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Effects of Resistin on Cardiovascular Structure and Function.

Glenda Norman

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

Johannesburg, South Africa

2019

Abstract

Beyond conventional risk factors, obesity is a well-recognised predictor of several major cardiovascular events. However, the exact mechanisms responsible for this effect are uncertain. A contemporary hypothesis explaining the adverse effects of obesity on the cardiovascular system is that through the production of several adipocytokines, obesity represents a state of low-grade inflammation. Although studies exploring relationships between most adipocytokines and events are inconsistent, more consistent relationships between circulating concentrations of resistin and several major events or their severity have been noted. However, the exact mechanisms responsible for the cardiovascular effects of resistin are uncertain. In the present thesis in a large community-based study of African ancestry, I explored relationships between resistin concentrations and several cardiovascular intermediate phenotypes known to cause events.

In 739 randomly selected participants of African ancestry from a community sample (43.6% obese), I assessed relations between circulating resistin concentrations and left ventricular mass index (LVMI), LVM beyond that predicted by stroke work (inappropriate LVMI [LVM_{inappr}]) and systolic and diastolic LV function (echocardiography). Although resistin concentrations were not independently associated with blood pressure (BP), they were associated with LVMI ($p<0.0005$), LVM_{inappr} ($p<0.0001$) and LV hypertrophy ($p<0.001$) independent of BP, body mass index (BMI), the homeostasis model of insulin resistance (HOMA-IR) and additional confounders including C-reactive protein (CRP). Resistin concentration ($p<0.002$ in all and $p<0.0005$ in untreated) was the only factor independently associated with LV midwall fractional shortening and these relations were enhanced at incremental concentrations of CRP, Thus, resistin in-part explains variations in LVM, hypertrophy and myocardial systolic dysfunction, and these effects are independent of insulin resistance and general inflammatory changes.

In 683 randomly selected participants of African ancestry from SOWETO, South Africa whom had never received antihypertensive therapy I determined independent relations between circulating resistin concentrations and aortic pulse wave velocity (PWV) and wave reflection. Resistin concentrations were not independently associated with office or 24-hour (n=492) BP. In a stepwise regression model with BMI included in the model, age ($p<0.0001$), mean arterial pressure ($p<0.0001$), plasma resistin concentrations ($p<0.005$), female gender ($p=0.01$) and creatinine concentrations ($p<0.01$) contributed independently to variations in PWV. Independent relations between resistin concentrations and PWV persisted with further adjustments for CRP ($p<0.005$), and HOMA-IR ($p<0.02$). Thus, resistin is independently associated with aortic stiffness and these effects occur beyond BP, insulin resistance and general inflammation.

In a cross-sectional community-based study in 1010 randomly selected participants of African ancestry I determined the extent to which obesity-associated metabolic and inflammatory changes are associated with renal damage and chronic kidney disease (CKD) as compared to modifiable conventional risk factors. In multivariate regression models with adjustments for conventional risk factors (including HbA1c, office BP or any adiposity index) and additional confounders, HOMA-IR (standardized β-coefficient [β-coeff]=-0.13±0.02, <0.0001) and plasma resistin concentrations (β-coeff=-0.13±0.02, <0.0001) were second only to age and equivalent to systolic BP (β-coeff=-0.09±0.03, <0.005) in the impact on eGFR, whilst alternative conventional risk factors, including BMI contributed little to eGFR. Similar results were obtained in relations with CKD. CRP was not independently associated with eGFR. Neither 24-hour BP, aortic BP, nor PWV, modified the relative contribution of HOMA-IR and resistin concentrations versus alternative parameters to eGFR. Thus, at a community level the combined impact of obesity-associated metabolic and inflammatory changes on glomerular filtration rate and CKD is greater than that of modifiable conventional risk factors. Targeting conventional risk factors alone is therefore likely to result in a marked residual impact on the development of CKD in communities with a high prevalence obesity.

In 739 randomly selected participants of African ancestry from a community sample I assessed the extent to which relationships between circulating resistin concentrations and LVM are explained by the adverse effects of resistin on aortic stiffness (ventriculo-vascular coupling) and renal function (cardio-renal syndrome). Independent of confounders, LVMI, LVM_{inappr} and LVH were independently associated with PWV and eGFR. However, adjustments for either PWV or eGFR failed to modify relations between resistin concentrations and LVMI, LVM_{inappr} or LVH. Moreover, in product of coefficient mediation analysis, neither eGFR nor PWV explained a significant proportion of the relationship between resistin and LV structural alterations. Thus, relationships between circulating concentrations of resistin and LVM are not explained by the impact of resistin on ventricular-vascular coupling or the cardio-renal syndrome. Resistin's effect on LVMI are therefore likely to be through direct actions on the myocardium.

In conclusion, in the present thesis I show that elevated circulating concentrations of the adipocytokine resistin, but not CRP are associated with LV hypertrophy and systolic dysfunction, aortic stiffness and glomerular dysfunction beyond conventional risk factors including adiposity indices and BP (office, aortic and ambulatory). Importantly, these effects were similarly independent of insulin resistance as indexed by the homeostasis model and relationships between resistin concentrations and eGFR could not be explained by increases in aortic stiffness and between resistin concentrations and LV hypertrophy could not be explained by the cardio-renal syndrome or ventriculo-vascular coupling. Thus, the present thesis suggests that resistin, possibly through direct effects on several organ systems, may contribute to

cardiovascular events through an impact on LV hypertrophy and dysfunction, increases in aortic stiffness and reductions in glomerular filtration.

Declaration

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

……

Glenda Norman …… day of ……., 2019

I certify that all studies contained in this thesis have the approval of the Committee for Research on Humans of the University of the Witwatersrand (clearance numbers: M17-08-84, M17-04-01, M12-04-108, M07-04-69, M02-04-72).

……

Glenda Norman …….. day of ……., 2019

....

Angela J Woodiwiss (supervisor) Gavin R Norton (supervisor)

Date..... Date.....

Dedication**:**

To my wonderful loving husband, Allan
and our two sons, Cameron and Donovan

Anything is possible when you believe
that you can do it

To my father George Kelly
Thank you for encouraging me to continue my studies

To my friend Jennifer Hind
Thank you for your encouragement to continue my studies

In memory of my mother Catherine Kelly (1940-2007)
and my aunt Mina Morris (1923-2017)

Thank you for believing that I could complete this study.

Publications

In support of the present thesis, the work included has resulted in the following publications in international journals.

1. Norman G, Norton GR, Libhaber CD, Michel F, Majane OHI, Millen AME, Sareli P, Woodiwiss AJ. Independent associations between resistin and left ventricular mass and myocardial dysfunction in a community sample with prevalent obesity. *Int J Cardiol* 2015; 196:81-87.
2. Norman G, Norton GR, Gomes M, Michel F, Majane OH, Sareli P, Millen AM, Woodiwiss AJ. Circulating resistin concentrations are independently associated with aortic pulse wave velocity in a community sample. *J Hypertens*. 2016; 34:274-281.

Presentations (Oral and Poster)

In support of the present thesis, the work was presented at the following conferences.

1. Distinct Independent Relationships between Insulin Resistance or Resistin Concentrations and Glomerular Filtration Rate in a Community Sample. Glenda Norman, Angela J Woodiwiss, Vernice Peterson, Monica Gomes, Pinhas Sareli, Gavin R Norton. Stroke and Hypertension Society South Africa, Stellenbosch 2018.
2. Do Increases in Aortic Forward or Backward Wave Pressures Account for Adrenergic-Induced Increases in Pulse Pressure in Rats? Glenda Norman, Angela J Woodiwiss, Grace Tade, Gavin R Norton. Physiology Society South Africa, Pretoria 2017.
3. In the Presence of Modest Independent Effects on Blood Pressure, Obesity Markedly Modifies the Impact of Age on Ambulatory Pulse Pressure in a Community Sample**.** Physiology Society South Africa, Pretoria 2017. Glenda Norman, Gavin R Norton, Monica Gomes, Olebogeng HI Majane, Pinhas Sareli, Angela J Woodiwiss

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Acknowledgements

I wish to thank the University of the Witwatersrand, School of Physiology and Heads of School, Prof David Gray and Prof William Daniels, and staff for affording me the opportunity to complete this degree whilst working in the department. I wish to thank my supervisors, Prof Gavin Norton and Prof Angela Woodiwiss, for their constant encouragement, support and guidance while I was doing these studies. I wish to thank the Cardiovascular Pathophysiology and Genomics Unit for the opportunity to work in this world-recognized unit. I also wish to thank my colleagues and friends, Dr. Vernice Peterson and Mrs. Grace Tade for their help and guidance throughout my studies. To my husband, Allan, and my sons, Cameron and Donovan, I wish to thank you for your patience, encouragement, and understanding while I was completing this degree.

List of Abbreviations

a’	peak velocity during late (atrial) diastole
A wave	trans-mitral blood flow velocity in the late (atrial-A) period
ACR	albumin to creatinine ratio
ß-coeff	standardized ß coefficient
BMI	body mass index
BP	blood pressure
CAD	coronary artery disease
CHD	coronary heart disease
CKD	chronic kidney disease
CRP	C-reactive protein
DBP	diastolic blood pressure
DM	diabetes mellitus
eGFR	estimated glomerular filtration rate
e’	peak velocity during early diastole at the mitral annulus
Ė	early
E/A	ratio of E wave to A wave velocity
E/e’	index of LV filling pressures
EF	ejection fraction
E wave	trans-mitral blood flow velocity in the early period of left ventricular diastolic filling
FSend	endocardial fractional shortening
FSmid	midwall (myocardial) fractional shortening
FSGS	focal segmental glomerular sclerosis
GTF	generalized transfer function
HbA1c	percentage glycated haemoglobin
HDL	high density lipoprotein
HOMA-IR	homeostasis model of insulin resistance
IL-1	interleukin -1
IL-1ß	interleukin -1 beta
IL-2	interleukin -2
IL-6	interleukin -6
IL-8	interleukin -8
IL-10	interleukin -10
IL-12	interleukin -12

IL-15	interleukin -15
IDMS	isotope dilution mass spectrometry
kg	kilogram
kg/m^2	kg per $meter^2$
LDL	low density lipoprotein
LPS	lipopolysaccharide
LV	left ventricular
LV DD	left ventricular diastolic dysfunction
LVH	left ventricular hypertrophy
LVM	left ventricular mass
LVMI	left ventricular mass indexed for $height^{2.7}$
LVM_{inappr}	inappropriate LVM
MAP	mean arterial pressure
MDRD	modification in diet in renal disease
NO	nitric oxide
ORG	obesity related glomerulopathy
PAI-1	plasminogen activator inhibitor-1
PPAR-γ	peroxisome proliferator-activated receptor- γ
Pf	forward wave pressure component
Pb	backward (reflected) pressure wave component
PP	pulse pressure
PWV	pulse wave velocity
RI	reflected wave index
ROS	reactive oxygen species
SOWETO	South Western Township
SBP	systolic blood pressure
SBPc	central aortic SBP
SD	standard deviation
TZD	thiazolidinedione
TDI	tissue doppler imaging
TNF-α	tumour necrosis factor alpha
WC	waist circumference

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Preface

The increasing prevalence of obesity in both developed and developing nations has had a major impact on the age of onset of cardiovascular events. There is substantial evidence that a significant proportion of the adverse effects of obesity on the cardiovascular system are mediated by conventional risk factors, such as age (men >/- 45 years, women >/- 55 years), hypertension (BP >140/90mmHg or on antihypertensive medication), type II diabetes mellitus, cigarette smoking, obesity / lack of exercise, hypercholesterolemia, low HDL cholesterol (<1.03mmol/l), and hypertriglyceridemia (>2.26mmol/l). Importantly, these risk factors are readily targeted by pharmacological agents. However, adjustments for these risk factors have a remarkably limited effect on the ability of obesity to predict events. Thus, alternative factors are thought to play a major role in mediating obesity-associated cardiovascular events.

An obvious solution to prevent obesity-associated cardiovascular events is to ensure the adequate implementation of weight reduction programs and to change lifestyles to prevent the development of obesity. However, despite considerable public health measures and media exposure world-wide on this topic, there is little evidence for a reduction in the high prevalence rates of obesity. Furthermore, once obesity is established weight reduction programs seldom achieve target body weights and are generally not sustainable. Thus, a better understanding of the independent relationships between obesity and cardiovascular disease is required. With this understanding alternative therapeutic approaches may be developed that could prevent cardiovascular events in obese individuals. In this regard, more recently the focus of attention has been on the possibility that through the synthesis and release of adipocytokines, obesity represents a state of low-grade inflammation and that obesity-associated inflammatory changes mediate cardiovascular disease either directly or through insulin resistance.

In the present thesis I focused my attention on the possible role of one of the adipocytokines, resistin, a molecule that has in comparison to alternative adipokines, provided the most consistent evidence to-date as a possible candidate for mediating cardiovascular events beyond all alternative risk factors and obesity *per se*. Much previous work has been published on the cellular and molecular effects of resistin on the cardiovascular system and on the ability of resistin to predict events. Hence, I concentrated my efforts on better understanding the possible effects of resistin on intermediate cardiovascular phenotypes at a community level. In this regard, at the time of initiating the present thesis, previous studies assessing the impact of resistin on intermediate cardiovascular phyenotypes had several limitations. They had been conducted in limited study samples; were confounded by the treatment of conventional risk factors; were insufficiently thorough to determine the exact extent of the contribution of resistin to these intermediate cardiovascular phenotypes, and were thus characterized by conflicting evidence. In the present thesis I provide strong evidence for a role of resistin in mediating

adverse effects on several cardiovascular end organs (sites of cardiovascular damage, previously termed target organs) including the heart, vasculature and kidney.

In chapter 1 of the present thesis I review the current understanding and controversies related to obesity effects on the cardiovascular system, thus arguing in favour of conducting the studies described herein. In this chapter I focus my attention on the possible importance of a role for resistin. In chapters 2 - 5, I describe each of the studies, their results and the implications thereof. These chapters are constructed as semi-independent chapters and each includes its own abstract, introduction, methods, results and discussion sections. The present thesis concludes with a chapter (chapter 6), which provides a summary of the findings reported; highlights the novelty of these findings and places them in context of our current understanding of obesity and resistin effects on the cardiovascular system. In support of this thesis, the data provided in chapter 2 has been published in the *International Journal of Cardiology* (Norman *et al*., 2015); chapter 3 has been published in the *Journal of Hypertension* (Norman *et al.,* 2016); chapter 4 is currently under review in the journal *Cardiovascular Journal of Africa* (Norman *et al.,* 2019) and chapter 5 is currently under review in the journal *Biomarkers* (Norman *et al*., 2019).

CHAPTER 1

Introduction

Cardiovascular Effects of Obesity and its Metabolic Consequences: Possible Role for Resistin

1.0 Introduction

Over the past three decades there has been a steady increase in the prevalence of obesity in both developed and developing countries (Bourne *et al*., 2002; Ogden *et al.*, 2007; Finucane *et al.*, 2011; Flegal *et al.*, 2013). This increase has in some countries, been dramatic. In the USA in 1986, 1 in 200 adults were noted to be morbidly obese. By 2000, approximately 20% of adults were reported to be obese (body mass index [BMI] ≥ 30kg/m²) (Mokdad *et al*., 2003). By 2004, the prevalence of obesity in the USA was reported to have increased to ~31% in men and ~33% in women (Ogden *et al.*, 2007), that is 1 in 50 people were obese. The extent of fat accumulation in obese individuals has been substantial with more recent reports of over 3% of the total population (adults and children) of the USA estimated to be morbidly obese (BMI ≥ 40kg/m² (Bastien *et al.*, 2014; Ng *et al.*, 2014). Currently in the USA, 1 in 5 adults are morbidly obese (BMJ Publishing Group Ltd, 2016). The USA is not the only developed country to experience striking increasers in the prevalence of obesity. Since 2000, obesity has increased from 10% to 40% in European countries, and has more than doubled in England (Agha and Agha, 2017). In the 2008 health survey conducted in England, 65% of men and 25% of women were reported to be obese, that is 1 in 4 adults (Department of Health, 2011). It is presently estimated that in some European countries 8% of 1–2-year-old children (Barker, 1999) and 80% of 10–14-year olds will become obese adults (Perez-Pastor *et al.*, 2009). The obesity epidemic has not been restricted to adulthood, but has been noted in early childhood. Indeed, in England, approximately 750,000 boys and 676,000 girls were reported to be obese in 2003, and in 2010, 792,321 (19%) of boys and 910,630 (22%) of girls were obese. Current evidence suggests that by 2050, in children aged 6–10 years, the number of obese boys will be greater than girls with an estimated 50% of boys becoming obese by 2050 (NHS Digital Statistics Team, 2018).

South Africa is following this worldwide trend of obesity with the largest increase occurring amongst those with a high socioeconomic status, rural dwellers, and young women where there is a decrease in physical activity (Cois and Day, 2015). In South Africa, during the last three decades, in a nationally representative population sample of 13 089 participants, ~29% of men and ~57% of women were estimated to be obese (Puoane *et al.*, 2002; *Bourne et al*., 2002; Mozaffarian *et al.*, 2015). A more recent report suggests that as compared to previous surveys (Puoane *et al.*, 2002; Bourne *et al*., 2002) an increase in the prevalence of obesity has occurred in South Africa, and irrespective of socioeconomic status a greater proportion of women are now obese and most wealthy men are obese (Alaba and Chola, 2014).

Importantly, although weight reduction programs have proved to be successful, they seldom result in obese individuals reaching target body weights and they fail to maintain this decrease in body weight (Anderson *et al.*, 2001; Narbro *et al.*, 2004; Lambert and Kolbe-Alexander, 2013 National Department of Health, 2013; Alaba and Chola, 2014). In this regard,

the perceptions of obesity related diseases amongst obese and overweight persons differs greatly with many failing to acknowledge the health risks of obesity and thus being unresponsive to weight reduction programs (Alaba and Chola, 2014; Joubert *et al.*, 2007). Thus, a better understanding of the mechanisms responsible for the health risk associated with obesity is required to identify possible obesity-associated changes that could be targeted.

1.1 Obesity as a risk factor for cardiovascular disease beyond conventional risk factors

Obesity is a well-recognised determinant of damage to cardiovascular structure (Alpert *et al.*, 2014; Murdolo *et al.*, 2015) and function (Seven, 2015; Mahajan *et al.*, 2015), leading to an increased risk for cardiovascular disease. This increased risk occurs for myocardial infarction (MI), stroke, heart failure and renal failure (Eckel *et al*., 2002; Klein *et al.*, 2004; Poirier *et al.*, 2006; Murphy *et al.*, 2006). Overall obesity is therefore associated with an increased risk of fatal and non-fatal cardiovascular events (Murphy *et al.*, 2006). Although much of this risk is determined by obesity-associated increases in blood pressure (BP), diabetes mellitus and changes in lipid concentrations, it is also acknowledged that this increased risk is also substantially beyond these conventional risk factors. Importantly, it is now well-recognized that an increased visceral or abdominal adipose tissue, which is closely associated with the metabolic disturbances (insulin resistance and disturbances of glucose and lipid metabolism) that occur in obesity, is more strongly associated with cardiovascular risk than the measure of general obesity, BMI (Larsson *et al.*, 1984). Indeed, waist circumference, a measure of abdominal obesity is a more consistent predictor of cardiovascular disease than BMI (Janssen *et al*., 2004). Although obesity has an adverse impact on the cardiovascular system, an obesity paradox is now well established, where although associated with the development of events, overweight is also related to an improved survival (Lavie *et al.*, 2014).

Several large studies including the INTERHEART study (Steyn *et al.*, 2005; MacMahon *et al.*, 2009) have demonstrated strong independent relationships between obesity and coronary heart disease (CHD) (Freedman *et al.*, 2001). Importantly, obesity causes CHD (Eckel and Krauss, 1998) including MI, irrespective of the presence or absence of the conventional risk factors hypertension, dyslipidemia and hyperglycemia (Thomsen and Nordestgaard, 2014). For example in a large cohort study with 111,847 participants**,** 6% of whom were morbidly obese, these individuals developed MI 16 years earlier than their lean counterparts (Madala *et al.*, 2008). In that study (Madala *et al.*, 2008), independent of conventional risk factors and medication use, obesity was strongly associated with the onset of first non-ST-segment elevation MI. Those with extreme obesity also present with ST segment elevation MI at younger ages (Das *et al.*, 2011). Although the younger age of presentation of ST segment elevation MI is

associated with less extensive coronary artery disease and better left ventricular (LV) systolic function (the obesity paradox), extreme obesity nevertheless remains independently associated with higher in-hospital mortality (Das *et al.*, 2011). The extent to which obesity associated CHD (Freedman *et al.*, 2001) is determined by the metabolic disturbances that accompany obesity (insulin resistance, impaired glucose metabolism and lipid disturbances) is uncertain. However, the risk of MI is more strongly associated with the presence of metabolic disorders than with overweight *per se* (Anderson *et al.*, 2015).

Independent of the conventional risk factors hypertension, diabetes mellitus and changes in cholesterol concentration; overweight and obesity also increase the risk for total, ischaemic and hemorrhagic stroke, (Kurth, 2002; Gu *et al.*, 2006; Go *et al.*, 2013; Wan, Vogel and Chen, 2014; S. *et al.*, 2015; Valma *et al.*, 2018; Kang *et al.*, 2018). Obesity has a particularly major impact on the risk of stroke in younger persons (Dehlendorff, KK and TS, 2014; Muldoon *et al.*, 2016). In South Africa 45% of ischaemic strokes are due to excess body weight and as the impact of obesity increases so too does the prevalence of stroke (Joubert *et al.*, 2007). There is also evidence of an obesity paradox in stroke, in that, a higher waist circumference on admission (Witberg *et al.*, 2016; Thomsen and Nordestgaard, 2014) associates with milder baseline stroke severity among patients with acute ischaemic stroke (Kang *et al.*, 2018). Moreover, obesity may protect patients from a second stroke (Dey *et al.*, 2003). Indeed, in the USA, a heterogeneous association between BMI and all-cause mortality occurs in the middle aged and older adults with acute ischaemic stroke, where obesity is protective whilst morbid obesity detrimental (Skolarus *et al.*, 2014). As with CHD, the extent to which obesity-associated stroke is determined by the metabolic disturbances that accompany obesity (insulin resistance, impaired glucose metabolism and lipid disturbances) is uncertain. The risk of ischaemic stroke is also more strongly associated with the presence of metabolic disorders than with overweight *per se* (Anderson *et al.*, 2015).

As with CHD and stroke, a relationship independent of conventional risk factors exists between the degree of adiposity and the development of heart failure (England, 1997a; Manuscript and Structures, 2009; Ingelsson, *et al.*, 2005; Ingelsson *et al.*, 2005; Bahrami *et al.*, 2008; Spies *et al.*, 2009; Nichols, 2014; He *et al*., 2001; Ärnlöv, *et al.*, 2005; Bastien *et al.*, 2014). This relationship has been demonstrated in the elderly (Chen *et al.*, 1999; Nicklas *et al.*, 2006; Lavie *et al.*, 2009), in the middle aged (Ingelsson, *et al.*, 2005), in men (Wilhelmsen *et al.*, 2001; Ingelsson *et al.*, 2005), and in women (He *et al*., 2001); in the general practice setting (Johansson *et al.*, 2001), in physicians (England, 1997) and in persons with established coronary artery disease (Spies *et al.*, 2009). In this regard, there is a 39 to 49% increase in the risk of heart failure with obesity (Bahrami *et al.*, 2008). The independent risk for heart failure in overweight or obese people is quantitatively comparable with the impact of the presence of hypertension, or diabetes mellitus in these studies depending on the study design and

population (Alpert, Lavie, *et al.*, 2014). Although adjustments for conventional cardiovascular risk factors, including hypertension, diabetes mellitus, cholesterol concentrations and adjustments for coronary events, modify the relationship between excess adiposity and the development of heart failure, the impact of these adjustments is modest. In obese and overweight persons the risk for heart failure is reduced by only 1-13% with these adjustments (He *et al*., 2001; Ingelsson *et al.*, 2005; Ingelsson *et al.*, 2005; Nicklas *et al.*, 2006; Spies *et al.*, 2009) and by 55% where obesity increased the risk for heart failure by 180% (Kenchaiah *et al*., 2009).

1.2 Obesity effects on cardiovascular structure and function beyond conventional risk factors.

1.2.1 Left ventricular structure and function

1.2.1.1 Importance of left ventricular hypertrophy

A number of clinical, community or population-based observational studies have demonstrated that LV hypertrophy (LVH) is an independent predictor of the development of heart failure (Casale *et al.,* 1986; Levy *et al.*, 1990; Levy *et al*., 1991; Gottdiener *et al*., 2000; Gardin *et al.,* 2001; Aurigemma 2001). Moreover, it is now well established that regression of LVH produced by antihypertensive therapy is independently associated with a reduction in cardiovascular events, including stroke and MI (Devereux *et al.*, 2004; Okin *et al.*, 2004; Wachtell *et al.*, 2007; Verdecchia *et al.*, 2003). Nevertheless, these relationships between LVH and heart failure or alternative events are not necessarily cause-effect relationships as there are no studies that have specifically targeted LVH therapeutically and demonstrated an ability to prevent the development of heart failure or other events.

Of all the cardiovascular events that LVH may mediate the most likely event that may be causally associated with LVH is heart failure. In this regard, in keeping with the classical tenet that LVH is a compensatory response to increases in LV load, earlier studies demonstrated that an increased LV mass (LVM) is associated with an unchanged LV chamber systolic function as indexed by ejection fraction (EF) (Aurigemma *et al.,* 1995; de Simone[b] *et al*., 1994, Shimizu *et al*.,1991). LVH may even be associated with an enhanced EF for that predicted by wall stress (Hartford *et al*. 1985). More contemporary studies have demonstrated that independent of traditional risk factors, including conventional (office) BP, LVH is associated with the development of a decreased LV EF (Drazner *et al.,* 2004) suggesting a pathophysiological rather than compensatory role in LV function. Indeed, as a reduced LV EF in asymptomatic patients is associated with the subsequent development of heart failure (Wang *et al.*, 2003), it is

possible that LVH may be a critical pathophysiological mechanism which drives the transition to heart failure. Importantly, in the Losartan Intervention For Endpoint reduction (LIFE) study, a greater regression of LVH occurred in the patient group treated with an angiotensin II receptor blocker in combination with other antihypertensive agents, as compared to the patient group receiving a β-adrenoreceptor blocker together with other antihypertensive agents (Okin *et al*., 2003). As compared to the β-adrenoreceptor blocker-treated group, patients receiving the angiotensin II receptor blocker experienced a greater reduction in overall cardiovascular mortality (Dahlof *et al*., 2002). Surprisingly though, the secondary end-point of heart failure was not reduced to a greater extent in the angiotensin II receptor blocker treated group (Dahlof *et al*., 2002). Moreover, in the echocardiographic subgroup of the LIFE study, on-treatment decreases in LVM were related to reductions rather than increases in indices of LV systolic chamber function, despite improvements in indices of myocardial systolic function (Wachtell *et al*., 2002). Thus, the LIFE Study provides contradictory evidence for a pathophysiological role for LVH as a cause of cardiac decompensation. In the Heart Outcomes Prevention Evaluation (HOPE) trial, angiotensin-converting enzyme inhibition both reduced LVM and improved LV function beyond BP control (Lonn *et al.,* 2004), providing some evidence that LVH may be causally related to LV dysfunction.

One possibility that may account for the transition from compensated LVH to LV systolic chamber decompensation is that LVH in-keeping with work load (i.e. stroke work=blood pressure x stroke volume) is compensatory, whilst that exceeding workload (i.e. “inappropriate” LVM [LVM_{inappr}]) (de Simone *et al*., 1998) contributes toward decompensation. Indeed a number of earlier studies had demonstrated an inverse relationship between LVM_{inappr} and LV systolic chamber function (Palmieri *et al.*, 1999; Palmieri *et al.*, 2001; Celentano *et al.*, 2001; De Simone *et al.*, 2004; Chinali *et al.*, 2006; Chinali *et al.*, 2007; Cioffi *et al.*, 2011). Whether LVM_{inappr} is associated with a reduced systolic chamber function independent of and more strongly than absolute or indexed LVM was at this time uncertain. Some prior studies conducted in select clinical samples demonstrated inverse correlations between LV systolic chamber function and both absolute LVM as well as LVM_{inappr} (Palmieri *et al.*, 1999; Palmieri *et al.*, 2001) and in one of these studies LVM was equally as strongly inversely correlated with EF as LVM_{inappr} (Palmieri *et al.*, 2001; de Simone *et al*., 1991) . This finding is at odds with the notion that it is only LVM beyond stroke work that explains decompensation. Moreover, prior relationships between LVM_{inappr} and LV systolic chamber function were demonstrated in cross-sectional studies (Palmieri *et al.*, 1999; Palmieri *et al.*, 2001; Celentano *et al.*, 2001; De Simone *et al.*, 2004; Chinali *et al.*, 2006; Chinali *et al.*, 2007; Cioffi *et al.*, 2011). Conclusions regarding cause and effect cannot be drawn from cross-sectional studies. Indeed, relationships between LVM_{inappr} and LV systolic chamber function may reflect residual confounding effects or compensatory increases in LVM as a consequence of systolic dysfunction (reverse causality). Although one

previous study at that time had reported that on-treatment regression but not persistence of LVM_{inappr} is associated with an improved EF (Muiesan *et al.*, 2007), whether increases in EF in the participants showing regression of LVM_{inappr} in that study were independent of or stronger than changes in LVM or LVMI was uncertain. More recent studies from our group have demonstrated in a large community-based sample a clear and strong relationship between LVM_{inappr} and EF beyond LVMI or LVM and conventional risk factors and confounders (Libhaber *et al.*, 2013), and a strong relationship between on-treatment decreases in LVM_{inappr}, but not LVMI or LVM and EF (Woodiwiss *et al.*, 2012). Thus, LVH is indeed an important cause of LV systolic decompensation.

1.2.1.2 Obesity and left ventricular hypertrophy

Obesity is a well-recognised cause of increases in cardiac loading conditions. Increases in cardiac loading will contribute to the development of LVH (Chinali *et al.*, 2006; Lavie *et al.*, 2013), and hence cardiac dysfunction and heart failure; possibly cardio-embolic stroke (through the development of heart failure or atrial fibrillation), and through the development of LVH, CHD (Lavie *et al.*, 2013). Although, the effects of obesity on cardiovascular events are independent of BP, the question arises as to whether obesity can promote LVH independent of BP? Obesity not only increases loading conditions through an impact on BP, but also increases blood volume, which enhances stroke volume and cardiac output and this increases workload on the heart (Landsberg *et al.*, 2013; Buchwald *et al.*, 2004; Lavie *et al.*, 2014; Skinner *et al.*, 2015; Alpert *et al.*, 2014; Alpert *et al*., 2016). Thus, consideration must also be given to whether LVH in obesity is through afterload effects on the LV mediated by increases in BP, an enhanced workload on the heart, or through cellular changes independent of both BP and workload. What is the evidence for obesity effects on LVH beyond BP or workload?

A 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). Importantly, LVM indices 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 indices 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 currently considered to be of pathophysiological significance. Obesity enhances the impact of BP on LVM (Avelar *et al.*, 2007; Norton *et al.*, 2009) potentially through the adverse effects of leptin (Norton *et al.*, 2009). As BP effects on the heart are of pathological relevance, these data support a view that obesity effects on LVM are of pathophysiological and not just physiological significance.

As an increased LVM exceeding that predicted from stroke work (LVM_{inappr}) is strongly related to LV systolic decompensation (see above), the obvious question is whether obesity plays a role in mediating LVM_{inappr}? Although several studies have demonstrated an independent association between BMI and LVM_{inappr} (Palmieri *et al.*, 2004; Lopez *et al.,* 2007; Palmieri *et al.*, 2001; de Simone *et al.*, 2004; Chinali *et al.*, 2007; Chinali *et al.*, 2006; Palmieri *et al.*, 1999; Celentano *et al.*, 2001; Ratto *et al.*, 2008). A number of studies have also failed to demonstrate these relationships (de Simone *et al*., 2001; Muiesan *et al.,* 2007; Mureddu *et al.,* 2001; Cioffi *et al.,* 2011). Thus, there is considerable uncertainty as to the role of obesity as a determinant of LVM_{inappr}. Discrepancies between studies may be attributed to several factors including the use of select clinical samples; adolescents; the lack of relationships assessed in continuous analysis with adjustments for confounders or the impact of alternative haemodynamic factors such as 24-hour BP or arterial stiffness being more pronounced in some than in other populations. Importantly, what should also be considered is that factors associated with obesity that may be more striking in some than other populations may account for obesity effects on LVM_{inappr}.

1.2.1.3 Obesity and left ventricular function

Although the impact of obesity on LVM beyond conventional risk factors is undisputed, the effects of obesity on LV function are less clear. Despite the effects of obesity on EF produced by increases in LVM_{inappr}, it is only with long-standing and more advanced obesity that obesity may be associated with reductions in EF beyond LVM_{inappr}. Indeed, most obese individuals have a normal EF. Although there is some evidence that obesity decreases more subtle indices of LV systolic myocardial function, such as tissue Doppler indices or longitudinal strain (Peterson *et al*., 2004; Wong *et al.,* 2004), these studies have generally been conducted in small samples of select individuals or have not adjusted for all confounders including LVM_{inappr} and have not been reproduced by subsequent larger studies Despite the limited evidence to support a role of obesity in mediating LV systolic dysfunction beyond LVH or additional confounders, the evidence to support a role for obesity in mediating LV diastolic dysfunction is more striking. Indices of excess adiposity have consistently been shown to be associated with several indices of LV diastolic function beyond BP and LVMI in large study samples of

hypertensives (Tsioufis *et al*., 2008), beyond ambulatory and central BP and LVMI or the degree of concentric LV remodelling in the general community (Libhaber *et al*., 2009) and beyond conventional risk factors and LVM in general or elderly community samples (Russo *et al*., 2011; AlJaroudi *et al,.* 2012; Çil *et al*., 2012). Although BP effects on LV diastolic function in a community sample are far more important than obesity effects on LV diastolic function (Millen *et al*., 2014), the metabolic consequences of obesity strongly interact with the degree of LV geometric remodeling to determine LV diastolic dysfunction (Peterson *et al*., 2015). As a consequence, the metabolic effects of obesity determine the extent to which diastolic dysfunction occurs in hypertensives (Bamaiyi *et al.,* 2019). Importantly, in a systematic review and meta-analysis of 23 studies assessing the effects of bariatric surgery on cardiac structure and function in obese patients with preserved systolic function, improvements in LV diastolic function were indeed a consequence of weight loss (Cuspidi *et al*., 2014).

1.2.2 Aortic dysfunction

1.2.2.1 Importance of aortic dysfunction.

Meta-analyses (Vlachopoulos *et al*., 2010; Ben-Shlomo *et al.,* 2014), including an individual patient meta-analysis (Ben-Shlomo *et al*., 2014) have demonstrated the ability of increases in carotid-femoral (aortic) pulse wave velocity (PWV), an index of aortic stiffness, to predict cardiovascular events beyond brachial BP. This relationship is noted for both MI and stroke and occurs across several subgroups including at a younger age (Ben-Shlomo *et al.,* 2014). The measurement of aortic PWV is recommended by hypertension guidelines to enhance risk prediction. The relationship between aortic PWV and cardiovascular events beyond brachial BP is to some extent surprising given the importance of aortic stiffness as a determinant of BP (Roman *et al.*, 2007; Vlachopoulos *et al.*, 2013). Aortic stiffness is a strong determinant of the impedance (resistance in a pulsatile system) to flow during ventricular ejection and characteristic impedance (impedance in the absence of wave reflection) is thought to be the main determinant of pulse pressure (PP) and hence systolic BP. The impact of aortic PWV on events beyond brachial BP was initially thought to be in-part accounted for by brachial BP being an inadequate index of central aortic BP (PP is amplified from the aorta to the peripheral arteries). Several lines of evidence including a meta-analysis (Vlachopoulos *et al.,* 2010) suggest that aortic PP may not predict events that much beyond brachial PP. Although this meta-analysis may have excluded key studies published in the same year and included some studies that employed approaches that may be criticized, the Framingham Heart Study demonstrated an ability of PWV but not aortic PP to predict events beyond brachial PP (Mitchell *et al*., 2010). There is significant evidence that aortic PWV predicts events beyond both brachial

as well as aortic PP. Nevertheless, the authors of the Framingham Heart Study subsequently reported an ability of the forward pressure wave component (Pf) of aortic PP, that pressure wave that is driven by aortic stiffness, to predict events (Cooper *et al*., 2015). However, there are several lines of evidence to suggest that changes in aortic stiffness may cause cardiovascular damage beyond pulsatile load, including Pf. What is this evidence?

An early hypothesis was that increases in large artery stiffness mediate atherosclerotic changes independent of pulsatile loads. This hypothesis arose from several studies suggesting effects on atherogenesis beyond pressure pulsatility. When endothelial cells are plated in a compliant versus a stiff conduit and then exposed to the same pulsatile loads, those in the stiff conduit show worse function as compared to those in the compliant conduit (Peng *et al*., 2003). Moreover, whilst collagen, which increases arterial stiffness has been demonstrated to directly activate endothelial cell adhesion molecules in the vascular wall, elastin, which improves aortic elasticity improves endothelial function (Orr *et al.,* 2009; Gonzales-Santiago *et al.,* 2002). As endothelial dysfunction is an essential step in the atherosclerotic process, these findings (Peng *et al*., 2003; Orr *et al*., 2009; Gonzales-Santiago *et al*., 2002) suggest that arterial stiffness promotes atherosclerosis beyond pulsatile loads. Nevertheless, this notion suggests that atherosclerosis is more likely in the vascular wall where stiffness occurs. Hence, these studies posit a mechanism through which aortic PWV could contribute to aortic aneurysm formation and related events (rupture or dissection). However, as aortic PWV is an index of stiffness in the aorta and not in smaller arteries, it will not explain either MI or stroke, which are produced by atherosclerotic changes in intra-or extracranial arteries or coronary vessels.

A more recent hypothesis that has emerged is that increases in aortic stiffness reduce the normal impedance mismatch that exists between the aorta (low impedance) and more distal arterial beds (higher impedance), and thus promotes an increased transmission of pulsatile energy into these distal beds (O'rourke *et al*., 2010; Smulyan *et al*., 2016**).** In this regard, this will increase pulsatile flow in and cause damage to the microvasculature of low resistance organs such as the brain and the kidney. This hypothesis has evidence to support it for white matter lesions and cognitive function, dementia, renal disease and endothelial function in the microvasculature of the forearm (Hashimoto *et al.,* 2011; Mitchell *et al*., 2011; Woodard *et al*., 5015; Cooper *et al*., 2016; Webb *et al*., 2012). However, once again there is evidence lacking for stroke itself and this hypothesis cannot explain relationships between aortic stiffness and coronary artery disease which occurs because of damage to major arteries and not microvascular damage.

Through ventricular-vascular coupling an increased proximal aortic stiffness may also impose a load on the left ventricle beyond aortic PP or its pressure wave components causing hypertrophy and dysfunction (Bell *et al*., 2015; Ohyama *et al*., 2016; Bello *et al.,* 2017). Through LVH an increased aortic stiffness may subsequently enhance the chances of MI beyond

pulsatile pressure loads (LVM is a risk factor for MI). Because of its elasticity, the healthy aorta has a high capacity to store energy during systole and thus through recoil in diastole, contribute to flow. It is well recognized that as the aorta increases its stiffness it loses its capacity for elastic recoil and hence the ability to contribute to systemic blood flow in diastole. As coronary flow mainly occurs in diastole, it is possible that an increased aortic stiffness contributes to a reduced myocardial blood flow. Several studies have indeed produced the evidence to show that increases in aortic stiffness are associated with coronary microcirculatory dysfunction (Watanabe *et al*., 1993; Ikonomidis *et al*., 2008).

