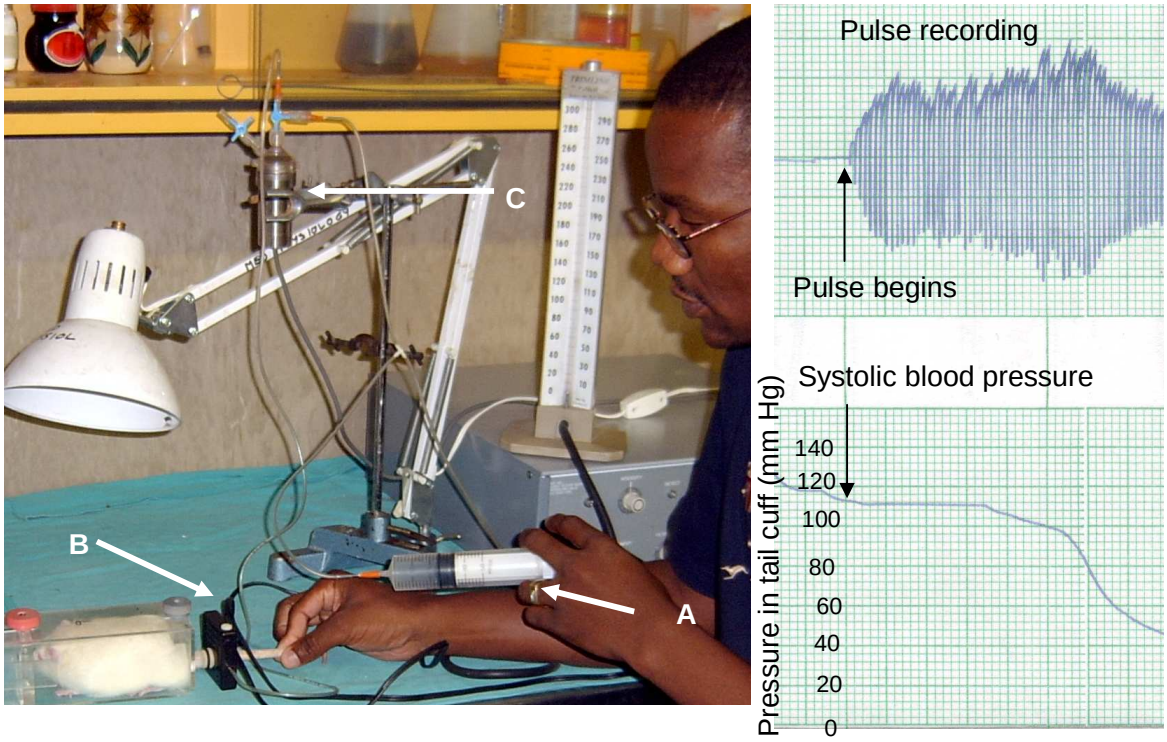
The experimental diet contained elevated carbohydrates and fats and resembles a Western-type diet (Pickavance et al 1999). The experimental diet consisted of 65% carbohydrate, 19% protein, and 16% fat in comparison to that of a control group which consisted of 60% carbohydrate, 30% protein, and 10% fat (du Toit et al 2008). As the diet is designed to induce hyperphagia (Pickavance et al 1999), the experimental groups consumed a greater quantity of food and consequently, energy intake was enhanced in

the experimental groups (570±23 kJ/day) as compared to the control group that consumed 371±18 kJ/day). Our group has previously shown that differences in micronutrient (vitamins and minerals) intake, produced by dilution of the diet by addition of carbohydrates and fats, does not modify either body size or cardiac function (du Toit et al 2008).

Assessment of dietary-induced effects on adipose tissue. To identify an impact of the diet on adipose tissue, body weights were measured weekly and visceral fat was weighed at the end of the study. Visceral fat included retroperitoneal and omental fat.

Blood pressure. Tail cuff systolic BP was determined on three separate occasions (beginning, middle and end of dietary period) during the study using a photoelectric diode to detect tail pulses (Figure 5.1). Rats were housed in restrainers for approximately 20 minutes and a lamp placed over the tail to dilate the tail artery in order to assist with the detection of the tail pulse (Figure 5.1). Once a pulse was detected with a photoelectric diode, a cuff placed proximal to the photoelectric diode was inflated until the pulse disappeared. The cuff was then allowed to slowly deflate until the pulse was detected again. The cuff pressure at which the pulse was detected on deflation of the cuff was considered systolic BP (Figure 5.1). Prior to the first measurement, rats were placed for two hours a day over two days in restrainers, and the tail cuff inflated to 200 mm Hg 5 times for 30 seconds each time to habituate rats to the procedure.

Echocardiography. Left ventricular systolic function and chamber dimensions were determined *in vivo* using two-dimensional targeted M-mode echocardiography performed using a 7.5 MHz transducer and a Hewlett Packard Sonos 2500 sector scanner according to the American Society of Echocardiography convention and as previously described (Norton et al 2002). Echocardiography was performed under anaesthesia induced by the administration of 50 mg/kg of ketamine and 3 mg/kg of xylazine (Norton et al 2002). Left



A = Syringe coupled to pressure transducer and to tail cuff. The syringe is used to inflate or deflate the tail cuff

B = Casing containing the photoelectric diode which is used to detect tail pulses (upper right recording) and the tail cuff used to occlude the tail artery.

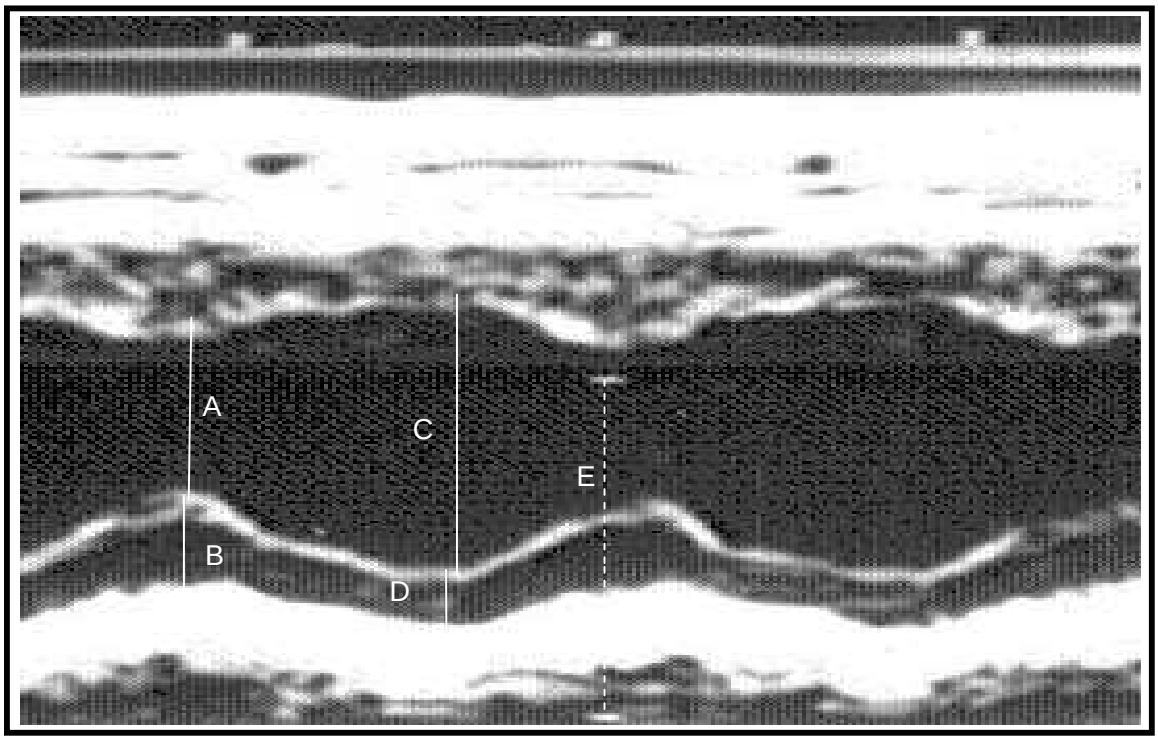
C = Pressure transducer used to record the pressures in the tail cuff as depicted in the lower right recording.

Figure 5.1. Photograph of the experimental apparatus used to measure tail artery systolic blood pressures in rats (left panel) and an example of a recording obtained (right panel). Systolic blood pressure was determined by inflating the tail cuff to pressures above that which abolished the pulse in the tail artery and then slowly releasing the tail cuff pressure until the pulse returned (see right panel). Systolic blood pressure was taken as the pressure in the tail cuff at which the pulse was first detected.

ventricular end-diastolic (LVEDD) and end-systolic (LVESD) internal diameter and end-diastolic (ED PWT) and end-systolic posterior wall thickness (ES PWT) were measured using the leading edge technique (Norton et al 2002) (Figure 5.2).

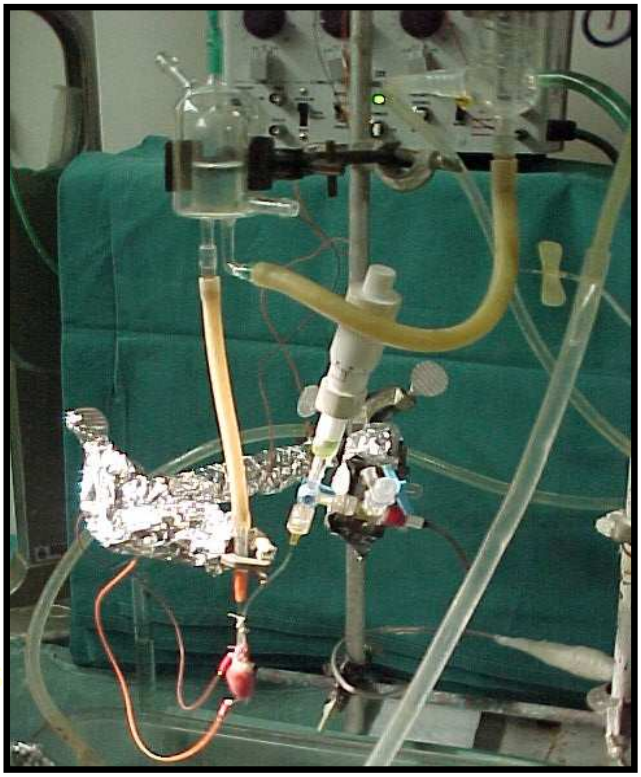
Left ventricular systolic chamber function (pump function) was determined from LV endocardial fractional shortening (LV FSend)(Norton et al 2002). Left ventricular FSend was determined from the equation (LVEDD-LVESD)/LVEDD x 100 (Norton et al 2002). LV remodelling was determined from LV EDD and ED PWT measurements as well as LV relative wall thickness values calculated from ED PWT/0.5 x LVEDD.

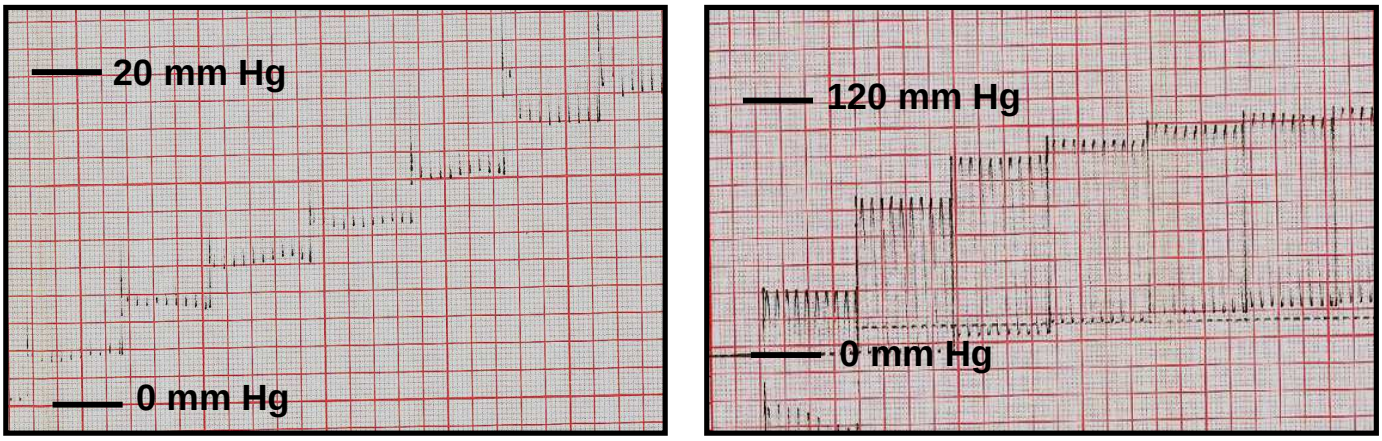
Isolated, perfused heart preparations. Following echocardiography, LV systolic function and LV remodelling was assessed *ex vivo* in isolated perfused heart preparations as previously described (Woodiwiss et al 2001, Norton et al 2002). To undertake these measurements, whilst rats were still anaesthetized hearts were removed from the thoracic cavity and placed in an ice-cold physiological saline solution until they were mounted on a perfusion apparatus illustrated in Figure 5.3. Hearts were perfused retrogradely via the aorta at a constant flow (12 $ml.min^{-1}$.g wet heart weight) with 37℃ physiological saline solution consisting of (in mM) 118.0 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 25.0 $NaHCO_3$, 1.2 KH_2PO_4, 1.2 $MgSO_4$ and 10.0 glucose with a pH of 7.4. The solution was saturated with 95% O_2 and 5% CO_2 gas and filtered through a size 0.45μm Millipore Durapore membrane filter. Hearts were paced at 360 $beats.min^{-1}$ with platinum electrodes attached to the left atrium and the apex of the heart. An empty latex balloon with a known wall volume, coupled to a Statham P23 pressure transducer and a micromanipulator (see Figure 5.3) via a polyethylene catheter, was inserted through the mitral valve into the LV lumen. A thin-walled latex balloon with zero pressure at filling volumes beyond maximum LV lumen capacities was selected for this study to avoid the stiffness of the balloon wall contributing to LV pressure at higher filling volumes. The volume of the balloon wall was assessed with a water-displacement technique, and the same balloon was used



A = Left ventricular end systolic diameter
B = Left ventricular end systolic posterior wall thickness
C = Left ventricular end diastolic diameter
D = Left ventricular end diastolic posterior wall thickness
E = Calibration showing 1 cm

Figure 5.2. Echocardiogram used to assess cardiac structure and function in rats. The figure shows internal dimensions and posterior wall thickness of the left ventricle recorded over ~three beats from an ultrasonic image obtained in the short axis of the heart. The lines (A-D) show where recordings of internal dimensions at end diastole and peak systole were obtained in order to determine systolic pump function and the degree of chamber dilatation (see text).





Left ventricular diastolic pressure

Left ventricular developed (systolic) pressure

Figure 5.3. Experimental apparatus for the isolated, perfused heart apparatus (upper panel) and typical recordings obtained (lower panel). See text for description. The left ventricular diastolic and developed (systolic) pressures are recorded at incremental volumes in the left ventricle (0.01 increments in volume).

throughout each of the studies. A micromanipulator was used to gradually increase LV volumes to values that resulted in no further change in LV developed pressure. LV pressures were determined at as many multiple small increments in volume as were practically possible to improve on the accuracy of curve fitting during analysis (Figure 5.3).

Load-independent LV systolic chamber performance (a measure of systolic pump function) was determined *in vitro* from the slope (Ees) of the linear portion of the LV peak systolic pressure-volume relation. Intrinsic myocardial systolic performance (a load-independent measure of intrinsic myocardial contractility) was assessed *in vitro* from the slope (E_n) of the systolic stress (σ)-strain relation (Norton et al 2002). Systolic σ and strain were calculated using previously described formulae (Weber et al 1988) assuming a thick-walled, spherical model of LV geometry as follows:

$$\sigma = [1.36\ P\ V^{2/3}]/[(V+V_m)^{2/3}-V^{2/3}]$$

$$\text{strain} = \{[V^{1/3}+(V+V_m)^{1/3}]/[V_0^{1/3}+(V_0+V_m)^{1/3}]\} - 1$$

where P=pressure, V=volume, V_m=left ventricular muscle volume, V_0=unstressed left ventricular volume

LV remodelling was assessed in isolated, perfused heart preparations from the volume intercept (V_0) of the LV diastolic P-V relation (Woodiwiss et al 2001, Norton et al 2002).

Cardiomyocyte necrosis. A longitudinal slice of the LV from the apex to the base through the LV free wall was obtained from all rats for histology. LV tissue was stored in formalin for subsequent histology. LV tissue was processed routinely for light microscopy and 50 μm-thick sections of the long axis circumference were cut through the full thickness of the LV wall. Ten slices were obtained at 1-mm intervals and stained with van Gieson's stain. After staining a pathological grade was assigned, where 0 indicates no damage; 1

and 2, patchy fibrosis in less than or more than 20% of the field respectively; 3 and 4, diffuse contiguous subendocardial fibrosis in less than or more than 50% of the field respectively and 5 and 6, full thickness fibrosis in less than or more than 50% of the field respectively (Teerlink et al 1994; Woodiwiss et al 2001). Representative slides are illustrated in Figure 5.4.

Cardiomyocyte apoptosis. The degree of apoptosis was quantified on myocardial tissue sections obtained from the same tissue blocks used to assess the pathological score. For each tissue block, 5 μm thick sections were stained and evaluated. Nuclear deoxyribonucleic acid (DNA) fragments in the tissue sections were detected using a non-radioactive *in situ* apoptotic cell death detection kit (DeadEnd^{TM} Colorimetric TUNEL system, Promega, Madison, WI, USA), where terminal deoxynucleotidyl transferase (TdT) was used to incorporate biotinylated nucleotide at the 3'-OH DNA ends. Using this technique horseradish-peroxidase-labeled streptavidin binds to biotinylated nucleotides, which subsequently stain dark brown in response to hydrogen peroxide and diaminobenzidine (Agarwala and Kalil 1998) (Figure 5.4). Both positive (DNase treated) and negative (no addition of TdT) control tissue sections were incorporated into each assay (Figure 5.5). The number of apoptotic cardiomyocyte nuclei and the total number of cardiomyocyte nuclei (haematoxylin and eosin stain) in each slide were counted on ten evenly spaced fields from the apex to the base using a computer-based image acquisition and analysis system at 400 times magnification (Axiovision 3, Carl Zeiss, Gottingen, Germany). Apoptotic cardiomyocyte nuclei were expressed as a percentage of the total number of cardiomyocyte nuclei. Representative examples of stained sections for the samples assessed and from positive and negative controls and from an SHR are illustrated in Figure 5.5.

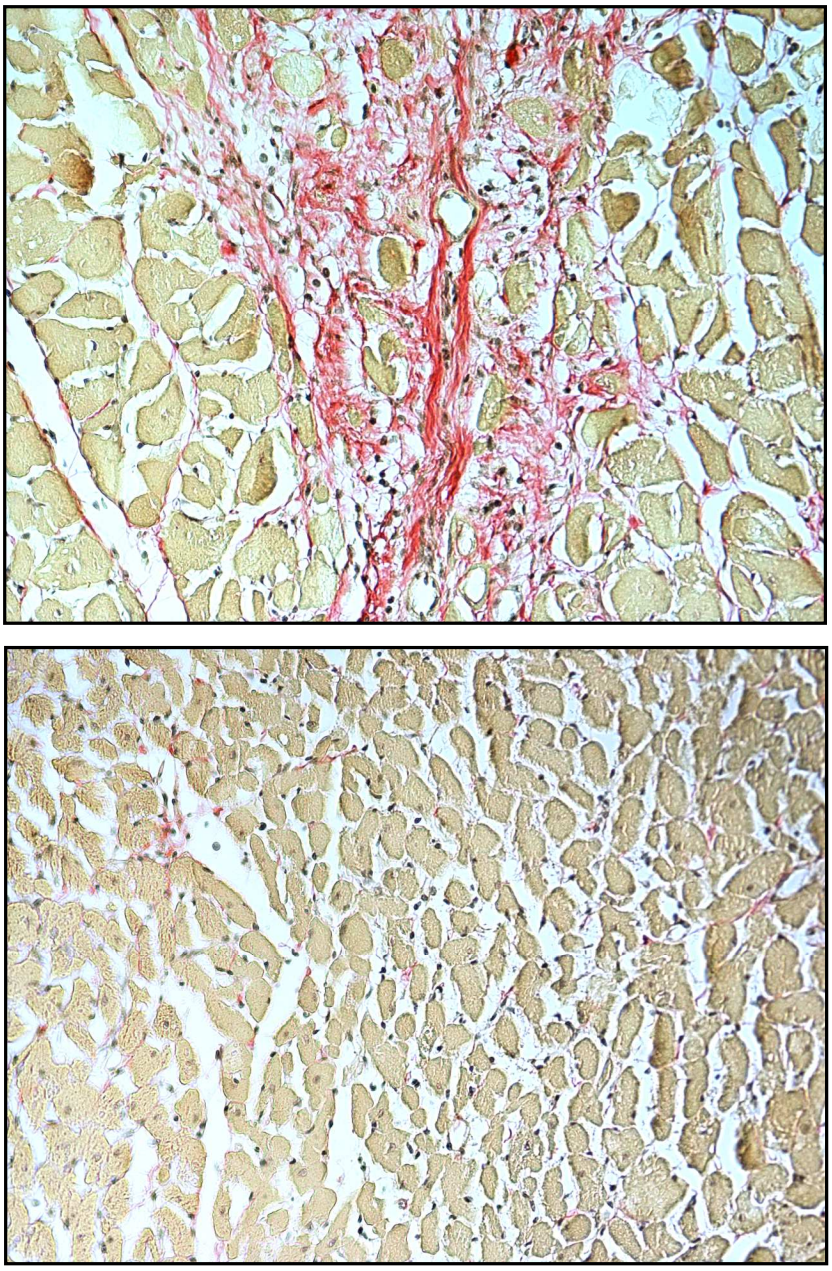


Figure 5.4. Histological images obtained using light microscopy from cross-sections of myocardial tissue stained with van Gieson's stain. The slides show portions of the heart with evidence of tissue necrosis and fibrosis following cell death (upper panel) as compared to normal portions of the heart (lower panel).