In short an increase in aortic stiffness may, through several mechanisms unrelated to changes in pulsatile load, contribute to cardiovascular events. What is the evidence that obesity or its metabolic effects cause increases in aortic stiffness?

1.2.2.2 Obesity and aortic dysfunction.

Numerous studies have reported on a relationship between excess adiposity and an increase in arterial stiffness as indexed by aortic PWV or alternative measures (Resnick *et al.,* 1997; Palmieri *et al.*, 1999; Sutton-Tyrrell et al, 2001; Selzer *et al.*, 2001; Wildman *et al.*, 2003; Ferreira *et al.*, 2004; Ferreira *et al.*, 2004; Snijder *et al.*, 2004; Cote *et al.*, 2015; García-Espinosa *et al.*, 2016) effects which are noted both in the young (Tounian *et al.*, 2001; Ho, 2009; Cote *et al.*, 2015) and the elderly (Ingelsson *et al.*, 2005; Sutton-tyrrell *et al.*, 2001; Wildman *et al.*, 2003). However, the extent to which these relationships are independent of haemodynamic factors, such as BP and heart rate or beyond diabetes mellitus (DM) or alternative risk factors such as dyslipidaemia is not clear. Indeed, some studies have demonstrated strong relationships between adiposity indices and PWV beyond conventional risk factors (Sutton-Tyrrell *et al.*, 2001; Wildman *et al.*, 2003; Ferreira, Twisk, *et al.*, 2004; Safar, 2006; Lurbe *et al.*, 2012). Yet, other studies failed to show similar strong independent relationships between adiposity indices and PWV beyond conventional risk factors (Amar *et al.*, 2001; Nakanishi, Suzuki and Tatara, 2003; A. *et al.*, 2003; Mitchell *et al.*, 2004; Zebekakis *et al.*, 2005, Majane *et al*., 2009). These discrepancies in relationships between obesity and aortic stiffness raises the question of whether factors associated with obesity rather than obesity *per se* explain obesity-aortic stiffness relationships.

1.2.3 Renal dysfunction

1.2.3.1 Importance of renal dysfunction as a determinant of cardiovascular events.

Chronic kidney disease, as defined by reductions in estimated glomerular filtration rate (eGFR), is in most countries, a major public health problem with substantial social and economic consequences (Levey *et al.*, 2007; Bello *et al.,* 2005). Chronic kidney disease progresses to end-stage renal disease and predicts cardiovascular events beyond conventional risk factors (Go *et al.*, 2004; Matsushita *et al.*, 2010; Mahmoodi *et al.*, 2012; Matsushita *et al.*, 2015; Waheed *et al.*, 2012; Hui *et al.*, 2013). Of importance to the present thesis however is that through the cardio-renal syndrome, well before the onset of renal failure, reductions in estimated glomerular filtration rate are strongly associated with LVM and these effects are not attributed to an impact of hemodynamic factors (Maunganidze *et al.*, 2014). The possible mechanisms of this effect have been extensively reviewed, but also go beyond the scope of the present thesis.

1.2.3.2 Obesity effects on glomerular filtration rate

The relationship between obesity and renal dysfunction has been recognized for over a century with a recent review (Bayliss *et al*., 2012) highlighting very early evidence of albuminuria on obese individuals described as early as 1923(Cobb and Blythe, 2010) with subsequent studies performed much later in the century confirming these findings (Weisinger *et al.*, 1974; Cohen, 1975). Although much of this relationship is attributed to co-morbidities in obesity including hypertension and type II diabetes mellitus, there is clear evidence that adiposity indices predict indices of renal dysfunction beyond conventional risk factors. In this regard, recent meta-analyses demonstrate an independent ability of BMI to predict CKD onset and albuminuria in 39 cohorts with 630,677 participants of general adult populations with a mean of 6.8 years of follow up (Garofalo *et al*., 2017). Importantly, weight loss results in a marked regression of proteinuria on a low calorie diet (Morales *et al.*, 2003). In the latter half of the last century renal dysfunction in obesity was attributed to the development of focal segmental glomerular sclerosis (Verani, 1992). In this regard, obesity is associated with larger glomeruli and glomerular hyperfiltration, changes attributed to the hyperdynamic circulation often noted in obesity (Bayliss *et al*.,2012). Indeed, a retrospective analysis of 6818 renal biopsies demonstrated that an increased BMI over time was associated with an obesity-related glomerulopathy (Kambham *et al.*, 2003). Despite strong evidence to suggest that obesity associated renal dysfunction is associated with glomerular hyperfiltration and hence destruction

of glomeruli. Several additional mechanisms may also explain the conventional risk factor-independent association between obesity and reductions in eGFR.

1.3 Mechanisms that may explain obesity effects on the cardiovascular system beyond conventional risk factors?

1.3.1 Cardiovascular effects of insulin resistance.

One possible mechanism that may account for cardiovascular disease in obesity beyond conventional cardiovascular risk factors is through the adverse actions of insulin resistance. Insulin resistance may occur as a consequence of excess adiposity (Zeyda and Stulnig, 2009), and 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, causing tyrosine auto phosphorylation and consequent intracellular signalling. This event culminates in cellular responses where translocation of glucose transporters to the cell surface allow glucose to be taken up for use or glycogen storage (Caro *et al.*, 1989). In obesity, insulin signalling may be defective, where Insulin-stimulated protein kinase activity of the insulin receptor, which mediates tyrosine auto phosphorylation, is reduced, and it is further reduced in patients with obesity-induced type II diabetes mellitus (Mykkänen *et al.*, 1998).

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 (Kriketos *et al.*, 2004). Therefore, insulin resistance goes undetected in these individuals (Monteiro and Azevedo, 2010; McArdle *et al.*, 2013). Hyperinsulinaemia in insulin resistance may promote adverse cardiovascular effects through several mechanisms. In this regard, insulin resistance is well recognised as activating the sympathetic and the renin-angiotensin-aldosterone systems (Whaley-Connell and Sowers, 2018; Smekal and Vaclavik, 2017), changes which can promote LVH and LV dysfunction through load-independent mechanisms (Norton *et al.*, 2018). Hyperinsulinaemia can induce hypertrophy of the vascular wall through an increased synthesis of collagen and proliferation of vascular smooth muscle cells and thus induce increases in arterial stiffness (Cameron and Cruickshank, 2007; DeFronzo and Ferrannini, 1991; Avena *et al.*, 1998; King and Wakasaki, 1999; Popele *et al.*, 2000) and insulin resistance can promote endothelial dysfunction (Arcaro *et al.*, 2002) and thus contribute toward and enhanced atherogenesis. Insulin resistance involves not only skeletal muscle, but myocardial muscle tissue, where it downregulates glucose uptake thus preventing the energy advantage provided by the glucose from being used as opposed to free fatty acid oxidation (Nikolaidis *et al.*, 2004; Ouwens *et al.*, 2005; Coort *et al.*, 2007). Insulin resistance is also

associated with an accumulation of intracellular triacylglycerol which is lipotoxic (Coort *et al.*, 2007). Obesity could therefore promote LV dysfunction through a reduced efficacy of energy utilisation or through apoptosis due to lipotoxicity. As insulin resistance is associated with leptin resistance (Smekal and Vaclavik, 2017), a reduced cardiac efficiency and reduced myocardial glucose metabolism may be seen in obesity with leptin resistance (Buchanan *et al.*, 2005; Myers *et al.*, 2010; Sáinz *et al.*, 2015; Sáinz *et al.*, 2015). These myocardial substrate metabolism changes may contribute to an increased cardio-myocyte apoptosis in obesity with leptin deficiency or leptin resistance (Barouch *et al.*, 2006). This cardio-myocyte apoptosis occurs through reduced cardio-protective action by leptin, which is induced by an increase in lipid overload in cardio-myocytes, as seen in an animal study of leptin resistant or leptin deficient animals (Zhou *et al.*, 2000; Wang *et al.*, 2017). Thus, when considering the adverse impact of obesity on the cardiovascular system the role of insulin resistance should be considered foremost as a potential mechanism mediating these effects. Moreover, molecules that alter insulin resistance should be considered as potentially important as determinants of cardiovascular damage beyond conventional risk factors.

1.3.2 Obesity-associated inflammatory changes

A more contemporary notion of the possible mechanisms that may account for cardiovascular disease in obesity beyond conventional risk factors is through the adverse actions of obesity-associated chronic inflammation (Zeyda and Stulnig, 2009). Obesity changes the morphology and composition of adipose tissue, leading to alterations in protein production and secretion, including several pro-inflammatory mediators, which are produced by macrophages resident in adipose tissue. The changes in inflammatory status of adipose tissue with obesity has rendered a growing recognition that obesity represents a state of chronic low-level inflammation (Stienstra *et al.*, 2007). Changes in adipose tissue with obesity (Zeyda and Stulnig, 2009) are associated with an increase in systemic circulating levels of the pro-inflammatory molecules including C-reactive protein (CRP), interleukin-6 (Il-6), tumour necrosis factor-α (TNF-α), plasminogen activator inhibitor-1 (PAI-1), interleukin -1 (IL-1), interleukin-8 (IL-8), interleukin-10 (IL-10), and interleukin-15 (IL-15), leukemia inhibitory factor, hepatocyte growth factor, SAA3, macrophage migration inhibitory factor, haptoglobin, complement factors B, D, C3, prostaglandin E2, and potential inflammatory modulators such as leptin and resistin produced by adipose tissue (Berg and Scherer, 2005; Forsythe *et al.*, 2008; Forsythe, Wallace and Livingstone, 2008). In addition, obesity is thought to result in a decrease in circulating concentrations of the anti-inflammatory adipokine adiponectin (Fantuzzi, 2013; Esfahani *et al.*, 2015; Smekal and Vaclavik, 2017). The primary function of several adipokines is to control fat metabolism, energy homeostasis, appetite, and insulin sensitivity. However, being inflammatory

molecules, these substances clearly have ubiquitous effects and these effects are often detrimental to the cardiovascular system.

Substantial work has been performed on assessing the role of the two most studied adipokines, adiponectin and leptin. In this regard, adiponectin is protective to the development of diseases associated with obesity and by reducing proliferation and migration of smooth muscle cells, inhibiting the conversion of macrophages into foam cells, suppressing the production of reactive oxygen species (ROS) and increasing the production of nitric oxide (NO), adiponectin is considered to be anti-atherosclerotic (Smekal and Vaclavik, 2017). The normal function of leptin is to suppress appetite, regulate food intake and increase metabolism, but with obesity and insulin resistance, circulating leptin concentrations are increased and this is partly explained by leptin resistance which results in higher levels of SOCS3, which is involved in negative regulation of cytokines that signal through the JAK/STAT pathway, thereby inhibiting the activity of JAK kinase (Smekal and Vaclavik, 2017). Although insulin resistance has been postulated to play a key role in the adverse effects of obesity on cardiovascular events (see aforementioned discussion), several of the more well-described adipokines, such as leptin and adiponectin have similarly been demonstrated to mediate either adverse or beneficial effects, but these data are inconsistent. I explored the role of the adipokine, resistin and in-part compared its association to that of adiponectin (an anti-inflammatory adipokine) on several obesity-associated cardiovascular structural and functional changes.

1.3.3 Obesity and resistin

Resistin is a low molecular weight, 12.5 kDa, cysteine-rich protein (murine 114 amino acids, human 104 amino acids) secreted from adipose tissue (Shojima *et al.*, 2002; Patel et al., 2003). The direct regulation of resistin secretion from adipose tissue by peroxisome proliferator-activated receptor γ (PPAR-γ) may provide one link between the protein and insulin signalling in type II diabetes mellitus (Savage *et al.*, 2001). Resistin was initially observed to be secreted by rodent fat cells, and noted to impair glucose metabolism and insulin action in mouse models of obesity and cultured adipocytes (Steppan *et al.*, 2001). Importantly, immuno-neutralization of resistin improves insulin action in mice with diet-induced obesity (Steppan *et al.*, 2001). Overexpression of resistin in metabolically healthy mice leads to insulin resistance and dysregulated lipid metabolism with an increased accumulation of triglycerides and cholesterol (Sato *et al.*, 2005; Satoh *et al.*, 2004). Plasma resistin concentrations are increased in genetic mice models of obesity (db/db, ob/ob) and in diet-induced obese mice (Steppan *et al.*, 2001), while resistin mRNA levels in adipose tissue of obese rodents are decreased (Rajala *et al.*, 2002). Notably, mice lacking resistin have an improved glucose tolerance as compared with wild type controls both in diet-induced obesity (Banerjee *et al.*, 2004) and in ob/ob mice (Qi *et al.*,

2006), suggesting a role for resistin in insulin resistance and the hyperglycemia associated with obesity. Loss- and gain-of-function studies have demonstrated that resistin modulates glucose metabolism through inhibition of AMP-activated protein kinase and increased expression of gluconeogenic enzymes in the liver (Banerjee *et al.*, 2004; Qi *et al.*, 2006; Qi *et al.*, 2008). However, resistin may also act centrally to regulate glucose homeostasis (Muse *et al.*, 2004; Singhal et al., 2007). Resistin is a target of the anti-diabetic drug thiazolidinedione (TZD) as TZD treatment suppresses resistin expression in adipocytes and in white adipose tissues of mice fed a high fat diet (Steppan *et al.*, 2001). Collectively, these findings provide evidence that resistin may function as a significant contributor to the development of insulin resistance and type II diabetes mellitus. In view of the possible role of resistin as a mediator of insulin resistance, and the aforementioned effects of insulin resistance on cardiovascular structure and function, one should consider the possibility that resistin may mediate adverse cardiovascular effects beyond conventional risk factors including diabetes mellitus. However, for several reasons the pathophysiological role of resistin in humans has been questioned.

The human homologue of resistin is only 59% identical to mouse resistin at the amino acid level and the source of resistin appears to differ between humans and mice (Steppan *et al.*, 2001; Ghosh *et al.*, 2003). Unlike mice, resistin in humans is undetectable in adipocytes but highly expressed in macrophages. Indeed, the major source of circulating resistin in humans is likely to be peripheral blood mononuclear cells (Kaser *et al.*, 2003). Nevertheless, in humans, resistin expression also occurs in myocytes (Nagaev and Smith, 2001; Dietze *et al.*, 2002; Savage *et al.*, 2001), and endothelial cells (Lu *et al.*, 2002; Nagaev and Smith, 2001; Savage *et al.*, 2001; Patel *et al.*, 2003). Importantly, TZD drugs down-regulate the expression of human resistin in macrophages and reduce serum resistin concentrations (Patel, *et al.*, 2003; Lehrke *et al.*, 2004; Samaha *et al.*, 2006). Resistin expression is found in the non-adipocyte stromal vascular fraction in white adipose tissue, in the fibrotic liver, and in atherosclerotic lesions in humans, indicating that macrophages are the main source of resistin in humans (Fain *et al.*, 2003; Bertolani *et al.*, 2006; Burnett *et al.*, 2005; Jung *et al.*, 2006a). Nevertheless, obese individuals who are likely to have a greater infiltration of macrophages in adipose tissue show increased expression of resistin in adipose tissue samples and higher circulating concentrations of resistin than lean individuals (Savage *et al.*, 2001; Pagano *et al.*, 2005). The relationship between obesity and circulating resistin concentrations is however highly variable. Indeed, in obese subjects undergoing weight reduction through diet or bariatric surgery, serum resistin concentrations are reduced, or remained unchanged, despite weight loss (Azuma *et al.*, 2003; Azuma *et al.*, 2003; de Luis *et al*., 2011). Moreover, several reports indicate that serum resistin concentrations are not associated with obesity or insulin resistance (McTernan *et al.*, 2003; Lee *et al.*, 2003; Lee *et al.*, 2003). However, circulating resistin concentrations have been associated with visceral, intrathoracic, and pericardial fat (Jain *et al.*, 2009). Thus, obesity-

associated changes in resistin may play a major role in the development of several disorders even if the relationship between adiposity indices and resistin concentrations are difficult to show. What is the role of resistin in mediating insulin resistance in humans?

Numerous clinical studies have examined a possible relationship between resistin and insulin resistance in obesity with or without diabetes. Since obesity and type II diabetes mellitus are both associated with chronic inflammation in white adipose tissue, and resistin is mainly produced by macrophages in humans, it is possible that hyper-resistinaemia is a contributing factor to these pathophysiological states. However, as discussed above, the relationship between human resistin concentrations and insulin resistance, or type II diabetes mellitus is inconsistent (Lazar, 2007; McTernan *et al.*, 2003; Lee *et al.*, 2003; Youn *et al.*, 2004; Hivert *et al.*, 2008; Tsiotra *et al.*, 2008). Nevertheless, transgenic mice expressing human resistin in macrophages develop white adipose tissue inflammation and insulin resistance (Qatanani *et al.*, 2009), and in prospective case-control studies, elevated baseline levels of resistin concentrations increase the risk of developing type II diabetes mellitus even after adjusting for conventional risk factors (Chen *et al*., 2009; Daniel *et al*., 2011). Importantly, the expression of human resistin in macrophages raises the question of what inflammation does to resistin and what resistin does to inflammation? Resistin is robustly induced in response to various pro-inflammatory stimuli such as lipopolysaccharide (LPS), TNF-α, IL-6, IL-1β, and resistin itself, suggesting a role for resistin in inflammation in humans (Kaser *et al.*, 2003; Lehrke *et al.*, 2004; Bokarewa *et al.*, 2005). Furthermore, resistin has been shown to up-regulate the expression of pro-inflammatory cytokines TNF-α, IL-6, IL-12, and monocyte chemoattractant protein (MCP)-1 in macrophages, through the nuclear factor-κB signalling pathway (Bertolani *et al.*, 2006; Bokarewa *et al.*, 2005; Silswal *et al.*, 2005). Consistent with these findings, circulating resistin concentrations associate with inflammatory markers such as C-reactive protein (CRP), TNF-α, IL-6, or plasminogen activator inhibitor (PAI)-1 (Reilly *et al.*, 2005; Tahara *et al*., 2007; Shetty *et al.*, 2004; Bo *et al.*, 2005; Kunnari *et al.*, 2006; Qi *et al.*, 2008; Fargnoli *et al.*, 2010; Konrad *et al.*, 2007) and increased resistin levels occur in chronic inflammatory conditions (Filková *et al.*, 2009; Šenolt *et al.*, 2007; Konrad *et al.*, 2007). Importantly several inflammatory substances up-regulated by human resistin are known to be involved in the development of insulin resistance. Therefore, it is possible that resistin may play a role in the development of insulin resistance in humans through inflammatory effects. Indeed in mice lacking murine resistin but transgenic for a bacterial artificial chromosome containing human resistin whose expression was similar to that in humans, developed hepatic insulin resistance under chronic endotoxemia, accompanied by inflammation in liver and skeletal muscle (Park *et al.*, 2011). The close relationship between insulin resistance or inflammation and cardiovascular effects has prompted the notion that at least some of the adverse effects of obesity on the cardiovascular system could be explained by the association of resistin. Emerging evidence now suggests that cardiovascular disease is

accompanied by changes in circulating resistin concentrations in humans. What is this evidence?

1.3.3.1 Adverse cellular effects of resistin on the cardiovascular system

Resistin produces several adverse effects on cardiovascular tissue. The data discussed below are summarized in Figure 1.1 for easy reference. Resistin is found in human atherosclerotic lesions and affects vascular endothelial function (Burnett *et al.*, 2005; Jung *et al.*, 2006a; Verma *et al.*, 2003). Increased proliferation and migration of human endothelial cells and vascular smooth muscle cells is induced by resistin through the extracellular signal-regulated kinase, PI3K, or p38 mitogen-activated protein kinase pathways (Mu *et al.*, 2006; Calabro *et al.*, 2004; Shen *et al.*, 2006; Tarkowski *et al.*, 2010). Resistin inhibits endothelial NO synthase, and increases oxidative stress in human aortic and coronary artery endothelial cells (Shen *et al.*, 2006; Chen *et al.*, 2010). Indeed, resistin reduces eNOS mRNA, protein and activity levels, and eNOS mRNA stability and cellular NO levels are reduced (Jamaluddin *et al.*, 2012). In addition, resistin up-regulates the expression of adhesion molecules such as intercellular adhesion molecule-1, vascular cell adhesion molecule-1, P-selectin, and fractalkine; increases proinflammatory MCP-1, PAI-1, endothelin-1, matrix metalloproteinases, and vascular endothelial growth factor receptors, and, thus, promotes monocyte adhesion in vascular endothelial cells (Barnes and Miner, 2009; Jung *et al.*, 2006b; Verma *et al.*, 2003; Mu *et al.*, 2006; Hsu *et al.*, 2011; Manduteanu *et al.*, 2010). Furthermore, resistin increases vascular wall lipid accumulation through the up-regulation of CD36 expression and an increase in cholesteryl ester deposition in human macrophages (Burnett *et al.*, 2005; Jung *et al.*, 2006b; Xu *et al.*, 2006; Rae *et al.*, 2007). Thus resistin modulates the transformation of macrophages into foam cells. In addition, resistin induces a prothrombotic phenotype by up-regulating tissue factor expression in human coronary artery endothelial cells (Calabro *et al.*, 2011; Jamaluddin *et al.*, 2012). Thus resistin plays an important role in the pathogenesis and progression of atherosclerosis in humans.

There is an increasing evidence for a role for resistin in adversely modifying cardiac structure and function. In this regard, there are several lines of evidence to support such a role. Cardiac tissues from those with diabetes mellitus express elevated levels of resistin (Rauch *et al.*, 2012; Karakikes *et al.*, 2009). Furthermore, myocardial resistin expression is up-regulated by cyclic stretch and aorta-caval shunt (Wang *et al.*, 2007). Resistin impairs glucose transport in isolated mouse cardiomyocytes (Graveleau *et al.*, 2005). Resistin may contribute to the adverse effects of myocardial ischaemia through endothelial dysfunction and the promotion of ischemia-reperfusion (IR) myocardial injury (Gao *et al.*, 2007; Gualillo *et al*., 2007; Rothwell et al., 2006). Nevertheless, whilst some studies show that resistin administered prior to ischemia worsened

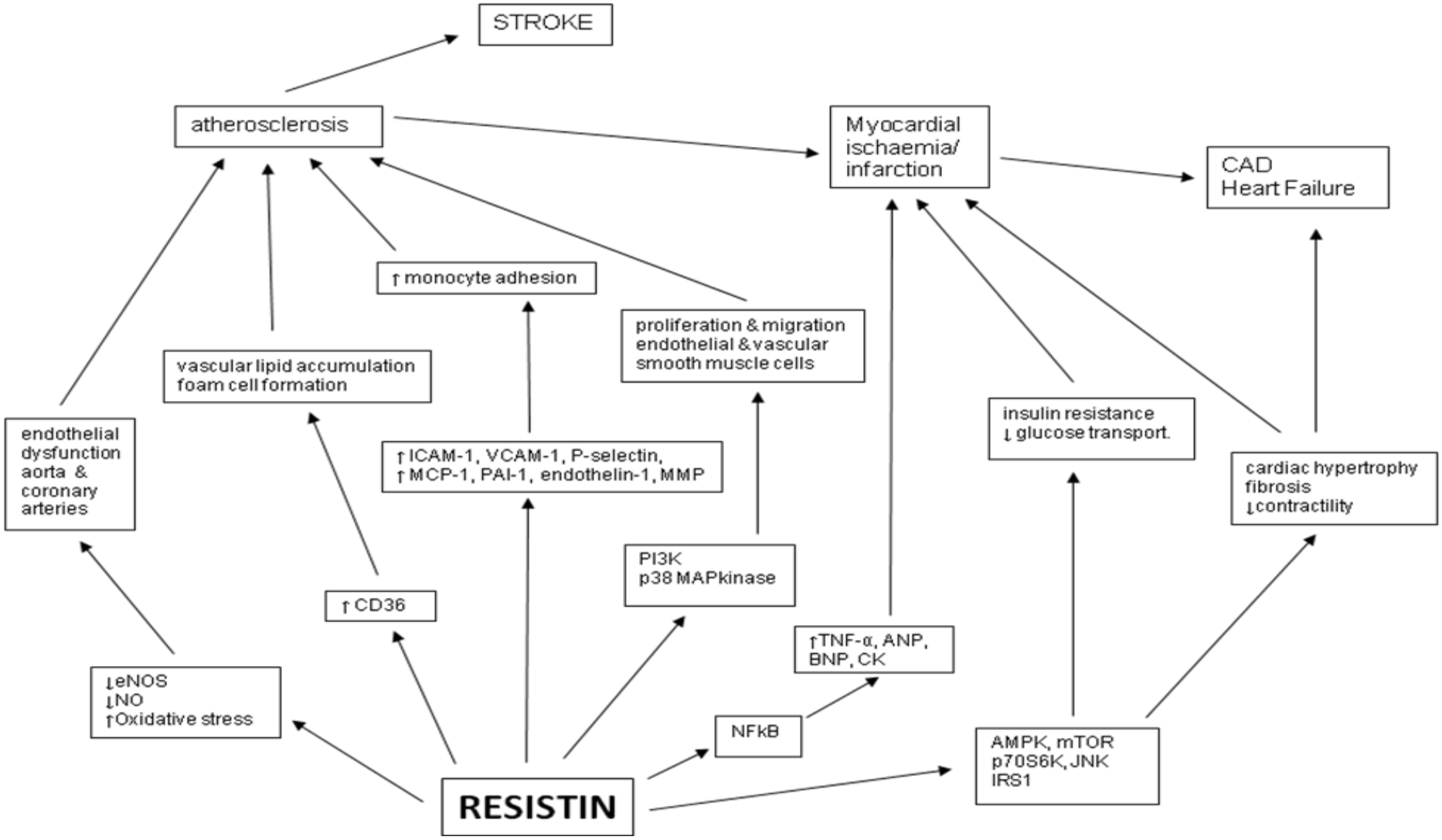


Figure 1.1. Schematic of how resistin mediates cellular effects.

I/R injury (Rothwell *et al*., 2006), Another study notes that pre-treatment with resistin reduces myocardial injury through PI3K/Akt/PKC-mediated pathways (Gao *et al.*, 2007). Overexpression of resistin promotes a hypertrophic response associated with an increased left ventricular wall thickness and end-diastolic pressure (Kim *et al.*, 2008). Resistin has been demonstrated to induce cardiac hypertrophy and myocardial insulin resistance, possibly via the AMP-activated kinase (AMPK) /activation of tuberous sclerosis complex 2/mammalian target of rapamycin (mTOR)/ p70S6K and apoptosis signal-regulating kinase 1/1/c-Jun N-terminal Kinase (JNK) pathway, a system known to stimulate insulin resistance (JNK)/reduced serine 307 phosphorylation of IRS1 (IRS1) pathways (Kang *et al.*, 2011). Adenoviral overexpression of resistin induces cardiac hypertrophy, contractile dysfunction with impaired Ca^{2+} handling (Kim *et al.*, 2008), together with insulin resistance in isolated rat cardiomyocytes (Kang *et al.*, 2011). Long-term cardiac specific overexpression of resistin *in vivo* using adeno-associated virus serotype 9 significantly decreases cardiac contractility and induces a complex phenotype of oxidative stress, fibrosis, apoptosis and myocardial remodelling (Chemaly *et al.*, 2011). In addition, recombinant human resistin exacerbates cardiac I/R injury, and stimulates TNF-α secretion and upregulates cardiac injury markers such as atrial natriuretic peptide, brain natriuretic peptide and creatine kinase through an NF-κB signalling pathway (Rothwell *et al*., 2006). Furthermore, animal models of cardiac hypertrophy associated with fibrosis (diabetes, pressure overload and heart failure) demonstrate elevated tissue levels of resistin compared to non-fibrosing hypertrophy (volume overload) where resistin is minimally or not elevated (Chemaly *et al.*, 2013). Resistin is expressed locally in infarct as opposed to remote myocardial areas (Chemaly *et al.*, 2013). In cardiac tissue from animals with pressure and volume overload, resistin inversely correlates with mRNA levels of angiotensin II receptor type 1. Further, an increased resistin expression occurs in cardiac myocytes and fibroblasts in response to neurohormonal stimuli (Chemaly *et al.*, 2013).

1.3.3.2 Resistin and cardiovascular events

At the clinical level, several studies have demonstrated associations between high circulating resistin concentrations and the development or severity of cardiovascular disease, including CAD, heart failure and stroke. With respect to disease of the coronary vasculature, plasma resistin concentrations are increased in CHD and MI (Pischon *et al.*, 2005; McTernan *et al.*, 2003; Korah *et al.*, 2011; Erer *et al.*, 2014). Moreover, in 26,490 healthy individuals, a relative risk of 2.09 for the development of MI was noted in those with the highest quartile of resistin concentrations (Weikert *et al.*, 2008). Higher plasma resistin concentrations predict not only the development of, but also the severity of CAD (Reilly *et al.*, 2005) and all-cause mortality in patients with acute MI (Lee *et al.*, 2007). In addition, circulating resistin concentrations are a

strong independent predictor of major adverse cardiac and cerebrovascular events in patients with stable multi-vessel coronary artery disease (Krecki *et al.*, 2011). A meta-analysis of prospective studies noted an association between elevated concentrations of resistin and increased mortality risk in high risk patients with diabetes mellitus and CAD (Fontana *et al.*, 2015). Further, resistin concentrations are independent predictors of recurrent coronary events following hospitalisation for acute coronary syndrome (Khera *et al.*, 2015).

As with relationships between resistin concentrations and CHD, strong independent relationships have been noted between circulating resistin concentrations and heart failure. In this regard, the Framingham Heart Study demonstrated that increased circulating resistin concentrations predict incident heart failure in 2739 offspring of the original Framingham study (Frankel *et al.*, 2009; Levy et al.,2010). Several studies published at a similar time or soon thereafter have similarly demonstrated that independent of conventional risk factors, circulating resistin concentrations predict the development of heart failure (Kang *et al.*, 2011; Butler *et al.*, 2009). Large cohorts studies have not only demonstrated that resistin concentrations predict incident heart failure; but in addition, increase the risk for heart failure hospitalization (Frankel *et al.*, 2009; Butler *et al.*, 2009) and all-cause mortality (Zhang *et al.*, 2010). Serum resistin concentrations are not only increased in patients with heart failure, but are also associated with an increased severity of heart failure (Takeishi *et al.*, 2007). Further, serum resistin concentrations are associated with a higher mortality rate in patients with heart failure and a reduced ejection fraction (Benjamin *et al.*, 2017).

As with CHD and heart failure, circulating resistin concentrations predict an increased risk for stroke (León *et al.*,2014) with this relationship noted for ischaemic stroke including lacunar and atherothrombotic infarction (Osawa *et al.*, 2007, 2009). In this regard, the resistin polymorphism at -420 (C→G) is also a risk marker for stroke susceptibility in type II diabetes mellitus (Tsukahara *et al.*, 2009). Resistin may also determine prognosis after first stroke as in patients with atherothrombotic strokes, plasma resistin concentrations are associated with an elevated risk of 5-year mortality (Efstathiou *et al.*, 2007). Plasma resistin concentrations are higher in those with ischaemic stroke and worse neurological deficits (Kochanowski *et al.*, 2012). Associations between resistin concentrations and stroke have not always been consistent. In a large sample (n=26 490) of middle aged subjects although associated with the risk for MI, circulating resistin concentrations did not predict ischaemic stroke (Weikert *et al.*, 2008). It is possible that resistin concentrations predict stroke better in combination with alternative risk factors. In this regard, increased resistin concentrations are more likely to be noted in diabetic patients who have suffered a stroke (Tsukahara *et al.*, 2009; Schwartz and Lazar, 2011). Elevated resistin concentrations have been reported to be a risk factor for cardiovascular disease and all-cause mortality in diabetic patients (Menzaghi *et al.*, 2013). The combination of high circulating resistin concentrations and the presence of either diabetes

mellitus or hypertension was noted to increase the risk of ischaemic stroke (Chin *et al.*, 2018; Osawa *et al.*, 2009). In the Women's Health Initiative Observational study cohort, in 972 women circulating resistin concentrations were associated with an increased risk of incident ischaemic stroke in menopausal women (Rajpathak *et al.*, 2011). Alternative risk factors are not a prerequisite for the impact of resistin on events. Circulating resistin concentrations add incremental value to traditional risk factors in predicting ischaemic stroke (Prugger *et al.*, 2013).

To some extent relationships between resistin concentrations and cardiovascular events may be mediated by insulin resistance as the treatment of diabetes mellitus may result in decreased plasma resistin concentrations (Bajaj *et al.*, 2004). Several studies have failed to show any association between circulating resistin concentrations and insulin resistance (Heilbronn *et al.*, 2004; Lee *et al.*, 2003). The inflammatory effects of resistin may also explain relationships with cardiovascular events. In the Health, Aging, and Body Composition Study of 3044 subjects resistin concentrations predicted cardiovascular events independently of clinical risk factors and metabolic markers, but these effects were attenuated by adjustments for the presence of inflammatory markers (Gencer et al.,2016).

1.3.3.3 End-organ effects of resistin

To better understand the ability of risk factors to predict cardiovascular events, it is important to understand not only the molecular, cellular and neurohumoral mechanisms involved, but also the structural and functional changes in the cardiovascular system that mediate the impact of these risk factors on events. The data discussed in the sections below are summarized in Figure 1.2 for easy reference. Although this can often be demonstrated in preclinical studies, associations in human studies are equally necessary to support a role for a risk factor. Preclinical information often does not translate at a clinical level. Although relationships between obesity, insulin resistance or associated metabolic abnormalities and end organ effects have been well described, the relationships between circulating adipokines and end organ changes have been less well researched. If clear independent relationships between resistin concentrations and LVH, or LV function are demonstrated then this will provide insights into the possible mechanisms that explain the ability of resistin concentrations to predict heart failure and to some degree CHD. Similarly, if clear independent relationships between resistin concentrations and aortic dysfunction are demonstrated then this will provide insights into the possible mechanisms that explain the ability of resistin concentrations to predict CHD or stroke. If clear independent relationships between resistin concentrations and eGFR are demonstrated then this will provide insights into the possible mechanisms that may explain any cardiovascular event or the development of end stage renal failure.

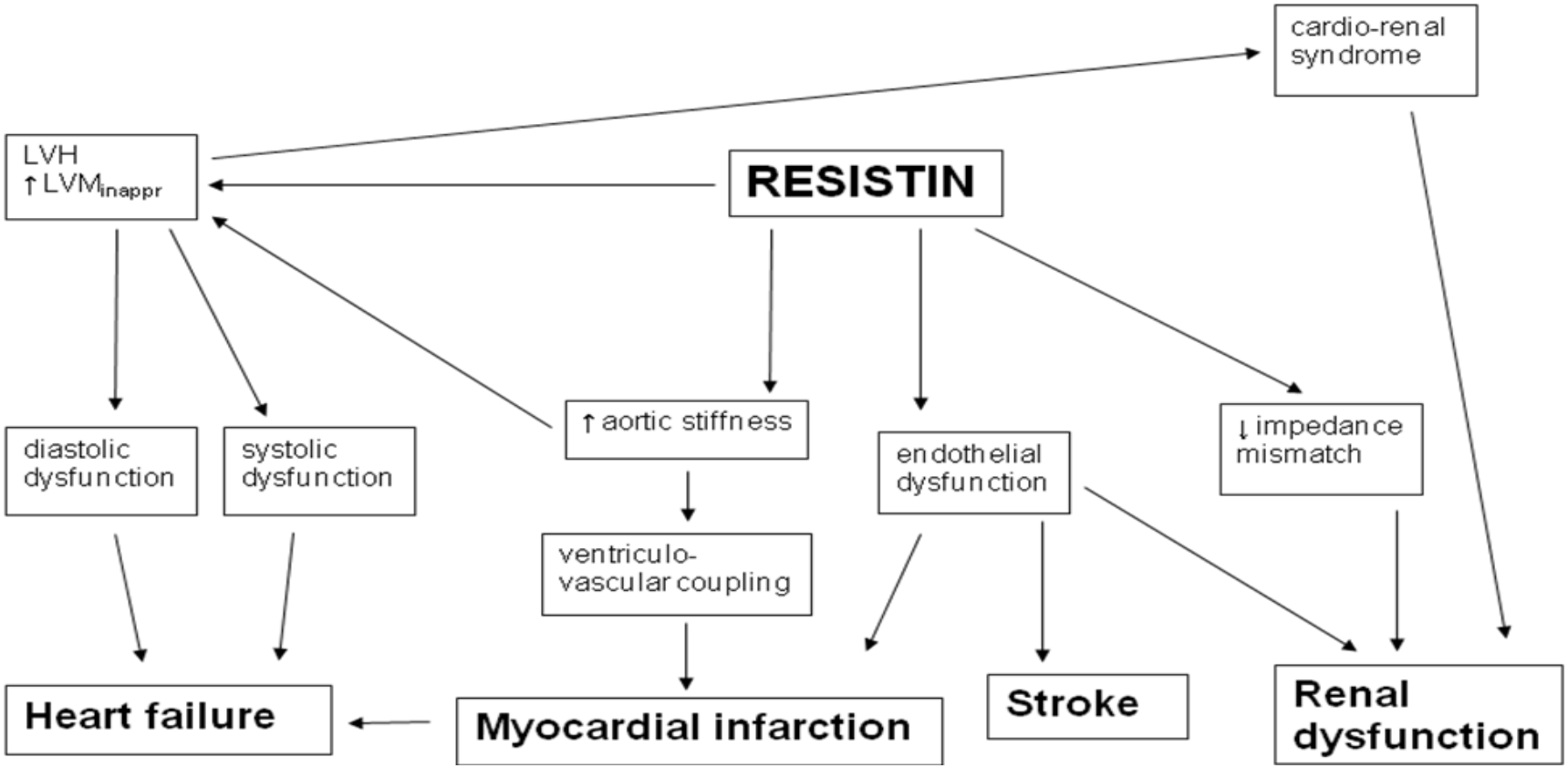


Figure 1.2. Schematic of proposed impacts of resistin on cardiovascular system.

1.3.3.3.1 Resistin and left ventricular hypertrophy and function

Associations between circulating resistin concentrations and LVM and myocardial function in humans were equivocal (Akinci *et al.*, 2013; McManus *et al.*, 2012; Allison *et al.*, 2013). Relationships with LVM had been demonstrated in a small study sample of children (Akinci *et al.*, 2013), but independent relationships were not reported in large adult population samples (McManus *et al.*, 2012; Allison *et al.*, 2013). Prior community-based studies that had failed to show independent associations between circulating resistin concentrations and LVM (McManus *et al.*, 2012; Allison *et al.*, 2013) had not assessed whether these relationships occur with LVM_{inappr}. Indeed, as discussed in aforementioned sections, increases in LVM_{inappr}, rather than LVMI are associated with LV ejection fraction (Woodiwiss *et al.*, 2012). It is possible that the transition to heart failure predicted by LVM is mediated by increases in LVM_{inappr} and consequently decreases in systolic function. Further, even in large samples only modest independent relationships between circulating resistin concentrations and LV systolic function were reported (McManus *et al.*, 2012; Allison *et al.*, 2013). In these prior studies these relationships showed either only trend effects ($p=0.04$) despite a large study sample ($n=2615$) (McManus *et al.*, 2012), or were not adjusted for confounders such as BP and diabetes mellitus (Allison *et al.*, 2013). In addition, blood adipokine concentrations were not contemporaneous with echocardiographic assessments (McManus *et al.*, 2012). I assessed whether independent relationships between circulating resistin concentrations and LVM, LVM_{inappr}, LV hypertrophy or LV function occur in a relatively large community-based sample with prevalent obesity and never-treated hypertension. This study was published in the *International Journal of Cardiology* (Norman *et al.*, 2015) and the findings and the implications thereof are described in chapter 2 of the present thesis.

1.3.3.3.2 Resistin and aortic dysfunction

One small ($n=271$) case-control study suggested that circulating resistin concentrations are associated with increases in brachial-ankle PWV (Yang *et al.*, 2009). In contrast, in a large ($n=749$) cross-sectional study conducted in a sample with a high prevalence of antihypertensive treatment, circulating resistin concentrations were inversely related to aortic PWV (Windham *et al.*, 2010), and in a small study ($n=141$) conducted in treated hypertensives, circulating resistin concentrations failed to predict changes in PWV over 24-months of follow-up (Youn *et al.*, 2013). The presence of antihypertensive treatment (Yang *et al.*, 2009; Windham *et al.*, 2010; Youn *et al.*, 2013), small study samples (Yang *et al.*, 2009; Youn *et al.*, 2013), and the use of brachial-ankle PWV measurements (Yang *et al.*, 2009), which assess stiffness of large and medium-sized arteries, are likely to have confounded the results of previous studies (Yang *et*

al., 2009; Windham *et al.*, 2010; Youn *et al.*, 2013). The assessment of independent relationships between circulating resistin concentrations and aortic PWV or wave reflection which occur in a relatively large community-based sample with prevalent obesity and never-treated hypertension, have been published in the *Journal of Hypertension* (Norman *et al.*, 2016), and the findings and the implications thereof are described in chapter 3. Another study demonstrating a similar association between circulating resistin concentrations and aortic PWV in patients with coronary artery disease, supports the view that the ability of resistin concentrations to predict MI is in-part mediated by increases in aortic stiffness (Wang *et al.*, 2017).

1.3.3.3.3 Resistin and glomerular filtration rate

Markers of inflammation are associated with a rise in serum creatinine concentrations and a decline in estimated GFR (Fried, 2004). Resistin is one such circulating inflammatory marker that is increased in concentration in renal disease. Circulating resistin concentrations are inversely associated with eGFR and directly correlated with urinary micro-albumin excretion in patients with both end-stage and mild-to-moderate renal disease (Axelsson and Stenvinkel, 2008; Axelsson, *et al.*, 2006; Risch *et al.*, 2007; Menzaghi *et al.*, 2012; Zhang *et al.*, 2015; Moreno *et al.*, 2015) and the relationships with GFR have been demonstrated using not only equations applied to serum creatinine concentrations, but also to serum Cystatin C concentrations (Zhang *et al.*, 2015). Resistin concentrations are inversely related to the proportion of individuals with chronic kidney disease as defined by an eGFR<60 ml/min/1.73m^2 (Moreno *et al.*, 2015). Although several studies have suggested that increases in resistin concentrations in chronic kidney disease may be attributed to a decreased renal excretion of resistin (Axelsson and Stenvinkel, 2008; Nusken *et al.*, 2006), resistin concentrations are independently related to eGFR in untreated, non-diabetic hypertensives subjects without chronic kidney disease and independent of alternative factors responsible for decreases in GFR (Ellington *et al.*, 2007; Dimitriadis *et al.*, 2009). Increases in circulating resistin concentrations are perceived as a risk factor for decreases in eGFR rather than as a consequence of reductions in resistin clearance. The adverse effect of resistin on the kidney are thought to be produced by a preglomerular microvasculopathy mediated by increases in the expression of endothelin, adhesion molecules, and matrix metalloproteinases (Dimitriadis *et al.*, 2009). However, there are no large community or population-based studies that have evaluated the extent to which resistin accounts for reductions in eGFR as compared to obesity *per se*, the metabolic abnormalities associated with obesity including insulin resistance or the haemodynamic effects of obesity, or resistin *per se*. If resistin accounts for aortic dysfunction, then resistin may mediate renal effects through a reduction in the impedance mismatch

between the aorta and smaller arteries in the kidney. Moreover, the extent to which obesity, insulin resistance and increases in pro-inflammatory adipokines such as circulating resistin either alone or together account for reductions in GFR as compared to traditional risk factors such as hypertension or diabetes mellitus has not been reported on.

1.3.3.4 Are relationships between resistin and end-organ measures determined by alternative factors?

Through effects that are independent of pulsatile load in the aorta, increases in aortic stiffness may reduce the normally high impedance mismatch that exists between the aorta and distal vessels, enhance pulsatile flow in distal vessels and hence cause damage to microvascular beds that normally show pulsatile flow such as the kidney. An important question that arises is whether the consistent relationships that have been described between resistin concentrations and eGFR can be ascribed to an enhanced aortic stiffness. Increases in aortic stiffness can also cause LVH or dysfunction without altering aortic pulsatile loads. This is thought to occur through ventricular-vascular coupling. A further important question that arises is whether relationships between resistin concentrations and LV structure or function can be accounted for by an enhanced aortic stiffness and hence ventriculo-vascular coupling. It is well recognized that beyond loading conditions reductions in renal function increases LVM, an effect attributed to the cardio-renal syndrome. The question that arises is whether relationships between resistin concentrations and LV structure or function can be accounted for by an impact of resistin on renal function.

1.3.4 Problem statement

Although there is substantial evidence that obesity has adverse effects on cardiovascular structure and function and hence contributes to cardiovascular events beyond conventional risk factors the exact mechanisms responsible for these effects are unknown. There is nevertheless considerable evidence to show that unlike alternative adipokines which have failed to produce consistent data, the pro-inflammatory adipokine resistin relatively consistently predicts several cardiovascular events including CHD, stroke and heart failure or determines their severity. However, the exact mechanisms of this effect are uncertain. Although there was some suggestion that resistin may be independently associated with several cardiovascular structural and functional changes known to play an important role in the development of events (LVH and dysfunction, and aortic dysfunction), these data were obtained in small study samples and were remarkably inconsistent. The extent to which resistin’s effects

on these cardiovascular structural and functional changes could be attributed to an indirect effect mediated by insulin resistance was unknown. The extent to which the consistent relationship between resistin and eGFR previously described, accounts for variations in eGFR at a community or population level as compared to the impact of alternative traditional risk factors such as hypertension or diabetes mellitus, was uncertain. In addition, whether relationships between resistin and eGFR could be accounted for by an impact of increases in aortic stiffness and hence on the impedance mismatch between the aorta and more distal vessels, was unknown. Further, whether relationships between resistin and LV structure could be accounted for by the impact of the cardio-renal syndrome or ventricular-vascular coupling was similarly unknown.

1.3.5 Aims

In the present thesis I therefore aimed

1. To determine the independent relationship between circulating concentrations of the adipokine resistin and LV structure and function in a large community-based sample with a high prevalence of obesity. These data have been published in the *International Journal of Cardiology*, 2015:196:81-87 and are described and discussed in chapter 2.
2. To determine the independent relationship between circulating concentrations of the adipokine resistin and aortic function in a large community-based sample with a high prevalence of obesity. These data have been published in the *Journal of Hypertension* 2016 Feb: 34: (2) 274-81 and are described and discussed in chapter 3.
3. To determine the extent to which insulin resistance and the pro-inflammatory marker, resistin account for variations in eGFR in a large community-based sample with a high prevalence of obesity. These data are under review in the *Cardiovascular Journal of Africa* and are described and discussed in chapter 4.
3. To determine the extent to which the cardio-renal syndrome or ventricular-vascular coupling account for independent relationship between circulating concentrations of the pro-inflammatory adipokine, resistin and LV structure in a large community-based sample with a high prevalence of obesity. These data are under review in *Biomarkers* and are described and discussed in chapter 5.

To address these aims I performed studies using a population sample of participants recruited from Soweto. In chapter 3 analysis was done in participants who were not receiving treatment for hypertension in order to avoid the possible confounding effects of antihypertensive

theraphyvoid the confounding effects of antihypertensive therapy. The sample size differed depending on what was analyzed as not all measurements were available in all participants.

CHAPTER 2

Independent Associations Between Resistin and Left Ventricular Mass and Myocardial Dysfunction in a Community Sample with Prevalent Obesity.

The data in this chapter have been published in
International Journal of Cardiology 2015; 196:81-87
Glenda Norman, Gavin R Norton, Carlos D Libhaber, Frederic Michel, Olebogeng HI Majane, Aletta ME Millen, Pinhas Sareli, Angela J Woodiwiss.
Independent associations between resistin and left ventricular mass and myocardial dysfunction in a community sample with prevalent obesity.

I was involved in data collection from 2014 until 2019. In addition I retrieved the samples from storage and aliquoted the samples before assisting with running the Elisas for the resistin, CRP and HOMA-IR. Furthermore, I did all the data analysis for the publications using SAS software, version 9.4.

2.0 Abstract

Aims: Although the adipokine resistin may play a role in heart failure, the mechanisms of this effect are uncertain. Relationships with left ventricular mass (LVM) and function are uncertain.
Methods: In 739 randomly selected participants from a community sample (43.6% obese), we assessed relationships between circulating resistin concentrations and LVM index (LVMI), LVM beyond that predicted by stroke work (inappropriate LVMI [LVM_{inappr}]) and systolic and diastolic LV function (echocardiography).
Results: Resistin concentrations were not independently associated with blood pressure (BP). However, resistin concentrations were associated with LVMI (partial r=0.12, p<0.0005), LVM_{inappr} (partial r=0.18, p<0.0001) and LV hypertrophy (partial r=0.13, p<0.001) independent of BP, BMI, the homeostasis model of insulin resistance and additional confounders. Independent relationships between resistin concentrations and LVMI and LVM_{inappr} persisted with further adjustments for C-reactive protein concentrations. Resistin concentration (partial r=-0.12, p<0.002 in all and partial r=-0.15, p<0.0005 in untreated) was the only factor independently associated with LV midwall fractional shortening and these relationships were enhanced at incremental concentrations of CRP. Resistin was not independently associated with transmitral and myocardial tissue Doppler indices of LV diastolic function.
Conclusions: Resistin in-part explains variations in LVM, hypertrophy and myocardial systolic dysfunction, and these effects are independent of insulin resistance and general inflammatory changes.

Key words: obesity, inappropriate left ventricular mass, myocardial systolic function, resistin.

2.1 Introduction

Independent of conventional risk factors and myocardial infarction, obesity predicts the development of heart failure (Kenchaiah *et al* ., 2002; Ingelsson *et al.*, 2005; Bahrami *et al.*, 2008). The mechanisms responsible for this effect are nonetheless uncertain. Although insulin resistance may play an important role in mediating obesity-induced heart failure, insulin resistance accounts for only a minor proportion of these effects (Ingelsson *et al.*, 2005; Bahrami *et al.*, 2008). More recently, several inflammatory substances released from adipocytes (adipocytokines or adipokines) have been suggested to contribute toward the adverse effects of obesity on the heart. However, at present the contribution of resistin, a circulating adipokine which in humans is predominantly expressed in monocytes and macrophages, and which is responsive to inflammatory stimuli (Patel *et al.*, 2003; Lu *et al.*, 200; Kaser *et al.*, 2003), is uncertain.

Cardiomyocyte overexpression of resistin promotes myocardial hypertrophy and dysfunction in mice (Chemaly *et al.*, 2011; Kang *et al.*, 2011; Kim *et al.*, 2008). Furthermore, mice transgenic for the human resistin gene develop worse cardiac function in response to a cardiotoxic intervention and these changes are associated with an increased resistin expression (Schwartz *et al.*, 2013). Moreover, circulating resistin concentrations predict the development of and prognosis in heart failure (Butler *et al.*, 2009; Frankel *et al.*, 2009; Zhang *et al.*, 2011; Wu *et al.*, 2012; Takeishi *et al.*, 2007; Muse *et al.*, 2015). Thus, resistin may in-part explain independent relationships between obesity and heart failure (Kenchaiah *et al.*, 2002; Ingelsson *et al.*, 2005; Bahrami *et al.*, 2008). However, the mechanisms that account for the ability of resistin to predict human heart failure are unclear. In this regard, associations between circulating resistin concentrations and left ventricular mass (LVM) and myocardial function in humans are equivocal (Akinci *et al.*, 2013; McManus *et al.*, 2012; Allison *et al.*, 2013). Indeed, relationships with LVM have been demonstrated in a small study sample of children (Akinci *et al.*, 2013), but independent relationships were not reported in large adult population samples (McManus *et al.*, 2012; Allison *et al.*, 2013). Further, even in large samples only modest independent relationships between circulating resistin concentrations and LV systolic function were reported (McManus *et al.*, 2012; Allison *et al.*, 2013). However, in these studies (McManus *et al.*, 2012; Allison *et al.*, 2013) low average blood pressure (BP) levels suggest that most participants had well-controlled BP on therapy, which is likely to confound relationships with LVM and function; blood adipokine concentrations were not contemporaneous with echocardiographic assessments (McManus *et al.*, 2012); and relationships were not adjusted for BP or diabetes mellitus (Allison *et al.*, 2013). In the present study we therefore aimed to assess whether independent relationships between circulating resistin concentrations

and LVM, LV hypertrophy or LV function occur in a relatively large community-based sample with prevalent obesity and never-treated hypertension.

2.2 Methods

2.2.1 Study group. The present study was conducted according to the principles outlined in the Helsinki declaration. The Committee for Research on Human Subjects of the University of the Witwatersrand approved the protocol (approval numbers: M02-04-72 renewed as M07-04-69 and M12-04-108). Participants gave informed, written consent. The study design has previously been described (Norton *et al.*, 2012; Woodiwiss *et al.*, 2009). Nuclear families of black African descent with siblings older than 16 years were randomly recruited from the South West Township (SOWETO) of Johannesburg, South Africa. Of the 1024 participants recruited, in a sub-study, 739 participants had echocardiography, of whom 554 were not receiving treatment for hypertension. Of the sample 498 had 24-hour ambulatory BP measurements that met with pre-specified quality control criteria (longer than 20 hours and more than 10 and 5 readings for the computation of day and night means, respectively).

2.2.2 Clinical, demographic and anthropometric measurements. Demographic and clinical data were obtained using a standardized questionnaire (Norton *et al.*, 2012; Woodiwiss *et al.*, 2009). Height, weight, waist (WC) and hip circumference were measured using standard approaches and participants were identified as being overweight if their body mass index (BMI) was ≥25 kg/m^2 and obese if their BMI was ≥30 kg/m^2. Central obesity was defined as an enlarged WC (≥88 cm in women and ≥102 cm in men). Laboratory blood tests of renal function, liver function, blood glucose, lipid profiles, hematological parameters, and percentage glycated hemoglobin (HbA1c)(Roche Diagnostics, Mannheim, Germany) were performed. Fasting plasma insulin concentrations were determined from an insulin immulite, solid phase, two-site chemiluminescent immunometric assay (Diagnostic Products Corporation, Los Angeles, CA, USA) and insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) using the formula (insulin [uU/ml] x glucose [mmol/l])/22.5. Diabetes mellitus (DM) or abnormal blood glucose control was defined as the use of insulin or oral hypoglycemic agents or an HbA1c value greater than 6.1% respectively.

2.2.3 Brachial and aortic blood pressure. Brachial BP measurements were obtained by a trained nurse-technician using a standard mercury sphygmomanometer and using SpaceLabs monitors (model 90207) (ambulatory 24-hour BP) as previously described (Woodiwiss *et al.*, 2009). Monitors were programmed to measure BP at 15-minute intervals from 06:00 to 22:00 and at 30-minute intervals from 22:00 to 06:00. Aortic BP was determined using radial applanation tonometry and SphygmoCor software as previously described (Norton *et al.*, 2012). After participants had rested for 15 minutes in the supine position, arterial waveforms at the

radial (dominant arm) artery were recorded during an 8-second period using a high-fidelity SPC-301 micromanometer (Millar Instrument, Inc., Houston, Texas) interfaced with a computer employing SphygmoCor, version 9.0 software (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia). To determine aortic BP, the pulse wave obtained from radial tonometer recordings was calibrated by manual measurement (auscultation) of brachial BP taken immediately before the recordings. The radial pressure waveform was converted into a central (aortic) waveform using a validated generalized transfer function (GTF) incorporated in SphymoCor software.

2.2.4 Echocardiography. Echocardiography was performed as previously described (Norton *et al.*, 2012; Woodiwiss *et al.*, 2009; Woodiwiss *et al.*, 2012). All measurements were recorded and analyzed off-line by experienced investigators whom were unaware of the clinical data of the participants. Left ventricular mass (LVM) was determined using a standard formula (Devereux *et al.*, 1986) and indexed (LVMI) to height$^{1.7}$. Left ventricular hypertrophy was defined as an LVMI≥60 g/m$^{1.7}$ in women and LVMI≥80 g/m$^{1.7}$ in men (Chirinos *et al*., 2010). Stroke volume was evaluated from the difference between LV end diastolic and systolic volumes determined using the Z-derived method (Woodiwiss *et al.*, 2012). Circumferential LV systolic wall stress was calculated as previously described (Woodiwiss *et al.*, 2012). The extent of LVM_{inappr} was determined from predicted LVM (Woodiwiss *et al.*, 2012), where predicted LVM was calculated as 55.37+(6.64 x height$^{2.7}$)+(0.64 x [systolic BP x stroke volume x 0.014]) – (18.07 x gender), where male gender=1 and female gender=2, and where stroke volume was calculated from LV volumes assessed from the Z-derived method. Inappropriate LVM was expressed either as actual-predicted LVM in grams, or % actual LVM/predicted LVM.

Left ventricular ejection fraction (EF)(biplane Simpson) was calculated to determine LV chamber systolic function using a standard formula (Woodiwiss *et al.*, 2012). Endocardial (FSend) (LV systolic chamber function) and midwall (FSmid)(LV systolic myocardial function) fractional shortening were similarly determined as previously described (Woodiwiss *et al.*, 2012). Left ventricular diastolic function was assessed from a pulsed wave Doppler examination of the mitral inflow at rest in all participants and using tissue Doppler indexes (TDI) in 442 participants (Millen *et al.*, 2014). Pulse wave Doppler recordings of transmitral velocity are obtained with the sample volume at the tip of the mitral valve in the apical 4-chamber view. Transmitral velocity measurements are obtained during the early (E) and late (atrial-A) period of left ventricular diastolic inflow and expressed as the E/A ratio. To perform TDI, the velocity of myocardial tissue lengthening at the level of the mitral annulus was recorded in the apical four-chamber view. The sample volume was positioned at the septal and lateral corners of the mitral annulus. To determine diastolic function using TDI, peak tissue velocity during early (e') diastole was measured and data are expressed as the E/e' ratio (an index of LV filling pressures).

2.2.5 Inflammatory markers and adipokines. Blood samples were centrifuged and immediately stored at -80^{o}C. Plasma concentrations of human resistin, adiponectin and high-sensitivity (range 0.01 to 50 ng/ml) C-reactive protein (CRP) concentrations were measured using enzyme-linked immunosorbent assays (Quantikine, R&D Systems Inc, Minneapolis, MN, USA). The resistin assay had a lower detection limit of 0.026 ng/ml and intra-assay and inter-assay coefficients of variation ranging from 3.8 to 5.3% and 7.8 to 9.2% respectively. The adiponectin assay had a lower detection limit of 0.246 ng/ml and intra-assay and inter-assay coefficients of variation ranging from 2.5 to 4.7% and 5.8 to 6.9% respectively. The CRP assay had a mean lower detection limit of 0.010 ng/ml and intra-assay and inter-assay coefficients of variation ranging from 3.8 to 8.3% and 6.0 to 7.0% respectively. CRP concentrations could not be determined on 27 participants whose concentrations were below the lower level of detection.

2.2.6 Data analysis. Database management and statistical analyses were performed with SAS software, version 9.1 (SAS Institute Inc., Cary, NC, USA). Continuous data are reported as mean±SD. Unadjusted means and proportions were compared by the large-sample z-test and the χ^2-statistic, respectively. As resistin concentrations were positively skewed (skewness=2.59, kurtosis=12.9; Shapiro-Wilk's statistic=0.80, p<0.0001) resistin concentrations were log transformed. Log transformation of resistin concentrations resulted in an improved distribution (skewness=0.15, kurtosis=0.37; Shapiro-Wilk's statistic=1.00). Moreover, as adiponectin concentrations were positively skewed (skewness=1.51, kurtosis=3.02, Shapiro-Wilk's statistic=0.88, p<0.0001) adiponectin concentrations were also log transformed. Log transformation of adiponectin concentrations resulted in an improved distribution (skewness=-0.63, kurtosis=0.79; Shapiro-Wilk's statistic=0.98). As CRP concentrations and HOMA-IR were positively skewed, these were also log transformed and log transformation resulted in an improved distribution (data not shown).

Bivariate correlations for continuous data were assessed from Pearson's correlation coefficients. Independent relationships were assessed from mulltivariate linear regression analysis with appropriate adjustors. Probability values were further adjusted for non-independence of family members using the method of maximum likelihood as implemented by the mixed procedure as defined in the SAS package. As antihypertensive treatment may influence LVM and function, sensitivity analysis was conducted in untreated participants.

2.3 Results

2.3.1 Participant characteristics. As compared to those without echocardiographic or TDI measurements, those with echocardiographic or TDI measurements were modestly older, and had only a moderately greater BMI, WC, % obese, % treated for hypertension and % treated for DM (Tables 2.1 and 2.2). In all participants and in participants whom had never received

antihypertensive therapy, a high proportion was overweight or obese and had central (abdominal) obesity (Table 2.3). Furthermore, a high proportion of all participants and of participants whom had never received antihypertensive therapy was hypertensive (Table 2.3). 47% of participants had LVH. Of the participants who were receiving treatment for hypertension, 80.0% were receiving diuretic agents; 18.9% blockers of the renin-angiotensin aldosterone system; 15.7% calcium channel blockers and only 1.6% were receiving beta blockers.

2.3.2 Relationships between adiposity indexes and circulating adipokine concentrations. In multivariate adjusted models, BMI, WC and waist-to-hip ratio were directly associated with CRP concentrations as well as HOMA-IR, but inversely associated with adiponectin concentrations (Table 2.4). BMI and WC were only modestly associated with resistin concentrations in untreated participants (Table 2.4).

2.3.3 Relationships between circulating adipokine concentrations and HOMA-IR or CRP. With adjustments for age, sex, and DM or HbA1c>6.1% (except for HOMA-IR), regular smoking, regular alcohol intake and treatment for hypertension, adiponectin concentrations were inversely associated with CRP concentrations (partial r=-0.12, p<0.005) and HOMA-IR (partial r=-0.16, p<0.0001). With adjustments for age, sex, and DM or HbA1c>6.1% (except for HOMA-IR), regular smoking, regular alcohol intake and treatment for hypertension, resistin concentrations were directly associated with CRP concentrations (partial r=0.18, p<0.0001), but not HOMA-IR (partial r=0.006, p=0.87).

2.3.4 Associations between adipokines and BP. On bivariate analysis, office, 24-hour and aortic BP were correlated with CRP (p<0.0005 for all), but not with resistin (p>0.14) concentrations. Only a trend for a bivariate correlation was noted between office DBP and adiponectin concentrations (r=-0.09, p<0.05). With adjustments for confounders, no independent associations were noted between BP and either CRP or resistin concentrations (Table 2.5). Multivariate adjusted direct relationships between adiponectin concentrations and office, aortic and 24-hour SBP were noted (Table 2.5).

2.3.5 Associations between adipokines and LVM. On bivariate analysis adiposity indexes, circulating resistin concentrations, CRP concentrations, and HOMA-IR, but not circulating adiponectin concentrations were associated with LVMI and LVM_{inappr} (p<0.0001 for all). In stepwise regression models with the inclusion of resistin, but not with the inclusion of adiponectin in the models; BMI, HOMA-IR, and resistin concentrations were directly associated with LVMI and LVM_{inappr} (Table 2.6). With adjustments including BMI, LVM_{inappr} increased across octiles of resistin concentrations with the highest 2 octiles showing markedly greater LVM_{inappr} than the lowest 2 octiles of resistin concentrations (Figure 2.1). Similar relationships were noted with WC rather than BMI in the models (Table 2.7). Independent of confounders including age, sex, BMI, SBP, regular smoking, regular alcohol consumption, treatment for hypertension and log HOMA-IR, resistin concentrations were also independent associated with LVH (partial

Table 2.1. Characteristics of study participants with and without echocardiography.

Echocardiography→	With (n=739)	Without (n=285)
% Women	63.3	63.2
Age (years)	44.6±18.1	41.3±18.4*
Body mass index (kg/m^2)	29.5±7.8	28.5±7.9
Waist circumference (cm)	91.1±16.8	88.3±16.5*
% Overweight/obese	23.7/43.6	22.8/36.1*
% Central obesity	42.6	40.4
% Hypertension	45.3	40.7
% Treated for hypertension	25.0	16.5*
% Treated for DM	8.8	4.2*
% DM or HbA1c >6.1%	26.3	23.9
% Regular smoking	15.0	16.1
% Regular alcohol	19.6	20.7
Office SBP/DBP (mm Hg)	128±22/84±13	128±23/84±12

All continuous data are expressed as mean±SD. DM, diabetes mellitus, SBP, systolic blood pressure; DBP, diastolic BP. *p<0.05 versus with.

Table 2.2. Characteristics of study participants with and without tissue Doppler indexes (TDI) of left ventricular function.

TDI→	With (n=442)	Without (n=582)
% Women	62.9	63.5
Age (years)	45.2±17.9	42.5±18.4*
Body mass index (kg/m^2)	29.8±8.1	28.8±7.7*
Waist circumference (cm)	91.8±17.4	89.1±16.1*
% Overweight/obese	22.4/45.3	24.3/38.6*
% Central obesity	43.9	40.4
% Hypertension	44.6	43.6
% Treated for hypertension	26.5	19.8*
% Treated for DM	9.9	5.7*
% DM or HbA1c >6.1%	26.5	24.9
% Regular smoking	17.2	13.9
% Regular alcohol	18.8	20.8
Office SBP/DBP (mm Hg)	127±23/84±13	129±23/84±12

All continuous data are expressed as mean±SD. DM, diabetes mellitus, SBP, systolic blood pressure; DBP, diastolic BP. *p<0.05 versus with.

Table 2.3. Characteristics of study participants.

	All (n=739)	Untreated (n=554)
% Women	63.3	58.7
Age (years)	44.6±18.1	40.0±16.1
Body mass index (kg/m^2)	29.5±7.8	28.0±7.4
Waist circumference (cm)	91.1±16.8	87.4±16.1
% Overweight/obese	23.7/43.6	24.4/35.9
% Central obesity	42.6	33.6
% Hypertension	45.3	27.1
% Treated for DM	8.8	2.0
% DM or HbA1c >6.1%	26.3	17.0
% Regular smoking	15.0	17.9
% Regular alcohol	19.6	22.6
Office SBP/DBP (mm Hg)	128±22/84±13	125±21/82±12
Pulse rate (beats/min)	66±11	65±11
24-hour SBP/DBP (mm Hg)	119±15/73±11 (n=498)	117±14/72±10 (n=383)
Aortic SBP (mm Hg)	120±22	116±21
LV mass (LVM)(g)	153±53	148±49
LVM index (g/m$^{1.7}$)	67.9±23.7	64.9±21.5
% LVH	46.7	41.5
% Actual LVM/Predicted LVM	134±35	132±33
Actual LVM-predicted LVM (g)	38.0±40.8	34.8±37.2
LV ejection fraction (%)	67±9	67±8
FSend (%)	40.0±8.1	39.7±7.8
FSmid (%)	23.4±5.4	23.4±5.1
E/A	1.29±0.49	1.41±0.48
E/e’	7.66±4.29 (n=442)	7.02±3.68 (n=325)
Adiponection (mg/l)	7.4 (range=0.08-36.6)	7.6 (range=0.08-36.6)
Resistin (ng/ml)	10.6 (range=1.4-83.0)	10.4 (range=1.4-62.2)
CRP (mg/l)	3.87 (range=0.04-53.8) (n=712)	3.05 (range=0.04-53.8) (n=529)

Continuous data are expressed as mean±SD or median (range). DM, diabetes mellitus, SBP, systolic blood pressure; DBP, diastolic BP; FSend, endocardial fractional shortening; FSmid, midwall fractional shortening; E/A, early-to-atrial transmitral blood flow velocity; e’, myocardial lateral wall tissue velocity in early diastole.

Table 2.4. Relationships between indices of adiposity and circulating concentrations of adipokines or C-reactive protein (CRP), or the homeostasis model of insulin resistance (HOMA-IR) in separate models.

	All participants† (n=739)		Untreated participants† (n=554)	
	Partial r* (CI)	p value	Partial r* (CI)	p value
Body mass index versus				
Log resistin	0.03 (-0.04 to 0.10)	=0.41	0.09 (0.01 to 0.17)	<0.05
Log adiponectin	-0.22 (-0.29 to -0.15)	<0.0001	-0.20 (-0.28 to -0.12)	<0.0001
Log CRP	0.32 (0.25 to 0.38)	<0.0001	0.31 (0.23 to 0.38)	<0.0001
Log HOMA-IR	0.16 (0.09 to 0.23)	<0.0001	0.17 (0.08 to 0.25)	<0.0001
Waist circumference versus				
Log resistin	0.03 (-0.04 to 0.11)	=0.39	0.09 (0.00 to 0.17)	<0.05
Log adiponectin	-0.28 (-0.35 to -0.22)	<0.0001	-0.25 (-0.32 to -0.16)	<0.0001
Log CRP	0.30 (0.23 to 0.36)	<0.0001	0.29 (0.21 to 0.37)	<0.0001
Log HOMA-IR	0.22 (0.14 to 0.28)	<0.0001	0.21 (0.13 to 0.29)	<0.0001
Waist-to-hip ratio versus				
Log resistin	0.06 (-0.02 to 0.13)	=0.12	0.08 (-0.006 to 0.17)	=0.07
Log adiponectin	-0.24 (-0.31 to -0.16)	<0.0001	-0.22 (-0.30 to -0.13)	<0.0001
Log CRP	0.12 (0.05 to 0.20)	<0.005	0.12 (0.03 to 0.20)	<0.01
Log HOMA-IR	0.22 (0.15 to 0.29)	<0.0001	0.18 (0.10 to 0.27)	<0.0001

*Adjustments are for age, sex, diabetes mellitus or an HbA1c>6.1% (except for log HOMA-IR), regular smoking, regular alcohol intake and treatment for hypertension (in all). †For CRP, n=712 for all participants and 529 for untreated participants.

Table 2.5. Relationships between circulating adipokine concentrations or inflammatory markers and blood pressure (BP) in a community sample.

	All participants			Untreated participants		
	n=	Partial r* (CI)	p value	n=	Partial r* (CI)	p value
Log resistin versus						
Office SBP	739	0.005 (-0.07 to 0.08)	=0.89	554	0.01 (-0.07 to 0.09)	=0.81
Office DBP	739	-0.06 (-0.13 to 0.01)	=0.09	554	-0.06 (-0.14 to 0.02)	=0.16
24-hour SBP	498	-0.004 (-0.09 to 0.08)	=0.93	383	-0.01 (-0.11 to 0.09)	=0.81
24-hour DBP	498	-0.08 (-0.16 to 0.01)	=0.09	383	-0.06 (-0.16 to 0.04)	=0.23
Aortic SBP	739	-0.03 (-0.10 to 0.05)	=0.50	554	-0.02 (-0.10 to 0.07)	=0.66
Log adiponectin versus						
Office SBP	739	0.14 (0.07 to 0.21)	<0.0005	554	0.15 (0.07 to 0.23)	<0.001
Office DBP	739	0.003 (-0.07 to 0.08)	=0.92	554	0.01 (-0.07 to 0.09)	=0.80
24-hour SBP	498	0.10 (0.01 to 0.19)	<0.05	383	0.10 (0.00 to 0.20)	=0.05
24-hour DBP	498	0.04 (-0.05 to 0.13)	=0.34	383	0.03 (-0.07 to 0.13)	=0.56
Aortic SBP	739	0.13 (0.06 to 0.20)	<0.001	554	0.14 (0.06 to 0.22)	<0.002
Log C-reactive protein versus						
Office SBP	712	-0.04 (-0.12 to 0.03)	=0.26	529	0.001 (-0.08 to 0.09)	=0.98
Office DBP	712	0.01 (-0.06 to 0.08)	=0.78	529	0.04 (-0.04 to 0.13)	=0.33
24-hour SBP	487	-0.01 (-0.10 to 0.08)	=0.85	372	0.01 (-0.09 to 0.11)	=0.83
24-hour DBP	487	0.02 (-0.07 to 0.11)	=0.66	372	0.04 (-0.07 to 0.14)	=0.50
Aortic SBP	712	-0.04 (-0.12 to 0.03)	=0.26	529	-0.002 (-0.09 to 0.08)	=0.96

SBP, systolic BP, DBP, diastolic BP. *Adjusted for age, sex, body mass index, diabetes mellitus or an HbA1c>6.1%, regular smoking, regular

alcohol intake and treatment for hypertension (in all).

Table 2.6. Relationships between circulating adipokine concentrations and left ventricular mass (LVM) in a community sample.

	All participants (n=739)		Untreated participants (n=554)	
	Partial r* (CI)	p value	Partial r* (CI)	p value
Models with resistin				
LVMI versus				
BMI	0.34 (0.27 to 0.42)	<0.0001	0.29 (0.21 to 0.37)	<0.0001
Log resistin	0.12 (0.05 to 0.19)	<0.0005	0.08 (0.005 to 0.16)	<0.05
SBP	0.26 (0.19 to 0.33)	<0.0001	0.38 (0.30 to 0.46)	<0.0001
Log HOMA-IR	0.11 (0.04 to 0.18)	<0.002	0.07 (-0.01 to 0.15)	=0.07
LVM_{inappr} versus				
BMI	0.31 (0.24 to 0.38)	<0.0001	0.33 (0.25 to 0.41)	<0.0001
Log resistin	0.18 (0.11 to 0.25)	<0.0001	0.13 (0.05 to 0.21)	<0.002
Log HOMA-IR	0.09 (0.02 to 0.16)	<0.005	0.07 (-0.01 to 0.15)	=0.08
Models with adiponectin				
LVMI versus				
BMI	0.34 (0.27 to 0.42)	<0.0001	0.29 (0.21 to 0.37)	<0.0001
Log adiponectin	0.08 (0.01 to 0.15)	<0.01	0.10 (0.02 to 0.18)	<0.01
SBP	0.26 (0.19 to 0.33)	<0.0001	0.38 (0.30 to 0.46)	<0.0001
Log HOMA-IR	0.11 (0.04 to 0.18)	<0.001	0.08 (0.004 to 0.16)	<0.05
LVM_{inappr} versus				
BMI	0.31 (0.24 to 0.38)	<0.0001	0.33 (0.25 to 0.41)	<0.0001
Log adiponectin	0.04 (-0.04 to 0.11)	=0.30	0.05 (-0.03 to 0.13)	=0.19
Log HOMA-IR	0.10 (0.03 to 0.17)	<0.005	0.07 (-0.01 to 0.15)	=0.10

LVMI, LVM indexed to $height^{1.7}$; LVM_{inappr}, inappropriate LVM; BMI, body mass index; SBP, office systolic blood pressure; HOMA-IR, homeostasis model of insulin resistance. *Additional factors included in the stepwise regression models include age, sex, regular smoking, regular alcohol intake, treatment for hypertension (in all) and office SBP in models for both LVMI and LVM_{inappr}.

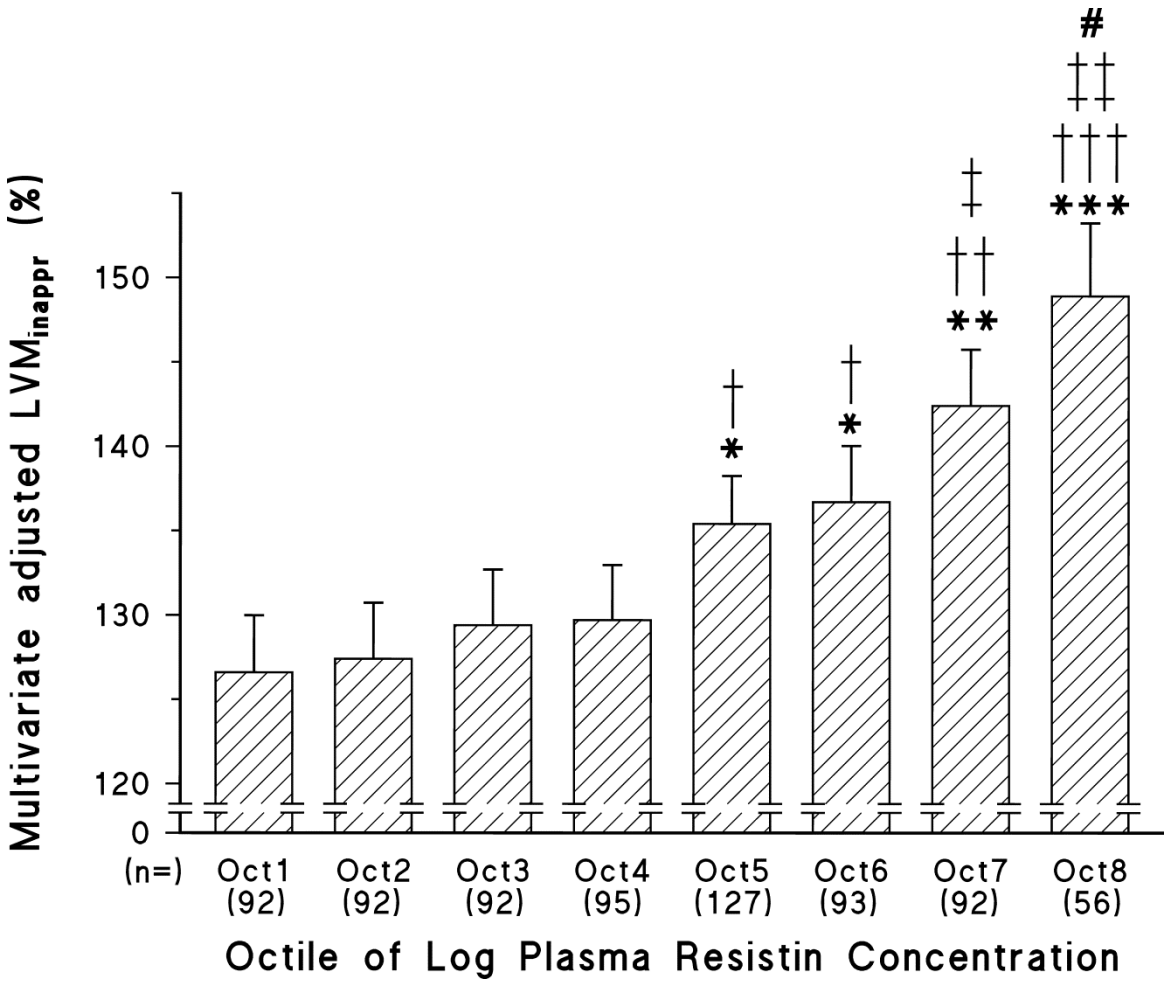


Figure 2.1. Multivariate adjusted left ventricular mass (LVM) beyond that predicted by stroke work (inappropriate LVM or LVM_{inappr}) across octiles (Oct) of log resistin concentrations. Adjustments are for age, sex, body mass index, office systolic blood pressure, treatment for hypertension, diabetes mellitus or an HbA1c>6.1%, regular smoking, and regular alcohol intake. *p<0.05, **p<0.001, ***p<0.0001 vs octile 1; †p<0.05, ††p<0.001, †††p<0.0001 vs octile 2; ‡p<0.01, ‡‡p<0.001 vs octiles 3 and 4; #p<0.05 vs octile 5.

Table 2.7. Relationships between circulating adipokine concentrations and left ventricular mass (LVM) in a community sample with waist circumference (WC) in the models.