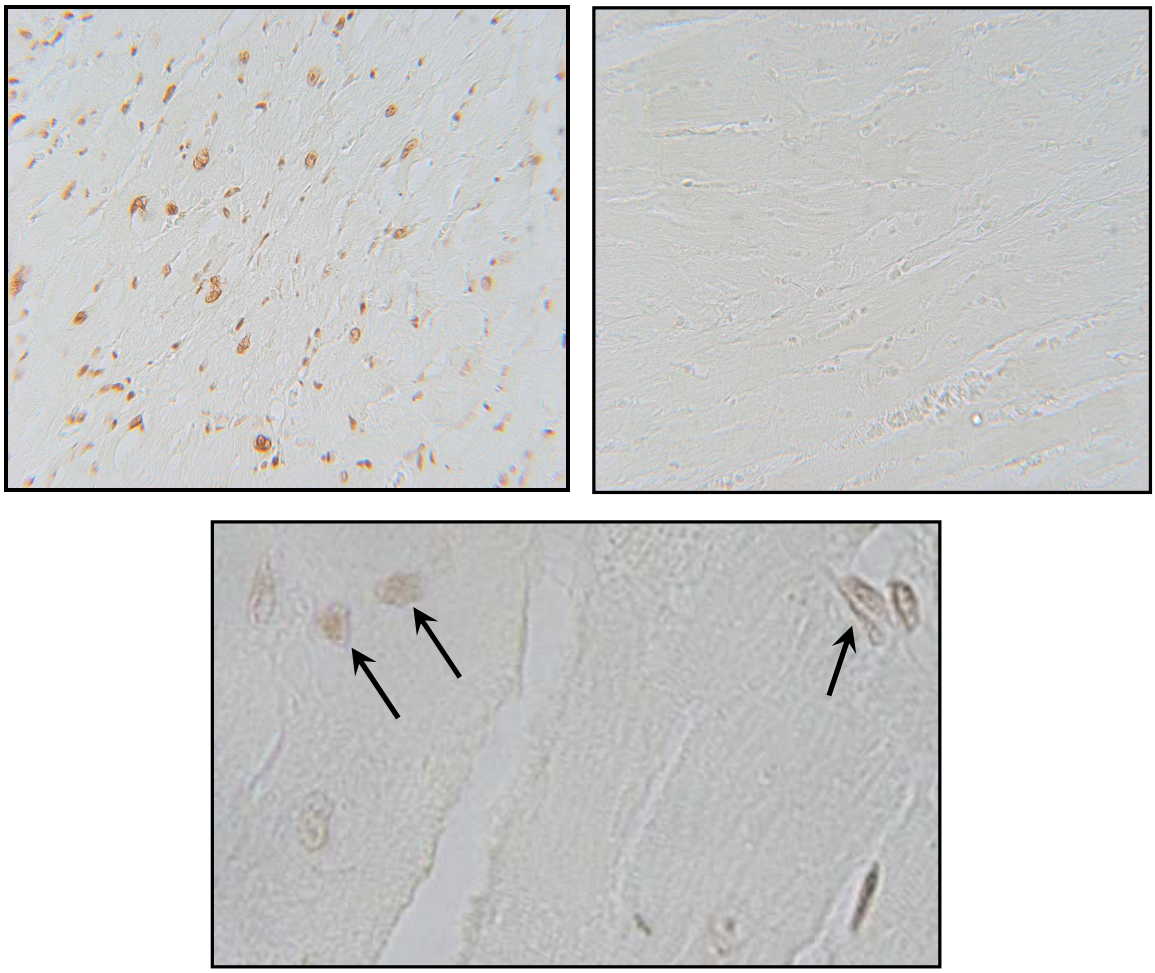


Figure 5.5. Histological sections of the myocardium stained for apoptotic nuclei. The upper panels illustrate sections obtained from a positive (left) and a negative (right) control and the lower panel a section from the myocardium of a heart showing apoptotic cardiomyocyte nuclei (arrows). Note the numerous apoptotic nuclei in the positive control section.

Matrix metalloproteinase activity. To avoid a potential impact of prolonged anaesthesia and perfusion of the myocardium with artificial solutions on MMP activity, gelatin zymography was performed on tissue collected from rats that had not undergone echocardiography or isolated, perfused heart studies. Gelatin zymography was performed as previously described (Tyagi et al 1993) in order to determine the activity of the gelatinase, MMP-2. For this purpose, tissue from the lateral wall of the LV was analyzed. Tissue samples used were frozen in liquid nitrogen within 5 minutes of removing hearts from the thoracic cavity and then stored at -70℃ until analysis. To extract cardiac tissue protein, tissue was ground to a powder under liquid nitrogen using a mortar and pestle. The ground tissue was weighed and 100 µl extraction buffer (50 mM Tris, 0.1% SDS) added per 100 mg tissue powder. The samples were incubated for 18 h at 4℃ and thereafter the samples were centrifuged at 12 000 rpm for 10 minutes and the supernatant containing the soluble extracted protein was decanted and stored at -70℃. The protein concentrations of the supernatants were estimated using a modified Lowry/Folin technique (Lowry et al 1951). In order to determine the relative activity of MMP-2 in each sample, 20 µg of protein was loaded into each well of a 10% polyacrylamide gel containing 1mg.ml^{-1} of type A gelatin. The proteins were separated electrophoretically over 1.5 hours at 30 mA. A single standard of rat MMP-2 (Sigma, purity >95% by SDS-PAGE visualized by silver staining) was included on each gel to locate the MMP-2 bands. The gels were then incubated overnight in substrate buffer (Tris 50 mM pH 8, $CaCl_2$ 5 mM) to allow degradation of gelatin. The gels were then stained for protein with Coomassie blue dye resulting in a gel with a dark background and light bands (Figure 5.6 upper panel), the intensity of which indicate the activity of MMP. The gels were scanned using a flat bed transmission scanner (Cano Scan 4200 F, Cannon Solutions, China). Images were inverted (Figure 5.6 lower panel) and the density of the MMP band analyzed compared to

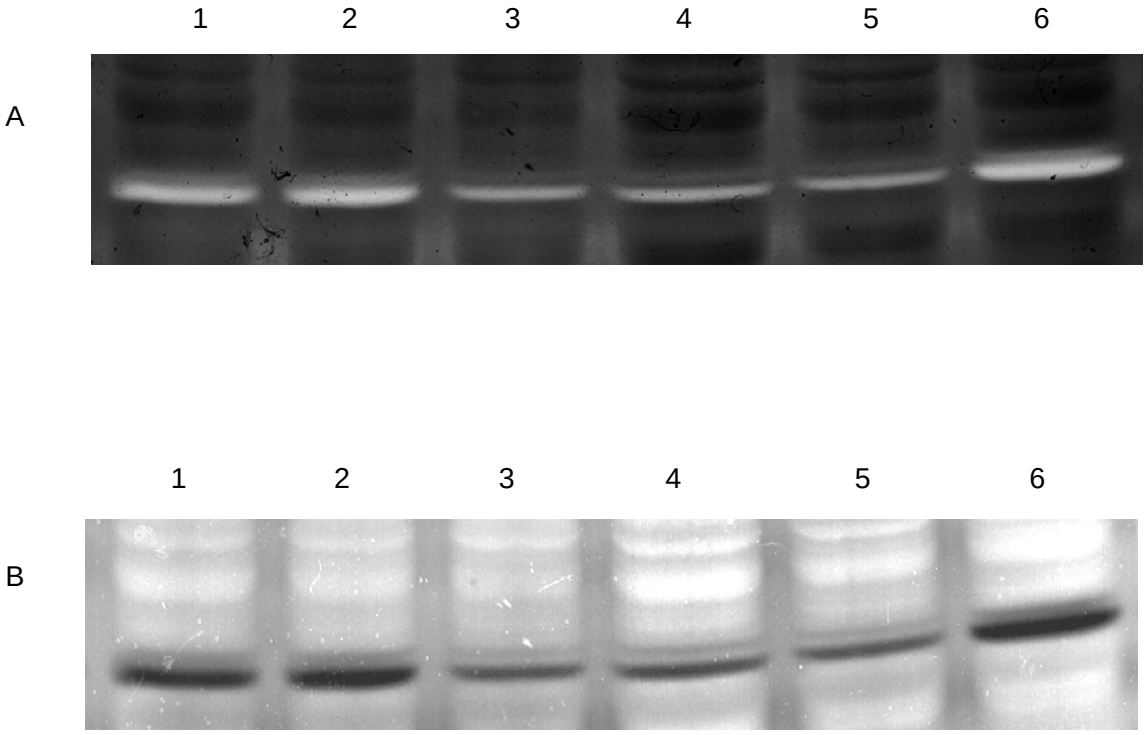


Figure 5.6. A representative example of a normal (A) and inverted (B) image of a zymogram illustrating banding patterns obtained for matrix metalloproteinase (MMP) 2 activity. Lanes 1-6 represent samples.

a standard extract MMP sample using digital densitometry with LabWorks Software Version 4.5 (UVP, Upland, USA).

Myocardial collagen. Samples of LV tissue were weighed and stored at -70℃ prior to tissue analysis. Myocardial hydroxyproline concentration ([HPRO]) was determined after acid (HCl) hydrolysis (Norton et al 1997, Tsotetsi et al 2001, Woodiwiss et al 2001, Norton et al 2002, Badenhorst et al 2003b). Myocardial collagen was also extracted and digested with cyanogen bromide (CNBr) (Norton et al 1997; Tsotetsi et al 2001; Woodiwiss et al 2001; Badenhorst et al 2003b). A portion of the CNBr digested collagen sample was subjected to acid hydrolysis and [HPRO] determination. The amounts of non-cross-linked (soluble) and cross-linked (insoluble) collagen in the myocardium were ascertained based on the solubility of myocardial collagen to CNBr digestion (Norton et al 1997, Tsotetsi et al 2001, Woodiwiss et al 2001, Badenhorst et al 2003b).

Blood analysis To determine the impact of the model of obesity on percentage glycosylated haemoglobin (HbA1c), blood samples were obtained from the thoracic cavity immediately after extirpation of the heart. HbA1c measurements were determined using HBA1C II Tina-quant kit (Cobas – Roche Diagnostics).

Data analysis. Regression analysis was used to determine the lines of best fit for the cardiac function relations. All data are presented as mean±SEM. Comparisons between groups were made with a two way ANOVA.

5.3 Results

Characteristics of the obesity model. Table 5.1 shows the effect of the experimental diet on body weights, visceral fat weight, systolic BP, and percentage HbA1c in SHR and WKY rats. At the initiation of the study, WKY rats were markedly heavier than SHR, but otherwise body weights were similar between groups of animals assigned to dietary groups (SHR assigned to control diet=360±17.9 g, SHR assigned to experimental

Table 5.1. Effect of an obesity-inducing diet (Experimental [Exp] diet) on morphological, blood and haemodynamic characteristics in spontaneously hypertensive (SHR) and Wistar Kyoto control (WKY) rats.