	All participants (n=739)		Untreated participants (n=554)	
	Partial r* (CI)	p value	Partial r* (CI)	p value
		Models with resistin		
LVMI versus				
WC	0.36 (0.29 to 0.43)	<0.0001	0.26 (0.18 to 0.34)	<0.0001
Log resistin	0.10 (0.03 to 0.17)	<0.005	0.07 (-0.01 to 0.15)	=0.07
SBP	0.23 (0.16 to 0.30)	<0.0001	0.38 (0.30 to 0.46)	<0.0001
Log HOMA-IR	0.10 (0.03 to 0.17)	<0.005	0.07 (-0.01 to 0.15)	=0.08
LVM_{inappr} versus				
WC	0.30 (0.23 to 0.37)	<0.0001	0.30 (0.22 to 0.38)	<0.0001
Log resistin	0.18 (0.11 to 0.25)	<0.0001	0.12 (0.04 to 0.20)	<0.005
Log HOMA-IR	0.07 (0.01 to 0.14)	<0.05	0.05 (-0.03 to 0.13)	=0.22
		Models with adiponectin		
LVMI versus				
WC	0.36 (0.29 to 0.43)	<0.0001	0.26 (0.18 to 0.34)	<0.0001
Log adiponectin	0.08 (0.01 to 0.15)	<0.05	0.08 (0.01 to 0.16)	<0.05
SBP	0.23 (0.16 to 0.30)	<0.0001	0.38 (0.30 to 0.46)	<0.0001
Log HOMA-IR	0.10 (0.03 to 0.17)	<0.005	0.08 (0.00 to 0.16)	<0.05
LVM_{inappr} versus				
WC	0.30 (0.23 to 0.37)	<0.0001	0.30 (0.22 to 0.38)	<0.0001
Log adiponectin	0.06 (-0.01 to 0.13)	=0.10	0.06 (-0.02 to 0.14)	=0.12
Log HOMA-IR	0.07 (0.01 to 0.14)	<0.05	0.05 (-0.03 to 0.13)	=0.20

LVMI, LVM indexed to $height^{1.7}$; LVM_{inappr}, inappropriate LVM; SBP, clinic systolic blood pressure; HOMA-IR, homeostasis model of insulin resistance. *Additional factors included in the stepwise regression models include age, sex, regular smoking, regular alcohol intake, treatment for hypertension (in all) and office SBP in models for both LVMI and LVM_{inappr}.

Table 2.8. Relationships between circulating C-reactive protein (CRP) concentrations and left ventricular mass (LVM) in a community sample.

	All participants (n=712)		Untreated participants (n=529)	
	Partial r* (CI)	p value	Partial r* (CI)	p value
LVMI versus				
BMI	0.34 (0.27 to 0.41)	<0.0001	0.27 (0.18 to 0.35)	<0.0001
Log CRP	-0.006 (-0.08 to 0.07)	=0.88	-0.01 (-0.09 to 0.08)	=0.72
SBP	0.26 (0.19 to 0.33)	<0.0001	0.39 (0.30 to 0.47)	<0.0001
Log HOMA-IR	0.11 (0.04 to 0.18)	<0.001	0.07 (-0.01 to 0.15)	=0.06
LVM_{inappr} versus				
BMI	0.29 (0.22 to 0.36)	<0.0001	0.31 (0.22 to 0.39)	<0.0001
Log CRP	0.05 (-0.02 to 0.12)	=0.15	0.05 (-0.03 to 0.13)	=0.21
Log HOMA-IR	0.11 (0.04 to 0.18)	<0.005	0.07 (-0.01 to 0.15)	=0.07

LVMI, LVM indexed to height$^{1.7}$; LVM_{inappr}, inappropriate LVM; BMI, body mass index; SBP, clinic systolic blood pressure; HOMA-IR, homeostasis model of insulin resistance. *Additional factors included in the stepwise regression models include age, sex, regular smoking, regular alcohol intake, treatment for hypertension (in all) and office SBP in models for both LVMI and LVM_{inappr}.

r=0.13, p<0.001). Although adiponectin concentrations were directly associated with LVMI, no independent associations were noted with LVM_{inappr} (Tables 2.6 and 2.7). CRP concentrations showed no independent relationships with LVMI or LVM_{inappr} (Table 2.8). Relationships between resistin concentrations and LVM_{inappr} persisted with the inclusion of LVMI, (partial r=0.15, p<0.0001), CRP (partial r=0.20, p<0.0001) or aortic rather than brachial SBP (partial r=0.19, p<0.0001) in the models. Relationships between resistin concentrations and LVMI (partial r=0.14, p<0.0005) or LVH (partial r=0.13, p<0.001) also persisted with the inclusion of CRP concentrations in the models.

2.3.6 Associations between adipokines and LV systolic function. On bivariate analysis no single factor was associated with either EF, or FSend (data not shown). However, resistin (r=-0.12, p<0.005) and a trend for HOMA-IR (r=-0.07, p=0.05) were inversely correlated with FSmid. In multivariate models, with the inclusion of adiposity indexes and additional possible confounders forced into the models, relationships between resistin concentrations and FSmid, but not between HOMA-IR and FSmid persisted (Table 2.9). Neither adiponectin nor CRP concentrations were independently associated with FSmid (Table 2.9). Nevertheless, an interaction between resistin and CRP concentrations was associated with FSmid independent of the individual terms (partial partial r=-0.10, p<0.01). This translated into an incremental increase in the strength (partial r) and magnitude (β-coefficient or slope) of the independent relation between resistin concentration and FSmid across tertiles of CRP concentrations (Figure 2.2).

2.3.7 Associations between adipokines and LV diastolic function. On bivariate analysis adipokine concentrations, adiposity indexes, HOMA-IR, and CRP concentrations were all associated with E/A (p<0.005 for all). Although adiposity indexes, HOMA-IR and CRP concentrations were correlated with E/e' (p<0.05 for all), neither resistin nor adiponectin concentrations were correlated with E/e' (p>0.28). In multivariate models, although WC and HOMA-IR (for E/A) were, neither resistin, nor adiponectin concentrations were independently associated with either E/A (Table 2.10) or E/e' (Table 2.11).

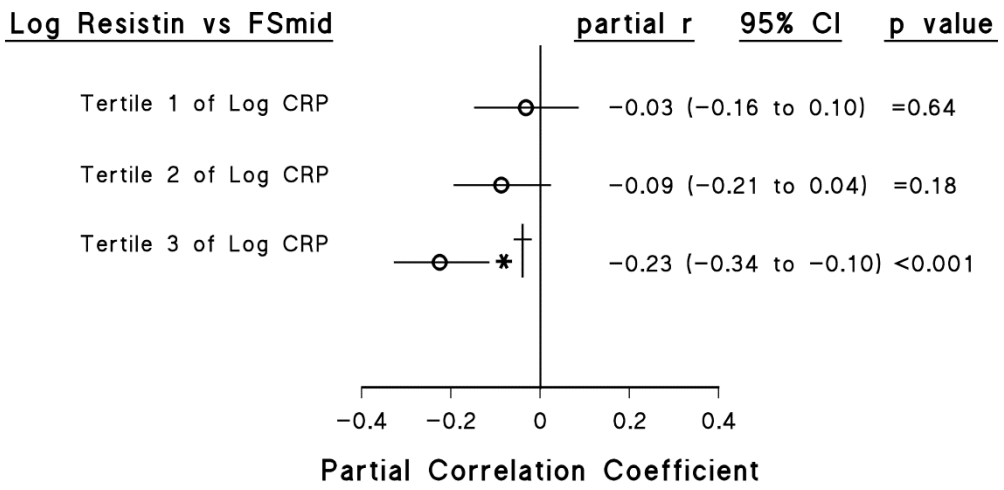
2.4 Discussion

The main findings of the present study are as follows: In a randomly selected community sample of African ancestry with a high prevalence of obesity and untreated hypertension, circulating resistin, but not adiponectin or CRP concentrations were associated with both LVMI and LVM_{inappr} beyond adiposity indexes, BP, HOMA-IR and additional confounders. Furthermore, circulating resistin concentrations were the only factor consistently and independently associated with variations in myocardial function, as determined from FSmid. Importantly however, the independent relationships between resistin concentrations and FSmid increased across tertiles of CRP concentrations.

Table 2.9. Relationships between circulating adipokine or C-reactive protein (CRP) concentrations and left ventricular midwall fractional shortening (FSmid) in a community sample.

	All participants† (n=739)		Untreated participants† (n=554)	
	Partial r* (CI)	p value	Partial r* (CI)	p value
		Model with resistin		
FSmid versus				
BMI	-0.04 (-0.11 to 0.03)	=0.25	-0.08 (-0.16 to -0.003)	=0.05
Log resistin	-0.12 (-0.19 to -0.05)	<0.002	-0.15 (-0.23 to -0.07)	<0.0005
SBP	0.01 (-0.06 to 0.08)	=0.80	-0.07 (-0.15 to 0.01)	=0.10
Log HOMA-IR	-0.07 (-0.14 to 0.002)	=0.07	-0.04 (-0.12 to 0.04)	=0.39
		Model with adiponectin		
FSmid versus				
BMI	-0.07 (-0.14 to 0.00)	=0.05	-0.11 (-0.19 to -0.03)	<0.02
Log adiponectin	-0.05 (-0.12 to 0.02)	=0.20	-0.05 (-0.13 to 0.03)	=0.25
SBP	0.02 (-0.05 to 0.09)	=0.62	-0.07 (-0.15 to 0.01)	=0.09
Log HOMA-IR	-0.07 (-0.14 to 0.002)	=0.06	-0.04 (-0.12 to 0.04)	=0.33
		Model with CRP		
FSmid versus				
BMI	-0.06 (-0.13 to 0.01)	=0.13	-0.10 (-0.18 to -0.01)	<0.02
Log CRP	-0.03 (-0.10 to 0.04)	=0.42	-0.01 (-0.10 to 0.08)	=0.87
SBP	-0.001 (-0.07 to 0.07)	=0.86	-0.08 (-0.16 to 0.005)	=0.06
Log HOMA-IR	-0.07 (-0.14 to 0.004)	=0.06	-0.03 (-0.12 to 0.06)	=0.43

BMI, body mass index; SBP, office systolic blood pressure; HOMA-IR, homeostasis model of insulin resistance. *Additional factors included in the stepwise regression models include age, sex, regular smoking, regular alcohol intake and treatment for hypertension (in all). †For CRP n=712 for all participants and 529 for untreated participants.



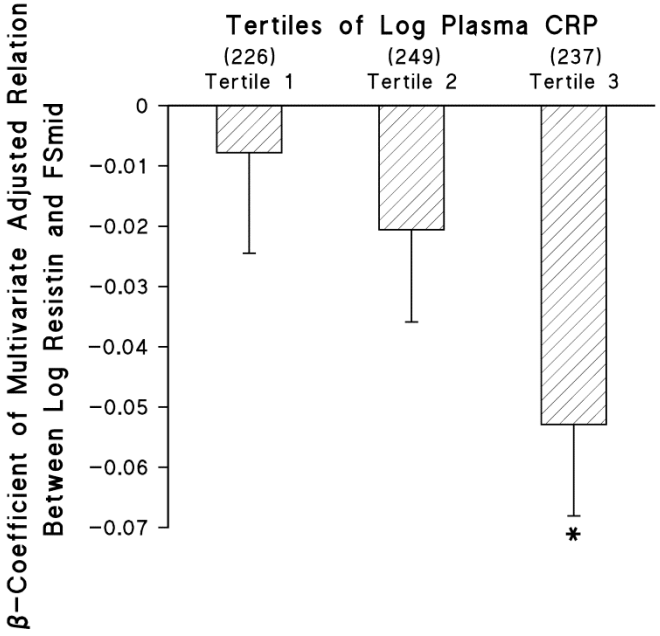


Figure 2.2. Multivariate adjusted relationships between log circulating resistin concentrations and left ventricular midwall fractional shortening (FSmid) across tertiles of C-reactive protein concentrations (CRP). A comparison of the strength of the relationships (partial r) and the magnitude of the effect of resistin on FSmid (β-coefficient) are shown. Adjustments are for age, sex, body mass index, office systolic blood pressure, treatment for hypertension, diabetes mellitus or an HbA1c>6.1%, regular smoking, and regular alcohol intake. *p<0.05 vs tertile 1; †p=0.05 vs tertile 2.

Table 2.10. Relationships between circulating adipokine or C-reactive protein (CRP) concentrations and left ventricular diastolic function as determined from transmitral blood flow velocity measurements in a community sample.

	All participants† (n=739)		Untreated participants† (n=554)	
	Partial r* (CI)	p value	Partial r* (CI)	p value
		Model with resistin		
E/A versus				
WC	-0.13 (-0.20 to -0.06)	<0.0001	-0.10 (-0.18 to -0.02)	<0.005
Log resistin	-0.04 (-0.11 to 0.03)	=0.15	-0.04 (-0.12 to 0.04)	=0.18
DBP	-0.09 (-0.17 to -0.02)	<0.001	-0.08 (-0.16 to -0.01)	<0.02
Log HOMA-IR	-0.07 (-0.14 to -0.01)	<0.01	-0.07 (-0.15 to -0.01)	<0.05
		Model with adiponectin		
E/A versus				
WC	-0.12 (-0.19 to -0.05)	<0.0001	-0.10 (-0.18 to -0.02)	<0.005
Log adiponectin	-0.02 (-0.09 to 0.05)	=0.55	-0.06 (-0.14 to 0.01)	=0.07
DBP	-0.09 (-0.17 to -0.02)	<0.001	-0.08 (-0.16 to -0.01)	<0.02
Log HOMA-IR	-0.07 (-0.14 to -0.01)	<0.01	-0.07 (-0.15 to -0.01)	<0.05
		Model with CRP		
E/A versus				
WC	-0.10 (-0.17 to -0.03)	<0.0005	-0.04 (-0.12 to 0.04)	=0.18
Log CRP	-0.005 (-0.08 to 0.07)	=0.89	-0.005 (-0.09 to 0.08)	=0.91
DBP	-0.12 (-0.19 to -0.05)	<0.0001	-0.10 (-0.18 to -0.02)	<0.005
Log HOMA-IR	-0.08 (-0.15 to -0.01)	<0.01	-0.09 (-0.17 to -0.01)	<0.02

E/A, ratio of early-to-late (atrial) transmitral blood flow velocity; WC, waist circumference; DBP, clinic diastolic blood pressure; HOMA-IR, homeostasis model of insulin resistance. *Additional factors included in the stepwise regression models include age, sex, pulse rate, regular smoking, regular alcohol intake, and treatment for hypertension (in all). †For CRP, n=712 for all participants and 529 for untreated participants.

Table 2.11. Relationships between circulating adipokine or C-reactive protein (CRP) concentrations and left ventricular diastolic function as determined from transmitral blood flow and myocardial tissue velocity measurements in a community sample.

	All participants† (n=442)		Untreated participants† (n=325)	
	Partial r* (CI)	p value	Partial r* (CI)	p value
		Model with resistin		
E/e' versus				
WC	0.09 (0.01 to 0.18)	<0.05	0.13 (0.02 to 0.24)	<0.02
Log resistin	-0.04 (-0.13 to 0.05)	=0.32	-0.06 (-0.17 to 0.05)	=0.21
SBP	0.18 (0.09 to 0.27)	<0.0001	0.19 (0.08 to 0.30)	<0.0005
Log HOMA-IR	0.05 (-0.04 to 0.14)	=0.27	0.01 (-0.10 to 0.11)	=0.88
		Model with adiponectin		
E/e' versus				
WC	0.09 (0.01 to 0.18)	<0.05	0.13 (0.02 to 0.24)	<0.02
Log adiponectin	0.007 (-0.09 to 0.10)	=0.88	-0.01 (-0.11 to 0.10)	=0.84
SBP	0.18 (0.09 to 0.27)	<0.0001	0.19 (0.08 to 0.30)	<0.0005
Log HOMA-IR	0.05 (-0.04 to 0.14)	=0.27	0.01 (-0.10 to 0.11)	=0.79
		Model with CRP		
E/e' versus				
WC	0.10 (0.01 to 0.19)	<0.05	0.34 (0.23 to 0.45)	<0.0001
Log CRP	-0.08 (-0.18 to 0.01)	=0.08	-0.10 (-0.21 to 0.01)	=0.07
SBP	0.16 (0.06 to 0.25)	<0.0005	0.19 (0.08 to 0.30)	<0.001
Log HOMA-IR	0.07 (-0.02 to 0.17)	=0.12	0.04 (-0.07 to 0.15)	=0.50

E, early transmitral blood flow velocity; e', early lateral wall myocardial tissue velocity; WC, waist circumference; SBP, clinic systolic blood pressure; HOMA-IR, homeostasis model of insulin resistance. *Additional factors included in the stepwise regression models include age, sex, pulse rate, regular smoking, regular alcohol intake, and treatment for hypertension (in all). †For CRP, n=416 for all participants and 301 for untreated participants.

Although circulating resistin concentrations predict the development of and prognosis in heart failure (Butler *et al.*, 2009; Frankel *et al.*, 2009; Zhang *et al.*, 2011; Wu *et al.*, 2012; Takeishi *et al.*, 2007; Muse *et al.*, 2015), the mechanisms that account for this effect are unclear. Although preclinical studies indicate that resistin produces cardiomyocyte hypertrophy (Kim *et al.*, 2008; Chemaly *et al.*, 2011; Kang *et al.*, 2011), and LV hypertrophyis a well-recognized predictor of outcomes, associations between circulating resistin concentrations and LVM in humans are equivocal (Akinci *et al.*, 2013; McManus *et al.*, 2012; Allison *et al.*, 2013). In large community-based studies, a lack of independent relationship between circulating resistin concentrations and LVM have been reported (McManus *et al.*, 2012; (Allison *et al.*, 2013). However, these studies are confounded by a high prevalence of participants receiving antihypertensive therapy (McManus *et al.*, 2012; Allison *et al.*, 2013), and a lack or contemporaneous blood and echocardiographic assessment (McManus *et al.*, 2012). In the present study we show independent relationships between resistin concentrations and LVMI, LVM_{inappr} and LVH in a community sample with a high prevalence of hypertension, but with a low prevalence of antihypertensive therapy, and these findings were reproduced in untreated participants. The present results are therefore the first to suggest that increases in LVM may be one pathway that explains the ability of circulating resistin concentrations to predict human heart failure.

Prior community-based studies that have failed to show independent associations between circulating resistin concentrations and LVM (McManus *et al.*, 2012; Allison *et al.*, 2013) have not assessed whether these relationships occur with LVM beyond that predicted by loading conditions (stroke work) (LVM_{inappr}). In this regard, in the present study relationships between resistin concentrations and LVM tended to be stronger for LVM_{inappr} than LVMI. As LVM_{inappr} and reductions in LVM_{inappr}, rather than LVMI or reductions in LVMI are associated with LV ejection fraction (Woodiwiss *et al.*, 2012), it is possible that the transition to heart failure predicted by circulating resistin is mediated in-part by increases in LVM_{inappr} and consequently decreases in systolic function. Indeed in the present study circulating resistin concentrations were independently and inversely associated with FSmid, an index of myocardial systolic function. However, these relationships did not translate into decreases in ejection fraction. Whether resistin produces myocardial systolic dysfunction and subsequently compensatory LVH, thus maintaining systolic chamber function, or increases in LVM and hence myocardial systolic dysfunction is uncertain. Further prospective studies are warranted to evaluate whether circulating resistin predicts the development of a reduced ejection fraction and consequently heart failure.

The inverse relationships between circulating resistin concentrations and myocardial systolic function noted in the present study are in keeping with large, community-based studies which have similarly reported on inverse relationships between circulating resistin

concentrations and LV systolic function (McManus *et al.*, 2012; Allison *et al.*, 2013). However, in these prior studies these relationships showed either only trend effects (p=0.04) despite a large study sample (n=2615) (McManus *et al.*, 2012), or were not adjusted for confounders such as BP and diabetes mellitus (Allison *et al.*, 2013). In both studies, a high proportion of participants were receiving antihypertensive therapy (McManus *et al.*, 2012; Allison *et al.*, 2013). In contrast, in the present study we show more convincing relationships between circulating resistin concentrations and myocardial systolic function ($p<0.005$) which were pronounced in participants not receiving antihypertensive therapy ($p<0.0005$) and these relationships were independent of multiple confounders including brachial BP and diabetes mellitus. In addition we show an association between myocardial systolic function and an interaction between circulating resistin and CRP concentrations, independent of the independent terms, an effect which translated into incremental relationships between resistin concentrations and myocardial function across tertiles of CRP concentrations. Hence, the present study provides more convincing evidence for a resistin effect on myocardial systolic function in the human heart, and suggests that these effects may depend on the overall level of inflammation that exists.

As insulin resistance has previously been demonstrated to be associated with LVM (Millen *et al.*, 201; Hwang *et al.*, 2012) and the development of heart failure (Ingelsson *et al.*, 2005), and resistin promotes myocardial insulin resistance (Kang *et al.*, 2011), an important consideration of the present study is whether relationships between circulating resistin concentrations and LVM or dysfunction are mediated through insulin resistance. In this regard, in the present study HOMA-IR was independently associated with increases in LVMI and LVM_{inappr}, but not FSmid. Relationships between circulating resistin concentrations and LVM, LVH or FSmid were nevertheless independent of HOMA-IR. Hence, it is possible that resistin versus LVM or LVH and resistin versus FSmid relationships are explained through effects other than insulin resistance. In this regard, resistin overexpression in the rat heart promotes myocardial oxidative stress, fibrosis and apoptosis (Chemaly *et al.*, 2011).

The limitations of the present study include the cross-sectional design which precludes us from drawing causal inferences. Second, TDI measures of diastolic function were available in 60% of participants with echocardiograms, albeit that the characteristics of this group were similar to those without TDI measurements. Thus, we cannot be certain that circulating resistin concentrations are not associated with E/e'. Third, our study sample was exclusively of African ancestry. Whether similar findings occur in other ethnic groups requires further consideration.

2.5 Conclusions

In the present study conducted in a community sample with a high prevalence of obesity and where antihypertensive therapy was limited, we show that circulating resistin concentrations are indeed independently associated with LVM and systolic function beyond adiposity indexes, diabetes mellitus, BP, HOMA-IR and general inflammation. The present study therefore supports the view that resistin may mediate the transition to heart failure in-part through increases in LVM and myocardial systolic dysfunction.

CHAPTER 3

Circulating Resistin Concentrations are Independently Associated with Aortic Pulse Wave Velocity in a Community Sample.

The data in this chapter have been published in
Journal of Hypertension 2016 Feb; 34 (2):274-81
Glenda Norman G, Gavin R Norton, Monica Gomes, Frederic Michel, Olebogeng HI Majane, Pinhas Sareli, Aletta ME Millen, Angela J Woodiwiss

Circulating resistin concentrations are independently associated with aortic pulse wave velocity in a community sample.

I was involved in data collection from 2014 until 2019. In addition I retrieved the samples from storage and aliquoted the samples before assisting with running the Elisas for the resistin, CRP and HOMA-IR. Furthermore, I did all the data analysis for the publications using SAS software, version 9.4.

3.0 Abstract

Aims: The role of the adipokine, resistin in mediating increases in aortic stiffness is uncertain. We aimed to determine independent relationships between circulating resistin concentrations and aortic pulse wave velocity (PWV) and wave reflection in a community-based sample with a high prevalence of untreated hypertension and obesity.

Methods: Plasma resistin, adiponectin and C-reactive protein (CRP) concentrations (ELISA); carotid-femoral (aortic) PWV and the aortic reflected wave index (RI)(applanation tonometry and SphygmoCor software) were determined in 683 randomly selected participants of African ancestry from SOWETO, South Africa whom had never received antihypertensive therapy.

Results: Resistin concentrations were not independently associated with office or 24-hour (n=492) blood pressure (BP). In a stepwise regression model with BMI included in the model, age ($p<0.0001$), mean arterial pressure ($p<0.0001$), plasma resistin concentrations ($p<0.005$), female gender ($p=0.01$) and creatinine concentrations ($p<0.01$) contributed independently to variations in PWV. Independent relationships between resistin concentrations and PWV persisted with further adjustments for C-reactive protein concentrations ($p<0.005$), and the homeostasis model of insulin resistance (HOMA-IR) ($p<0.02$). Similar relationships were noted with waist circumference rather than BMI in the model. Resistin concentrations were not independently associated with RI or aortic BP.

Conclusion: Resistin is independently and directly associated with aortic stiffness and these effects occur beyond BP, insulin resistance and general inflammation.

Key words: obesity, aortic pulse wave velocity, aortic wave reflection, resistin.

3.1 Introduction

The steady increase in the global prevalence of obesity (Finucane *et al.*, 2011) and its associated cardiometabolic risk factors is a critical obstacle to the effective prevention of cardiovascular disease. Although a half to three-quarters of obesity-related cardiovascular disease may be attributed to conventional risk factors, the remaining one quarter to a half remains unaccounted for (Lu *et al.,* 2014; Lu *et al.*, 2015). More recently, several inflammatory substances released from adipocytes (adipocytokines or adipokines) have been suggested to contribute toward the adverse effects of obesity on the cardiovascular system. However, at present the contribution of resistin, a circulating adipokine, which in humans is predominantly expressed in monocytes and macrophages, and which is responsive to inflammatory stimuli (Patel *et al.*, 2003; Lu *et al.*, 2002; Kaser *et al.*, 2003), is uncertain.

Although some studies suggest that circulating resistin concentrations predict the development of cardiovascular events (Menzaghi *et al.*, 2013; Muse *et al.*, 2015; Butler *et al.*, 2009; Frankel *et al.*, 2009), these findings are not supported by all studies (Luc *et al.*, 2010; Lim *et al.*, 2008). A fundamental mechanism that may explain relationships between circulating resistin concentrations and vascular disease has only recently begun to emerge. Importantly, few studies have evaluated relationships between resistin concentrations and large artery function, including aortic (carotid-femoral) pulse wave velocity (PWV) and reflected waves, both of which predict cardiovascular outcomes beyond conventional risk factors (Vlachopoulos *et al.,* 2010; Ben-Shlomo *et al.*, 2014; Wang *et al.*, 2010; Chirinos *et al.*, 2012; Weber *et al.*, 2012). One small (n=271) case-control study suggests that circulating resistin concentrations are associated with increases in brachial-ankle PWV (Yang *et al.*, 2009). In contrast, in a large (n=749) cross-sectional study conducted in a sample with a high prevalence of antihypertensive treatment, circulating resistin concentrations were inversely related to aortic PWV (Windham *et al.*, 2010), and in a small study (n=141) conducted in treated hypertensives, circulating resistin concentrations failed to predict changes in PWV over 24-months of follow-up (Youn *et al.*, 2013). The presence of antihypertensive treatment (Yang *et al.*, 2009; Windham *et al.*, 2010; Youn *et al.*, 2013), small study samples (Yang *et al.*, 2009;Youn *et al.*, 2013), and the use of brachial-ankle PWV measurements (Yang *et al.*, 2009), which assess stiffness of large and medium-sized arteries, are likely to have confounded the results of previous studies (Yang *et al.*, 2009; Windham *et al.*, 2010; Youn *et al.*, 2013) . Therefore, in the present study we aimed to assess whether independent relationships between circulating resistin concentrations and aortic PWV or wave reflection occur in a relatively large community-based sample with prevalent obesity and never-treated hypertension.

3.2 Methods

3.2.1 Study group. The present study was conducted according to the principles outlined in the Helsinki declaration. The Committee for Research on Human Subjects of the University of the Witwatersrand approved the protocol (approval numbers: M02-04-72 renewed as M07-04-69 and M12-04-108). Participants gave informed, written consent. The study design has previously been described (Norton *et al.*, 2012; Woodiwiss *et al.*, 2009). Nuclear families of black African descent with siblings older than 16 years were randomly recruited from the South West Township (SOWETO) of Johannesburg, South Africa. Of the 1211 participants recruited, 1041 participants had aortic PWV measurements of whom 683 were not receiving treatment for hypertension. 492 of the sample had 24-hour ambulatory BP measurements that met with pre-specified quality control criteria (longer than 20 hours and more than 10 and 5 readings for the computation of day and night means, respectively).

3.2.2 Clinical, demographic and anthropometric measurements. Demographic and clinical data were obtained using a standardized questionnaire (Norton *et al.*, 2012; Woodiwiss *et al.*, 2009). Regular tobacco use was defined as daily cigarette smoking and regular alcohol consumption as either one beer a day, a bottle of wine (750 mls) a week or 250 mls of spirits a week. Height, weight and waist circumference (WC) were measured using standard approaches and participants were identified as being overweight if their body mass index (BMI) was ≥25 kg/m^2 and obese if their BMI was ≥30 kg/m^2. Central obesity was defined as an enlarged WC (≥88 cm in women and ≥102 cm in men). Laboratory blood tests of renal function, liver function, blood glucose, lipid profiles, haematological parameters, and percentage glycated haemoglobin (HbA1c) (Roche Diagnostics, Mannheim, Germany) were performed. Fasting plasma insulin concentrations were determined from an insulin immulite, solid phase, two-site chemiluminescent immunometric assay (Diagnostic Products Corporation, Los Angeles, CA, USA) and insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) using the formula (insulin [µU/ml] x glucose [mmol/l])/22.5. Diabetes mellitus (DM) or abnormal blood glucose control was defined as the use of insulin or oral hypoglycaemic agents or an HbA1c value greater than 6.5% respectively.

3.2.3 Blood pressure. Nurse-derived conventional BP was measured after 10 minutes of rest in the seated position as previously described (Norton *et al.*, 2012; Woodiwiss *et al.,* 2009) within a half hour of obtaining blood samples and in the opposite arm to that subjected to venesection. These measurements were performed to an accuracy of 2 mm Hg by a trained nurse using a mercury sphygmomanometer and an appropriate sized cuff according to the European Society of Hypertension guidelines (O'Brien *et al.*, 2003). Five consecutive BP readings were obtained 30 to 60 seconds apart. The average of the five readings was taken as the BP. Only 0.29 % of visits had fewer than the planned BP recordings. The frequency of

identical consecutive recordings was 0.44% for systolic BP (SBP) and 1.46% for diastolic BP (DBP). No BP values were recorded as an odd number. Hypertension was diagnosed as a conventional BP≥140/90 mm Hg.

Ambulatory 24-hour, day and night BP were determined using SpaceLabs monitors (model 90207; Spacelabs, Redmond, Washington, USA) as previously described (Woodiwiss *et al.*, 2009). The size of the cuff was the same as that used for conventional BP measurements. Monitors were programmed to measure 24-hour BP at 15-min intervals from 06:00 to 22:00 hours and at 30-min intervals from 22:00 to 06:00 hours. Intra-individual means of the ambulatory measurements were weighted by the time interval between successive readings (Woodiwiss *et al.*, 2009). The average (±SD) number of BP readings obtained was 60.8±12.2 (range=24 to 81) for the 24-h period.

3.2.4 Central aortic haemodynamics. Central aortic haemodynamics were determined as previously described (Norton *et al.*, 2012; Woodiwiss *et al.*, 2009). After participants had rested for 15 minutes in the supine position, arterial waveforms at the radial (dominant arm), carotid and femoral artery pulses were recorded by applanation tonometry. Pressure waveforms were recorded during an 8-second period using a high-fidelity SPC-301 micromanometer (Millar Instrument, Inc., Houston, Texas) interfaced with a computer employing SphygmoCor, version 9.0 software (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia). Aortic PWV was determined from sequential waveform measurements at carotid and femoral sites as previously described (Woodiwiss *et al.*, 2009). The time delay in the pulse waves between the carotid and femoral sites was determined using an electrocardiograph-derived R wave as a fiducial point. Pulse transit time was taken as the average of 10 consecutive beats. The distance which the pulse wave travels was determined as the difference between the distance from the femoral sampling site to the suprasternal notch, and the distance from the carotid sampling site to the suprasternal notch. Aortic PWV was calculated as the ratio of the distance to the transit time (m/sec). To derive an approximate standard aortic PWV ("converted standard PWV") for comparative purposes, we multiplied travel distance derived from the subtracted distance by the ratio (50.7:45.5=1.114) of mean reference distance/mean tape measure distance using the "subtraction" approach given in Table 1 of the recent consensus document (Van Bortel *et al.*, 2012). However, as advised by the consensus document (Van Bortel *et al.*, 2012) in order to perform our primary analysis we did not employ formulae published to convert subtracted distance to direct distance as these formulae introduce additional errors.

To determine aortic wave reflection and aortic BP the pulse wave obtained from the radial tonometer recordings was calibrated by manual measurement (auscultation) of brachial BP taken immediately before the recordings. The radial pressure waveform was converted into a central (aortic) waveform using a validated generalized transfer function incorporated in SphymoCor software. The aortic backward (reflected) (Pb) and the aortic forward (Pf) wave

pressures were determined from the aortic pulse wave using SphygmoCor software which separates the aortic waveform using a triangular flow wave (Westerhof *et al.*, 2006). Reflected wave index (RI) was calculated as Pb/Pf x 100, as previously described (Chirinos *et al.*, 2012). Central aortic SBP (SBPc) was derived from the aortic waveform and central aortic pulse pressure (PPc) was calculated from the difference between SBPc and DBP.

3.2.5 Inflammatory markers and adipokines. Blood samples were centrifuged and immediately stored at -80^{o}C. Plasma concentrations of human resistin, adiponectin and high-sensitivity (range 0.01 to 50 ng/ml) C-reactive protein (CRP) concentrations were measured using enzyme-linked immunosorbent assays (Quantikine, R&D Systems Inc, Minneapolis, MN, USA). The resistin assay had a lower detection limit of 0.026 ng/ml and intra-assay and inter-assay coefficients of variation ranging from 3.8 to 5.3% and 7.8 to 9.2% respectively. The adiponectin assay had a lower detection limit of 0.246 ng/ml and intra-assay and inter-assay coefficients of variation ranging from 2.5 to 4.7% and 5.8 to 6.9% respectively. The CRP assay had a mean lower detection limit of 0.010 ng/ml and intra-assay and inter-assay coefficients of variation ranging from 3.8 to 8.3% and 6.0 to 7.0% respectively. CRP concentrations could not be determined on 15 participants whose concentrations were below the lower level of detection.

3.2.6 Data analysis. Database management and statistical analyses were performed with SAS software, version 9.3 (SAS Institute Inc., Cary, NC, USA). Continuous data are reported as mean±SD or mean (range). Unadjusted means and proportions were compared by the large-sample z-test and the χ^2-statistic, respectively. As HOMA-IR was positively skewed (skewness=4.39, kurtosis=26.05; Shapiro-Wilk's statistic=0.56, p<0.0001) HOMA-IR was log transformed which resulted in an improved distribution (skewness=0.44, kurtosis=-0.29; Shapiro-Wilk's statistic=0.97). Similarly, as resistin concentrations were positively skewed (skewness=2.97, kurtosis=18.78; Shapiro-Wilk's statistic=0.80, p<0.0001) resistin concentrations were log transformed which resulted in an improved distribution (skewness=0.07, kurtosis=0.77; Shapiro-Wilk's statistic=0.99). Moreover, as adiponectin concentrations were positively skewed (skewness=1.18, kurtosis=1.72; Shapiro-Wilk's statistic=0.92, p<0.0001) adiponectin concentrations were similarly log transformed which resulted in an improved distribution (skewness=-0.68, kurtosis=0.61; Shapiro-Wilk's statistic=0.97). Bivariate correlations for continuous data were assessed from Pearson's correlation coefficients. Independent relationships were assessed from mulltivariate linear regression analysis with appropriate adjustors (see legends of Tables). As aortic functional changes not only contribute toward increases in PP and SBP, but increases in PP and SBP are risk factors for aortic functional changes, in separate models adjustments for clinic and 24-hour PP and SBP were made when assessing independent relationships between circulating adiponectin or resistin concentrations and aortic function. Probability values were further

adjusted for non-independence of family members using the method of maximum likelihood as implemented by the mixed procedure as defined in the SAS package.

3.3 Results

3.3.1 Participant characteristics. Table 3.1 shows the characteristics of the participants in the study sample. More women than men participated and a high prevalence of hypertension (27%) and obesity (30.8%) was noted. Of the regular smokers, only 4 participants smoked 1 cigarette per day. None of the sample were receiving lipid lowering therapy (they are not available at a primary care level in the public healthcare sector of South Africa).

3.3.2 Relationships between adiposity indices and circulating adipokine concentrations. On bivariate analysis, BMI and WC were directly associated with resistin and CRP concentrations as well as HOMA-IR, but inversely associated with adiponectin concentrations ($p<0.0001$). However, with adjustments for confounders although adiposity indices remained associated with adiponection and CRP concentrations and HOMA-IR, no independent relationships were noted with resistin concentrations (Table 3.2).

3.3.3 Relationships between circulating adipokine concentrations and metabolic factors and CRP. In multivariate adjusted models adiponectin concentrations were inversely associated with HOMA-IR and triglyceride concentrations and directly related to HDL cholesterol concentrations (Table 3.3). In contrast, in multivariate adjusted models, resistin concentrations were directly associated with CRP and creatinine concentrations, but unrelated to other metabolic factors including lipid concentrations (Table 3.3).

3.3.4 Associations between adipokines and BP. Although office, 24-hour and aortic BP were correlated with circulating adiponectin, resistin or CRP concentrations ($p<0.0001$ for all), with adjustments for confounders, only modest associations were noted between adiponectin concentrations and clinic and aortic SBP (Table 3.4).

3.3.5 Associations of plasma adipokine concentrations with aortic PWV. On bivariate analysis and in multivariate adjusted models including adjustments for brachial office or 24-hour mean arterial pressure (MAP)(distending pressures), PP or SBP in separate models, plasma resistin (Table 3.5), but not adiponectin (Table 3.6) concentrations were associated with aortic PWV. These relationships were independent of either BMI or WC (Table 3.7). These relationships were noted in women, but failed to achieve significance in men (Tables 3.5 and 3.8). In stepwise regression models, plasma resistin concentrations were third only to age and MAP and similar to creatinine and female gender in the impact (standardized β-coefficient) of the relationships with PWV (Table 3.7).

Table 3.1. Characteristics of study sample having never received antihypertensive therapy.

Number (% Women)	683 (58.1)
Age (years)	37.8±16.4
Body mass index (kg/m^2)	27.1±6.7
% Overweight/obese	24.5/30.8
% Regular tobacco*	18.7
% Regular alcohol*	23.1
% DM or HbA1c>6.5%	6.3
% Hypertension	27.2
Total cholesterol (mmol/l)	4.41±1.08
LDL cholesterol (mmol/l)	2.47±0.90
Creatinine (µmol/l)	73.6±15.8
Office SBP/DBP (mm Hg)	125±20/82±12
24-Hour SBP/DBP (mm Hg)(n=492)	116±14/72±10
Aortic pulse wave velocity (PWV)(m/sec)	5.79±2.21
Converted standard aortic PWV (m/sec)†	6.45±2.47
Aortic pulse pressure (mm Hg)	33±14
Aortic SBP (mm Hg)	116±22
Aortic forward wave pressure (mm Hg)	22.8±8.3
Aortic backward wave pressure (mm Hg)	15.7±7.5
Aortic reflected wave index (%)	69±20
Adiponectin (mg/l)	8.4 (range=0.08 to 36.6)
Resistin (ng/ml)	10.5 (range=1.4 to 82.5)
C-reactive protein (mg/l)	2.86 (range=0.04 to 53.8)
HOMA-IR	1.58 (range=0.21 to 42.0)

DM, diabetes mellitus; HbA1c, glycated haemoglobin; LDL, low density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostasis model of insulin resistance. *See methods for definitions. †Calculated from 1.114 x subtracted wave travel distance (see methods for explanation).

Table 3.2. Factors independently associated with adiposity indices.

	BMI vs		WC vs	
	β-coefficient* (±SEM)	p-values	β-coefficient* (±SEM)	p-values
Age	0.17±0.04	<0.0001	0.30±0.04	<0.0001
Mean arterial pressure	0.19±0.04	<0.0001	0.14±0.04	<0.0002
Female gender	0.35±0.04	<0.0001	0.13±0.04	<0.005
DM or HbA1c>6.5%	-0.002±0.03	=0.96	0.02±0.03	=0.61
Regular smoking	0.13±0.04	<0.0005	0.10±0.04	<0.005
Regular alcohol	0.02±0.03	=0.55	-0.03±0.03	=0.35
Pulse rate	-0.07±0.03	<0.05	-0.04±0.03	=0.27
LDL cholesterol	0.07±0.03	<0.05	0.06±0.03	=0.09
Creatinine	-0.004±0.04	=0.92	-0.04±0.04	=0.29
Log resistin	0.04±0.03	=0.28	0.02±0.03	=0.58
Log adiponectin	-0.17±0.03	<0.0001	-0.21±0.03	<0.0001
Log CRP	0.22±0.04	<0.0001	0.22±0.04	<0.0001
Log HOMA-IR	0.09±0.03	<0.01	0.14±0.03	<0.0001

*Indicates standardized β-coefficient. BMI, body mass index; WC, waist circumference; DM, diabetes mellitus; HbA1c, glycated haemoglobin; CRP, C-reactive protein concentrations; HOMA-IR, homeostasis model of insulin resistance.

Table 3.3. Relationships between plasma adipokine concentrations and metabolic and inflammatory indices.

	Partial r (95% CI)*	p value
Log adiponectin vs		
Log CRP	-0.03 (-0.11 to 0.04)	=0.39
Log HOMA-IR	-0.12 (-0.20 to -0.04)	<0.005
Total cholesterol	0.02 (-0.06 to 0.09)	=0.69
LDL cholesterol	-0.06 (-0.14 to 0.01)	=0.10
HDL cholesterol	0.26 (0.19 to 0.33)	<0.0001
Triglyceride	-0.19 (-0.26 to -0.11)	<0.0001
Creatinine	0.06 (-0.01 to 0.14)	=0.11
Log resistin vs		
Log CRP	0.22 (0.15 to 0.29)	<0.0001
Log HOMA-IR	-0.06 (-0.14 to 0.02)	=0.13
Total cholesterol	-0.02 (-0.09 to 0.06)	=0.67
LDL cholesterol	-0.02 (-0.10 to 0.05)	=0.57
HDL cholesterol	-0.005 (-0.08 to 0.07)	=0.90
Triglyceride	-0.06 (-0.14 to 0.01)	=0.10
Creatinine	0.15 (0.07 to 0.22)	<0.0002

CI, confidence intervals; CRP, C-reactive protein concentrations; HOMA-IR, homeostasis model of insulin resistance. *Adjustments are for age, gender, body mass index, regular smoking, regular alcohol intake, diabetes mellitus or an HbA1c>6.5%. Probability values are further adjusted for non-independence of family members.

Table 3.4. Relationships between circulating adipokine concentrations and blood pressure (BP) in participants having never received antihypertensive therapy.

	Partial r* (95% CI)	p value
Log resistin versus		
Clinic SBP (n=683)	-0.01 (-0.08 to 0.07)	=0.81
Clinic DBP (n=683)	-0.04 (-0.11 to 0.04)	=0.32
24-hour SBP (n=492)	0.01 (-0.08 to 0.10)	=0.88
24-hour DBP (n=492)	-0.04 (-0.12 to 0.05)	=0.43
Aortic SBP (n=683)	-0.01 (-0.08 to 0.07)	=0.82
Log adiponectin versus		
Clinic SBP (n=683)	0.08 (0.01 to 0.15)	<0.05
Clinic DBP (n=683)	-0.01 (-0.08 to 0.07)	=0.82
24-hour SBP (n=492)	0.08 (-0.01 to 0.17)	=0.06
24-hour DBP (n=492)	0.02 (-0.06 to 0.11)	=0.60
Aortic SBP (n=683)	0.09 (0.02 to 0.17)	<0.02

SBP, systolic BP, DBP, diastolic BP. *Adjusted for age, sex, body mass index, diabetes mellitus or an HbA1c>6.5%, regular smoking, and regular alcohol intake.

Table 3.5. Relationships between plasma resistin concentrations and carotid-femoral (aortic) pulse wave velocity (PWV) in participants having never received antihypertensive therapy.

	Log resistin vs log aortic PWV		
Adjustments	Partial r (95% CI)	p value	Model r^2
	(n=683)		
Unadjusted	0.17 (0.09 to 0.24)	<0.0001	0.027
*	0.10 (0.03 to 0.17)	<0.01	0.396
* + office MAP	0.11 (0.04 to 0.19)	<0.005	0.451
* + office PP	0.09 (0.02 to 0.17)	<0.02	0.432
* + office SBP	0.11 (0.04 to 0.19)	<0.005	0.453
	with 24-hour BP (n=492)		
Unadjusted	0.18 (0.10 to 0.27)	<0.0001	0.033
*	0.11 (0.02 to 0.19)	<0.02	0.407
* + 24-hour PP	0.10 (0.01 to 0.19)	<0.05	0.411
* + 24-hour SBP	0.11 (0.02 to 0.20)	<0.02	0.432
	Women (n=397)		
Unadjusted	0.16 (0.07 to 0.26)	=0.001	0.027
*	0.12 (0.02 to 0.22)	<0.02	0.417
* + office MAP	0.12 (0.02 to 0.21)	<0.05	0.487
* + office PP	0.12 (0.02 to 0.21)	<0.05	0.446
	Men (n=286)		
Unadjusted	0.18 (0.06 to 0.28)	<0.005	0.031
*	0.07 (-0.04 to 0.19)	=0.21	0.388
* + office MAP	0.10 (-0.01 to 0.22)	=0.09	0.431
* + office PP	0.06 (-0.05 to 0.18)	=0.296	0.432

CI, confidence intervals; office, brachial blood pressure; MAP, mean arterial pressure; PP, pulse pressure; SBP, systolic BP. *Adjustments are for BP as indicated in separate models, age, gender, pulse rate, body mass index, regular smoking, regular alcohol intake, diabetes mellitus or an HbA1c>6.5%, LDL cholesterol, and creatinine concentrations. Probability values are further adjusted for non-independence of family members.

Table 3.6. Unadjusted relationships between plasma adiponectin concentrations and carotid-femoral (aortic) pulse wave velocity (PWV).

	Log adiponectin vs log aortic PWV		
	n=	Partial r (95% CI)	p value
All	683	0.04 (-0.03 to 0.12)	=0.25
Women	397	0.04 (-0.06 to 0.14)	=0.44
Men	286	0.07 (-0.04 to 0.18)	=0.23

CI, confidence intervals.

Table 3.7. Factors independently associated with carotid-femoral (aortic) pulse wave velocity (PWV) in 683 participants having never received antihypertensive therapy.

	With BMI in model		With WC in model	
Log PWV vs	β-coefficient* (±SEM)	p-values	β-coefficient* (±SEM)	p-values
Age	0.47±0.04	<0.0001	0.47±0.04	<0.0001
Mean arterial pressure	0.28±0.03	<0.0001	0.28±0.03	<0.0001
Log resistin	0.09±0.03	<0.005	0.08±0.03	<0.01
Body mass index	-0.01±0.04	=0.83	-	-
Waist circumference	-	-	-0.01±0.03	=0.74
Female gender	0.10±0.04	=0.01	0.11±0.04	<0.01
DM or HbA1c>6.5%	0.01±0.03	=0.91	-0.001±0.03	=0.98
Regular smoking	0.01±0.03	=0.63	0.01±0.03	=0.66
Regular alcohol	-0.07±0.03	<0.05	-0.08±0.03	<0.02
Pulse rate	-0.03±0.03	=0.30	-0.03±0.03	=0.31
LDL cholesterol	-0.10±0.03	<0.005	-0.10±0.03	<0.002
Creatinine	0.10±0.03	<0.01	0.10±0.04	<0.01

*Indicates standardized β-coefficient. BMI, body mass index; WC, waist circumference; DM, diabetes mellitus; HbA1c, glycated haemoglobin; LDL, low density lipoprotein.

Table 3.8. Sex-specific relationships between plasma resistin concentrations and carotid-femoral (aortic) pulse wave velocity (PWV) with adjustments for 24-hour blood pressure.

Log resistin vs log aortic PWV

Adjustments	Partial r (95% CI)	p value	Partial r (95% CI)	p value
	Women		Men	
with 24-hour BP (n=282)			with 24-hour BP (n=210)	
Unadjusted	0.18 (0.07 to 0.29)	<0.005	0.19 (0.06 to 0.32)	<0.01
*	0.14 (0.03 to 0.26)	<0.02	0.06 (-0.08 to 0.19)	=0.41
* + 24-hour PP	0.14 (0.02 to 0.26)	<0.02	0.05 (-0.09 to 0.19)	=0.46
* + 24-hour SBP	0.15 (0.03 to 0.27)	<0.02	0.06 (-0.08 to 0.19)	=0.42

CI, confidence intervals; MAP, mean arterial pressure; PP, pulse pressure; SBP, systolic BP. *Adjustments are for age, blood pressure as indicated in separate models, pulse rate, body mass index, regular smoking, regular alcohol intake, diabetes mellitus or an HbA1c>6.5%, LDL cholesteroland creatinine concentrations. Probability values are further adjusted for non-independence of family members.

3.3.6 *Associations of plasma adipokine concentrations with aortic reflected wave and aortic BP*. In multivariate adjusted models neither plasma resistin (Table 3.9), nor adiponectin (data not shown) concentrations were independently associated with RI or PPc.

3.3.7 Associations of plasma resistin concentrations with aortic PWV are independent of CRP and HOMA-IR. In bivariate models, CRP concentrations (Pearson's r=0.22, CI=0.14 to 0.29, p<0.0001), but not HOMA-IR (p=0.50) were correlated with aortic PWV. However, with adjustments for confounders, CRP concentrations failed to associate with PWV (partial r=0.01, CI=-0.07 to 0.08, p=0.83). With multivariate adjustments including CRP concentrations and HOMA-IR in the models, circulating resistin concentrations remained independently associated with aortic PWV (Table 3.10).

3.4 Discussion

The main findings of the present study are as follows: In a relatively large randomly selected community sample of black African ancestry with a high prevalence of obesity and untreated hypertension, circulating resistin, but not adiponectin or CRP concentrations were associated with aortic PWV beyond adiposity indexes, BP, HOMA-IR, general inflammation (as indexed by CRP concentrations) and additional confounders. However, neither adiponectin, nor resistin concentrations were independently associated with aortic BP or aortic backward waves.

Although circulating resistin concentrations have been noted to predict cardiovascular events beyond conventional risk factors (Menzaghi *et al.*, 2013; Muse *et al.*, 2015; Butler *et al.*, 2009; Frankel *et al.*, 2009), the mechanisms that account for this effect are unclear. In the present study we show that circulating resistin concentrations are directly associated with an increased aortic PWV independent of adiposity indices, concentrations of the inflammatory marker CRP, insulin resistance as indexed by HOMA-IR, and conventional cardiovascular risk factors. These data are in sharp contrast to a similarly large (n=749) cross-sectional study conducted in a sample with a high prevalence of antihypertensive treatment (which will decrease PWV), where circulating resistin concentrations were inversely related to aortic PWV (Windham *et al.*, 2010), and in a small longitudinal study (n=141) conducted in treated hypertensives, where circulating resistin concentrations failed to predict changes in PWV over 24-months of follow-up (Youn *et al.*, 2013). Only one prior case-control study conducted in a small study sample (n=271) suggests that circulating resistin concentrations are directly associated with increases in PWV (Yang *et al.*, 2009). However, that study (Yang *et al.*, 2009) assessed brachial-ankle PWV which is determined by a composite of elastic and muscular artery stiffness. Hence, the present study provides the first clear evidence that circulating resistin concentrations are independently and directly associated with aortic stiffness and these

Table 3.9. Relationships between plasma resistin concentrations and aortic reflected wave index (RI) or aortic pulse pressure in participants having never received antihypertensive therapy.

Adjustments	Partial r (95% CI)	p value	Model r^2
	Log resistin vs aortic reflected wave index (n=683)		
Unadjusted	-0.03 (-0.11 to 0.04)	=0.38	0.001
*	-0.06 (-0.14 to 0.01)	=0.09	0.419
* + office MAP	-0.06 (-0.14 to 0.01)	=0.11	0.438
	Log resistin vs aortic pulse pressure (n=683)		
Unadjusted	0.07 (-0.002 to 0.15)	=0.06	0.005
*	0.03 (-0.04 to 0.11)	=0.41	0.362
* + office MAP	0.04 (-0.03 to 0.12)	=0.24	0.478

CI, confidence intervals; MAP, mean arterial pressure. *Adjustments are for age, MAP as indicated, gender, pulse rate, body mass index, regular smoking, regular alcohol intake, diabetes mellitus or an HbA1c>6.5%, LDL cholesterol and creatinine concentrations. Probability values are further adjusted for non-independence of family members.

Table 3.10. Relationships between plasma resistin concentrations and carotid-femoral (aortic) pulse wave velocity (PWV) independent of C-reactive protein (CRP) concentrations or the homeostasis model of insulin resistance (HOMA-IR) in participants having never received antihypertensive therapy.

	Log resistin vs log PWV			
Adjustments	n=	Partial r (95% CI)	p value	Model r^2
<u>Adjusted for CRP</u>				
* + office MAP + log CRP	668	0.12 (0.04 to 0.19)	<0.005	0.449
* + office SBP + log CRP	668	0.11 (0.04 to 0.19)	<0.005	0.450
* + 24-hour SBP + log CRP	481	0.11 (0.02 to 0.20)	<0.02	0.434
<u>Adjusted for HOMA-IR</u>				
* + office MAP + HOMA-IR	683	0.10 (0.02 to 0.18)	<0.02	0.65
* + office SBP + HOMA-IR	683	0.10 (0.02 to 0.17)	<0.02	0.466
* + 24-hour SBP + HOMA-IR	492	0.10 (0.01 to 0.19)	<0.05	0.441

CI, confidence intervals; MAP, mean arterial pressure; SBP, systolic blood pressure. *Adjustments are for age, BP as indicated in separate models, gender, pulse rate, body mass index, regular smoking, regular alcohol intake, diabetes mellitus or an HbA1c>6.5%, LDL cholesterol, and creatinine concentrations. Probability values are further adjusted for non-independence of family members.

relationships are not explained by obesity, insulin resistance or increases in general inflammation.

As aortic (carotid-femoral) pulse wave velocity (PWV) predicts cardiovascular outcomes beyond conventional risk factors (Vlachopoulos *et al.*, 2010; Ben-Shlomo *et al.*, 2014), the present study suggests a possible mechanism that may explain the ability of circulating resistin concentrations to predict cardiovascular events beyond conventional risk factors (Menzaghi *et al.*, 2013; Muse *et al.*, 2015; Butler *et al.*, 2009; Frankel *et al.*, 2009).

In the present study relationships between resistin concentrations and aortic PWV were independent of several BP values including office and 24-hour brachial mean arterial pressure, SBP and PP. However, adjustments for brachial PP tended to reduce the strength of the relation. In this regard, caution should be exercised in interpreting these results as increases in aortic PWV occur as a consequence of increases in PP, and aortic PWV also determines PP. Hence, resistin versus PWV relationships are likely to have been over-adjusted for with PP included in the model, thus artificially diminishing the strength of the relation.

In the present study the independent relationship between resistin and aortic PWV failed to translate into associations with aortic backward waves (RI), aortic pulse pressure, aortic SBP, or 24-hour brachial BP. These findings are in accordance with a prior large study reporting on a lack of association of circulating resistin concentrations with brachial PP (Coutinho *et al.*, 2012). Hence, resistin is unlikely to produce cardiovascular damage through an impact on BP. However, increases in aortic PWV may mediate adverse cardiovascular effects through facilitation of transmission of pulsatile energy into end-organ circulatory systems (Mitchell *et al.*, 2011). Moreover, increases in aortic stiffness are associated with a reduced baroreceptor responsiveness, the consequence of which may be an increased vascular stress (Michas *et al.*, 2012). Alternatively, increases in PWV may reflect the extent of arteriosclerosis and therefore herald the onset of vascular events.

Explanations for independent relationships between circulating resistin concentrations and aortic PWV in the present study, although speculative, require consideration. Resistin promotes endothelial activation by upregulating endothelin-1, adhesion molecules, monocyte chemoattractant factors and pro-inflammatory cytokines (Kawanami *et al.*, 2004; Verma *et al.*, 2003), the possible consequence being enhanced arteriosclerosis. In this regard, the arteriosclerotic process is associated with vascular fibrosis and degeneration of elastic tissue, the possible result being an increase in large artery stiffness. Alternatively, as resistin is expressed in macrophages in atheroma (Jung *et al.*, 2006) and increases in aortic stiffness may reflect the extent of atherosclerosis, atherosclerotic lesions may be the source of circulating resistin (reverse causality). Importantly, in the present study resistin concentrations were independently associated with CRP and creatinine concentrations. Hence, it may be argued that resistin-PWV relationships are attributed to the previously described resistin versus CRP or

creatinine relationships (Zhu *et al*., 2012; Wang *et al.*, 2014). However, in the present study, resistin-PWV relationships were independent of CRP and creatinine concentrations and additional confounders.

The limitations of the present study warrant consideration. As this was a cross-sectional study no conclusions can be drawn regarding cause and effect. Second, the present study was conducted in a group of black African ancestry and hence the present results require validation in alternative ethnic groups. Third, PWV measurements were obtained from pulse transit distance using subtracted distances (difference between the distance from the femoral sampling site to the suprasternal notch, and the distance from the carotid sampling site to the suprasternal notch), prior to the recommendation that a standardized approach of 80% of the direct carotid-femoral distance be used (Van Bortel *et al.*, 2012). Hence, our PWV values are likely to differ from standard non-invasive measurements (see converted standard aortic PWV values given in Table 3.1). Fourth, although independent relationships between resistin concentrations and PWV were noted in women, we were not statistically powered to show independent relationships between resistin concentrations and PWV in men. Hence, further studies in men are required. Fifth, the calibration of aortic pressure from radial tonometry employs brachial pressure measurements and ignores possible amplification of pressures from the brachial to the radial artery (Picone *et al.*, 2015). However, although this would affect relations between resistin concentrations and aortic PP, relationships with aortic PWV or RI, the principle outcomes of the present study, would not have been influenced by this possible error as neither PWV nor RI depend on errors in calibration. Last, aortic wave separation was performed using a ‘triangular’ flow waveform rather than a measured or ‘physiological’ aortic flow waveform, and hence RI should be considered as an approximate at best.

3.5 Conclusions

In the present study we provide the first evidence derived from a relatively large, randomly selected community-based sample of participants having never been treated with antihypertensive therapy and with a high prevalence of obesity, that independent of confounders, circulating resistin concentrations are independently associated with aortic PWV. These relationships persisted with further adjustments for CRP and HOMA-IR. These findings therefore suggest a potential mechanism that may explain relationships between circulating resistin concentrations and cardiovascular disease.

CHAPTER 4

Impact in a Community Sample of Obesity-Associated Metabolic and Inflammatory Changes on Glomerular Function is Greater than Modifiable Conventional Risk Factors.

This data chapter is currently under review in *Cardiovascular Journal of Africa*.

Impact of Obesity-Associated Metabolic and Inflammatory Changes on Glomerular Filtration Rate is Greater than that of Modifiable Conventional Risk Factors.

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I was involved in data collection from 2014 until 2019. In addition I retrieved the samples from storage and aliquoted the samples before assisting with running the Elisas for the resistin, CRP and HOMA-IR. Furthermore, I did all the data analysis for the publications using SAS software, version 9.4.

4.0 Abstract

Introduction: Although obesity-associated metabolic and inflammatory changes are associated with renal damage and chronic kidney disease (CKD), the extent to which these effects account for decreases in renal function as compared to modifiable conventional risk factors, is uncertain.
Methods: In a cross-sectional community-based study in 1010 randomly selected (2002 to 2017) participants over the age of 16 years in an urban, developing community with a high prevalence of obesity (41.8%), we assessed plasma resistin and C-reactive protein (CRP) concentrations (ELISA); the homeostasis model of IR (HOMA-IR) and associated metabolic changes, estimated glomerular filtration rate (eGFR), ambulatory blood pressure (BP)(n=688), aortic BP and pulse wave velocity (PWV)(n=896).
Results: In multivariate regression models with adjustments for conventional risk factors (including HbA1c, office BP or any adiposity index) and additional confounders, HOMA-IR (standardized β-coefficient [β-coeff]=-0.13±0.02, <0.0001) and plasma resistin concentrations (β-coeff=-0.13±0.02, <0.0001) were second only to age and equivalent to systolic BP (β-coeff=-0.09±0.03, <0.005) in the impact on eGFR, whilst alternative conventional risk factors contributed little to eGFR. Similar results were obtained in relationships with CKD. CRP was not independently associated with eGFR. Neither 24-hour BP, aortic BP, nor PWV, modified the relative contribution of HOMA-IR and resistin concentrations versus alternative parameters to eGFR.
Conclusions: In a community sample with a high prevalence of obesity, the combined impact of obesity-associated metabolic and inflammatory changes on glomerular filtration rate and CKD is greater than that of modifiable conventional risk factors. Targeting conventional risk factors alone is therefore likely to result in a marked residual impact on the development of CKD in communities with a high prevalence obesity.

Key words: chronic kidney disease, resistin, insulin resistance.

4.1 Introduction

Chronic kidney disease (CKD), as defined by reductions in estimated glomerular filtration rate (eGFR), is in most countries, a major public health problem with substantial social and economic consequences (Levey *et al.*, 2007; Bello *et al.,* 2005). Chronic kidney disease progresses to end-stage renal disease and predicts cardiovascular events beyond conventional risk factors (Go *et al.*, 2004; Matsushita *et al.*, 2010; Mahmoodi *et al.*, 2012; Matsushita *et al.*, 2015; Waheed *et al.*, 2012; Hui *et al.*, 2013). Although poor control of hypertension and diabetes mellitus accounts for a substantial portion of reductions in eGFR, a significant proportion of decreases in eGFR may be attributed to obesity (Iseki *et al.*, 2004; Kramer *et al.*, 2006; Ejerblad *et al.*, 2006). Importantly, several studies have demonstrated relationships between the obesity associated metabolic change, insulin resistance (Mykkänen *et al.*, 1998; Chen *et al.*, 2003; Whaley-Connell *et al.*, 2010) or increased circulating concentrations of pro-inflammatory adipokines (Fried *et al.*, 2004; Cabré *et al.*, 2007; Axelsson *et al.,* 2008; Bash *et al.*, 2009; Keller *et al.*, 2010; Kawamura *et al.*, 2010; Shankar *et al.*, 2011; Upadhyay *et al.*, 2011; Hiramoto *et al.*, 2012; Menzaghi *et al.*, 2012; Mills *et al.*, 2013; An *et al.*, 2015; Moreno *et al.*, 2015; Liu *et al.*, 2016) and renal dysfunction independent of diabetes mellitus or conventional blood pressure (BP). These effects may be through direct actions on the kidney (Whaley-Connell *et al.*, 2017; Tomosugi *et al.*, 1989; Bertani *et al.*, 1989) and as a consequence the adverse effects of these metabolic and inflammatory changes may not be amenable to preventative strategies by targeting conventional risk factors alone. However, the extent to which insulin resistance or adipocytokine changes contribute to decreases in eGFR as compared to modifiable conventional risk factors is uncertain (Whaley-Connell *et al*., 2017).

Obesity-associated insulin resistance or adipocytokine changes may contribute to the development of type II diabetes mellitus or hypertension and hence the impact of these changes on renal function may not be distinct from these conventional risk factors (Whaley-Connell *et al.*, 2017). Targeting conventional risk factors may therefore have major benefits to preventing obesity-associated metabolic of inflammatory effects on CKD. Importantly, the adverse impact of obesity-associated metabolic or inflammatory effects on BP may be best indexed by increases in ambulatory BP (Hänninen *et al.*, 2011) or aortic stiffness (Bäckdahl *et al.*, 2018). In this regard, the extent to which insulin resistance and adipocytokine changes as compared to modifiable conventional cardiovascular risk factors, including alterations in ambulatory BP or aortic stiffness contribute to decreases in renal function (Mykkänen *et al.*, 1998; Chen *et al.*, 2003; Whaley-Connell *et al.*, 2010; Fox *et al.*, 2004; Fried *et al*., 2004; Cabré *et al.*, 2007; Axelsson *et al.,* 2008; Bash *et al.*, 2009; Keller *et al.*, 2010; Kawamura *et al.*, 2010; Shankar *et al.*, 2011; Upadhyay *et al.*, 2011; Hiramoto *et al.*, 2012; Menzaghi *et al.*, 2012; Mills *et al.*, 2013; An *et al.*, 2015; Moreno *et al.*, 2015; Liu *et al.*, 2015) is unclear. To determine the extent to

which targeting conventional risk factors will contribute to preventing the adverse effects of obesity-associated metabolic and inflammatory changes on CKD, in a large sample of a community with prevalent obesity (Norman *et al.*, 2016; Peterson *et al.*, 2016) we assessed the relative impact on eGFR and CKD of insulin resistance and inflammatory changes as compared to that of modifiable conventional risk factors, including ambulatory BP and aortic stiffness.

4.2 Methods

The present study was conducted according to the principles outlined in the Helsinki declaration. The Committee for Research on Human Subjects of the University of the Witwatersrand approved the protocol (approval numbers: M02-04-72 renewed as M07-04-69, M12-04-108 and M17-04-01). Participants gave informed, written consent. The study design has previously been described (Norton *et al.*, 2012; Woodiwiss *et al.*, 2009). Nuclear families of black African descent with siblings older than 16 years were randomly recruited (2002 to 2017) from the South West Township (SOWETO) of Johannesburg, South Africa. Of the 1010 participants studied, 896 participants had aortic PWV measurements and 688 had 24-hour ambulatory BP measurements that met with pre-specified quality control criteria (longer than 20 hours and more than 10 and 5 readings for the computation of day and night means, respectively).

4.2.1 Clinical, demographic and anthropometric measurements. Demographic and clinical data were obtained using a standardized questionnaire (Norton *et al.*, 2012; Woodiwiss *et al.*, 2009). Regular tobacco use was defined as daily cigarette smoking and regular alcohol consumption as either one beer a day, a bottle of wine (750 mls) a week or 250 mls of spirits a week. Height, weight, waist circumference (WC) and hip circumference were measured using standard approaches and participants were identified as being overweight if their body mass index (BMI) was ≥25 kg/m^2 and obese if their BMI was ≥30 kg/m^2. Central obesity was defined as an enlarged WC (≥88 cm in women and ≥102 cm in men). Fasting laboratory blood tests of renal function, blood glucose, lipid profiles, and percentage glycated hemoglobin (HbA1c)(Roche Diagnostics, Mannheim, Germany) were performed. Fasting plasma insulin concentrations were determined from an insulin immulite, solid phase, two-site chemiluminescent immunometric assay (Diagnostic Products Corporation, Los Angeles, CA, USA) and insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) using the formula (insulin [µU/ml] x glucose [mmol/l])/22.5 (Norman *et al.,* 2016; Peterson *et al.,* 2016). Diabetes mellitus (DM) was defined as the use of insulin or oral hypoglycemic agents or an HbA1c value greater than 6.5%.

4.2.2 Blood pressure. Nurse-derived conventional BP was measured after 10 minutes of rest in the seated position as previously described (Norton *et al.*, 2012; Woodiwiss *et al.*, 2009)

within a half hour of obtaining blood samples and in the opposite arm to that subjected to venesection. These measurements were performed to an accuracy of 2 mm Hg by a trained nurse using a mercury sphygmomanometer and an appropriate sized cuff according to guidelines. Five consecutive BP readings were obtained 30 to 60 seconds apart. The average of the five readings was taken as the BP. Hypertension was diagnosed in those receiving antihypertensive therapy or having a conventional BP≥140/90 mm Hg. Ambulatory 24-hour BP was determined using SpaceLabs monitors (model 90207; Spacelabs, Redmond, Washington, USA) as previously described (Woodiwiss *et al.*, 2009). The size of the cuff was the same as that used for conventional BP measurements. Monitors were programmed to measure 24-hour BP at 15-min intervals from 06:00 to 22:00 hours and at 30-min intervals from 22:00 to 06:00 hours. Intra-individual means of the ambulatory measurements were weighted by the time interval between successive readings (Woodiwiss *et al.*, 2009). The average (±SD) number of BP readings obtained was 60.7±12.2 (range=24 to 81) for the 24-h period.

4.2.3 Estimated GFR. Serum creatinine concentrations were measured using the Advia Chemistry systems (Siemens) with calibration traceable to isotope dilution mass spectrometry (IDMS). The 4-variable MDRD and CKD-EPI equations were employed to estimate GFR. The ethnicity factor as recommended in African Americans when calculating the MDRD and CKD-EPI eGFR was not applied in the present study as the use results in overestimation of kidney function in black Africans (Van Deventer *et al.*, 2011; Eastwood *et al.*, 2010).

4.2.4 Inflammatory markers. Blood samples were centrifuged and immediately stored at -80^{o}C. Plasma concentrations of human resistin and high-sensitivity (range 0.01 to 50 ng/ml) CRP concentrations were measured using enzyme-linked immunosorbent assays (Quantikine, R&D Systems Inc, Minneapolis, MN, USA). Resistin was selected as an adipocytokine with possible adverse effects on renal function beyond that of obesity *per se* and CRP as a marker of general inflammation. The resistin assay had a lower detection limit of 0.026 ng/ml and intra-assay and inter-assay coefficients of variation ranging from 3.8 to 5.3% and 7.8 to 9.2% respectively. The CRP assay had a mean lower detection limit of 0.010 ng/ml and intra-assay and inter-assay coefficients of variation ranging from 3.8 to 8.3% and 6.0 to 7.0% respectively.

4.2.5 Central aortic haemodynamics. Central aortic haemodynamics were determined as previously described (Norton *et al.*, 2012; Woodiwiss *et al.*, 2009). After participants had rested for 15 minutes in the supine position, arterial waveforms at the radial (dominant arm), carotid and femoral artery pulses were recorded by applanation tonometry. Pressure waveforms were recorded during an 8-second period using a high-fidelity SPC-301 micromanometer (Millar Instrument, Inc., Houston, Texas) interfaced with a computer employing SphygmoCor, version 9.0 software (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia). To determine aortic BP the pulse wave obtained from the radial tonometer recordings was calibrated by manual measurement (auscultation) of brachial BP taken immediately before the recordings.

The radial pressure waveform was converted into a central (aortic) waveform using a validated generalized transfer function incorporated in SphygmoCor software. Central aortic SBP was derived from the aortic waveform. Aortic PWV was determined from sequential waveform measurements at carotid and femoral sites. The time delay in the pulse waves between the carotid and femoral sites was determined using an electrocardiograph-derived R wave as a fiducial point. Pulse transit time was taken as the average of 10 consecutive beats. The distance which the pulse wave travels was determined as the difference between the distance from the femoral sampling site to the suprasternal notch, and the distance from the carotid sampling site to the suprasternal notch. Aortic PWV was calculated as the ratio of the distance to the transit time (m/sec).

4.2.6 Data analysis. Database management and statistical analyses were performed with SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA). Continuous data are reported as mean±SD or SEM, or median and interquartile range. Unadjusted means and proportions were compared by the large-sample z-test and the χ^2-statistic, respectively. As HOMA-IR and circulating concentrations of inflammatory markers were non-normally distributed, they were transformed to improve on the distribution (Table 4.1). Bivariate correlations were assessed from Pearson's correlation coefficients. Independent relationships were assessed from linear, logistic or stepwise regression analysis with appropriate adjustors. Probability values were further adjusted for non-independence of family members using the method of maximum likelihood as implemented by the mixed procedure as defined in the SAS package.

4.3 Results

4.3.1 Participant characteristics. Table 4.2 shows the characteristics of the participants in the study sample. More women than men participated and a high prevalence of hypertension and obesity was noted with many hypertensives being untreated or uncontrolled. 34.9% of the sample had an eGFR=60-90 mls/min/1.73m^2 and 6.4% an eGFR=20-60 mls/min/1.73m^2.

4.3.2 Adiposity indexes or associated metabolic abnormalities and eGFR. Except for LDL cholesterol concentrations that showed only weak independent relationships with eGFR, no adiposity index, nor obesity-associated metabolic abnormality (glucose or lipids) independently associated with eGFR or creatinine concentrations (Table 4.3).

4.3.3 Associations between resistin or insulin resistance and eGFR. In multivariate regression models, independent of conventional risk factors including HbA1c values (blood glucose control), HOMA-IR, and plasma resistin concentrations (Figure 4.1), but not CRP concentrations (Table 4.4) were independently associated with creatinine concentrations or eGFR and these effects were unaffected by adjustments for adiposity indexes or obesity-

Table 4.1 Distribution of circulating inflammatory markers or the homeostasis model of insulin resistance (HOMA-IR) before and after transformation of data.

Parameter	Skewness	Kurtosis	Shapiro-Wilk
Un-transformed			
C-reactive protein	4.22	31.2	0.64
Resistin	2.81	15.8	0.80
HOMA-IR	4.33	26.3	0.57
Logarithm of:			
C-reactive protein	-0.37	0.04	0.99
Resistin	0.12	0.52	1.00
HOMA-IR	0.36	-0.24	0.98

Table 4.2. Participant characteristics.

	All	With 24-hour BP	With PWV
Number (% female)	1010 (63.6)	688 (62.9)	896 (62.2)
Age (years)	44.0±18.2	43.9±18.1	43.5±18.4
Body mass index (kg/m^2)	29.3±7.9	28.7±7.6	28.6±7.4
% overweight/obese	23.5/41.8	25.4/38.2	24.3/38.8
Waist circumference (cm)	90.6±16.8 (n=984)	89.7±16.4 (n=669)	89.5±16.1 (n=876)
% abdominal obesity	44.9	41.9	41.8
Waist/hip ratio (WHR)	0.84±0.10 (n=984)	0.84±0.10 (n=669)	0.83±0.10 (n=876)
Regular tobacco (% subjects)	14.7	14.8	16.1
Regular alcohol (% subjects)	19.9	20.3	20.5
% Females postmenopausal	43.8	41.8	42.9
% Diabetes mellitus	13.7	12.8	13.1
% Glucose lowering agents	7.5	7.0	7.3
% Hypertension	44.7	43.3	43.8
Current antihypertensive meds (%)	22.9	21.1	22.1
Glucose (mmol/l)	5.26±2.46	5.30±2.60	5.26±2.54
LDL cholesterol (mmol/l)	2.63±0.94	2.62±0.97	2.63±0.95
HDL cholesterol (mmol/l)	1.40±0.41	1.41±0.41	1.41±0.42
Triglycerides (mmol/l)	1.24±1.12	1.25±1.28	1.23±1.15
Insulin (μU/ml)	8.45 (4.15 to 15.32)	8.92 (4.32 to 17.2)	8.32 (4.02 to 15.00)
HOMA-IR	1.80 (0.84 to 3.74)	1.86 (0.88 to 4.19)	1.74 (0.82 to 3.65)
Resistin (ng/ml)	10.8 (7.7 to 15.7)	10.5 (7.5 to 15.2)	10.7 (7.7 to 15.5)
C-reactive protein (ng/ml)	3.80 (1.42 to 8.44)	3.45 (1.24 to 8.37)	3.65 (1.31 to 8.06)
Office SBP/DBP (mm Hg)	128±22/84±13	128±22/83±12	128±22/83±12
24-hour SBP/DBP (mm Hg)	118±15/72±10 (n=688)	118±15/72±10	118±15/72±10
Aortic pulse wave velocity (m/sec)	6.26±2.67 (n=896)	6.39±2.78 (n=608)	6.26±2.67
Aortic SBP (mm Hg)	120±22	119±22	119±22
Creatinine (μmol/l)	75.1±22.0	75.6±19.9	75.0±22.3
eGFR (MDRD) (mls/min/1.73m^2)	94.3±26.1	93.8±25.9	95.0±25.8
eGFR (CKD-EPI) (mls/min/1.73m^2)	95.0±22.2	94.5±22.3	95.7±22.1

Data shown are mean±SD, median and interquartile range and proportions. LDL, low density lipoprotein; HDL, high density lipoprotein; HOMA-IR, homeostasis model of insulin resistance; SBP, systolic blood pressure; DBP, diastolic BP; eGFR, estimated glomerular filtration rate;

MDRD, Modification of Diet in Renal Disease equation; CKD-EPI- Chronic Kidney Disease Epidemiology equation.

Table 4.3. Multivariate-adjusted relationships between adiposity indexes or obesity-related metabolic changes and estimated glomerular filtration rate (eGFR) in a community sample.

	MDRD eGFR		CKD-EPI eGFR	
eGFR versus	Partial r (95% CI)	p value	Partial r (95% CI)	p value
Body mass index	-0.011 (-0.07 to 0.05)	=0.77	0.010 (-0.05 to 0.07)	=0.75
Waist circumference	0.006 (-0.06 to 0.07)	=0.85	0.030 (-0.04 to 0.09)	=0.42
Waist-to-hip ratio	-0.007 (-0.07 to 0.06)	=0.84	0.002 (-0.06 to 0.07)	=0.95
Glucose	-0.05 (-0.11 to 0.01)	=0.10	-0.05 (-0.11 to 0.01)	=0.14
Triglycerides	-0.002 (-0.06 to 0.06)	=0.96	0.01 (-0.05 to 0.07)	=0.80
HDL cholesterol	0.04 (-0.02 to 0.10)	=0.23	0.03 (-0.03 to 0.10)	=0.31
LDL cholesterol	-0.09 (-0.15 to -0.02)	<0.01	-0.08 (-0.14 to -0.02)	<0.05

See table 4.2 for abbreviations. *Adjustments are for age, sex, conventional systolic blood pressure, regular tobacco use, regular alcohol consumption, diabetes mellitus, and HbA1c.