	WKY		SHR	
	Control (n=10)	Exp diet (n=9)	Control (n=10)	Exp diet (n= 10)
Final body weight (BW)(g)	484±9	527±8**	362±5†	400±6**†
Tibial length (cm)	4.61±0.12	4.39±0.12	4.68±0.17	4.37±0.12
Body weight/tibial length (/10)	10.6±0.3	12.2±0.5**	7.6±0.3†	8.7±0.2**†
Visceral fat (g)	19.4±1.5	29.4±1.3**	9.6±0.7†	17.2±0.7**†
Systolic BP (mm Hg)	124±4	122±4	184±5†	186±3†
Glycated haemoglobin (%)	4.61±0.22	4.42±0.06	4.78±0.08	4.66±0.09
Heart weight (g)	1.41±0.15	1.52±0.07	1.52±0.07	1.70±0.23#
LV weight (g)	1.17±0.15	1.23±0.05	1.22±0.08	1.34±0.14#
Heart weight/BW (X100)	2.91±0.004	2.88±0.005	4.24±0.004†	4.52±0.002†
LV weight/BW (X100)	2.40±0.003	2.33±0.004	3.41±0.004†	3.55±0.002†
LV weight/tibial length (x10)	2.53±0.10	2.82±0.11	2.63±0.008	3.08±0.15†*

BP, blood pressure; LV, left ventricle. * $p<0.05$ ** $p<0.005$ vs SHR Control diet group or WKY Control diet group, † $p<0.001$ vs respective WKY group, # $p<0.05$ vs WKY Control group.

diet=361±19.0 g, WKY assigned to control diet=427±21.5 g, WKY assigned to experimental diet=414±19.1 g). The diet produced a modest increase in body weight in both SHR and WKY groups (Table 5.1), but almost doubled visceral fat content (Table 5.1). At the initiation of the study, systolic BP values were higher in SHR as compared to WKY rats. However, systolic BP values were otherwise similar between groups of animals assigned to dietary groups (SHR assigned to control diet=179±21.6 mm Hg, SHR assigned to experimental diet=177±20.5 mm Hg, WKY assigned to control diet=14±14.2 mm Hg, WKY assigned to experimental diet=111±11.7 mm Hg). The experimetal diet failed to modify systolic BP in either group either during the course of the study (data not shown) or at the termination of the study (Table 5.1). Furthermore, blood glucose control, as indexed by HbA1c measurements, was unchanged in either SHR or WKY rats receiving the experimental diet (Table 5.1).

Cardiac weight. Table 5.1 also shows the effect of the experimental diet on heart and LV weights in SHR and WKY rats. Despite SHR having increased systolic BP values, as SHR were considerably smaller than WKY rats, they had similar absolute heart and LV weights as compared to WKY rats. However, when normalised for body weight differences, SHR had marked increases in heart and LV weights as compared to WKY rats. The experimental diet increased LV weight in SHR, with this effect achieving statistical significance when LV weights were normalised for growth effects as determined from tibial length measurements ($p<0.05$ for the interaction between the presence of hypertension and the diet). However, the diet failed to significantly increase either heart or LV weights in WKY rats even after normalising LV weight for tibial length.

Left ventricular pump function. Figure 5.7 shows the effect of the experimental diet on LV systolic chamber function in SHR and WKY rats. In keeping with concentric LV remodelling (see data on LV remodelling below), SHR receiving the control diet had a greater FSend (Figure 5.7 left lower panel) and a left shift in the LV systolic pressure-

volume relationship (Figure 5.7-upper panel) as compared to WKY rats receiving the control diet. However, pump function, as assessed using the load and heart rate independent index of function, LV Ees (Figure 5.7-right lower panel), was unchanged in SHR as compared to WKY rats receiving a control diet. The experimental diet given to SHR resulted in a decrease in LV systolic chamber function as indicated by a decline in both FSend, and a right shift in the LV systolic pressure-volume relationship (upper panel of Figure 5.7), a change attributed to a decrease in the slope of this relationship (LV Ees). However, the experimental diet failed to modify LV systolic chamber function in WKY rats. Indeed, neither FSend nor the LV systolic pressure-volume relationship or its slope (LV Ees) was altered by feeding the experimental diet to WKY rats.

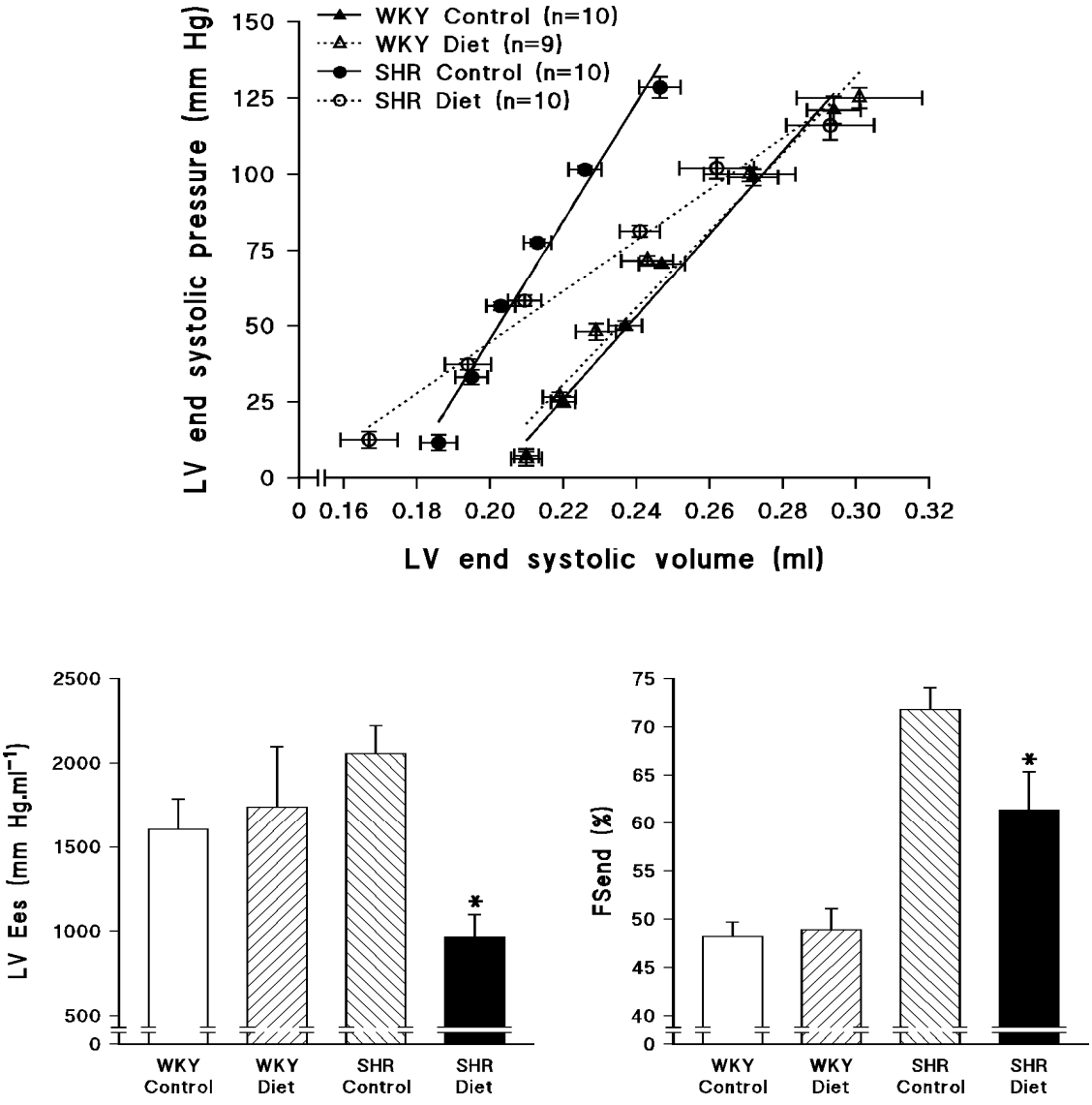


Figure 5.7. Effect of an obesity-inducing diet (Experimental [Exp] diet) on left ventricular (LV) pump function in spontaneously hypertensive (SHR) and Wistar Kyoto control (WKY) rats as determined *ex vivo* from LV systolic pressure-volume relations (upper panel) and the slope of these relations (LV Ees) (lower left panel) and *in vivo* from LV endocardial fractional shortening (FSend) measurements (lower right panel). * $p<0.05$ for an interaction between the presence of hypertension (SHR vs WKY) and diet category.

Left ventricular instrinsic myocardial systolic function. Figure 5.8 shows the effect of the experimental diet on intrinsic myocardial systolic function in SHR and WKY rats as indexed by LV systolic stress-strain relations and their load, heart rate and LV geometry-independent slope (LV En). SHR and WKY rats receiving the control diet had a similar intrinsic myocardial systolic function (systolic stress-strain relationships and LV En were comparable). The experimental diet given to SHR produced a striking decrease in intrinsic myocardial systolic function as indicated by right shift in the LV systolic stress-strain relation, an effect that was attributed to a decrease in the slope of this relationship (LV En). However, the experimental diet failed to modify intrinsic myocardial systolic function in WKY rats.

Left ventricular chamber remodelling. Figure 5.9 shows the effect of the obesity-inducing diet on LV diastolic pressure-volume relations assessed *ex vivo*, the volume intercept at a diastolic pressure of 0 mm Hg (LV V_0) of the LV diastolic pressure-volume relationship, and LV chamber dimensions assessed *in vivo* using echocardiography in SHR and WKY rats. Consistent with either concentric LV remodelling in SHR or with a smaller body size, despite having similar LV weights as WKY rats (Table 5.1), SHR had a left shift in the LV diastolic pressure-volume relationship, and a decrease in LV V_0, and LV EDD. Moreover, also consistent with concentric LV remodelling in SHR, despite having similar LV weights as age-matched WKY rats, SHR had increased LV end diastolic posterior and relative wall thickness values. The experimental diet given to SHR resulted in a right shift in the LV diastolic pressure-volume relationship, an increase in LV V_0 and LV EDD and a decrease in LV posterior and relative wall thickness. In contrast, the experimental diet given to WKY rats had no effect on the LV diastolic pressure-volume relationship, LV V_0, LV EDD, or LV posterior and relative wall thickness.