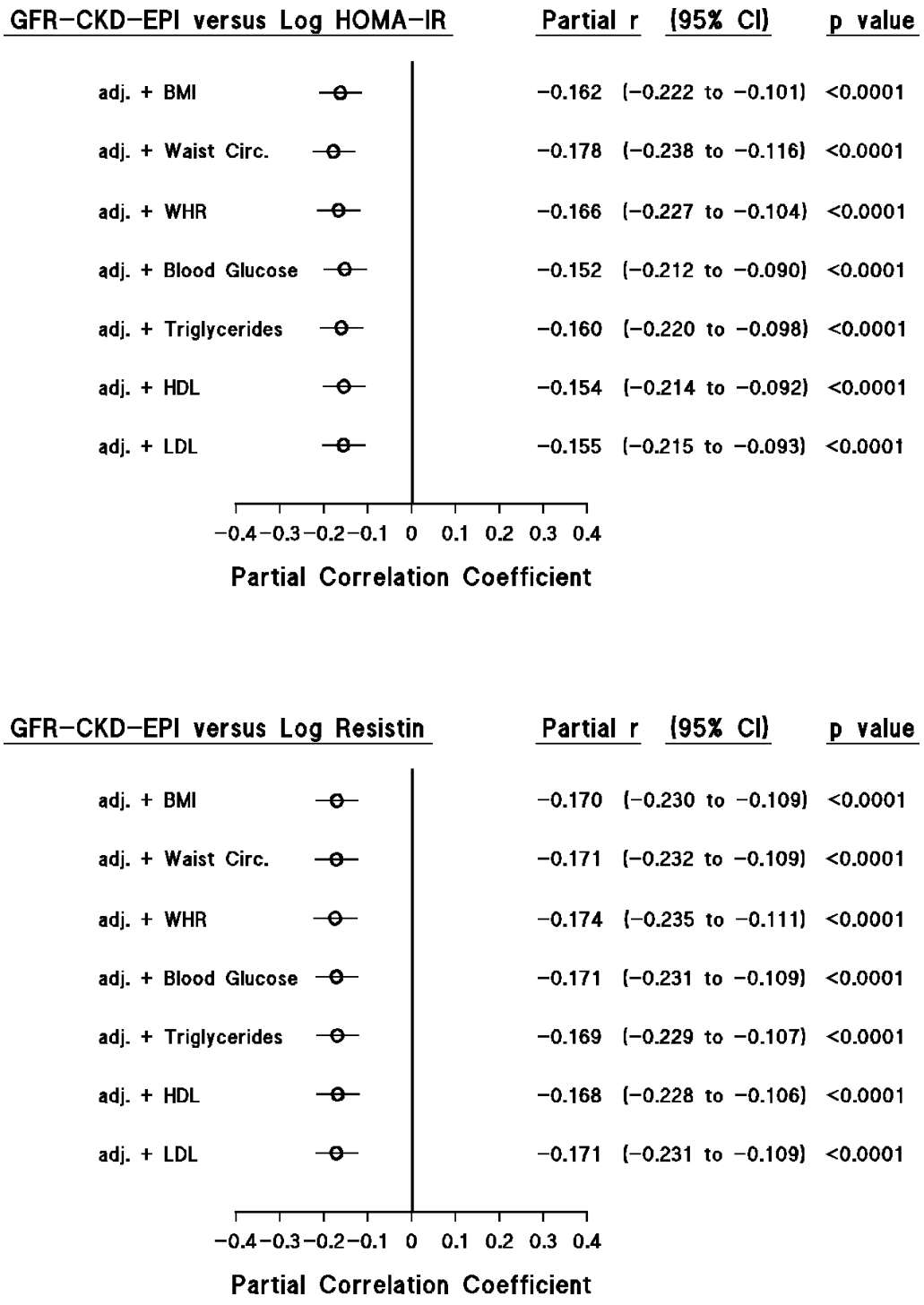


Figure. 4.1. Independent relationships between the homeostasis model of insulin resistance (HOMA-IR), or plasma resistin concentrations and estimated glomerular filtration rate (eGFR-CKD-EPI) beyond indexes of adiposity or obesity-associated metabolic features in a community sample. See table 4.2 for abbreviations. Adjustments are for age, sex, conventional systolic blood pressure, regular tobacco use, regular alcohol consumption, diabetes mellitus, HbA1c and the adiposity index or metabolic feature indicated.

Table 4.4. Multivariate-adjusted (partial r) relationships between C-reactive protein (CRP) concentrations and estimated glomerular filtration rate (eGFR- Chronic Kidney Disease Epidemiology equation) in the community sample.

CRP versus	Partial r (95% CI)	p value
MDRD eGFR	-0.05 (-0.11 to 0.01)	=0.11
CKD-EPI eGFR	-0.04 (-0.10 to 0.02)	=0.19

See table 4.2 for abbreviations. *Adjustments are for age, sex, conventional systolic blood pressure, regular tobacco use, regular alcohol consumption, diabetes mellitus, and HbA1c.

associated metabolic abnormalities (Figure 4.1). The independent relationships between HOMA-IR or resistin concentrations and eGFR or creatinine concentrations were noted in several subgroups of the sample including men and women separately (Table 4.5), and across tertiles of adiposity indexes (data not shown). Moreover, the independent relationships between resistin concentrations and eGFR were independent of CRP concentrations (Table 4.6).

4.3.4 Relative impact of resistin or insulin resistance on eGFR and CKD. In stepwise regression models, HOMA-IR and resistin concentrations were second and third only to age and at least as strong as BP or PWV in the impact (standardized β-coefficient) on eGFR and CKD (Tables 4.7 and 4.8). The impact of HOMA-IR and resistin concentrations together on eGFR or CKD was markedly greater than the impact of conventional risk factors combined. The relative impact of HOMA-IR or resistin concentrations on eGFR or CKD were similar irrespective of whether conventional brachial BP, 24-hour BP, aortic BP or aortic PWV were included in the regression models (Tables 4.7 and 4.8). The independent relationships between insulin resistance or resistin concentrations and eGFR were independent of each other (Tables 4.7 and 4.8) and of CRP concentrations (Table 4.6). Independent relationships between insulin resistance or resistin concentrations and creatinine concentrations or eGFR translated into comparable stepwise decreases in eGFR across octiles of HOMA-IR or resistin concentrations as did changes in eGFR across octiles of systolic BP (Figure 4.2).

4.4 Discussion

The main findings of the present study are as follows: In a large community sample with prevalent obesity (41.8% of sample), independent of confounders, insulin resistance and resistin concentrations combined produced a greater impact on creatinine concentrations, eGFR and CKD than modifiable conventional risk factors. These effects were noted irrespective of whether conventional BP or alternative obesity-associated hemodynamic changes (including 24-hour or aortic BP, and aortic PWV) were considered.

Obesity contributes to the development and progression of CKD (Iseki *et al.*, 2004; Kramer *et al.,* 2006; Ejerblad *et al.,* 2006). Animal-based studies suggest a distinct action of insulin resistance in contributing to these effects (Whaley-Connell *et al.*, 2017). Whilst a number of prior studies have demonstrated independent relationships between indexes of insulin resistance and CKD (Mykkänen *et al.*, 1998; Chen *et al.*, 2003; Whaley-Connell *et al.*, 2010; Chen *et al.*, 2004) these studies also show relationships that are stronger in the presence of obesity (Mykkänen *et al.*, 1998; Chen *et al.*, 2003). In addition, several of these prior studies have demonstrated that relationships between insulin resistance and glomerular function are

Table 4.5. Sex-specific multivariate-adjusted (partial r) relationships between the homeostasis model of insulin resistance (HOMA-IR) or resistin concentrations and estimated glomerular filtration rate (eGFR-Chronic Kidney Disease Epidemiology equation) in the community sample.

	Women (n=642)		Men (n=368)	
eGFR versus	partial r* (95% CI)	p value	partial r* (95% CI)	p value
HOMA-IR	-0.149 (-0.224 to -0.071)	<0.0005	-0.167 (-0.266 to -0.064)	<0.002
Resistin	-0.144 (-0.220 to -0.066)	<0.0005	-0.209 (-0.306 to -0.107)	<0.0001

*Adjustments are for age, sex, conventional systolic blood pressure, regular tobacco use, regular alcohol consumption, diabetes mellitus, and HbA1c.

Table 4.6. Impact of adjustments for C-reactive protein (CRP) on multivariate-adjusted relationships between resistin concentrations and estimated glomerular filtration rate (eGFR-Chronic Kidney Disease Epidemiology equation) in the community sample.

	MDRD eGFR		CKD-EPI eGFR	
Resistin versus Adjustments	Partial r (95% CI)	p value	Partial r (95% CI)	p value
eGFR *	-0.160 (-0.221 to -0.099)	<0.0001	-0.170 (-0.230 to -0.108)	<0.0001
* + CRP	-0.153 (-0.214 to -0.092)	<0.0001	-0.166 (-0.226 to -0.104)	<0.0001

See table 4.2 for abbreviations. *Adjustments are for age, conventional systolic blood pressure, regular tobacco use, regular alcohol consumption, diabetes mellitus, HbA1c and CRP as indicated.

Table 4.7. Relative impact (standardized slopes [β-coefficients]) of factors accounting for variations in estimated glomerular filtration rate (eGFR)(Chronic Kidney Disease Epidemiology equation) in a community sample.

Models with→	Brachial SBP		24-Hour SBP		Aortic SBP		Aortic PWV	
n=	984		669		977		876	
eGFR vs	β-coeff.±SEM	p value	β-coeff.±SEM	p value	β-coeff.±SEM	p value	β-coeff.±SEM	p value
Age	-0.67±0.03	<0.0001	-0.67±0.04	<0.0001	-0.66±0.03	<0.0001	-0.64±0.04	<0.0001
HOMA-IR	-0.13±0.02	<0.0001	-0.12±0.03	<0.0001	-0.13±0.02	<0.0001	-0.14±0.02	<0.0001
Resistin	-0.12±0.02	<0.0001	-0.12±0.03	<0.0001	-0.12±0.02	<0.0001	-0.12±0.02	<0.0001
Diabetes mellitus	0.005±0.030	=0.88	0.009±0.036	=0.80	0.003±0.030	=0.93	-0.006±0.032	=0.85
Hypertension	0.02±0.03	=0.42	-0.02±0.03	=0.61	0.02±0.03	=0.51	-0.002±0.030	=0.94
HbA1c	0.009±0.031	=0.77	-0.004±0.037	=0.90	0.008±0.031	=0.80	0.033±0.033	=0.32
Waist circumference	0.05±0.03	=0.06	0.05±0.03	=0.13	0.05±0.03	=0.06	0.04±0.03	=0.16
Brachial SBP	-0.09±0.03	<0.005	-	-	-	-	-	-
24-hour SBP	-	-	-0.04±0.03	=0.21	-	-	-	-
Aortic SBP	-	-	-	-	-0.08±0.03	=0.005	-	-
Aortic PWV	-	-	-	-	-	-	-0.09±0.03	<0.005

β-coeff., standardized β-coefficient; HOMA-IR, homeostasis model of insulin resistance; SBP, systolic blood pressure; PWV, pulse wave velocity. Also included in the regression models were sex, regular tobacco use, and regular alcohol consumption.

Table 4.8. Relative impact (standardized slopes [β-coefficients]) of factors accounting for chronic kidney disease (CKD) in a community sample.

Models with→	Brachial SBP		24-Hour SBP		Aortic SBP		Aortic PWV	
n= CKD/Total	404/984		279/669		400/977		348/876	
CKD vs	β-coeff.±SEM	p value	β-coeff.±SEM	p value	β-coeff.±SEM	p value	β-coeff.±SEM	p value
Age	0.58±0.04	<0.0001	0.56±0.04	<0.0001	0.59±0.04	<0.0001	0.55±0.04	<0.0001
HOMA-IR	0.12±0.03	<0.0001	0.08±0.03	<0.05	0.12±0.03	<0.0001	0.11±0.03	<0.0005
Resistin	0.07±0.03	<0.02	0.08±0.03	<0.02	0.06±0.03	<0.05	0.07±0.03	<0.02
Diabetes mellitus	0.05±0.04	=0.19	0.02±0.04	=0.63	0.05±0.04	=0.19	0.04±0.04	=0.34
Hypertension	0.02±0.04	=0.53	-0.01±0.04	=0.71	0.01±0.04	=0.72	0.03±0.04	=0.40
HbA1c	-0.03±0.04	=0.47	-0.04±0.04	=0.42	-0.03±0.04	=0.45	-0.03±0.04	=0.43
Waist circumference	-0.07±0.03	<0.05	-0.04±0.04	=0.32	-0.07±0.03	<0.05	-0.07±0.04	=0.06
Brachial SBP	0.02±0.03	=0.60	-	-	-	-	-	-
24-hour SBP	-	-	0.004±0.037	=0.91	-	-	-	-
Aortic SBP	-	-	-	-	0.004±0.036	=0.91	-	-
Aortic PWV	-	-	-	-	-	-	0.07±0.04	=0.07

β-coeff., standardized β-coefficient; HOMA-IR, homeostasis model of insulin resistance; SBP, systolic blood pressure; PWV, pulse wave velocity. Also included in the regression models were sex, regular tobacco use, and regular alcohol consumption. CKD was identified as eGFR values <90 mls/min/1.73m^2 from eGFR determined using the Chronic Kidney Disease Epidemiology equation.

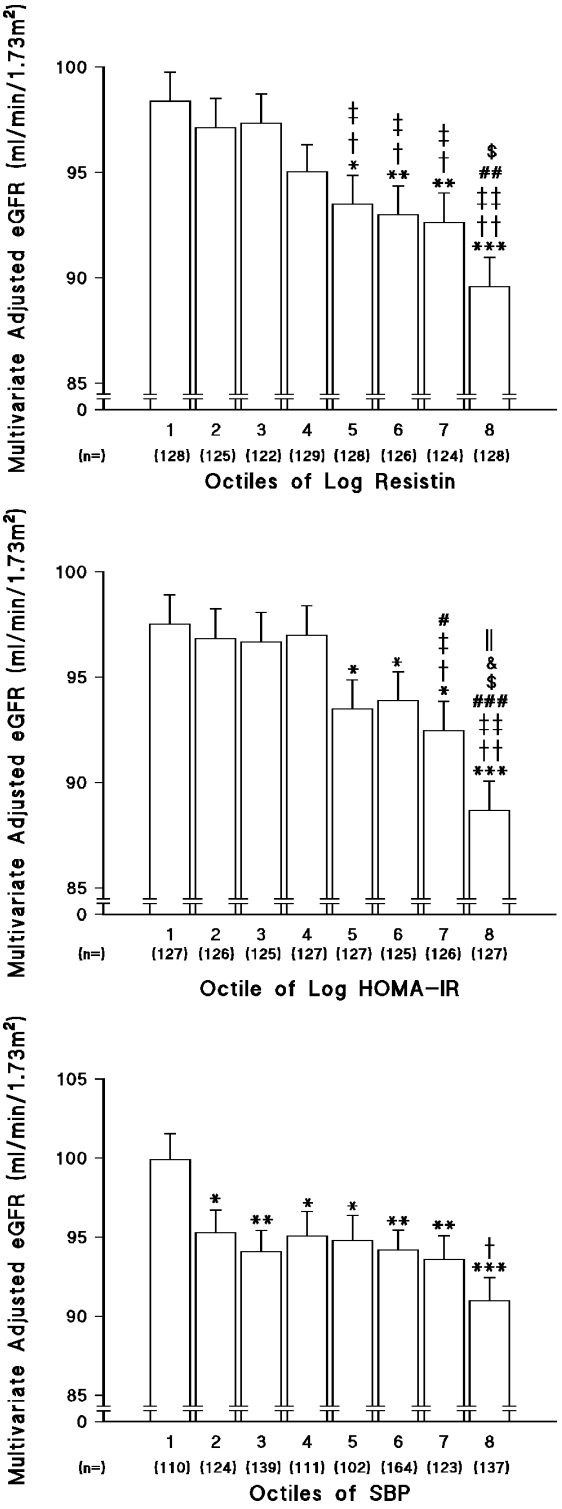


Figure 4.2. Multivariate adjusted estimated glomerular filtration rates (eGFR-CKD-EPI) across octiles of insulin resistance (homeostasis model-HOMA-IR) or plasma resistin concentrations as compared to systolic blood pressure (SBP) in a community sample. Adjustments are for age, sex, waist circumference, conventional systolic blood pressure (for HOMA-IR and resistin), regular tobacco use, regular alcohol consumption, diabetes mellitus, and HbA1c.*p<0.05,**p<0.005,***p<0.0001 vs octile 1,†p<0.05, ††p<0.0001 versus octile 2, ‡p<0.05, ‡‡p<0.0001 versus octile 3, $^{\#}$p<0.05, $^{\#\#}$p<0.005, $^{\#\#\#}$p<0.0001 versus octile 4, $^{\$}$p<0.05 versus octile 5, $^{\&}$p<0.05 versus octile 6, $^{\|}$p<0.05 versus octile 7.

strengthened by increasing components of the metabolic syndrome such as central obesity, hypertension, and the presence of dyslipidemia (Mykkänen *et al.*, 1998; Chen *et al.*, 2004). Thus, whilst a relationship between insulin resistance and CKD is accepted, the impact of insulin resistance relative to obesity-associated conventional risk fators on eGFR is unknown (Whaley-Connell *et al*., 2017). In contrast to previous findings (Mykkänen *et al.*, 1998; Chen *et al.*, 2003; Chen *et al.*, 2004), we show that relationships between HOMA-IR and eGFR are unaffected by the extent of obesity *per se* or the presence of metabolic features (glucose or lipid abnormalities), neither of which were independently correlated with eGFR. In addition, these effects were independent of BP. These data therefore support a view that the adverse renal effects of insulin resistance are distinct from that of obesity or associated metabolic features and that the impact is at least as strong as that of modifiable conventional risk factors (BP). Importantly, in combination with circulating resistin concentrations the impact of insulin resistance is markedly stronger than the combined impact of modifiable conventional risk factors. Consequently, targeting conventional risk factors alone may result in a marked residual impact on the development of CKD in communities with a high prevalence obesity.

There is also evidence to support a role for adipocytokines (obesity-associated inflammatory changes) in the development and progression of CKD (Axelsson *et al.,* 2008; Kawamura *et al.*, 2010; Menzaghi *et al.*, 2012; Mills *et al.*, 2013; Moreno *et al.,* 2015; Liu *et al.*, 2016). Animal-based studies similarly suggest a distinct action of inflammatory substances in contributing to these effects (Tomosugi *et al.*, 1989; Bertani *et al.*, 1989). However, clinical data are again unclear as to the relative impact of adipocytokines as compared to that of conventional risk factors on renal function. Whilst a number of prior studies have demonstrated relationships between circulating concentrations of adipocytokines and CKD (Axelsson *et al.,* 2008; Kawamura *et al.*, 2010; Menzaghi *et al.*, 2012; Mills *et al.*, 2013; Moreno *et al.,* 2015; Liu *et al.*, 2016), whether these relationships are stronger in the presence of obesity or obesity-associated metabolic features and the impact of adipocytokines relative to that of conventional risk factors, has not been determined. In this regard, we show that relationships between circulating resistin concentrations and eGFR are unaffacted by the extent of obesity, or the presence of obesity-associated metabolic features and that the relative impact of resistin concentrations on eGFR or CKD is at least as strong as modifiable conventional risk factors. Importantly, the combined impact of HOMA-IR and circulating resistin concentrations on eGFR is substantially greater than the combined impact of modifiable conventional risk factors.

Although relationships between either insulin resistance or circulating adipocytokine concentrations and eGFR have been demonstrated on several previous occasions (Mykkänen *et al.*, 1998; Chen *et al.*, 2003; Whaley-Connell *et al.*, 2010; Axelsson *et al.,* 2008;,Kawamura *et al.*, 2010; Menzaghi *et al.*, 2013; Mills *et al.*, 2013; Moreno *et al.*, 2015; Liu *et al.*, 2016; Chen *et al.*, 2004), these relationships have employed office conventional BP as the hemodynamic

adjustor. In this regard, none of these studies have considered the possibility that adjustments for office conventional BP measurements are inadequate for discounting the hemodynamic actions of insulin resistance, inflammatory changes or obesity *per se*. In this regard, obesity effects on BP, which are likely to be induced in-part by insulin resistance, are not detected using office BP measurements alone. Indeed, obesity results in masked effects on in-office BP (Hänninen *et al.*, 2011). Moreover, both insulin resistance (Bäckdahl *et al.*, 2018) and inflammatory adipocytokines (Norman *et al.*, 2016) may cause increases in aortic stiffness, and through an impact on aortic impedance may produce renal microvascular damage beyond brachial BP. Hence, it is uncertain whether relationships between insulin resistance or adipocytokines and eGFR (Mykkänen *et al.*, 1998; Chen *et al.*, 2003; Whaley-Connell *et al.*, 2010; Axelsson *et al*., 2008; Kawamura *et al.*, 2010; Menzaghi *et al.*, 2012; Mills *et al.*, 2013; Moreno *et al.*, 2015; Liu *et al.*, 2016; Chen *et al.*, 2004) are indeed beyond the adverse hemodynamic effects of these changes. However, in the present study relationships between HOMA-IR or resistin concentrations and eGFR were largely unaffected by adjustments for 24-hour BP, aortic BP or aortic PWV. The present study therefore suggests that the actions of insulin resistance or resistin are distinct from that of the adverse hemodynamic consequences of these alterations.

There are several limitations to the present study. The present study is cross-sectional in design and hence reverse causality may account for several of the relationships noted. In this regard, insulin resistance may be a consequence of CKD rather than a cause (Whaley-Connell *et al.,* 2017) and reductions in eGFR may result in a reduced clearance of circulating substances and increases in circulating resistin concentrations. However, being a community-based study few participants had late stages of CKD, which is more likely to result in insulin resistance or a reduced clearance of circulating resistin. Indeed, the relationships between insulin resistance or resistin concentrations and eGFR were largely in the early CKD stage range. Second, the appropriate formula for calculating eGFR from serum creatinine concentrations in groups of black African ancestry is uncertain and ethnic-specific formulae have not been identified in Africa. Consequently, validation of the present study is required in other populations where obesity or the associated lipid or glucose abnormalities contribute little to reductions in eGFR, but where insulin resistance and adipocytokines may play a role. Third, we assessed the impact of resistin and CRP alone on eGFR and failed to evaluate the several additional adipocytokine changes that may influence eGFR. Consequently, we are likely to have underestimated the contribution of obesity-associated inflammatory changes to reductions in eGFR. Despite this possibility we nevertheless still show a combined effect of resistin and insulin resistance on eGFR which is substantially greater than that of the combined impact of conventional risk factors including adiposity indices *per se*.

4.5 Conclusions

We show in a large community-based sample with a high prevalence of obesity that the combined impact of insulin resistance, as indexed by HOMA-IR and circulating resistin concentrations on eGFR or CKD is greater than that of all modifiable conventional cardiovascular risk factors together. Importantly these effects were beyond even ambulatory or aortic BP and aortic stiffness. These data suggest that targeting conventional risk factors alone may result in a marked residual impact on the development of CKD in communities with a high prevalence obesity. To adequately address the public health burden of CKD, approaches may therefore be required that influence insulin resistance and the adverse effects of resistin. Importantly, obesity per se was not independently associated with eGFR or CKD in the present community sample and hence targeting obesity is unlikely to effectively address the adverse impact of insulin resistance or resistin on CKD. It is therefore important that the cause of insulin resistance and enhanced circulating concentrations of resistin beyond obesity effects are identified and strategies put in-place to modify these changes.

CHAPTER 5

Associations between Resistin and Left Ventricular Mass are Not Accounted for by Effects on Aortic Stiffness or the Cardio-Renal Syndrome.

The data for this chapter have been submitted to the journal *BMC Cardiovascular Disorders f*or consideration.

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I was involved in data collection from 2014 until 2019. In addition I retrieved the samples from storage and aliquoted the samples before assisting with running the Elisas for the resistin, CRP and HOMA-IR. Furthermore, I did all the data analysis for the publications using SAS software, version 9.4.

5.0 Abstract

Background: Although, in-part through an impact on left ventricular mass (LVM), the adipokine resistin may play a role in heart failure, whether this is explained by the adverse effects of resistin on aortic stiffness and renal function is unknown.

Methods: In 739 randomly selected participants from a community sample, we assessed relationships between circulating resistin concentrations and LVM index (LVMI), and LVM beyond that predicted by stroke work (inappropriate LVMI [LVM_{inappr}]) (echocardiography) and evaluated, in product of coefficient mediation analysis, the extent to which these relationships could be explained by aortic pulse wave velocity (PWV) or estimated glomerular filtration rate (eGFR).

Results: Independent of confounders, resistin concentrations were independently associated with LVMI, LVM_{inappr}, LV hypertrophy (LVH), PWV and eGFR. Furthermore, independent of confounders, LVMI, LVM_{inappr} and LVH were independently associated with PWV and eGFR. However, adjustments for either PWV or eGFR failed to modify relationships between resistin concentrations and LVMI, LVM_{inappr} or LVH. Moreover, in product of coefficient mediation analysis, neither eGFR nor PWV explained a significant proportion of the relationship between resistin and LV structural alterations.

Conclusions*:* Independent relationships between circulating concentrations of the adipocytokine resistin and LVM are not explained by the impact of resistin on ventricular-vascular coupling or the cardio-renal syndrome. Resistin's effect on LVMI are therefore likely to be through direct actions on the myocardium.

Key words: obesity, inappropriate left ventricular mass, resistin, aortic stiffness, renal function.

5.1 Introduction

Independent of conventional risk factors and myocardial infarction, obesity predicts the development of heart failure.(Kenchaiah *et al.*, 2002; Ingelsson *et al*., 2005; Bahrami *et al.*, 2008). Although insulin resistance may play an important role in mediating obesity-induced heart failure, insulin resistance accounts for only a minor proportion of these effects (Ingelsson *et al.*, 2005; Bahrami *et al.*, 2008). Importantly, beyond adiposity indexes and insulin resistance, circulating concentrations of the adipocytokine resistin, which is predominantly expressed in monocytes and macrophages, and which is responsive to inflammatory stimuli (Patel *et al.*, 2003; Lu *et al.*, 2002; Kaser *et al.*, 2003), predicts the development of and prognosis in heart failure (Butler *et al.*, 2009; Frankel *et al.*, 2009; Zhang *et al.*, 2011; Wu *et al.*, 2012; Takeishi *et al.*, 2007; Muse *et al.*, 2015). Thus, resistin may in-part explain independent relationships between obesity and heart failure. However, at present the mechanisms responsible for resistin effects on heart failure are uncertain.

Recently, we reported the presence of strong, independent relationships between circulating resistin concentrations and left ventricular mass (LVM) in a large community-based sample (Norman *et al.*, 2015). As LVM is a well-recognized determinant of the progression to heart failure, the impact of resistin may in-part be explained by effects on LVM. Relationships between resistin and LVM may nevertheless be accounted for by several mechanisms. In this regard, effects on LVM may be through direct actions on the myocardium as cardiomyocyte overexpression of resistin promotes myocardial hypertrophy in mice (Kim *et al.*, 2008; Chemaly *et al.*, 2011; Kang *et al.*, 2011; Schwartz *et al.*, 2013). However, resistin may also mediate increases in LVM through indirect actions. Indeed, resistin independently associates with decreases in glomerular function (Mills *et al.*, 2013) and through the cardio-renal syndrome, increases in LVM are strongly associated with renal dysfunction beyond haemodynamic effects (Maunganidze *et al.*, 2013). Alternatively, beyond blood pressure effects, resistin may also enhance afterload to the LV through ventricular-vascular coupling. Ventricular-vascular coupling may account for resistin's effect on LVM through an enhanced aortic stiffness (Norman *et al.*, 2015), as aortic stiffness associates with LVM beyond blood pressure (Bello *et al.*, 2017). As it is uncertain whether resistin's effects on LVM and hence possibly heart failure may be accounted for by indirect (cardio-renal syndrome or ventricular-vascular coupling) or direct myocardial effects, using product of coefficient mediation analysis, in the present study we assessed the extent to which independent relationships between resistin and LVM in a large community-based sample are explained by an impact of resistin on renal or aortic function.

5.2 Methods

5.2.1 Study group. The present study was conducted according to the principles outlined in the Helsinki declaration. The Committee for Research on Human Subjects of the University of the Witwatersrand approved the protocol (approval numbers: M02-04-72 renewed as M07-04-69, M12-04-108 and M17-04-01). Participants gave informed, written consent. The study design has previously been described (Norton *et al.*, 2012; Woodiwiss *et al.*, 2009). Nuclear families of black African descent with siblings older than 16 years of age were randomly recruited from the South West Township (SOWETO) of Johannesburg, South Africa. Of the 1024 participants recruited, in a sub-study 739 participants had echocardiography, of whom 554 were not receiving treatment for hypertension. Of the sample 647 had aortic pulse wave velocity.

5.2.2 Clinical, demographic, anthropometric and blood measurements. Demographic and clinical data were obtained using a standardized questionnaire (Norton *et al.*, 2012; Woodiwiss *et al.*, 2009). Height, weight, waist (WC) and hip circumference were measured using standard approaches and participants were identified as being overweight if their body mass index (BMI) was ≥25 kg/m^2 and obese if their BMI was ≥30 kg/m^2. Central obesity was defined as an enlarged WC (≥88 cm in women and ≥102 cm in men). Laboratory blood tests of renal function, liver function, blood glucose, lipid profiles, hematological parameters, and percentage glycated hemoglobin (HbA1c) (Roche Diagnostics, Mannheim, Germany) were performed. Diabetes mellitus (DM) or abnormal blood glucose control was defined as the use of insulin or oral hypoglycemic agents or an HbA1c value greater than 6.1% respectively. Brachial BP measurements were obtained by a trained nurse-technician using a standard mercury sphygmomanometer.

Serum creatinine concentrations were measured using the Advia Chemistry systems (Siemens) with calibration traceable to isotope dilution mass spectrometry (IDMS). The 4-varible CKD-EPI equation was employed to estimate glomerular filtration rate (GFR) (Kolkenbeck-Ruh *et al.*, 2018). Blood samples for the measurement of insulin and resistin concentrations were centrifuged and immediately stored at -80^{o}C. Fasting plasma insulin concentrations were determined from an insulin immulite, solid phase, two-site chemiluminescent immunometric assay (Diagnostic Products Corporation, Los Angeles, CA, USA) and insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) using the formula (insulin [µU/ml] x glucose [mmol/l])/22.5 Plasma concentrations of human resistin was measured using enzyme-linked immunosorbent assay (Quantikine, R&D Systems Inc, Minneapolis, MN, USA). The resistin assay had a lower detection limit of 0.026 ng/ml and intra-assay and inter-assay coefficients of variation ranging from 3.8 to 5.3% and 7.8 to 9.2% respectively.

5.2.3 Echocardiography. Echocardiography was performed as previously described (Norton *et al.*, 2012; Woodiwiss *et al.*, 2009; Woodiwiss *et al.*, 2012). All measurements were recorded and analyzed off-line by experienced investigators whom were unaware of the clinical data of the participants. Left ventricular mass was determined using a standard formula (Devereux *et al.*, 1986) and indexed (LVMI) to height$^{1.7}$. Left ventricular hypertrophy was defined as an LVMI≥60 g/m$^{1.7}$ in women and LVMI≥80 g/m$^{1.7}$ in men (Chirinos *et al.*, 2010). Stroke volume was evaluated from the difference between LV end diastolic and systolic volumes determined using the Z-derived method (Woodiwiss *et al.*, 2012). To determine the impact of resistin on LVM beyond work load, we determined inappropriate LVM (LVM_{inappr}). The extent of LVM_{inappr} was determined from predicted LVM (Woodiwiss *et al.*, 2012), where predicted LVM was calculated as 55.37+(6.64 x height$^{2.7}$)+(0.64 x [systolic BP x stroke volume x 0.014]) – (18.07 x gender), where male gender=1 and female gender=2, and where stroke volume was calculated from LV volumes assessed from the Z-derived method. Inappropriate LVM was expressed either as actual-predicted LVM in grams, or % actual LVM/predicted LVM.

5.2.4 Aortic stiffness. Aortic stiffness was assessed using carotid-femoral (aortic) pulse wave velocity (PWV) as previously described (Norman *et al.*, 2016; Bello *et al.*, 2017). In this regard, after participants had rested for 15 minutes in the supine position, sequential arterial waveforms at the carotid and femoral pulse were recorded by applanation tonometry during an 8-second period each using a high-fidelity SPC-301 micromanometer (Millar Instrument, Inc., Houston, Texas) interfaced with a computer employing SphygmoCor, version 9.0 software (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia). The time delay in the pulse waves between the carotid and femoral sites was determined using an electrocardiograph-derived R wave as a fiducial point. Pulse transit time was obtained from the average of 10 consecutive beats. The distance which the pulse wave travels was determined as the difference between the distance from the femoral sampling site to the suprasternal notch, and the distance from the carotid sampling site to the suprasternal notch.

5.2.5 Data analysis. Database management and statistical analyses were performed with SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA). Continuous data are reported as mean±SD. Unadjusted means and proportions were compared by the large-sample z-test and the χ^2-statistic, respectively. Log transformation of resistin concentrations was performed as data were non-normally distributed and transformation improved distribution (Norman *et al.*, 2015). Bivariate correlations for continuous data were assessed from Pearson's correlation coefficients. Independent relationships were assessed from multivariate linear regression analysis with appropriate adjustors. To determine the contribution of eGFR or aortic PWV to the impact of circulating resistin concentrations and LVM, multivariate adjusted product of coefficient mediation analysis was performed. Probability values were further adjusted for non-independence of family members using the method of maximum likelihood as implemented

by the mixed procedure as defined in the SAS package.

5.3 Results

5.3.1 Participant characteristics. A high proportion of participants were overweight or obese and had central (abdominal) obesity (Table 5.1). Furthermore, a high proportion of participants were hypertensive (Table 5.1). 46.7% of participants had LVH. No differences were noted in the characteristics of the cohort with PWV data as compared to whole cohort (Table 5.1).

5.3.2 Associations between resistin concentrations and metabolic abnormalities. With adjustments for age, sex, regular smoking, regular alcohol intake and treatment for hypertension, BMI (partial r=0.05, p=0.18) and WC (partial r=0.04, p=0.29) were only modestly associated with resistin concentrations. Only modestly associated in untreated group (BMI: partial r=0.10, p<0.05; WC; partial r=0.09, p<0.05). No independent relationships were noted between resistin concentrations and HOMA-IR (p=0.89).

5.3.3 Associations between resistin concentrations and eGFR or aortic stiffness. With adjustments for age, sex, BMI, regular smoking, regular alcohol intake, treatment for hypertension, systolic, diastolic or mean arterial blood pressure, the presence of diabetes mellitus or an HbA1c>6.5%, circulating resistin concentrations were independently associated with eGFR (partial r=-0.21, p<0.0001). With the same adjustments, circulating resistin concentrations were also independently associated with aortic PWV (partial r=0.080, p<0.05).

5.3.4 Associations between eGFR or aortic stiffness and LVM. With adjustments for age, sex, body weight, regular smoking, regular alcohol intake, treatment for hypertension, systolic blood pressure, the presence of diabetes mellitus or an HbA1c>6.5%, eGFR was independently associated with LVMI (Table 5.2), LVM_{inappr}. (Table 5.2) and LVH (Odds ratio (95% CI) = 0.981 (0.971 to 0.992, p<0.001). With the same adjustments, aortic PWV was similarly independently associated with LVMI (Table 5.2), LVM_{inappr}. (Table 5.2) and LVH (Odds ratio (95% CI) = 1.116 (1.021 to 1.219, p<0.02).

5.3.5 Independent relationships between resistin concentrations and LVM. On bivariate analysis circulating resistin concentrations, were associated with LVMI and LVM_{inappr} (p<0.0001 for all). In stepwise regression models BMI, HOMA-IR, and resistin concentrations were all directly and independently associated with LVMI and LVM_{inappr} (Tables 5.2 and 5.3). These relationships were not affected by the inclusion of either eGFR or aortic PWV in the models (Tables 5.2 and 5.3). With adjustments including BMI, LVM_{inappr} increased across octiles of resistin concentrations with the highest 2 octiles showing markedly greater LVM_{inappr} than the lowest 2 octiles of resistin concentrations (Figure 5.1). These relationships were similarly unaffected by adjustments for either eGFR or aortic PWV (Figure 5.1).

Table 5.1. Characteristics of study participants.

	(n=739)	(n=647) with PWV
% Women	63.3	62.8
Age (years)	44.6±18.1	44.5±18.2
Body mass index (kg/m^2)	29.5±7.8	28.9±7.5
Waist circumference (cm)	91.1±16.8	90.3±16.3
% Overweight/obese	23.7/43.6	27.0/40.7
% Central obesity	42.6	42.5
% Hypertension	45.3	47.4
% Treated for DM	8.8	8.6
% DM or HbA1c >6.1%	26.3	25.0
% Regular smoking	15.0	15.8
% Regular alcohol	19.6	20.2
Office SBP/DBP (mm Hg)	128±22/84±13	128±22/83±12
LV mass (LVM)(g)	153±53	151±52
LVM index (g/m$^{1.7}$)	67.9±23.7	67.0±23.8
% LVH	46.7	45.4
% Actual LVM/Predicted LVM	134±35	133±36
Actual LVM-predicted LVM (g)	38.0±40.8	37.2±39.2
Aortic PWV (m/sec)	-	6.10±2.76
eGFR (mls/min/1.73m^2)	93.9±21.9	94.8±21.7
Resistin (ng/ml)	10.6 (range=1.4 to 83.0)	10.5 (range=1.4 to 83.0)

Continuous data are expressed as mean±SD or median (range). DM, diabetes mellitus, SBP, systolic blood pressure; DBP, diastolic BP; PWV, pulse wave velocity; eGFR, estimated glomerular filtration rate.

Table 5.2. Impact of adjustments for estimated glomerular filtration rate (eGFR) on the relative contribution of circulating resistin concentrations to variations left ventricular mass (LVM) in a community sample (n=739).

	LVM_{inappr}				LVMI			
Models with→	*		* + eGFR		*		* + eGFR	
	β-coeff ±SEM	p value	β-coeff±SEM	p value	β-coeff±SEM	p value	β-coeff±SEM	p value
Age	-0.010±0.047	=0.83	-0.103±0.055	=0.07	0.100±0.045	=0.025	0.026±0.053	=0.62
Male gender	0.052±0.041	=0.21	0.056±0.041	=0.18	0.110±0.037	<0.005	0.106±0.037	<0.005
BMI/Weight	0.234±0.043	<0.0001	0.238±0.043	<0.0001	0.233±0.037	<0.0001	0.232±0.037	<0.0001
SBP	-0.151±0.040	<0.0005	-0.165±0.040	<0.0001	0.193±0.038	<0.0001	0.181±0.039	<0.0001
Log resistin	0.143±0.035	<0.0001	0.120±0.036	<0.001	0.116±0.033	<0.0005	0.094±0.034	<0.005
eGFR	-	-	-0.154±0.049	<0.005	-	-	-0.125±0.047	<0.01
HOMA-IR	0.102±0.036	<0.005	0.083±0.037	<0.05	0.111±0.035	<0.005	0.096±0.035	<0.01

β-coeff, standardized β-coefficient; BMI, body mass index; SBP, office systolic blood pressure; eGFR, estimated glomerular filtration rate; HOMA-IR, homeostasis model of insulin resistance. *Additional factors included in the stepwise regression models include regular smoking, regular alcohol intake, treatment for hypertension and diabetes mellitus or an HbA1c>6.5%.

Table 5.3. Impact of adjustments for aortic pulse wave velocity (PWV) on the relative contribution of circulating resistin concentrations to variations left ventricular mass (LVM) in a community sample (n=647).

	LVM_{inappr}				LVMI			
Models with→	*		* + PWV		*		* + PWV	
	β-coeff ±SEM	p value	β-coeff±SEM	p value	β-coeff±SEM	p value	β-coeff±SEM	p value
Age	-0.022±0.051	=0.67	-0.022±0.055	=0.69	0.095±0.048	<0.05	0.052±0.052	=0.32
Male gender	0.091±0.045	<0.05	0.091±0.044	<0.05	0.079±0.040	=0.05	0.077±0.040	=0.05
BMI/Weight	0.244±0.046	<0.0001	0.257±0.049	<0.0001	0.226±0.039	<0.0001	0.242±0.041	<0.0001
SBP	-0.088±0.043	<0.05	-0.089±0.046	<0.05	0.207±0.042	<0.0001	0.183±0.044	<0.0001
Log resistin	0.119±0.038	<0.002	0.118±0.038	<0.002	0.076±0.036	<0.05	0.072±0.036	<0.05
PWV	-	-	0.002±0.050	=0.98	-	-	0.097±0.048	<0.05
HOMA-IR	0.114±0.039	<0.005	0.083±0.037	<0.005	0.107±0.038	<0.005	0.103±0.037	<0.01

β-coeff, standardized β-coefficient; BMI, body mass index; SBP, office systolic blood pressure; PWV, aortic pulse wave velocity; HOMA-IR, homeostasis model of insulin resistance. *Additional factors included in the stepwise regression models include regular smoking, regular alcohol intake, treatment for hypertension and diabetes mellitus or an HbA1c>6.5%.

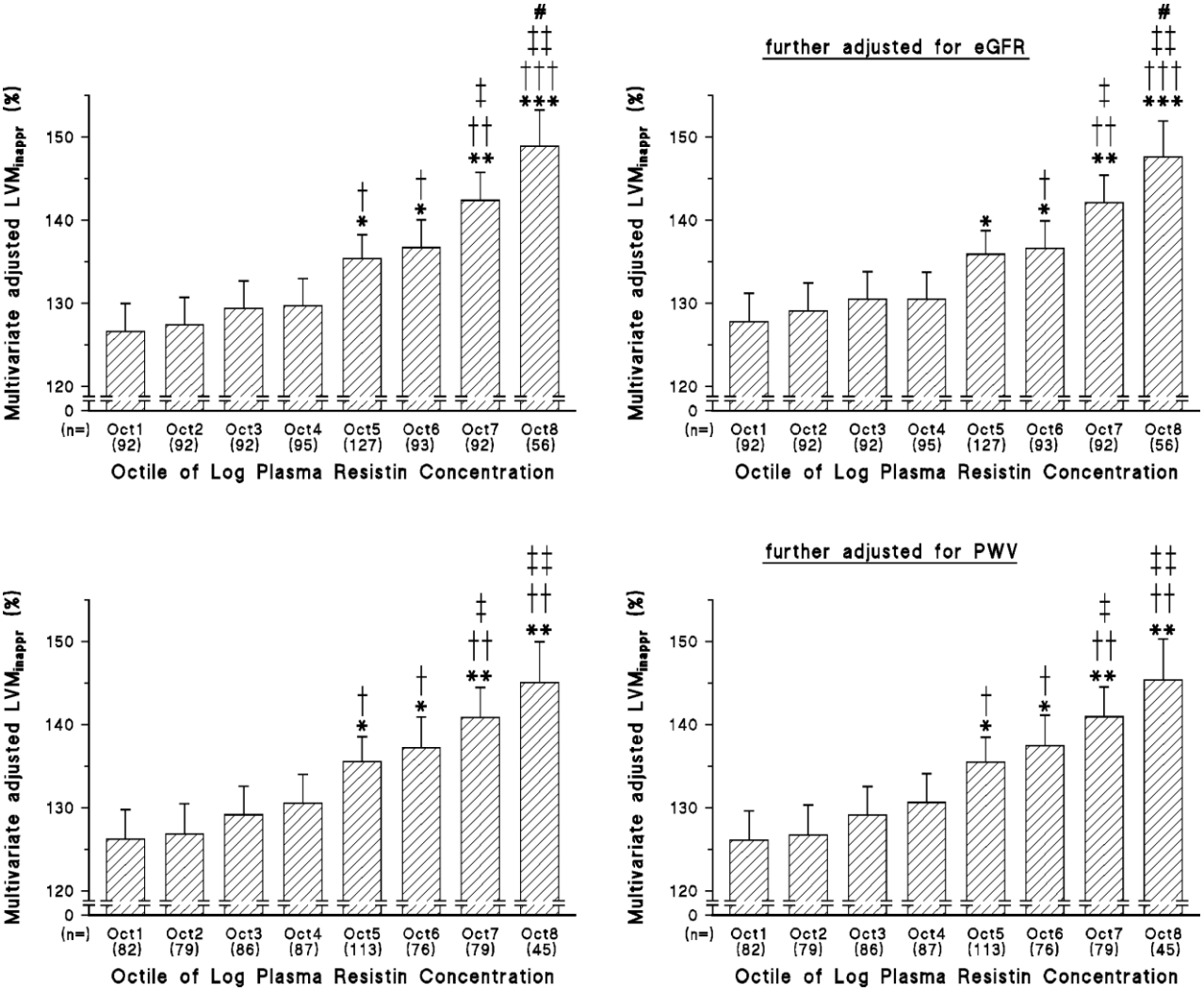


Figure 5.1. Impact of adjustments for aortic pulse wave velocity (PWV) or estimated glomerular filtration rate (eGFR) on multivariate adjusted left ventricular mass (LVM) beyond that predicted by stroke work (inappropriate LVM or LVM_{inappr}) across octiles (Oct) of log resistin concentrations. Additional adjustments are for age, sex, body mass index, office systolic blood pressure, treatment for hypertension, diabetes mellitus or an HbA1c>6.1%, regular smoking, and regular alcohol intake. *p<0.05, **p<0.005, ***p<0.0005 vs octile 1; †p<0.05, ††p<0.005, †††p<0.0005 vs octile 2; ‡p<0.05, ‡‡p<0.005 vs octiles 3 and 4; $^{\#}$p<0.05 vs octile 5.

5.3.6 Independent relationships between resistin concentrations and LVH. Independent of confounders including age, sex, BMI, SBP, regular smoking, regular alcohol consumption, treatment for hypertension, diabetes mellitus or an HbA1c>6.5% and log HOMA-IR, resistin concentrations were also independently associated with LVH (Figure 5.2). Importantly, these relationships were not affected by the inclusion of either eGFR or aortic PWV in the models (Figure 5.2).

5.3.7 Contribution of eGFR or aortic PWV to relationships between resistin concentrations and LVM. In product of coefficient mediation analysis, neither eGFR nor aortic PWV could account for a significant proportion of the relationship between resistin concentrations and LVM_{inappr} or LVMI (Figure 5.3).

5.4 Discussion

The main findings of the present study are as follows: In a large, randomly selected, community-based sample, circulating resistin concentrations were independently associated with indexes of structural changes in the LV including LVMI, LVM_{inapp}, and LVH, as well as with both eGFR and aortic stiffness (carotid-femoral PWV). In this regard, consistent with the well-recognized impact of ventricular-vascular coupling and the cardio-renal syndrome on LVM, both eGFR and PWV were also independently associated with LVM and LVH. However, in multivariate regression and product of coefficient mediation analysis, neither eGFR nor aortic PWV could account for independent relationships between resistin and LVM or LVH.

The mechanisms that may explain the ability of circulating resistin to predict the development and prognosis in heart failure (Butler *et al.*, 2009; Frankel *et al.*, 2009; Zhang *et al.*, 2010; Wu *et al.*, 2012; Takeishi *et al.*, 2007; Muse *et al.*, 2015) are uncertain. Whilst overexpression of resistin mediates several adverse effects on the myocardium in mice (Chemaly *et al.*, 2011; Kang *et al.*, 2011), resistin may produce several alternative changes that may mediate increases in LVM through a multitude of indirect mechanisms. In this regard, resistin was originally identified as a molecule with the ability to mediate insulin resistance in mice and insulin resistance in obesity is a well-recognized cause of cardiac hypertrophy and dysfunction. However, the role of resistin in mediating insulin resistance in humans is unclear and as we have previously described (Norman *et al.*, 2015) and similarly demonstrated in the present study, resistin is not associated with HOMA-IR in the present study sample. Moreover, HOMA-IR does not explain independent relationships between resistin and LVM (Norman *et al.*, 2015). Thus, an impact of resistin on LVM through direct myocardial effects mediated by inflammatory changes (Patel *et al.*, 2003; Lu *et al.*, 2002; Kaser *et al.*, 2003), requires consideration Nevertheless, several alternative indirect effects of resistin need to be excluded.

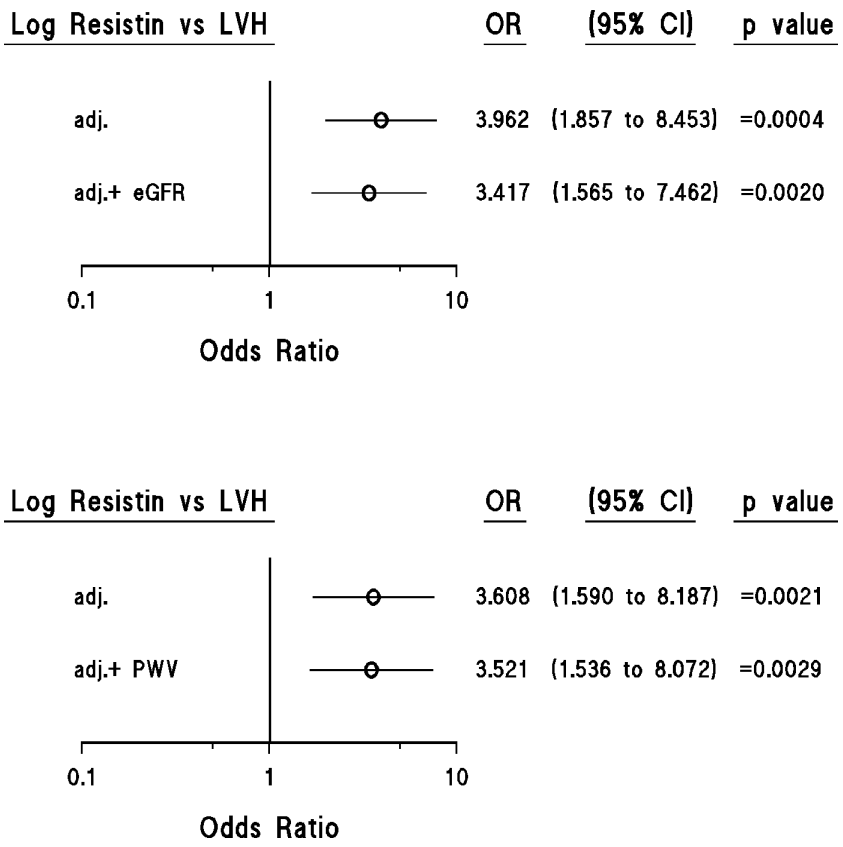


Figure 5.2. Impact of adjustments for aortic pulse wave velocity (PWV) or estimated glomerular filtration rate (eGFR) on multivariate adjusted relationships between log circulating resistin concentrations and left ventricular hypertrophy (LVH) in a community sample (n=345 with LVH). Additional adjustments are for age, sex, body mass index, systolic blood pressure, treatment for hypertension, diabetes mellitus or an HbA1c>6.5%, regular smoking, and regular alcohol intake.

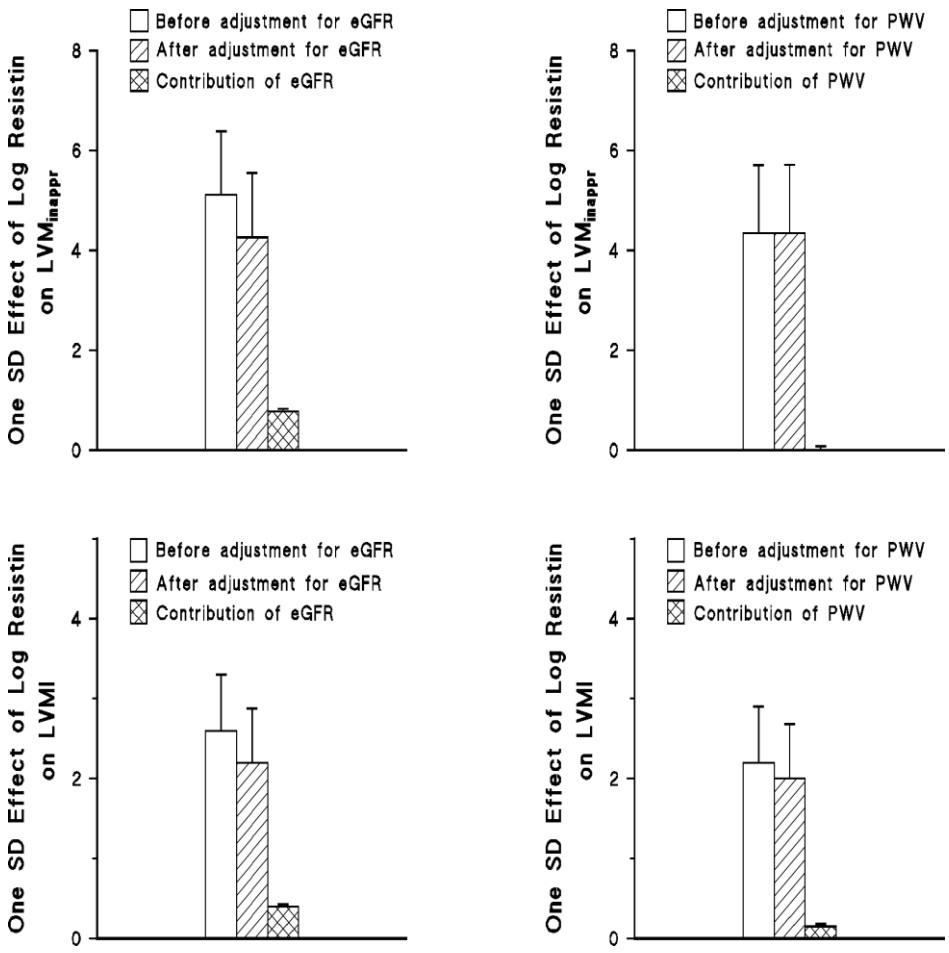


Figure 5.3. Contribution of aortic pulse wave velocity (PWV) or estimated glomerular filtration rate (eGFR) to the relationship between log circulating concentrations of resistin and left ventricular mass (LVM) beyond that predicted by stroke work (inappropriate LVM or LVM_{inappr}) or LVM index (LVMI) in a community sample (n=739 for eGFR and 647 for PWV). The figure shows one standard deviation (SD) effect of log resistin concentrations before and after adjustments (adj.) for PWV or eGFR and the contribution of PWV or eGFR to the one SD effect of log resistin on LVM_{inappr} or LVM (product of coefficient mediation analysis). Additional adjustments are for age, sex, body mass index (LVM_{inappr}) or body weight (LVMI), systolic blood pressure, treatment for hypertension, diabetes mellitus or an HbA1c>6.5%, regular smoking, and regular alcohol intake.

In the present study we addressed the possibility that two important effects of resistin (increases in aortic stiffness or renal dysfunction) may explain the adverse cardiac actions of resistin.

Increases in aortic stiffness are strongly associated with LVM (Bello *et al.*, 2017; Devereux *et al.*, 1986) and these relationships are independent of brachial and aortic pulse pressure as well as the aortic wave component that is driven by increases in aortic stiffness (forward wave pressure)(Bello *et al.*, 2017) In this regard, stiffness of the proximal aorta is thought to increase afterload to the left ventricle through ventricular-vascular coupling (Devereux *et al.*, 1986). As resistin is independently associated with aortic PWV, but not associated with aortic pulse pressure, nor the forward wave pressure (Norman *et al.*, 2016), the possibility that resistin may mediate increases in LVM through ventricular-vascular coupling requires consideration. Importantly, in the present study, as previously described (Norman *et al.*, 2016; Bello *et al.*, 2017), circulating resistin concentrations were independently associated with aortic PWV and PWV was independently associated with LVM. However, adjustments for PWV failed to influence resistin-LVM relationships and in product of coefficient mediation analysis, PWV failed to account for a significant proportion of the relationships between resistin and LVM. Thus, together with the fact that resistin concentrations are not independently associated with brachial, central aortic or ambulatory BP (Norman *et al.*, 2015), independent relationships between resistin concentrations and LVM are unlikely to be accounted for by load-dependent effects, including an effect of resistin on aortic stiffness. Modifying afterload to the left ventricle is thus not a therapeutic option in preventing the adverse effects of resistin on the heart.

Through the cardio-renal syndrome, well before the onset of renal failure, reductions in estimated glomerular filtration rate are strongly associated with LVM and these effects are not attributed to an impact of hemodynamic factors (Maunganidze *et al.*, 2014). As resistin is a well-recognized determinant of renal damage (Mills *et al.*, 2013), and in the present study was independently associated with a decrease in eGFR, the possibility that resistin mediates increases in LVM through the cardio-renal syndrome was considered. Importantly however, although eGFR was indeed strongly and independently associated with LVM, adjustments for eGFR did not modify resistin-LVM relationships and in product of coefficient mediation analysis eGFR failed to explain even a small proportion of resistin-LVM relationships. Thus, in risk-prediction, the assessment of eGFR, a simple and cost-effective assessment of cardiovascular risk, will not adequately identify the adverse effects of resistin on the LV. In this regard, the best approach to evaluating the adverse effects of resistin on the heart is through the assessment of LVM_{inappr}, a measure of LVM most closely associated with LV systolic dysfunction.[25]

Resistin is induced in response to various pro-inflammatory stimuli such as tumor necrosis factor-α (TNF-α), interleukin (IL)-6, and IL-1β (Patel *et al.*, 2003; Lu *et al.*, 2002; Kaser *et al.*, 2003). Furthermore, resistin has been shown to up-regulate the expression of pro-

inflammatory cytokines TNF-α, IL-6, IL-12, and monocyte chemoattractant protein-1 in macrophages. This raises the question of whether the independent relationships between resistin and LVM can be attributed to general inflammatory effects. In this regard however, whilst resistin is independently associated with LVM, the general inflammatory molecule C-reactive protein is not (Norman *et al.*, 2015). Furthermore, although circulating concentrations of TNF-α, and IL-6 independently associate with concentric LV remodeling, there is no relationship between these molecules and LVM (Norton *et al.*, 2019). Hence, it is unlikely that the resistin-LVM relationships can be explained by general inflammatory effects. Whether resistin has local effects on macrophage function in the myocardium is nevertheless possible.

There are several limitations to the present study that require consideration. In this regard, the present study was cross-sectional in design and hence no conclusions regarding causality can be drawn. However, as previously emphasized (Norman *et al.*, 2015), the present results are supported by extensive preclinical findings suggesting a myocardial hypertrophic effect of resistin (Kim *et al.*, 2008; Chemaly *et al.*, 2011; Kang *et al.*, 2011; Schwartz *et al.*, 2013). Second, the present study was conducted in one ethnic group in a specific community selected because of the high prevalence of obesity, and the limited confounding effects of antihypertensives on LVM (Norman *et al.*, 2015). Hence, whether resistin-LVM relationships are consistent across populations, is uncertain.

5.5 Conclusions

In the present study conducted in a large community-based sample with a high prevalence of obesity, we show that despite striking relationships noted between circulating resistin concentrations and both aortic stiffness and glomerular function, these effects do not account for the independent relationships between resistin and LVM or LVH. Thus, neither ventricular-vascular coupling nor the cardio-renal syndrome are likely to explain the impact of resistin on LV hypertrophy.

CHAPTER 6

Contextual Narrative and Conclusions

6.0 Introduction

The increasing prevalence of obesity in both developed and developing nations is likely to translate into a trend for a rising incidence of cardiovascular disease (CVD) worldwide. Although it is well accepted that through several conventional risk factors including type II diabetes mellitus, hypertension and dyslipidaemia, obesity causes CVD, adjustments for these conventional risk factors has a surprisingly modest impact on the ability of adiposity indices to predict cardiovascular events. Thus, for several decades there has been a search for alternative mechanisms through which obesity may cause CVD. Initial efforts in earlier years were on attempting to identify whether insulin resistance without overt type II diabetes mellitus mediated these events. Although several large studies have indeed demonstrated relationships between insulin resistance and associated metabolic abnormalities without overt type II diabetes mellitus and CVD, once again these relationships were insufficiently strong to explain the impact of obesity on CVD. The discovery that obesity is a state of low-grade inflammation and that chronic low-grade inflammation can cause CVD has resulted in the more contemporary hypothesis that inflammatory effects contribute to the impact of obesity on CVD. In the present thesis I focused my efforts on a role for the inflammatory molecule resistin as a possible important determinant of obesity-induced CVD. In the present chapter I will provide an over-arching synthesis of my findings and how they have contributed to our understanding of the possible role of the obesity-related inflammatory molecule resistin in mediating CVD.

6.1 Does resistin explain obesity-associated end organ changes?

In the present thesis I have demonstrated that circulating resistin concentrations are consistently associated with several end-organ measures, including cardiac (LVMI and FSmid), vascular (aortic stiffness), and renal (estimated glomerular filtration rate [eGFR]) end organs independent of conventional risk factors. In this regard, despite the high prevalence of obesity in the community sample that I studied, adiposity indices failed to show similar consistent and independent relationships with end organ measures. Indeed, adiposity indices (either BMI or WC) were independently associated only with LVMI, but not with FSmid, aortic PWV or eGFR. Consequently, a dissociation between obesity and resistin effects were noted in the present thesis. These data suggest that the adverse effects of resistin on cardiovascular end organs described in the present thesis are through effects beyond adipose tissue. Indeed, as underscored in the introductory chapter of the present thesis unlike mice, resistin in humans is often undetectable in adipocytes but highly expressed in macrophages. Indeed, the major source of circulating resistin in humans is likely to be peripheral blood mononuclear cells (Kaser *et al.*, 2003). Moreover, the relationship between obesity and circulating resistin

concentrations is highly variable. Indeed, in obese subjects undergoing weight reduction through diet or bariatric surgery, circulating resistin concentrations may be reduced, but often remain unchanged, despite weight loss (Azuma *et al.*, 2003; Moschen *et al.*, 2009; de Luis *et al*., 2011). Moreover, as noted with the studies described in the present thesis, several reports indicate that circulating resistin concentrations are not associated with obesity (McTernan *et al.*, 2003; Lee *et al.*, 2003; Reilly *et al.*, 2005). Importantly, the lack of relationship between adiposity indices and circulating resistin concentrations in the present study are not attributed to inaccuracies in measures of excess adiposity as expected relationships between adiposity indices and both circulating CRP and adiponection concentrations were noted. However, circulating resistin concentrations have been associated with visceral, intrathoracic, and pericardial fat (Jain *et al.*, 2009), and although we assessed waist circumference and waist-to-hip ratio, we did not perform more accurate assessments (e.g. computer assisted tomography) of visceral fat and we did not assess intrathoracic or pericardial fat. Moreover, obese individuals who are likely to have a greater infiltration of macrophages in adipose tissue show increased expression of resistin in adipose tissue samples and higher circulating concentrations of resistin than lean individuals (Savage *et al.*, 2001; Pagano *et al.*, 2005). Consequently, although the impacts of resistin on end organ measures described in the present thesis are indeed independent of indices of either general or central (abdominal) obesity, it is possible that the circulating resistin concentrations may in some way be associated with alterations in adipose tissue. The exact adipose tissue changes responsible for the resistin effects described in the present thesis however require further study.

6.2 Are associations between resistin and end organ measures explained by insulin resistance?

Resistin was originally named because it is secreted from murine adipose tissue and acts on skeletal muscle myocytes, hepatocytes, and adipocytes themselves, reducing their sensitivity to insulin (Shojima *et al.*, 2002; Patel *et al.*, 2003). In this regard, as described in the introductory chapter to the present thesis and shown in several of the data chapters, insulin resistance in the absence of type II diabetes mellitus may cause several adverse cardiovascular changes which could lead to CVD, Consequently, consideration must be given to the possibility that relationships between resistin and cardiovascular structural and functional changes may be accounted for by insulin resistance. Importantly however, the consistent independent relationships between circulating resistin concentrations and several end organ measures described in the present thesis were largely independent of the homeostasis model of insulin resistance (HOMA-IR), a well-accepted index of insulin resistance. Indeed, adjustments for HOMA-IR hardly modified the independent relationships between resistin concentrations and

end organ measures. Moreover, in the studies described in the present thesis, despite relationships noted between adiposity indices or adiponectin concentrations and HOMA-IR, circulating resistin concentrations were not independently associated with HOMA-IR. These findings are compatible with the inconsistent relationship noted between human resistin concentrations and insulin resistance in several studies (Lazar MA, 2007; McTernan *et al.*, 2003; Lee *et al.*, 2003; Youn *et al.*, 2004; Hivert *et al.*, 2008; Tsiotra *et al.*, 2008).

Nevertheless, as highlighted in the introductory chapter, transgenic mice expressing human resistin in macrophages develop white adipose tissue inflammation and insulin resistance (Qatanani *et al.*, 2009) and in prospective case-control studies, elevated baseline resistin concentrations increase the risk of developing type II diabetes mellitus even after adjusting for conventional risk factors (Chen *et al*.,2009; Schwartz and Lazar, 2011). As HOMA-IR provides an approximation of the extent of insulin resistance, it is possible that if I had employed more sensitive and accurate indices of insulin resistance such as a euglycaemic insulin clamp, that I may have demonstrated a role for insulin resistance. However, in the present study, HOMA-IR was independently associated with both LVMI and eGFR beyond all risk factors as well as adiposity indices, but these relationships failed to explain independent relationships between circulating resistin concentrations and end organ measures. Hence I assume that HOMA-IR was a sufficiently robust index of insulin resistance to detect adverse effects on end organ measures and that insulin resistance indeed does not explain resistin-end organ measure relationships.

As outlined in the introduction to the present thesis, I nevertheless cannot exclude the possibility that the relationships between circulating resistin concentrations and LVMI or FSmid are not explained by myocardial-specific insulin resistance. Indeed, resistin impairs glucose transport in isolated mouse cardiomyocytes (Hsu *et al.*, 2011). Furthermore, resistin has been demonstrated to induce cardiac hypertrophy and myocardial insulin resistance, possibly via the AMP-activated kinase (AMPK)/activation of tuberous sclerosis complex 2/ mammalian target of rapamycin (mTOR)/p70S6K and apoptosis signal-regulating kinase 1/1/c-Jun N-terminal Kinase (JNK) pathway, a system known to stimulate insulin resistance (JNK)/reduced serine 307 phosphorylation of IRS1 (IRS1) pathways (Kang *et al.*, 2011). In addition, adenoviral overexpression of resistin induces cardiac hypertrophy, contractile dysfunction with impaired Ca^{2+} handling (Qatanani *et al.*, 2009), together with insulin resistance in isolated rat cardiomyocytes (Park *et al.*, 2011). All of the aforementioned studies were however conducted in rodents and it is now well recognized that resistin has a different role to play in rodents as compared to humans (see introduction).

6.3 Other possible explanations for relationships between resistin and end organ measures

As underscored in the introduction to the present thesis, there are several possible mechanisms other than insulin resistance, through which resistin could promote end organ changes and hence mediate the development of CVD, In this regard, chronic low-grade inflammation is now a well-recognised cause of CVD and resistin up-regulates the expression of the pro-inflammatory cytokines TNF-α, IL-6, IL-12, and monocyte chemoattractant protein-1 in macrophages, through the nuclear factor-κB signalling pathway (Bertolani *et al.*, 2006; Bokarewa *et al.*, 2005; Silswal *et al.*, 2005). Indeed, circulating resistin concentrations have been shown to be associated with inflammatory markers such as CRP, TNF-α, IL-6, or plasminogen activator inhibitor (Reilly *et al.*, 2005; Osawa *et al*., 2007; Bo *et al.*, 2005; Kunnari *et al.*, 2006; Qi *et al.*, 2008; Fargnoli *et al.*, 2010; Konrad *et al.*, 2007) and in the studies described in the present thesis, resistin was indeed independently associated with CRP concentrations. In this regard, although we have not been able to show similar correlations, circulating concentrations of these inflammatory markers have previously been demonstrated to be associated with aortic stiffness (Yang *et al.*, 2009; Windham *et al.*, 2010; Youn *et al.*, 2013; Wang *et al.*, 2009) and with renal dysfunction (Fried, 2004; Axelsson and Stenvinkel, 2008; Axelsson, *et al.*, 2006; Risch *et al.*, 2007; Nusken *et al.*, 2006; Menzaghi *et al.*, 2012; Zhang *et al.*, 2015; Moreno *et al.*, 2015). Thus, it is indeed possible that the relationships noted between circulating resistin concentrations and aortic PWV or eGFR, could be explained by the adverse impact of low-grade inflammatory changes on these end organs. With respect to the impact on the heart, in the present thesis I demonstrated that the relationship between circulating resistin concentrations and FSmid, an index of myocardial systolic function, increased over incremental tertiles of circulating CRP concentrations. In this regard, low-grade inflammation has been well described as contributing toward myocardial apoptosis, fibrosis and dysfunction. Hence, it is also possible that low-grade inflammation mediates the adverse effects of resistin on cardiac function at least. With respect to a possible impact of resistin on cardiac hypertrophy mediated by inflammatory changes, recent data from our laboratory show that circulating concentrations of TNF-α, and IL-6 strongly and independently associate with the extent of left ventricular concentric geometric remodeling, but not with hypertrophy *per se* (Norton *et al*., 2019). In contrast, in the present thesis I have demonstrated that resistin concentrations associate with LVMI and not the extent of geometric remodeling of the left ventricle. Thus, it is unlikely that the relationship between circulating resistin concentrations and LVMI can be explained by inflammatory changes.

With respect to cardiac effects, resistin has repeatedly been demonstrated to produce hypertrophic responses in preclinical studies in rodents. Indeed, cardiomyocyte overexpression

of resistin promotes myocardial hypertrophy and dysfunction in mice (Chemaly *et al.*, 2011; Kang *et al.*, 2011; Kim *et al.*, 2008). Furthermore, mice transgenic for the human resistin gene develop worse cardiac function in response to a cardiotoxic intervention and these changes are associated with an increased resistin expression (Schwartz *et al.*, 2013). Moreover, long-term cardiac specific overexpression of resistin decreases cardiac contractility and induces a complex phenotype of oxidative stress, fibrosis, apoptosis and myocardial remodelling (Graveleau *et al.*, 2005). Furthermore, animal models of cardiac hypertrophy associated with fibrosis (diabetes mellitus, pressure overload and heart failure) demonstrate elevated tissue levels of resistin compared to non-fibrosing hypertrophy (volume overload) where resistin is minimally or not elevated (Sheng *et al.*, 2013). Further, an increased resistin expression occurs in cardiac myocytes and fibroblasts in response to neurohormonal stimuli (Sheng *et al.*, 2013). Thus, it is possible that resistin produces direct adverse effects on the myocardium that may not be entirely attributed to an inflammatory response, but do involve oxidative stress, apoptosis and fibrosis.

6.4 Consistency of relationships demonstrated between resistin and end organ measures

It is important to note that there are few examples where circulating concentrations of one molecule show such consistent independent relationships with so many end organ changes including the heart, vessels and kidney. These relationships are almost as consistent as relationships noted between conventional, well-established risk factors such as hypertension and diabetes mellitus and end organ measures. In addition, in the present community these relationships are far more consistent than variations in LDL cholesterol concentrations, which seldom associate with any end organ measure. Although being a cross-sectional study the present results do not imply causality, they certainly provide confidence that adipokines, at least resistin, may play an important role in mediating CVD. In this regard, there are several reasons to question the pathophysiological relevance of resistin. What are these reasons?

Although animal-based studies provide strong evidence for a role for rodent resistin as a mediator of several adverse cardiovascular effects, as described in the introductory chapter, human resistin differs markedly in both structure and function as compared to rodent resistin. Thus, although studies in mice have provided invaluable insights into the impact of resistin on the cardiovascular system, whether these data are translatable to the human condition is a matter of debate. Clinical studies are limited in many respects, the most important of which is that intervention studies targeting a single molecule are difficult to perform. Thus, human studies rely on associations. Although the ability of resistin concentrations to predict events is relatively consistent, human studies have previously provided inconsistent results with respect

to relationships between resistin concentrations and end organ measures. These inconsistencies have been described in chapters 1 - 3 and have been detailed in these chapters. These inconsistencies therefore challenge the concept that relationships between circulating resistin concentrations and CVD are cause and effect. However, inconsistencies in human studies are often attributed to small sample sizes and confounding effects such as the use of community-based or clinical samples where the majority of those with risk factors are receiving therapy to control these risk factors. Moreover, adjustments are frequently not made for all possible confounding effects. In contrast, in the present large community-based sample that I studied, only half of all of those with risk factors were receiving therapy and those receiving therapy do not have risk factors controlled to target levels. Moreover, adjustments in the present study were for several confounders including 24-hour BP values and blood glucose control as indexed by glycated haemoglobin. Hence, the results of the present study provide a level of confidence that human resistin may have pathophysiological relevance with regards to CVD. Nevertheless, the role of resistin will only be established as a cause of CVD once approaches to targeting human resistin become available.

6.5 Mechanistic insights

As discussed in the introductory chapter and aforementioned sections of the present chapter, resistin may mediate adverse cardiovascular effects through several potential mechanisms including insulin resistance and chronic, low-grade inflammatory effects. However, the exact adverse effects that resistin may produce have been poorly described. This is largely due to the fact that, as discussed in the previous section, studies evaluating relationships between circulating resistin concentrations and end-organ measures have produced inconsistent data. What have the results of the present thesis contributed to our understanding of how human resistin could determine CVD?

As discussed in the introductory chapter and again in chapter 2 of the present thesis, increased circulating resistin concentrations predict incident heart failure (Frankel *et al.*, 2009; Levy, Wang and Meigs, 2010; Kang *et al.*, 2011; Butler *et al.*, 2009) and are associated with an increased severity of heart failure (New York Heart Association functional classification) (Lee *et al.*, 2007). Moreover, serum resistin concentrations are associated with a higher mortality in patients with heart failure and a reduced ejection fraction (Wu *et al.*, 2012). However, the mechanisms responsible for these relationships had not, until our recent work published in the *Int J Cardiol* (Norman *et al*., 2015) and described in chapter 2 of the present thesis, been identified. The strong relationships noted between resistin concentrations and LVMI, LVM_{inappr} and FSmid in that study (Norman *et al*., 2015, chapter 2), provided strong evidence to support several preclinical studies published prior to this and discussed above suggesting that murine

resistin at least has direct effects on the heart to promote hypertrophy and systolic dysfunction. As highlighted in the introductory chapter, LV hypertrophy and systolic dysfunction are well recognized precursors for heart failure. Thus, the data published by our group (Norman *et al.,* 2015), suggest that resistin could contribute to the development of heart failure by promoting LV hypertrophy and systolic dysfunction.

As also discussed in the introductory chapter and again in later chapters of the present thesis, increased circulating resistin concentrations predict not only heart failure, but also arterial vascular events. These events include those related to the coronary circulation such as MI (Benomar *et al.*, 2013; Khera *et al.*, 2015; Rothwell *et a.l*, 2006; Schwartz and Lazar, 2011; Erer *et al.*, 2014), the severity of CAD (Menzaghi *et al.*, 2006); all-cause mortality in patients with acute MI (Osawa et al., 2007); mortality in high risk patients with diabetes mellitus and CAD (Fontana *et al.*, 2015);and recurrent coronary events following hospitalisation for acute coronary syndrome (Khera *et al.*, 2015). Moreover circulating resistin concentrations predict an increased risk for stroke (León *et al.*, 2014) with this relationship noted for ischaemic stroke including lacunar and atherothrombotic infarction (Osawa *et al.*, 2009); 5-year mortality in those with atherothrombotic strokes (Ding *et al.*, 2011); and worse neurological deficits in those with ischaemic stroke (Kochanowski *et al.*, 2012). The exact mechanisms responsible for the ability of resistin to predict arterial vascular events are uncertain. As described in the introductory chapter there is substantial evidence to show that resistin promotes atherogenesis. In addition, resistin induces a prothrombotic phenotype by up-regulating tissue factor expression in human coronary artery endothelial cells (Calabro *et al.*, 2011; Jamaluddin *et al.*, 2012). Thus, resistin is likely to induce arterial vascular events through atherosclerosis. However, the ability of resistin to predict lacunar strokes is difficult to understand as this is caused largely by a microvascular abnormality. Consequently, one has to consider what else could explain resistin's adverse effects on the arterial system?

As highlighted in the introductory chapter, a recent hypothesis that has emerged is that arteriosclerotic changes in the aorta can decrease the impedance mismatch that exists between the aorta and more distal vessels and that this can increase flow pulsatility in distal vessels, the consequence being increased loads in the microvasculature. As resistin promotes large artery inflammatory changes and chronic inflammation is well-recognised as contributing to increases in aortic stiffness, in the present thesis I therefore hypothesized that resistin could associate with increases in aortic stiffness. As demonstrated in chapter 3 and published in the *J Hypertens* (Norman *et al*., 2016) resistin was indeed independently associated with aortic PWV and similar relationships with aortic forward wave pressure or aortic or brachial PP were not observed. Subsequent work by alternative authors has demonstrated a similar relationship between resistin and aortic PWV in patients with CHD (Wang *et al.*, 2009). Importantly, resistin-induced increases in aortic stiffness could induce not only microvascular effects, but may

contribute toward atherosclerotic events (stroke or CHD) through several mechanisms described in the introductory chapter. Thus, the results of the present thesis provide strong evidence in support of a vascular mechanism that may explain the ability of resistin to predict arterial vascular events.

As also highlighted in the introductory chapter and again in chapter 4 of the present thesis, through several mechanisms discussed in that chapter, a strong risk factor for CVD is chronic kidney disease (CKD). In this regard, CKD predicts several cardiovascular events including heart failure, MI and stroke. The potential impact of resistin on renal function and the development of CKD, and thus potentially on CVD, has similarly been highlighted in the introductory chapter and again in chapter 4. Until the present thesis however, the exact contribution of resistin or alternative obesity-associated changes to CKD had not been appropriately described. In the present thesis in chapter 4 I show in a large community-based study with a high prevalence of obesity that even when adiposity indices account for little of the variation in eGFR and the presence of CKD, that resistin concentrations and insulin resistance account for substantially more of the variation in eGFR and the presence of CKD than conventional risk factors alone. These data therefore highlight a potential mechanism through which resistin could contribute to a substantial portion of CVD in community samples with a high prevalence of obesity.

An important consideration when interpreting relationships between any molecule and end organ measures is whether these relationships can be explained by alternative factors other than the usual conventional risk factors. In this regard, as highlighted in the introduction relationships between resistin and eGFR could be explained by an impact of aortic stiffness which reduces the impedance mismatch between the aorta and more distal vessels, thus increasing pulsatile flow in the microvasculature, including in the kidney. Moreover, relationships between resistin and LV mass could also be accounted for by increases in aortic stiffness through ventriculo-vascular coupling. In either situation, the effects of increases in aortic stiffness will not be mirrored by similar changes in either peripheral or central arterial pulse pressure. Furthermore, the relationships between resistin and LV mass could also be explained by an impact of resistin on renal function which may enhance LV mass independent of haemodynamic factors through the cardio-renal syndrome. However, in chapter 4 of the present thesis I show that the relationship between circulating resistin concentrations and eGFR is independent of aortic stiffness and using product of coefficient mediation analysis in chapter 5 that the relationship between circulating resistin concentrations and LV mass is not accounted for by resistin effects on either aortic stiffness and eGFR. Thus, the present thesis provides evidence that the impact of resistin on renal function cannot be accounted for by an enhanced aortic stiffness and that neither ventriculo-vascular coupling nor the cardio-renal syndrome

account for the impact of resistin on LV mass. These data provide further evidence in support of direct rather than indirect adverse effects of resistin on the kidney and heart.

6.6 Implications for healthcare

Of all of the adipokines studied to date, this study suggests that resistin, has a role in mediating CVD. Together with the substantial evidence suggesting an ability to predict events beyond conventional risk factors; and the innumerable studies that have provided preclinical data showing adverse effects of resistin; the results of the present thesis showing consistent independent relationships between resistin concentrations and several end-organ measures often similar in impact to conventional risk factors, highlights an urgent need to identify methods of reducing these adverse effects. Whether in high-risk patients with insulin resistance the presence of high circulating resistin concentrations warrants the use of thiazolidinedione (TZD) agents (which reduce resistin) at doses that decrease circulating insulin, without affecting blood glucose concentrations, requires consideration. For this to occur however, initially preclinical studies with animal models of CVD associated with resistin effects would need to be performed. Until such studies are performed, strategies that involve modifying health behaviors that effectively reduce circulating resistin concentrations (if any exist) require urgent identification. In this regard, behavioral strategies (diet and exercise) that alter visceral, intrathoracic, or pericardial fat, which are associated with resistin concentrations (Jain *et al.*, 2009), require identification. Whether modifying diet and increasing exercise can produce these effects and hence decrease circulating resistin concentrations, is uncertain.