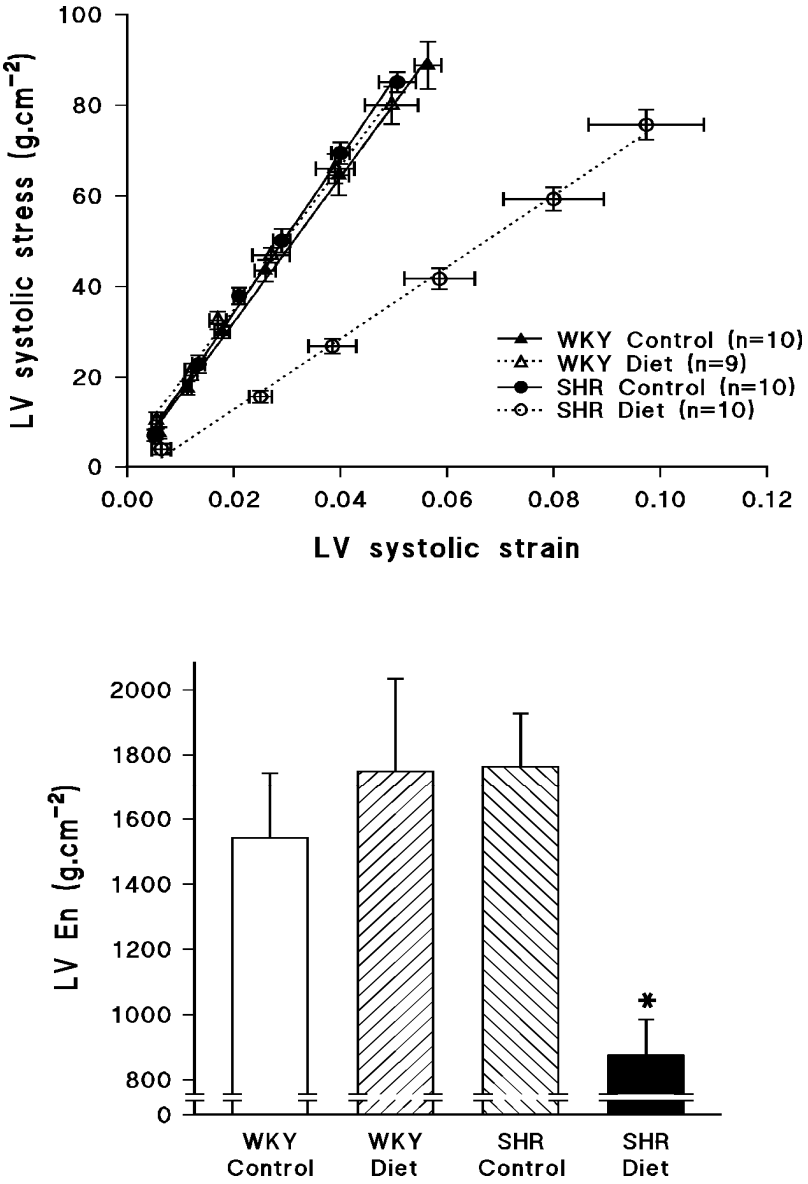


Figure 5.8. Effect of an obesity-inducing diet (Experimental [Exp] diet) on left ventricular (LV) intrinsic myocardial systolic function in spontaneously hypertensive (SHR) and Wistar Kyoto control (WKY) rats as determined *ex vivo* from LV systolic stress-strain relations (upper panel) and the slope of these relations (LV En) (lower panel). * $p<0.005$ for an interaction between the presence of hypertension (SHR vs WKY) and diet category.

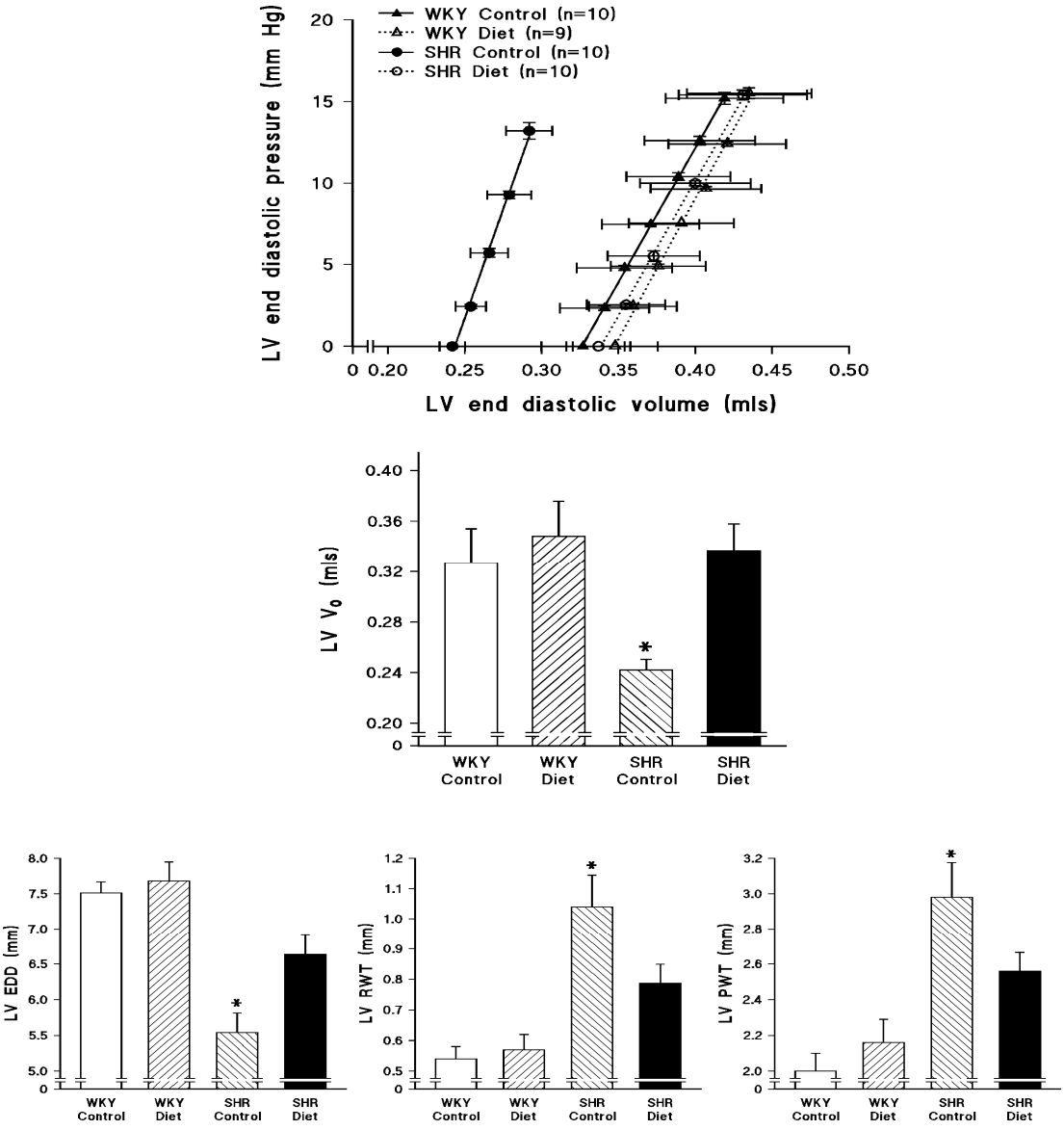


Figure 5.9. Effect of an obesity-inducing diet (Experimental [Exp] diet) on left ventricular (LV) diastolic pressure-volume relations (upper panel), the volume intercept of these relations (lower panel) and LV dimensions determined using echocardiography (lower panels) in spontaneously hypertensive (SHR) and Wistar Kyoto control (WKY) rats. * $p<0.005$ for an interaction between the presence of hypertension (SHR vs WKY) and diet category. EDD, end diastolic diameter; LV PWT, LV end diastolic posterior wall thickness; LV RWT, LV end diastolic relative wall thickness.

To assess whether the degree of adverse LV remodelling in SHR exceeded that which would be expected to occur with increases in body weight, LV diastolic pressure-volume relations were also constructed after normalizing all LV volumes to 100 g body weight (Figure 5.10). After adjustments of LV volumes for body weight, SHR receiving the control diet had similar LV diastolic pressure-volume relations and LV V_0 values as WKY rats (Figure 5.10). The experimental diet given to SHR resulted in a right shift in the LV diastolic pressure-volume relationship, and an increase in LV V_0. In contrast, the experimental diet given to WKY rats had no effect on the LV diastolic pressure-volume relationships or LV V_0 (Figure 5.10).

Myocardial apoptosis, necrosis and interstitial changes. Figure 5.11 shows the effect of the experimental diet on cardiomyocyte apoptosis and matrix metalloproteinase 2 activity in SHR and WKY rats. Table 5.2 shows the effect of the experimental diet on cardiomyocyte necrosis (pathological score), myocardial hydroxyproline concentrations and the degree of myocardial collagen cross-linking in SHR and WKY rats. SHRs receiving a control diet had a similar percentage cardiomyocyte apoptotic nuclei as WKY rats receiving a control diet. Administration of the experimental diet to SHR however produced an increase in cardiomyocyte apoptosis as compared to SHR receiving the control diet (Figure 5.11). In contrast, the experimental diet failed to modify the degree of cardiomyocyte apoptosis in WKY rats (Figure 5.11). In contrast to effects on the index of cardiomyocyte apoptosis, the experimental diet failed to influence the myocardial pathological score in either SHR or WKY rats (Table 5.2).

SHRs receiving a control diet had a similar myocardial MMP-2 activity as WKY rats receiving a control diet. Administration of the experimental diet to SHR however also produced an increase in MMP-2 activity as compared to SHR receiving the control diet (Figure 5.11). In contrast, the experimental diet failed to modify myocardial MMP-2 activity in WKY rats (Figure 5.11).

SHRs receiving a control diet had a marked increase in myocardial collagen concentrations, as indexed by HPRO measurements, as compared to WKY rats receiving a control diet (Table 5.2). The increase in myocardial collagen concentrations in SHR as compared to WKY rats receiving control diets was of the cross-linked form as indicated by an increased insoluble HPRO concentration (Table 5.2). The preferential accumulation of cross-linked myocardial collagen in SHR was attributed to an increase in myocardial collagen cross-linking as detected by a marked decrease in myocardial collagen solubility to digestion by cyanogen bromide (Table 5.2). As a consequence of a decrease in myocardial collagen solubility in SHR, the non-cross-linked form of HPRO was unchanged in SHR as compared to WKY rats receiving the control diet (Table 5.2). Administration of the experimental diet to either SHR or WKY rats failed to modify HPRO concentrations or to alter the degree of myocardial collagen cross-linking (Table 5.2).

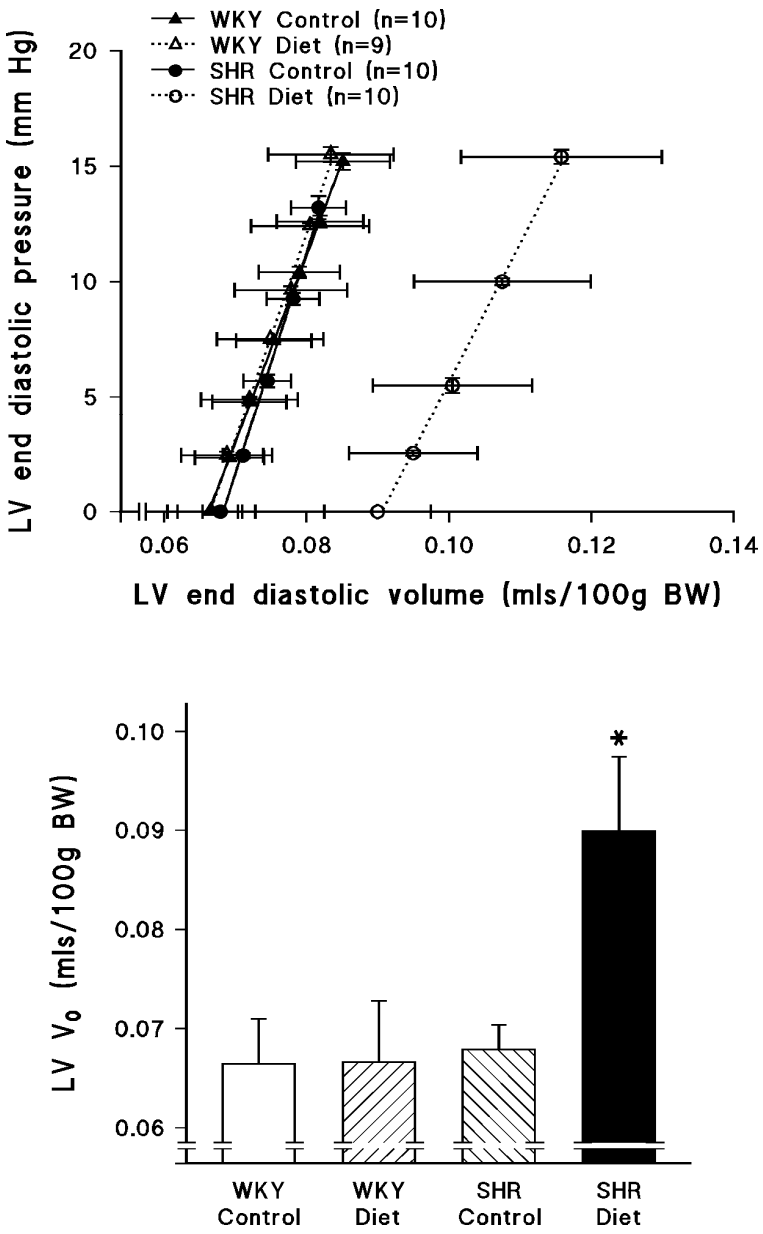


Figure 5.10. Effect of an obesity-inducing diet (Experimental [Exp] diet) on left ventricular (LV) diastolic pressure-volume relations (upper panel), and the volume intercept of these relations (lower panel) after normalising LV volumes to 100 g body weight, in spontaneously hypertensive (SHR) and Wistar Kyoto control (WKY) rats. * $p<0.005$ for an interaction between the presence of hypertension (SHR vs WKY) and diet category.

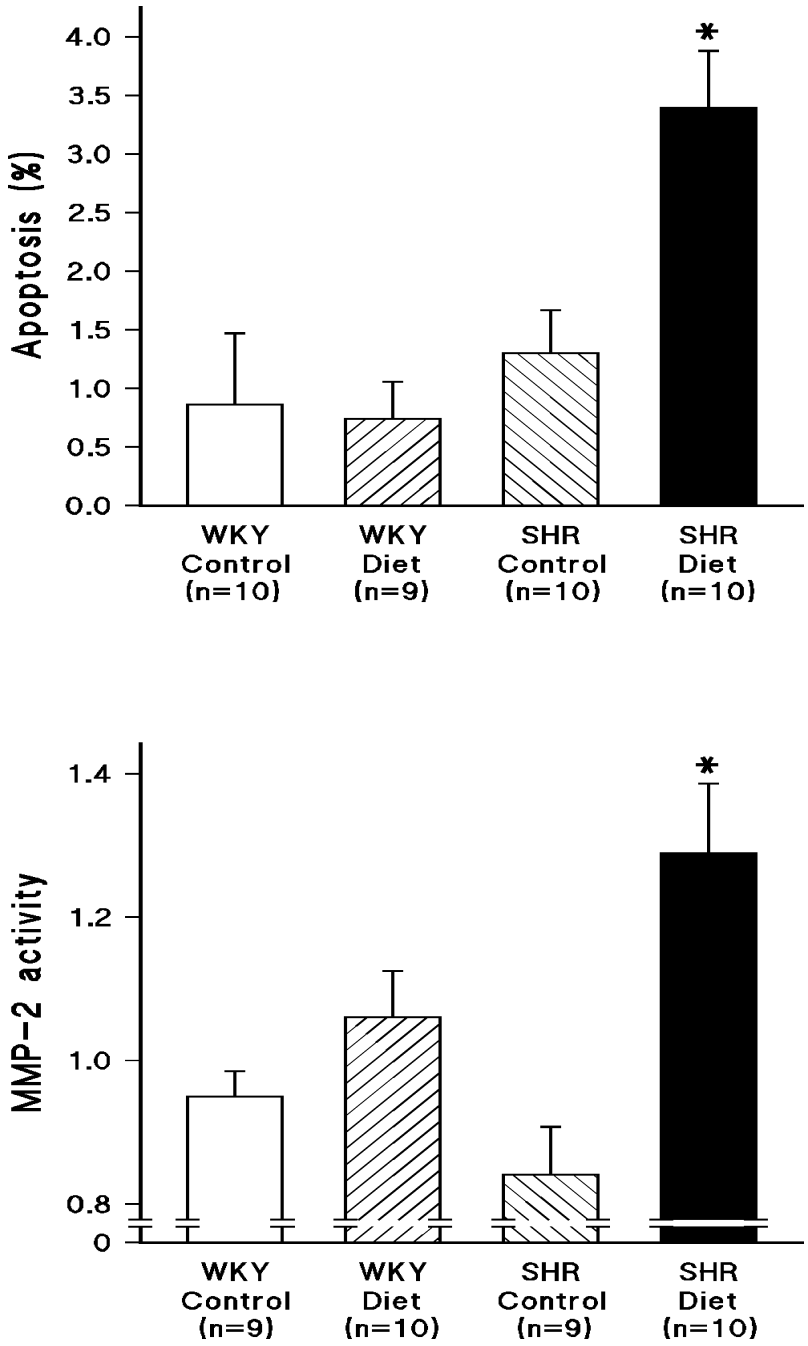


Figure 5.11. Effect of an obesity-inducing diet (Experimental [Exp] diet) on left ventricular cardiomyocyte apoptosis (TUNEL) (upper panel) and myocardial matrix metalloproteinase 2 activity (lower panel) in spontaneously hypertensive (SHR) and Wistar Kyoto control (WKY) rats. * $p<0.005$ for an interaction between the presence of hypertension (SHR vs WKY) and diet category.

Table 5.2. Effect of an obesity-inducing diet (Experimental [Exp] diet) on left ventricular (LV) necrosis (pathological score) and interstitial characteristics in spontaneously hypertensive (SHR) and Wistar Kyoto control (WKY) rats.

	WKY		SHR	
	Control (n=10)	Exp diet (n=9)	Control (n=10)	Exp diet (n=10)
Pathological score	0.88±0.26	0.50±0.18	0.94±0.18	0.88±0.19
[HPRO] (µg.mg^{-1} dry LV)	3.33±0.21	3.16±0.31	8.04±0.54†	9.22±0.43†
% CNBr solubility	28.6±4.33	35.4±4.8	12.3±1.5†	11.0±1.2†
Soluble HPRO (µg.mg^{-1} dry LV)	0.95±0.12	1.08±0.11	0.96±0.12	0.98±0.09
Insolube HPRO(µg.mg^{-1} dry LV)	2.37±0.20	2.07±0.28	7.16±0.56†	8.23±0.43†

[HPRO], myocardial hydroxyproline concentration; % CNBr solubility, solubility of myocardial collagen to cyanogens bromide digestion. † $p<0.001$ vs respective WKY groups.

5.4 Discussion

The main findings of the present study are as follows: Predominantly visceral obesity induced by feeding a diet designed to produce an increased caloric intake, was associated with the premature progression from compensated LVH to LV pump dysfunction in SHR, but not in WKY rats. The dietary-induced change in pump function in SHR occurred in the absence of further peripheral systolic BP changes or changes in blood glucose control (HbA1c). Dietary-induced pump dysfunction in SHR was noted both *in vivo* (endocardial fractional shortening) and *ex vivo* using a load-independent assessment of chamber function (end systolic chamber elastance). Further, dietary-induced pump dysfunction in SHR was associated with excessive LVH, a reduced intrinsic myocardial contractile function (end systolic myocardial elastance) and marked LV dilatation (increased chamber diameters and a right shift in the LV diastolic pressure-volume relationship), changes that were associated with increases in cardiomyocyte apoptosis and myocardial MMP-2 activation in SHR, but not in WKY rats. However, dietary-induced pump dysfunction in SHR was not associated with excessive myocardial necrosis, changes in myocardial collagen concentrations, or alterations in the quality of myocardial collagen.

The present study provides insight into the potential mechanisms that may explain the relationship between obesity and the development of heart failure even after adjustments for the presence of diabetes mellitus (Bahrami et al 2008, Ingelsson et a 2005, Kenchaiah et al 2002, Kenchaiah et al 2004, Poirier et al 2006, Wong and Marwick 2007, Contaldo et al 2002). The present study is the first prospective study to show that dietary-induced obesity, in the absence of hyperglycaemia, can promote myocardial contractile disturbances which translate into pump dysfunction. Although sensitive myocardial tissue Doppler techniques conducted in case-control studies indicate that obesity is associated with myocardial systolic abnormalities independent of blood glucose

control (Peterson et al 2004, Wong et al 2004), whether this is a cause-effect relationship is uncertain as weight loss produced either by lifestyle modification or gastric bypass does not influence myocardial systolic function of the LV (Skilton et al 2007, Wong et al 2006). Moreover, whether obesity-associated systolic myocardial abnormalities translate into pump dysfunction independent of conventional cardiovascular risk factors is controversial (Peterson et al 2004, Wong et al 2004, Pascual et al 2003, Scaglione et al 1992, de Divitiis et al 1981, Chakko 1998, Zarich et al 1991, Stoddard et al 1992, de Simone et al 1996, Mureddu et al 1996, Iacobellis et al 2002, Karason et al 1998, Carroll et al 2006, du Toit et al 2008). Indeed, although animal models of obesity without diabetes mellitus are associated with a reduced cardiomyocyte contractile function (Carroll et al 1997, Relling et al 2006, Dong et al 2006, Ren et al 2000), these animals have a preserved pump function (Carroll et al 2006, du Toit et al 2008). In contrast to previous studies, the present study clearly shows that obesity can promote both myocardial contractile disturbances and pump dysfunction even in the absence of hyperglycaemia, but that for this effect to occur over a relatively short period, the presence of hypertension is required. Whether longer periods of dietary-induced obesity induce similar changes in contractile and pump function in the absence of hypertension still requires elucidation.

Although obesity-induced pump dysfunction in SHR in the present study cannot be attributed to further increments in BP as measured in the periphery by tail-cuff techniques I cannot exclude the possibility that increases in central BP may have occurred. Indeed, excess adiposity is an independent predictor of increases in arterial stiffness (Majane et al 2008) and changes in arterial stiffness which influence central BP through early reflective waves are not closely reflected by peripheral BP values (Karamanoglu et al 1993, Williams et al 2006).

As an increased LVM is a risk factor for the development of pump dysfunction (Drazner et al 2004) and cardiomyocyte size is associated with the progression to pump dysfunction in hypertensive rats (Gerdes et al 1996), one potential mechanism responsible for the obesity-induced effect on pump dysfunction in SHR noted in the present study is an interactive effect between obesity and BP to promote increases in LVM. Indeed, in agreement with previous studies (see preceding chapter, Avelar et al 2007), in the present study an interaction between hypertension and obesity was noted to contribute toward increases in LVM. In this regard LVM normalised to tibial length (which accounts for growth but not excess adiposity) was augmented in SHR receiving the obesity-inducing diet, but not in WKY rats receiving the obesity-inducing diet or in SHR on a normal diet.

In agreement with previous studies conducted in euglycemic models of obesity in rodents (Barouch et al 2006, Zhou et al 2000), dietary-induced increases in cardiomyocyte apoptosis was noted in SHR in the present study. In this regard, the excessive cardiomyocyte apoptosis closely tracked intrinsic myocardial and pump dysfunction in that it only occurred in the SHR group receiving the obesity-inducing diet. Hence, dietary-induced pump dysfunction may be attributed in-part to excessive cardiomyocyte apoptosis, a change that is now considered to be an important pathophysiological process in the development of heart failure (Foo et al 2005, Wencker et al 2003). As LVH is associated with cardiomyocyte apoptosis (Wang et al 1998), the mechanism of the cardiomyocyte apoptosis in obese SHR may occur as a consequence of an augmented LVH. Alternatively, excessive cardiomyocyte apoptosis in obesity may relate to changes in myocardial substrate metabolism (Barouch et al 2006) with a resultant ectopic lipid overload in cardiomyocytes (lipoapoptosis) (Zhou et al 2000) or through increases in circulating aldosterone concentrations that occur in obese SHR (Nagase et al 2006), as aldosterone may induce apoptotic changes in the myocardium (de Angelis et al 2002).

Although not assessed, dietary-induced decreases in intrinsic myocardial systolic function noted in SHR in the present study could occur, in-part, as a consequence of reductions in cardiomyocyte contractile function as opposed to cell death (apoptosis and necrosis). As the dietary-induced decline in intrinsic myocardial systolic function was noted in isolated, perfused heart preparations in the absence of circulating factors, an attenuation in contractile responsiveness to myocardial adrenergic stimuli (Carroll et al 1997) or insulin and insulin-like growth factor-1 (Ren et al 2000) is unlikely to explain this change. Nevertheless, a reduced cardiomyocyte contractile function in the absence of circulating stimuli has been noted in dietary-induced obese animals (Relling et al 2006), and in leptin deficient animals (Dong et al 2006) without diabetes mellitus and these changes may occur as a consequence of a reduced myocardial contractile efficiency and an altered myocardial substrate metabolism (Buchanan et al 2005).

In the present study an interaction between obesity and hypertension promoted LV dilatation. Importantly, the degree of dietary-induced LV dilatation in SHR far exceeded that which could be explained by increases in body size, as marked right shifts in LV diastolic pressure-volume relations were noted in SHR fed the experimental diet even after normalising LV volumes to 100 g body weight. Whether obesity-induced cardiac dilatation noted in SHR in the present study is an indirect consequence of decreases in intrinsic myocardial contractile function or through direct effects of the diet could not be determined. However, irrespective of the cause, as previously demonstrated (Norton et al 2002), cardiac dilatation is likely to contribute toward progressive pump dysfunction in pressure overload states. Importantly, with regard to acknowledged mechanisms of cardiac dilatation, the cellular changes responsible for cardiac dilatation in the present study, appear to occur through the well recognised actions of activated collagenases (MMP-2)(Spinale 2002) as opposed to changes in myocardial collagen concentrations (Badenhorst et al 2003b), or reductions in myocardial collagen cross-linking (Woodiwiss et

al 2001, Tsotetsi et al 2001, Badenhorst et al 2003a and 2003b). As cardiac dilatation in pressure-overload LVH is associated with alterations in collagenases (Polyakova et al 2004) as well as increases in myocardial collagen concentrations and reductions in myocardial collagen cross-linking (Tsotetsi et al 2001, Badenhorst et al 2003b), it is possible that the cellular changes associated with cardiac dilatation in the present study indicate a unique effect of the obesity-inducing diet together with hypertension, rather than primarily a hypertensive effect.

From a clinical perspective the present study suggests that an obesity-inducing diet promotes the development of pump dysfunction, but that this effect is dependent on a simultaneous pressure-overload on the heart. Further studies are therefore required to determine whether careful BP control in obese individuals is sufficient to either prevent or reverse pump dysfunction in obesity.

In conclusion, the present study indicates that in the absence of a further increase in peripheral BP or the presence of hyperglycaemia, an obesity-inducing diet can promote the premature progression from compensated cardiac hypertrophy to pump dysfunction. This effect occurs through both decreases in intrinsic myocardial systolic function and through cardiac dilatation, changes associated with excessive LVH, cardiomyocyte apoptosis and myocardial MMP-2 activation, but not through cardiomyocyte necrosis, nor alterations in the quantitative or qualitative characteristics of the myocardial interstitium. Further studies are required to determine whether BP control alone or alternative interventions are required to prevent or reverse pump dysfunction induced by obesity.

CHAPTER 6

Summary and Conclusions.

Epidemiological trends suggest that obesity is becoming a major public health problem in both developed and developing countries ((Ogden et al 2007, Flegal et al 2002, Bourne et al 2002). Obesity carries a considerable health risk (Pi-Sunyer 1993, Bellanger and Bray 2005, McGee 2005, Giusti 2007, Allison et al 1997, Formiguera and Cantón 2004, Heitmann et al 2000, Lahmann et al 2002) and a large component of the morbidity and mortality associated with obesity is through an increased risk for cardiovascular events (Eckel et al 2002, Klein et al 2004a, Bahrami et al 2008, Ingelsson et al 2005, Kenchaiah et al 2002, Kenchaiah et al 2004, Poirier et al 2006, Wong and Marwick 2007, Contaldo et al 2002, Yusuf et al 2005, Steyn et al 2005, Kurth et al 2002, Ahluwalia et al 2003, Suk et al 2003).

In the present thesis I provide evidence to clarify some outstanding issues regarding the impact of excess adiposity on a number of cardiovascular changes including BP, target organs and cardiac dysfunction. In this regard, although there is now considerable evidence from previous and recent studies to suggest that obesity is associated with an increased conventional BP, arterial stiffness, an increased LVM and cardiac dysfunction, a number of outstanding issues nevertheless remain. In the present thesis I have provided substantial evidence in favour of the use of waist circumference as opposed to alternative adiposity indexes, as the most appropriate clinical index of adiposity when predicting adiposity effects on both conventional and ambulatory BP. Further, I have provided clarity on the independent role of excess adiposity as a predictor of large artery dysfunction as determined by indexes of arterial stiffness. Moreover, I have provided evidence to suggest that excess adiposity promotes pathological LVH in-part by enhancing the impact of BP on LV growth. Last, I have provided the first prospective data to suggest that dietary-induced excess adiposity may promote cardiac pump dysfunction and the mechanisms thereof. The following section summarises these data in the context

of our current understanding of the role of obesity in cardiovascular disease, highlighting the novel features and implications of these findings.

Although clinical indexes of central fat, such as waist circumference may be more closely associated with conventional BP or account for a greater proportion of the variability of BP than BMI in some studies (Okosun 1998, Williams et al 1987, Peiris et al 1989, Kanai et al 1990, Raison et al 1992, Boyko et al 1995, Lerario et al 1997, Ho et al 2001, Okosun et al 1999, Hayashi et al 2003, Ding et al 2004, Zhu et al 2005), not all studies support this notion. Indeed, in the ARIC study conducted in 15 063 partipants, BMI was as closely associated with conventional BP as was waist circumference (Harris et al 2000). Moreover, there have been outstanding issues regarding the relative impact of indexes of central fat and BMI on ambulatory BP, a measurement of BP that is considered a better index of cardiovascular outcomes (Verdecchia et al 1994, Clement et al 2003, Staessen et al 1999 and Okhubo et al 1998) and target organ effects (Mancia and Parati 2000) than conventional BP values. In this regard, to my knowledge, up until the time of publishing the data described in chapter 2 of the present thesis, studies that have compared the relative impact of indexes of central fat and BMI on ambulatory BP have either recruited small study samples, have been non-random, have focussed on waist-to-hip ratio rather than waist circumference as the index of central fat, and have produced ambiguous results (Guagnano et al 1994, Guagnano et al 1997, Steptoe et al 1999, Lurbe et al 1998 and Feldstein et al 2005). As described in chapter 2 and published in the *Journal of Hypertension* (Majane et al 2007), I was able to show in the first study conducted in a relatively large, randomly selected population sample with a high prevalence of excess adiposity (65%), that waist circumference is the only clinical index of adiposity that was associated with an increased conventional and ambulatory systolic and diastolic BP, independent of other indexes of adiposity. However, I did not observe any

independent relationship between waist-to-hip ratio and ambulatory systolic or diastolic BP.

With respect to the potential explanation for the independent relationship between waist circumference and ambulatory BP beyond alternative adiposity indexes, as discussed in chapter 2, visceral fat mass may be more important in mediating BP changes than peripheral fat (subcutaneous or other fat areas) (Williams et al 1987, Peiris et al 1989, Kanai et al 1990, Raison et al 1992, Boyko et al 1995, Lerario et al 1997, Okosun et al 1999, Ho et al 2001, Hayashi et al 2003, Ding et al 2004). The lack of consistent relationship between waist-to-hip ratio and ambulatory BP may be explained by the poor relationship between visceral fat mass and waist-to-hip ratio (Kvist et al 1988, Seidell et al 1988, Pouliot et al 1994, Wajchenberg et al 2000, Stewart et al 2003). With respect to the importance of the data described in chapter 2, presently there is no consensus as to which clinical index of adiposity should be employed in cardiovascular risk assessment. In this regard, the present data support an important role for the use of waist circumference rather than BMI or waist-to-hip ratio when predicting the BP effects of excess adiposity. However, prior to advocating the use of waist circumference rather than BMI as the preferred clinical index when predicting BP effects, further prospective, longitudinal analysis in sex-specific groups is required to identify the most appropriate clinical index of adiposity to employ when predicting increases in conventional and ambulatory BP over time. This study is presently being conducted by our group under the umbrella of the study described in chapter 2.

Having identified the preferred clinical index of adiposity that most closely reflects adiposity effects on BP, I then focussed on the role of obesity in promoting target organ changes. As indicated in the introductory chapter, the present thesis was largely designed to address outstanding issues with respect to the role of obesity in promoting organ changes through mechanisms that cannot be attributed solely to conventional

cardiovascular risk factors. One target organ change that I studied was large vessel disease as indexed by increases in large artery stiffness. In this regard, increases in the stiffness of the wall of large arteries have consistently been shown to be a risk factor for cardiovascular events independent of conventional cardiovascular risk factors (Benetos et al 1997, Blacher et al 1999a, Blacher et al 1999b, de Simone et al 1999, Laurent et al 2001, London et al 2001, Guerin et al 2001, Boutouyrie et al 2002, Safar et al 2002, Blacher and Safar 2005, Dolan et al 2006, Sutton-Tyrrell et al 2005, Cruickshank et al 2002, Meaume et al 2001). However, despite the consistency in the reports demonstrating relations between indexes of adiposity and arterial stiffness, there is controversy as to whether this relationship is independent of factors such as BP, heart rate and diabetes mellitus. Indeed, although adiposity is associated with pulse wave velocity (PWV), a simple and reliable index of arterial stiffness, some studies have (Sutton-Tyrrell et al 2001, Mackey et al 2002, Wildman et al 2003, Ferreira et al 2004), whilst most others have not (Ferreira et al 2004, Zebekakis et al 2005, Taquet et al 1993, Amar et al 2001, Nakanishi et al 2003, Oren et al 2003, Mitchell et al 2004, Czernichow et al 2005) been able to demonstrate strong relations between adiposity indexes and PWV independent of haemodynamic factors and diabetes mellitus.

To address the controversial issue of whether adiposity is independently associated with large artery stiffness, in chapter 3 and published in the *American Journal of Hypertension* (Majane et al 2008), I hypothesized that age may determine whether an excess adiposity promotes increases in arterial stiffness independent of confounders. This hypothesis was derived from the controversies in data showing convincing independent relations between clinical indexes of adiposity and PWV in elderly populations (Sutton-Tyrell et al 2001, Mackey et al 2002), whilst substantial evidence against a strong independent relationship between adiposity and PWV was noted in samples that are predominantly young-to-middle aged (Ferreira et al 2004, Zebekakis et al 2005, Taquet et

al 1993, Amar et al 2001, Nakanishi et al 2003, Oren et al 2003, Mitchell et al 2004, Czernichow et al 2005). In chapter 3 (Majane et al 2008) I was able to show that age markedly influenced the independent relationship between indexes of central adiposity (waist circumference and waist-to-hip ratio) and PWV in women but not in men after adjusting for confounders including BP, HR, and diabetes mellitus. More specifically, in women older than the median age of the sample (~42 years), waist circumference and waist-to-hip ratio were strongly and independently associated with PWV, whereas in women younger than the median age a trend for a negative relationship was noted. The effect of waist-to-hip ratio and waist circumference on increases in PWV was ~5-fold higher in older than in younger women after adjustments for confounders.

Thus, in chapter 3, I provide the first evidence to show that in a predominantly young-to-middle aged population sample with a high prevalence of excess adiposity, age markedly influences the independent relationship between indexes of central adiposity. These data provide insights into discrepancies in studies assessing relations between adiposity and PWV. From a clinical perspective these data also suggest that although in younger women the impact of adiposity on PWV is likely to be mediated principally through conventional haemodynamic factors such as BP, the effect of adiposity in older persons may be mediated by additional factors not normally included in cardiovascular risk assessment.

With respect to obesity-induced effects on cardiovascular target organ changes, a second target organ change that I studied in the present thesis was that of increases in LVM. In this regard, LVM is a strong independent predictor of cardiovascular events (Casale et al 1986, Devereux et al 2004, Ghali et al 1998, Koren et al 1991, Levy et al 1990, Levy et al 1994, Verdecchia et al 1996). Unlike normal growth effects on LVM which are physiological in nature, obesity-induced LVH may be of pathophysiological significance. One potential mechanism that may explain the potential pathological effects

of obesity-induced increases in LVM is through an enhanced impact of BP on LVM as demonstrated in severe obesity (body mass index>35 kg/m^2) (Avelar et al 2007). However, obesity may not increase the impact of BP on LVM in milder forms of obesity (Fox et al 2004, Lauer et al 1992). Nevertheless, in these studies only participants with a narrow range of BP levels (Lauer et al 1992) or where ~50% of the sample was receiving antihypertensive treatment at the time (Fox et al 2004) were studied. Therefore, in the present thesis I assessed in a population sample with a high prevalence of mild-to-moderate obesity and with a high prevalence of hypertension (~41%), but in whom antihypertensive use was limited (~17%), whether adiposity is associated with an enhanced impact of BP, or one of the determinants of BP, namely arterial stiffness, on LVMI and wall thickness.

As described in chapter 4 of the present thesis I was able to show marked synergistic effects between indexes of adiposity (waist circumference and skin-fold thickness) and haemodynamic variables (conventional or 24-hour systolic BP or indexes of large artery dysfunction [PWV and pulse pressure]) associated with LVM index and LV wall thickness. These data provide the first evidence to indicate that mild-to-moderate forms of excess adiposity not only promote the development of increases in BP as is well recognized (Majane et al 2007, chapter 2 and as summarized in chapter 1), but also enhance the impact of BP on LVM index. In addition, as systolic BP, pulse pressure and PWV are all affected by large artery dysfunction, these data suggest that excess adiposity not only promotes large artery dysfunction (chapter 3), but also enhances the impact of large artery dysfunction on LV growth via systolic BP effects. These data lend further support to the recent finding from our group demonstrating a strong BP-independent relationship between PWV and LVM and wall thickness in women (Libhaber et al 2008). As discussed in chapter 4, the synergy between adiposity and large artery dysfunction or BP to promote LV growth clearly represents a pathological afterload effect on the LV. This

finding may therefore provide insight into why adiposity-induced increases in LVM are associated with deleterious cardiovascular outcomes independent of conventional cardiovascular risk factors (de Simone et al 2005), in direct contrast to normal growth-induced increases in LVM that are considered to be entirely benign.

Although obesity is risk factor for heart failure independent of other conventional cardiovascular risk factors (Bahrami et al 2008, Ingelsson et al 2005, Kenchaiah et al 2002, Kenchaiah et al 2004, Poirier et al 2006, Wong and Marwick 2007, Contaldo et al 2002), the mechanisms of this relationship are uncertain. As adjustments for baseline cardiac systolic chamber function abolish the independent relationship between obesity and heart failure (Bahrami et al 2008), it is possible that heart failure in obesity is mediated by pump dysfunction. Nevertheless, whether obesity is associated with cardiac pump (chamber) dysfunction independent of conventional cardiovascular risk factors is controversial (Peterson et al 2004, Wong et al 2004, Pascual et al 2003, Scaglione et al 1992, de Divitiis et al 1981, Chakko 1998, Zarich et al 1991, Stoddard et al 1992, de Simone et al 1996, Mureddu et al 1996, Iacobellis et al 2002, Karason et al 1998, Carroll et al 2006, du Toit et al 2008). Moreover, although sensitive myocardial tissue Doppler techniques indicate that obesity is associated with myocardial systolic abnormalities independent of BP, age, gender, blood glucose control and LVM (Peterson et al 2004, Wong et al 2004); weight loss produced either by lifestyle modification or gastric bypass does not influence myocardial systolic function of the LV (Skilton et al 2007, Wong et al 2006). Furthermore, although animal models of obesity without diabetes mellitus are associated with a reduction in isolated cardiomyocyte contractile function (Carroll et al 1997, Relling et al 2006, Dong et al 2006, Ren et al 2000), euglycaemic animal models of obesity have a preserved pump function (Carroll et al 2006, du Toit et al 2008). As a reduced cardiac pump function is a well recognized feature of the development of heart

failure (Hunt et al 2005), it is therefore clear that further clarity on the role of obesity in the development of pump dysfunction independent of conventional risk factors is required.

In studies demonstrating a relationship between obesity and myocardial systolic dysfunction, BP is greater in obese as compared to lean participants (Skilton et al 2007, Peterson et al 2004, Wong et al 2004). It is therefore possible that obesity may promote the development of myocardial systolic and hence pump dysfunction through an interaction with BP effects. As obesity may enhance the impact of BP on LVM (see chapter 4, Avelar et al 2007) and an increased LVM is a risk factor for the development of pump dysfunction (Drazner et al 2004), in the present thesis I therefore hypothesized that one potential mechanism that may explain the relationship between obesity and the development of heart failure (Kenchaian et al 2002) is that obesity may enhance the detrimental effects of BP on the heart. To test this hypothesis I assessed whether dietary-induced obesity promotes the development of left ventricular systolic chamber and myocardial dysfunction in spontaneously hypertensive rats (SHR) as compared to normotensive Wistar Kyoto control (WKY) rats and the mechanisms thereof. I chose to perform this study in an animal model of hypertension as a similar prospective study conducted in humans would be both unethical and unfeasible within the constraints of current resources and a PhD thesis.

In chapter 5 of the present thesis I describe data to show that predominantly visceral obesity induced by feeding a diet designed to produce an increased caloric intake, was associated with the premature progression from compensated LVH to LV pump dysfunction in SHR, but not in WKY rats. The dietary-induced change in pump function in SHR occurred in the absence of further peripheral systolic BP changes or changes in blood glucose control (as determined from glycated haemoglobin measurements). Dietary-induced pump dysfunction in SHR was noted both *in vivo* (endocardial fractional shortening) and *ex vivo* using a load-independent assessment of chamber function (end

systolic chamber elastance). Further, dietary-induced pump dysfunction in SHR was associated with excessive LVH (LV weight was normalized for growth effects by indexing LV weight to tibial length), a reduced intrinsic myocardial contractile function (end systolic myocardial elastance) and marked LV dilatation (increased chamber diameters and a right shift in the LV diastolic pressure-volume relationship even after normalising LV volumes for the greater body sizes in the obese rats), changes that were associated with increases in cardiomyocyte apoptosis and myocardial matrix metalloproteinase-2 activation in SHR, but not WKY rats. However, dietary-induced pump dysfunction in SHR was not associated with excessive myocardial necrosis, changes in myocardial collagen concentrations, or alterations in the quality of myocardial collagen.

Importantly, the data described in chapter 5 of the present thesis are the first prospective data to show that dietary-induced obesity, in the absence of hyperglycaemia or further peripheral BP changes, can promote myocardial contractile disturbances which translate into pump dysfunction. Moreover, the data described in chapter 5 suggest potential mechanisms of the interactive effects of dietary-induced obesity and hypertension to promote pump dysfunction. The study described in chapter 5 clearly shows that obesity can promote both myocardial contractile disturbances and pump dysfunction even in the absence of hyperglycaemia, but that for this effect to occur over a relatively short period, the presence of hypertension is required. Whether longer periods of dietary-induced obesity induce similar changes in contractile and pump function in the absence of hypertension still requires elucidation. Although in the study described in chapter 5 an interaction between obesity and hypertension was noted when assessing the impact of these factors on LV weight, whether this is the mechanism of the deleterious effect of the diet on pump function cannot be determined. Moreover, although the diet failed to promote further increases in peripheral BP beyond what would normally be expected to occur in SHR at this age, whether increases in large artery stiffness and

hence central BP may still contribute to the outcome requires further studies conducted in unanaesthetised, unrestrained rats.

The specific strengths and limitations of each of the studies described in the present thesis have largely been addressed in individual semi-independent chapters. From a general perspective however, the strengths of the population-based studies was the random recruitment procedures conducted in studies with fairly large study samples, and the high quality laboratory and phenotypic assessments. The major limitation of the population-based studies is the lack of prospective or intervention data, data that is presently being obtained under the umbrella of the studies described in the present thesis. With respect to the animal-based study described in the preceding chapter, this study has a number of strengths including the careful assessment of cardiac function using well-published techniques established in our laboratory over many years and the number of potential cellular and interstitial mechanisms explored. The weakness of the animal-based study is the absence of a number of assessments that may have provided insights into the mechanisms of the dietary effects, including ectopic lipid overload in cardiomyocytes, isolated cardiomyocyte function, arterial stiffness, and central BP measurements in awake, unanaesthetised rats. Furthermore, whether obesity promotes pump dysfunction through central hypertensive effects or through excessive LVH could have been assessed by including a group of rats receiving hydralazine therapy, a potent antihypertensive agent with little impact on LV weight in SHR (Norton et al 1997). Moreover, the role of aldosterone in the ability of dietary-induced obesity to promote the progression from compensated cardiac hypertrophy to cardiac decompensation could have been assessed by evaluating the impact of spironolactone on obesity-induced cardiac effects in SHR (Veliotes et al 2005). The latter two studies are obviously studies that are presently being conducted in our laboratory.

In conclusion therefore, data described in the present thesis provide substantial evidence to clarify a number of outstanding issues with respect to the impact of excess adiposity on cardiovascular disease. First, I provide strong evidence to suggest that waist circumference, rather than BMI, skin-fold thickness or waist-to-hip ratio, is the preferred clinical index of adiposity when assessing BP effects. Second, I describe strong cross-sectional data to suggest that the conventional cardiovascular risk factor-independent effect of excess adiposity on large artery dysfunction depends on age in that this effect only occurs in people over the median age for the population sample that I studied (~43 years). Third, in the present thesis I provide evidence to indicate that obesity promotes LVH in-part by potentiating the impact of haemodynamic factors such as systolic BP and large artery stiffness on LVM. These data therefore provide a mechanism to explain the potential pathological effects of obesity-induced LVH. Finally, in a pre-clinical study I provide the first prospective evidence to show that dietary-induced obesity is associated with the progression from compensated cardiac hypertrophy to cardiac dilatation and pump dysfunction and a number of potential mechanisms thereof.

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