6.7 Limitations

The major limitations of the present thesis have largely been described and discussed in each of the data chapters where appropriate. There are nevertheless several overarching additional limitations that are worthy of further mention. As frequently mentioned throughout the present thesis associations do not necessarily imply causal relationships and may be explained by reverse causality or residual confounding. However, the associations that I selected to study were with a molecule which has extensive preclinical data to suggest that any relationship noted is likely to be causal and which has similarly extensive clinical evidence to suggest an ability to predict events. Thus, the associations reported on are more likely to represent cause and effect. Second, there are innumerable adipocytokines that may contribute to cardiovascular events, whilst in the present thesis I focused on largely a single molecule, resistin. Hence, a considerable proportion of the inflammatory effects of obesity on cardiovascular structure and function may have gone undetected. However, when evaluating the role of multiple biomarkers

simultaneously, very large study samples are required to achieve statistical power because of the need for Bonferroni corrections or alternative statistical procedures. Hence, further evaluation of related adipocytokines, and the possible assessment of interactive effects, would have required study sample sizes too large for a single center to adequately evaluate. Third, the assessment of relationships between circulating concentrations of substances and cardiovascular phenotypes is out of necessity a once off sampling approach which fails to account for variations in concentrations over a single day or over several days or months. Thus, whether relationships noted are either under or overestimated using this approach is unknown. Fourth, as the present study was conducted in one ethnic group (individuals of African descent) in a specific community selected because of the high prevalence of obesity, whether the relationships between resistin and LV structure and systolic dysfunction, aortic stiffness and glomerular dysfunction are consistent across populations, is uncertain.

6.8 Conclusions

In conclusion, in the present thesis I show that circulating concentrations of the adipocytokine resistin, but not CRP are associated with LV hypertrophy and systolic dysfunction, aortic stiffness and glomerular dysfunction beyond conventional risk factors including adiposity indices and BP (office, aortic and ambulatory). Importantly, these effects were similarly independent of insulin resistance as indexed by the homeostasis model and relationships between resistin concentrations and eGFR could not be explained by increases in aortic stiffness and between resistin concentrations and LV hypertrophy could not be explained by the cardio-renal syndrome or ventriculo-vascular coupling. Thus, the present thesis suggests that resistin, possibly through direct effects on several organ systems, may contribute to cardiovascular events through an impact on LV hypertrophy and dysfunction, increases in aortic stiffness and reductions in glomerular filtration. The present thesis therefore provides substantial further evidence to support a role of resistin as an essential target for drug development in the prevention of cardiovascular events and as a possible biomarker for enhancing cardiovascular risk prediction. As the present study was conducted in a group of black African ancestry, the present results require validation in alternative ethnic groups. In addition, further studies are required to assess the role of resistin as an essential target for drug development in the prevention of cardiovascular events and as a possible biomarker for enhancing cardiovascular risk prediction.

APPENDICES

Appendix A

Ethical clearance certificates

R14/49 Ms Glenda Norman et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170884

NAME: (Principal Investigator)	Ms Glenda Norman et al
DEPARTMENT:	Physiology University of the Witwatersrand Cardiovascular Pathophysiology and Genomics Research Unit School of Physiology
PROJECT TITLE:	Novel Effect of Adipocytokines on Cardiovascular Structure and Function
DATE CONSIDERED:	Adhoc
DECISION:	Approved unconditionally
CONDITIONS:	Sub-study under Primary Study (M170401)
SUPERVISOR:	Prof Gavin Norton
APPROVED BY:	Professor CB. Penny Co-Chairperson, HREC (Medical)
DATE OF APPROVAL:	22/09/2017

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

DECLARATION OF INVESTIGATORS

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

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

R14/49 Prof Angela Woodiwiss and Prof Gavin Norton

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170401

NAME: (Principal Investigator)	Prof Angela Woodiwiss and Prof Gavin Norton
DEPARTMENT:	School of Physiology
PROJECT TITLE:	Gene Candidates as Determinants of Blood Pressure and Intermediary Phenotypes in the Pathogenesis of Hypertension in Black South Africans
DATE CONSIDERED:	26/04/2002 (Initial Approval)
DECISION:	Approved unconditionally
CONDITIONS:	Renewal for 5 Years Valid for the Period 01 April 2017 - 30 April 2022 Previously M1204108, M070469 and M020472
SUPERVISOR:	Prof Angela Woodiwiss
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	07/04/2017

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

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in April and will therefore be due in the month of April each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

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

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

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

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

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

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

DECLARATION OF INVESTIGATOR(S)
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UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

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

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

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

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

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Woodiwiss A Prof

DECLARATION OF INVESTIGATOR(S)

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PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

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

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

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

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

DEPARTMENT School of Physiology, Wits Medical School

DATE CONSIDERED 02-04-26

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

DECISION OF THE COMMITTEE *

Approved unconditionally

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

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

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

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

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DECLARATION OF INVESTIGATOR(S)

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

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

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Appendix B

“Turn-it-in” Plagiarism report

Ms

by

Norman

Glenda

Submission date: 26- Mar- 2019 09:13AM (UTC+0200)

Submission ID: 1100011908

File name: Glenda_Norman.docx (393.3K)

Word count: 16003

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Appendix C

Publications

International Journal of Cardiology 196 (2015) 81–87

Contents lists available at ScienceDirect

International Journal of Cardiology

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Independent associations between resistin and left ventricular mass and myocardial dysfunction in a community sample with prevalent obesity☆,☆☆

Glenda Norman [a,1], Gavin R. Norton [a,*,1], Carlos D. Libhaber [b,2], Frederic Michel [a,2], Olebogeng H.I. Majane [a,2], Aletta M.E. Millen [a,2], Pinhas Sareli [a,2], Angela J. Woodiwiss [a,*,1]

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ARTICLE INFO

Article history:
Received 30 April 2015
Accepted 27 May 2015
Available online 5 June 2015

Keywords:
Obesity
Inappropriate left ventricular mass
Myocardial systolic function
Resistin

ABSTRACT

Background: Although the adipokine resistin may play a role in heart failure, the mechanisms of this effect are uncertain. Relations with left ventricular mass (LVM) and function are uncertain.
Methods: In 739 randomly selected participants from a community sample (43.6% obese), we assessed relations between circulating resistin concentrations and LVM index (LVMI), LVM beyond that predicted by stroke work (inappropriate LVMI [LVM_{inappr}]) and systolic and diastolic LV function (echocardiography).
Results: Resistin concentrations were not independently associated with blood pressure (BP). However, resistin concentrations were associated with LVMI (partial $r = 0.12$, $p < 0.0005$), LVM_{inappr} (partial $r = 0.18$, $p < 0.0001$) and LV hypertrophy (partial $r = 0.13$, $p < 0.001$) independent of BP, BMI, the homeostasis model of insulin resistance and additional confounders. Independent relations between resistin concentrations and LVMI and LVM_{inappr} persisted with further adjustments for C-reactive protein concentrations. Resistin concentration (partial $r = -0.12$, $p < 0.002$ in all and partial $r = -0.15$, $p < 0.0005$ in untreated) was the only factor independently associated with LV midwall fractional shortening and these relations were enhanced at incremental concentrations of CRP. Resistin was not independently associated with transmitral and myocardial tissue Doppler indices of LV diastolic function.
Conclusions: Resistin in-part explains variations in LVM, hypertrophy and myocardial systolic dysfunction, and these effects are independent of insulin resistance and general inflammatory changes.
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1. Introduction

Independent of conventional risk factors and myocardial infarction, obesity predicts the development of heart failure [1–3]. The mechanisms responsible for this effect are nonetheless uncertain. Although insulin resistance may play an important role in mediating obesity-induced heart failure, insulin resistance accounts for only a minor proportion of these effects [2,3]. More recently, several inflammatory substances released from adipocytes (adipocytokines or adipokines) have been suggested to contribute toward the adverse effects of obesity on the heart. However, at present the contribution of resistin, a circulating adipokine which in humans is predominantly expressed in monocytes and macrophages, and which is responsive to inflammatory stimuli [4–6], is uncertain.

Cardiomyocyte overexpression of resistin promotes myocardial hypertrophy and dysfunction in mice [7–9]. Furthermore, transgenic mice for the human resistin gene develop worse cardiac function in response to a cardiotoxic intervention and these changes are associated with an increased resistin expression [10]. Moreover, circulating resistin concentrations predict the development of and prognosis in heart failure [11–16]. Thus, resistin may in-part explain independent relations between obesity and heart failure [1–3]. However, the mechanisms that account for the ability of resistin to predict human heart failure are unclear. In this regard, although associations between circulating resistin concentrations and left ventricular mass (LVM) have been

☆ GN, GRN and AJW contributed equally to this work.
☆☆ This work was supported by the Medical Research Council of South Africa, the Circulatory Disorders Research Trust, the University Research Council of the University of the Witwatersrand, the National Research Foundation of South Africa (grant numbers: 73713, 77423, 81026, 87768, 90527), and the Carnegie Programme.
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[1] This author takes responsibility for drafting of the manuscript, critically revising the manuscript for intellectual content, conception and design of the study, analysis and interpretation of the data and final approval of the manuscript submitted.
[2] This author takes responsibility for drafting of the manuscript, critically revising the manuscript for intellectual content, analysis and interpretation of the data and final approval of the manuscript submitted.

http://dx.doi.org/10.1016/j.ijcard.2015.05.184
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demonstrated in a small study sample of children [17], no independent relations were noted in large adult population samples [18,19]. Further, in large samples only modest independent relations between circulating resistin concentrations and LV systolic function were reported [18,19]. However, in these studies [18,19] low average blood pressure (BP) levels suggest that most participants had well-controlled BP on therapy, which is likely to confound relations with LVM and function; blood adipokine concentrations were not contemporaneous with echocardiographic assessments [18]; and relations were not adjusted for BP or diabetes mellitus [19]. In the present study we therefore aimed to assess whether independent relations between circulating resistin concentrations and LVM, LV hypertrophy or LV function occur in a large community-based sample with prevalent obesity and never-treated hypertension.

2. Methods

2.1. Study group

The present study was conducted according to the principles outlined in the Helsinki declaration. The Committee for Research on Human Subjects of the University of the Witwatersrand approved the protocol (approval numbers: M02-04-72 renewed as M07-04-69 and M12-04-108). Participants gave informed, written consent. The study design has previously been described [20,21]. Nuclear families of black African descent with siblings older than 16 years were randomly recruited from the South West Township (SOWETO) of Johannesburg, South Africa. Of the 1024 participants recruited, in a sub-study 739 participants had echocardiography, of which 554 were not receiving treatment for hypertension. Of the sample 498 had 24-hour ambulatory BP measurements that met with pre-specified quality control criteria (longer than 20 h and more than 10 and 5 readings for the computation of day and night means, respectively).

2.2. Clinical, demographic and anthropometric measurements

Demographic and clinical data were obtained using a standardized questionnaire [20,21]. Height, weight, waist (WC) and hip circumference were measured using standard approaches and participants were identified as being overweight if their body mass index (BMI) was ≥25 kg/m^2 and obese if their BMI was ≥30 kg/m^2. Central obesity was defined as an enlarged WC (≥88 cm in women and ≥102 cm in men). Laboratory blood tests of renal function, liver function, blood glucose, lipid profiles, hematological parameters, and percentage glycated hemoglobin (HbA1c) (Roche Diagnostics, Mannheim, Germany) were performed. Fasting plasma insulin concentrations were determined from an insulin immulite, solid phase, two-site chemiluminescent immunometric assay (Diagnostic Products Corporation, Los Angeles, CA, USA) and insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) using the formula (insulin [uU/ml] × glucose [mmol/l])/22.5. Diabetes mellitus (DM) or abnormal blood glucose control was defined as the use of insulin or oral hypoglycemic agents or an HbA1c value greater than 6.1% respectively.

2.3. Brachial and aortic blood pressure

Brachial BP measurements were obtained by a trained nurse-technician using a standard mercury sphygmomanometer and using SpaceLabs monitors (model 90207) (ambulatory 24-hour BP) as previously described [21]. Monitors were programmed to measure BP at 15-minute intervals from 06:00 to 22:00 and at 30-minute intervals from 22:00 to 06:00. Aortic BP was determined using radial applanation tonometry and SphygmoCor software as previously described [20]. After participants had rested for 15 min in the supine position, arterial waveforms at the radial (dominant arm) artery were recorded during an 8-second period using a high-fidelity SPC-301 micromanometer (Millar Instrument, Inc., Houston, Texas) interfaced with a computer employing SphygmoCor, version 9.0 software (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia). To determine aortic BP, the pulse wave obtained from radial tonometer recordings was calibrated by manual measurement (auscultation) of brachial BP taken immediately before the recordings. The radial pressure waveform was converted into a central (aortic) waveform using a validated generalized transfer function (GTF) incorporated in SphymoCor software.

2.4. Echocardiography

Echocardiography was performed as previously described [20–22]. All measurements were recorded and analyzed off-line by experienced investigators whom were unaware of the clinical data of the participants. Left ventricular mass (LVM) was determined using a standard formula [23] and indexed (LVMI) to height$^{1.7}$. Left ventricular hypertrophy was defined as an LVMI ≥ 60 g/m$^{1.7}$ in women and LVMI ≥ 80 g/m$^{1.7}$ in men [24]. Stroke volume was evaluated from the difference between LV end diastolic and systolic volumes determined using the Z-derived method [22]. Circumferential LV systolic wall stress was calculated as previously described [22]. The extent of LVM$_{inappr}$ was determined from predicted LVM [22], where predicted LVM was calculated as $55.37 + (6.64 \times \text{height}^{2.7}) + (0.64 \times [\text{systolic BP} \times \text{stroke volume} \times 0.014]) - (18.07 \times \text{gender})$, where male gender = 1 and female gender = 2, and where stroke volume was calculated from LV volumes assessed from the Z-derived method. Inappropriate LVM was expressed either as actual-predicted LVM in grams, or % actual LVM/predicted LVM.

Left ventricular ejection fraction (EF) (biplane Simpson) was calculated to determine LV chamber systolic function using a standard formula [22]. Endocardial (FSend) (LV systolic chamber function) and midwall (FSmid) (LV systolic myocardial function) fractional shortening was similarly determined as previously described [22]. Left ventricular diastolic function was assessed from a pulsed wave Doppler examination of the mitral inflow at rest in all participants and using tissue Doppler indices (TDI) in 442 participants [25]. Pulse wave Doppler recordings of transmitral velocity are obtained with the sample volume at the tip of the mitral valve in the apical 4-chamber view. Transmitral velocity measurements are obtained during the early (E) and late (atrial-A) periods of left ventricular diastolic inflow and expressed as the E/A ratio. To perform TDI, the velocity of myocardial tissue lengthening at the level of the mitral annulus was recorded in the apical four-chamber view. The sample volume was positioned at the septal and lateral corners of the mitral annulus. To determine diastolic function using TDI, peak tissue velocity during early (e′) diastole was measured and data are expressed as the E/e′ ratio (an index of LV filling pressures).

2.5. Inflammatory markers and adipokines

Blood samples were centrifuged and immediately stored at −80 °C. Plasma concentrations of human resistin, adiponectin and high-sensitivity (range 0.01 to 50 ng/ml) C-reactive protein (CRP) concentrations were measured using enzyme-linked immunosorbent assays (Quantikine, R&D Systems Inc., Minneapolis, MN, USA). The resistin assay had a lower detection limit of 0.026 ng/ml and intra-assay and inter-assay coefficients of variation ranging from 3.8 to 5.3% and 7.8 to 9.2% respectively. The adiponectin assay had a lower detection limit of 0.246 ng/ml and intra-assay and inter-assay coefficients of variation ranging from 2.5 to 4.7% and 5.8 to 6.9% respectively. The CRP assay had a mean lower detection limit of 0.010 ng/ml and intra-assay and inter-assay coefficients of variation ranging from 3.8 to 8.3% and 6.0 to 7.0% respectively. CRP concentrations could not be determined on 27 participants whose concentrations were below the lower level of detection.

2.6. Data analysis

Database management and statistical analyses were performed with SAS software, version 9.3 (SAS Institute Inc., Cary, NC, USA). Continuous data are reported as mean ± SD. Unadjusted means and proportions were compared by the large-sample z-test and the χ^2-statistic, respectively. As resistin concentrations were positively skewed (skewness = 2.59, kurtosis = 12.9; Shapiro–Wilk's statistic = 0.80, $p < 0.0001$) resistin concentrations were log transformed. Log transformation of resistin concentrations resulted in an improved distribution (skewness = 0.15, kurtosis = 0.37; Shapiro–Wilk's statistic = 1.00). Moreover, as adiponectin concentrations were positively skewed (skewness = 1.51, kurtosis = 3.02, Shapiro–Wilk's statistic = 0.88, $p < 0.0001$) adiponectin concentrations were also log transformed. Log transformation of adiponectin concentrations resulted in an improved distribution (skewness = −0.63, kurtosis = 0.79; Shapiro-Wilk's statistic = 0.98). As CRP concentrations and HOMA-IR were positively skewed, these were also log transformed and log transformation resulted in an improved distribution (data not shown).

Bivariate correlations for continuous data were assessed from Pearson's correlation coefficients. Independent relations were assessed from multivariate linear regression analysis with appropriate adjustors. Probability values were further adjusted for non-independence of family members using the method of maximum likelihood as implemented by the mixed procedure as defined in the SAS package. As antihypertensive treatment may influence LVM and function, sensitivity analysis was conducted in untreated participants.

3. Results

As compared to those without echocardiographic or TDI measurements, those with echocardiographic or TDI measurements were modestly older, and had only a moderately greater BMI, WC, % obese, % treated for hypertension and % treated for DM (Tables S1 and S2). In all participants and in participants whom had never received antihypertensive therapy, a high proportion was overweight or obese and had central (abdominal) obesity (Table 1). Furthermore, a high proportion of all participants and of participants whom had never received antihypertensive therapy was hypertensive (Table 1). 47% of participants had LVH.

In multivariate adjusted models, BMI, WC and waist-to-hip ratio were directly associated with CRP concentrations as well as HOMA-IR, but inversely associated with adiponectin concentrations (Table S3). BMI and WC were only modestly associated with resistin concentrations in untreated participants (Table S3).

With adjustments for age, sex, and DM or HbA1c > 6.1% (except for HOMA-IR), regular smoking, regular alcohol intake and treatment for hypertension, adiponectin concentrations were inversely associated with CRP concentrations (partial $r = -0.12$, $p < 0.005$) and HOMA-IR (partial $r = -0.16$, $p < 0.0001$). With adjustments for age, sex, and DM or HbA1c > 6.1% (except for HOMA-IR), regular smoking, regular alcohol intake and treatment for hypertension, resistin concentrations were directly associated with CRP concentrations (partial $r = 0.18$, $p < 0.0001$), but not HOMA-IR (partial $r = 0.006$, $p = 0.87$).

On bivariate analysis, office, 24-hour and aortic BP were correlated with CRP ($p < 0.0005$ for all), but not with resistin ($p > 0.14$) concentrations. Only a trend for a bivariate correlation was noted between office DBP and adiponectin concentrations ($r = -0.09$, $p < 0.05$). With adjustments for confounders, no independent associations were noted between BP and either CRP or resistin concentrations (Table 2). Multivariate adjusted direct relations between adiponectin concentrations and office, aortic and 24-hour SBP were noted (Table 2).

On bivariate analysis adiposity indices, circulating resistin concentrations, CRP concentrations, and HOMA-IR, but not circulating adiponectin concentrations were associated with LVMI and LVM_{inappr} ($p < 0.0001$ for all). In stepwise regression models with the inclusion of resistin, but not with the inclusion of adiponectin in the models; BMI, HOMA-IR, and resistin concentrations were directly associated with LVMI and LVM_{inappr} (Table 3). With adjustments including BMI, LVM_{inappr} increased across ostiles of resistin concentrations with the highest 2 ostiles showing markedly greater LVM_{inappr} than the lowest 2 ostiles of resistin concentrations (Fig. 1). Similar relations were noted with WC rather than BMI in the models (Table S4). Independent of confounders including age, sex, BMI, SBP, regular smoking, regular alcohol consumption, treatment for hypertension and log HOMA-IR, resistin concentrations were also independently associated with LVH (partial $r = 0.13$, $p < 0.001$). Although adiponectin concentrations were directly associated with LVMI, no independent associations were noted with LVM_{inappr} (Tables 3 and S4). CRP concentrations showed no independent relations with LVMI or LVM_{inappr} (Table S5). Relations between resistin concentrations and LVM_{inappr} persisted with the inclusion of LVMI, (partial $r = 0.15$, $p < 0.0001$), CRP (partial $r = 0.20$, $p < 0.0001$) or aortic rather than brachial SBP (partial $r = 0.19$, $p < 0.0001$) in the models. Relations between resistin concentrations and LVMI (partial $r = 0.14$, $p < 0.0005$) or LVH (partial $r = 0.13$, $p < 0.001$) also persisted with the inclusion of CRP concentrations in the models.

On bivariate analysis no single factor was associated with either EF, or FSend (data not shown). However, resistin ($r = -0.12$, $p < 0.005$) and a trend for HOMA-IR ($r = -0.07$, $p = 0.05$) were inversely correlated with FSmid. In multivariate models, with the inclusion of adiposity indices and additional possible confounders forced into the models, relations between resistin concentrations and FSmid, but not between HOMA-IR and FSmid persisted (Table 4). Neither adiponectin nor CRP concentrations were independently associated with FSmid (Table 4). Nevertheless, an interaction between resistin and CRP concentrations was associated with FSmid independent of the individual terms (partial partial $r = -0.10$, $p < 0.01$). This translated into an incremental

Table 1
Characteristics of study participants.

	All (n = 739)	Untreated (n = 554)
% women	63.3	58.7
Age (years)	44.6 ± 18.1	40.0 ± 16.1
Body mass index (kg/m²)	29.5 ± 7.8	28.0 ± 7.4
Waist circumference (cm)	91.1 ± 16.8	87.4 ± 16.1
% overweight/obese	23.7/43.6	24.4/35.9
% central obesity	42.6	33.6
% hypertension	45.3	27.1
% treated for DM	8.8	2.0
% DM or HbA1c > 6.1%	26.3	17.0
% regular smoking	15.0	17.9
% regular alcohol	19.6	22.6
Office SBP/DBP (mm Hg)	128 ± 22/84 ± 13	125 ± 21/82 ± 12
Pulse rate (beats/min)	66 ± 11	65 ± 11
24-hour SBP/DBP (mm Hg)	119 ± 15/73 ± 11 (n = 498)	117 ± 14/72 ± 10 (n = 383)
Aortic SBP (mm Hg)	120 ± 22	116 ± 21
LV mass (LVM) (g)	153 ± 53	148 ± 49
LVM index (g/m$^{1.7}$)	67.9 ± 23.7	64.9 ± 21.5
% LVH	46.7	41.5
% actual LVM/Predicted LVM	134 ± 35	132 ± 33
Actual LVM-predicted LVM (g)	38.0 ± 40.8	34.8 ± 37.2
LV ejection fraction (%)	67 ± 9	67 ± 8
FSend (%)	40.0 ± 8.1	39.7 ± 7.8
FSmid (%)	23.4 ± 5.4	23.4 ± 5.1
E/A	1.29 ± 0.49	1.41 ± 0.48
E/e'	7.66 ± 4.29 (n = 442)	7.02 ± 3.68 (n = 325)
Adiponectin (mg/l)	7.4 (range = 0.08–36.6)	7.6 (range = 0.08–36.6)
Resistin (ng/ml)	10.6 (range = 1.4–83.0)	10.4 (range = 1.4–62.2)
CRP (mg/l)	3.87 (range = 0.04–53.8) (n = 712)	3.05 (range = 0.04–53.8) (n = 529)

Continuous data are expressed as mean ± SD or median (range). DM, diabetes mellitus, SBP, systolic blood pressure; DBP, diastolic BP; FSend, endocardial fractional shortening; FSmid, midwall fractional shortening; E/A, early-to-atrial transmitral blood flow velocity; e', myocardial lateral wall tissue velocity in early diastole.

2.6. Data analysis

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In multivariate adjusted models, BMI, WC and waist-to-hip ratio were directly associated with CRP concentrations as well as HOMA-IR, but inversely associated with adiponectin concentrations (Table S3). BMI and WC were only modestly associated with resistin concentrations in untreated participants (Table S3).

With adjustments for age, sex, and DM or HbA1c > 6.1% (except for HOMA-IR), regular smoking, regular alcohol intake and treatment for hypertension, adiponectin concentrations were inversely associated with CRP concentrations (partial $r = -0.12$, $p < 0.005$) and HOMA-IR (partial $r = -0.16$, $p < 0.0001$). With adjustments for age, sex, and DM or HbA1c > 6.1% (except for HOMA-IR), regular smoking, regular alcohol intake and treatment for hypertension, resistin concentrations were directly associated with CRP concentrations (partial $r = 0.18$, $p < 0.0001$), but not HOMA-IR (partial $r = 0.006$, $p = 0.87$).

On bivariate analysis, office, 24-hour and aortic BP were correlated with CRP ($p < 0.0005$ for all), but not with resistin ($p > 0.14$) concentrations. Only a trend for a bivariate correlation was noted between office DBP and adiponectin concentrations ($r = -0.09$, $p < 0.05$). With adjustments for confounders, no independent associations were noted between BP and either CRP or resistin concentrations (Table 2). Multivariate adjusted direct relations between adiponectin concentrations and office, aortic and 24-hour SBP were noted (Table 2).

On bivariate analysis adiposity indices, circulating resistin concentrations, CRP concentrations, and HOMA-IR, but not circulating adiponectin concentrations were associated with LVMI and LVM_{inappr} ($p < 0.0001$ for all). In stepwise regression models with the inclusion of resistin, but not with the inclusion of adiponectin in the models; BMI, HOMA-IR, and resistin concentrations were directly associated with LVMI and LVM_{inappr} (Table 3). With adjustments including BMI, LVM_{inappr} increased across octiles of resistin concentrations with the highest 2 octiles showing markedly greater LVM_{inappr} than the lowest 2 octiles of resistin concentrations (Fig. 1). Similar relations were noted with WC rather than BMI in the models (Table S4). Independent of confounders including age, sex, BMI, SBP, regular smoking, regular alcohol consumption, treatment for hypertension and log HOMA-IR, resistin concentrations were also independently associated with LVH (partial $r = 0.13$, $p < 0.001$). Although adiponectin concentrations were directly associated with LVMI, no independent associations were noted with LVM_{inappr} (Tables 3 and S4). CRP concentrations showed no independent relations with LVMI or LVM_{inappr} (Table S5). Relations between resistin concentrations and LVM_{inappr} persisted with the inclusion of LVMI, (partial $r = 0.15$, $p < 0.0001$), CRP (partial $r = 0.20$, $p < 0.0001$) or aortic rather than brachial SBP (partial $r = 0.19$, $p < 0.0001$) in the models. Relations between resistin concentrations and LVMI (partial $r = 0.14$, $p < 0.0005$) or LVH (partial $r = 0.13$, $p < 0.001$) also persisted with the inclusion of CRP concentrations in the models.

On bivariate analysis no single factor was associated with either EF, or FSend (data not shown). However, resistin ($r = -0.12$, $p < 0.005$) and a trend for HOMA-IR ($r = -0.07$, $p = 0.05$) were inversely correlated with FSmid. In multivariate models, with the inclusion of adiposity indices and additional possible confounders forced into the models, relations between resistin concentrations and FSmid, but not between HOMA-IR and FSmid persisted (Table 4). Neither adiponectin nor CRP concentrations were independently associated with FSmid (Table 4). Nevertheless, an interaction between resistin and CRP concentrations was associated with FSmid independent of the individual terms (partial partial $r = -0.10$, $p < 0.01$). This translated into an incremental

Table 1
Characteristics of study participants.

	All (n = 739)	Untreated (n = 554)
% women	63.3	58.7
Age (years)	44.6 ± 18.1	40.0 ± 16.1
Body mass index (kg/m^2)	29.5 ± 7.8	28.0 ± 7.4
Waist circumference (cm)	91.1 ± 16.8	87.4 ± 16.1
% overweight/obese	23.7/43.6	24.4/35.9
% central obesity	42.6	33.6
% hypertension	45.3	27.1
% treated for DM	8.8	2.0
% DM or HbA1c > 6.1%	26.3	17.0
% regular smoking	15.0	17.9
% regular alcohol	19.6	22.6
Office SBP/DBP (mm Hg)	128 ± 22/84 ± 13	125 ± 21/82 ± 12
Pulse rate (beats/min)	66 ± 11	65 ± 11
24-hour SBP/DBP (mm Hg)	119 ± 15/73 ± 11 (n = 498)	117 ± 14/72 ± 10 (n = 383)
Aortic SBP (mm Hg)	120 ± 22	116 ± 21
LV mass (LVM) (g)	153 ± 53	148 ± 49
LVM index (g/m$^{1.7}$)	67.9 ± 23.7	64.9 ± 21.5
% LVH	46.7	41.5
% actual LVM/Predicted LVM	134 ± 35	132 ± 33
Actual LVM-predicted LVM (g)	38.0 ± 40.8	34.8 ± 37.2
LV ejection fraction (%)	67 ± 9	67 ± 8
FSend (%)	40.0 ± 8.1	39.7 ± 7.8
FSmid (%)	23.4 ± 5.4	23.4 ± 5.1
E/A	1.29 ± 0.49	1.41 ± 0.48
E/e'	7.66 ± 4.29 (n = 442)	7.02 ± 3.68 (n = 325)
Adiponectin (mg/l)	7.4 (range = 0.08–36.6)	7.6 (range = 0.08–36.6)
Resistin (ng/ml)	10.6 (range = 1.4–83.0)	10.4 (range = 1.4–62.2)
CRP (mg/l)	3.87 (range = 0.04–53.8) (n = 712)	3.05 (range = 0.04-53.8) (n = 529)

Continuous data are expressed as mean ± SD or median (range). DM, diabetes mellitus, SBP, systolic blood pressure; DBP, diastolic BP; FSend, endocardial fractional shortening; FSmid, midwall fractional shortening; E/A, early-to-atrial transmitral blood flow velocity; e', myocardial lateral wall tissue velocity in early diastole.

Table 2
Relations between circulating adipokine concentrations or inflammatory markers and blood pressure (BP) in a community sample.

	All participants			Untreated participants		
	n=	Partial r[a] (CI)	p value	n=	Partial r[a] (CI)	p value
Log resistin versus						
Office SBP	739	0.005 (−0.07 to 0.08)	=0.89	554	0.01 (−0.07 to 0.09)	=0.81
Office DBP	739	−0.06 (−0.13 to 0.01)	=0.09	554	−0.06 (−0.14 to 0.02)	=0.16
24-hour SBP	498	−0.004 (−0.09 to 0.08)	=0.93	383	−0.01 (−0.11 to 0.09)	=0.81
24-hour DBP	498	−0.08 (−0.16 to 0.01)	=0.09	383	−0.06 (−0.16 to 0.04)	=0.23
Aortic SBP	739	−0.03 (−0.10 to 0.05)	=0.50	554	−0.02 (−0.10 to 0.07)	=0.66
Log adiponectin versus						
Office SBP	739	0.14 (0.07 to 0.21)	<0.0005	554	0.15 (0.07 to 0.23)	<0.001
Office DBP	739	0.003 (−0.07 to 0.08)	=0.92	554	0.01 (−0.07 to 0.09)	=0.80
24-hour SBP	498	0.10 (0.01 to 0.19)	<0.05	383	0.10 (0.00 to 0.20)	=0.05
24-hour DBP	498	0.04 (−0.05 to 0.13)	=0.34	383	0.03 (−0.07 to 0.13)	=0.56
Aortic SBP	739	0.13 (0.06 to 0.20)	<0.001	554	0.14 (0.06 to 0.22)	<0.002
Log C-reactive protein versus						
Office SBP	712	−0.04 (−0.12 to 0.03)	=0.26	529	0.001 (−0.08 to 0.09)	=0.98
Office DBP	712	0.01 (−0.06 to 0.08)	=0.78	529	0.04 (−0.04 to 0.13)	=0.33
24-hour SBP	487	−0.01 (−0.10 to 0.08)	=0.85	372	0.01 (−0.09 to 0.11)	=0.83
24-hour DBP	487	0.02 (−0.07 to 0.11)	=0.66	372	0.04 (−0.07 to 0.14)	=0.50
Aortic SBP	712	−0.04 (−0.12 to 0.03)	=0.26	529	−0.002 (−0.09 to 0.08)	=0.96

SBP, systolic BP, DBP, diastolic BP.
[a] Adjusted for age, sex, body mass index, diabetes mellitus or an HbA1c > 6.1%, regular smoking, regular alcohol intake and treatment for hypertension (in all).

increase in the strength (partial r) and magnitude (β-coefficient or slope) of the independent relation between resistin concentration and FSmid across tertiles of CRP concentrations (Fig. 2).

On bivariate analysis adipokine concentrations, adiposity indices, HOMA-IR, and CRP concentrations were all associated with E/A ($p < 0.005$ for all). Although adiposity indices, HOMA-IR and CRP concentrations were correlated with E/e′ ($p < 0.05$ for all), neither resistin nor adiponectin concentrations were correlated with E/e′ ($p > 0.28$). In multivariate models, although WC and HOMA-IR (for E/A) were, neither resistin, nor adiponectin concentrations were independently associated with either E/A (Table S6) or E/e′ (Table S7).

Table 3
Relations between circulating adipokine concentrations and left ventricular mass (LVM) in a community sample.

	All participants (n = 739)		Untreated participants (n = 554)	
	Partial r[a] (CI)	p value	Partial r[a] (CI)	p value
Models with resistin				
LVMI versus				
BMI	0.34 (0.27 to 0.42)	<0.0001	0.29 (0.21 to 0.37)	<0.0001
Log resistin	0.12 (0.05 to 0.19)	<0.0005	0.08 (0.005 to 0.16)	<0.05
SBP	0.26 (0.19 to 0.33)	<0.0001	0.38 (0.30 to 0.46)	<0.0001
Log HOMA-IR	0.11 (0.04 to 0.18)	<0.002	0.07 (−0.01 to 0.15)	=0.07
LVM_{inappr} versus				
BMI	0.31 (0.24 to 0.38)	<0.0001	0.33 (0.25 to 0.41)	<0.0001
Log resistin	0.18 (0.11 to 0.25)	<0.0001	0.13 (0.05 to 0.21)	<0.002
Log HOMA-IR	0.09 (0.02 to 0.16)	<0.005	0.07 (−0.01 to 0.15)	=0.08
Models with adiponectin				
LVMI versus				
BMI	0.34 (0.27 to 0.42)	<0.0001	0.29 (0.21 to 0.37)	<0.0001
log adiponectin	0.08 (0.01 to 0.15)	<0.01	0.10 (0.02 to 0.18)	<0.01
SBP	0.26 (0.19 to 0.33)	<0.0001	0.38 (0.30 to 0.46)	<0.0001
log HOMA-IR	0.11 (0.04 to 0.18)	<0.001	0.08 (0.004 to 0.16)	<0.05
LVM_{inappr} versus				
BMI	0.31 (0.24 to 0.38)	<0.0001	0.33 (0.25 to 0.41)	<0.0001
Log adiponectin	0.04 (−0.04 to 0.11)	=0.30	0.05 (−0.03 to 0.13)	=0.19
Log HOMA-IR	0.10 (0.03 to 0.17)	<0.005	0.07 (−0.01 to 0.15)	=0.10

LVMI, LVM indexed to height$^{1.7}$; LVM_{inappr}, inappropriate LVM; BMI, body mass index; SBP, office systolic blood pressure; HOMA-IR, homeostasis model of insulin resistance.
[a] Additional factors included in the stepwise regression models include age, sex, regular smoking, regular alcohol intake, treatment for hypertension (in all) and office SBP in models for both LVMI and LVM_{inappr}.

4. Discussion

The main findings of the present study are as follows: In a randomly selected community sample of African ancestry with a high prevalence of obesity and untreated hypertension, circulating resistin, but not adiponectin or CRP concentrations was associated with LVMI, LVM_{inappr} and LVH beyond adiposity indices, BP, HOMA-IR and additional confounders. Furthermore, circulating resistin concentrations were the only factor consistently and independently associated with variations in myocardial function, as determined from FSmid. Importantly however, the independent relationships between resistin concentrations and FSmid increased across tertiles of CRP concentrations.

Although circulating resistin concentrations predict the development of and prognosis in heart failure [11–16], the mechanisms that account for this effect are unclear. Although preclinical studies indicate that resistin produces cardiomyocyte hypertrophy [7–9], and LV

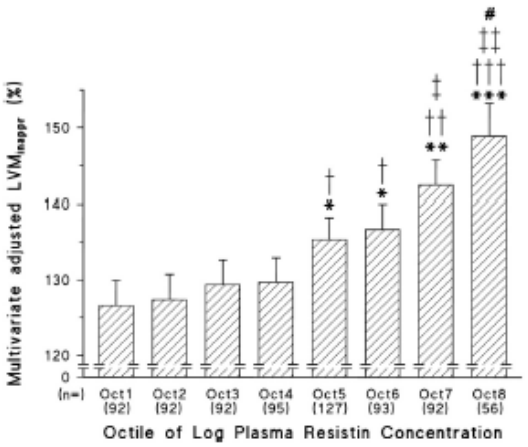
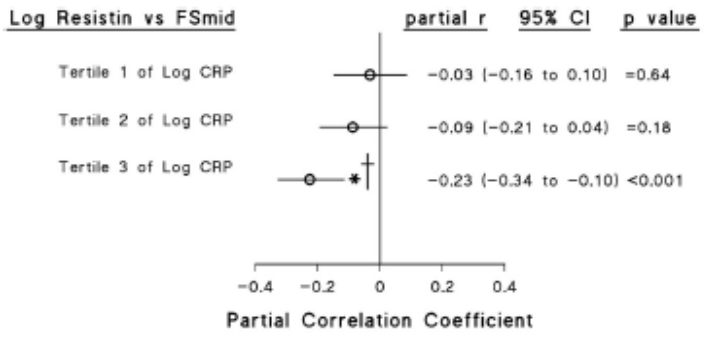


Fig. 1. Multivariate adjusted left ventricular mass (LVM) beyond that predicted by stroke work (inappropriate LVM or LVM_{inappr}) across octiles (Oct) of log resistin concentrations. Adjustments are for age, sex, body mass index, office systolic blood pressure, treatment for hypertension, diabetes mellitus or an HbA1c > 6.1%, regular smoking, and regular alcohol intake. *p< 0.05, **p < 0.001, *** p< 0.0001 vs octile 1; †p < 0.05, ††p < 0.001, †††p < 0.0001 vs octile 2; ‡p < 0.01, ‡‡p < 0.001 vs octiles 3 and 4; #p < 0.05 vs octile 5.

Table 4
Relations between circulating adipokine or C-reactive protein (CRP) concentrations and left ventricular midwall fractional shortening (FSmid) in a community sample.

	All participants[a] (n = 739)		Untreated participants[a] (n = 554)	
	Partial r[b] (CI)	p value	Partial r[b] (CI)	p value
Model with resistin				
FSmid versus				
BMI	−0.04 (−0.11 to 0.03)	=0.25	−0.08 (−0.16 to −0.003)	=0.05
log resistin	−0.12 (−0.19 to −0.05)	<0.002	−0.15 (−0.23 to −0.07)	<0.0005
SBP	0.01 (−0.06 to 0.08)	=0.80	−0.07 (−0.15 to 0.01)	=0.10
log HOMA-IR	−0.07 (−0.14 to 0.002)	=0.07	−0.04 (−0.12 to 0.04)	=0.39
Model with adiponectin				
FSmid versus				
BMI	−0.07 (−0.14 to 0.00)	=0.05	−0.11 (−0.19 to −0.03)	<0.02
log adiponectin	−0.05 (−0.12 to 0.02)	=0.20	−0.05 (−0.13 to 0.03)	=0.25
SBP	0.02 (−0.05 to 0.09)	=0.62	−0.07 (−0.15 to 0.01)	=0.09
log HOMA-IR	−0.07 (−0.14 to 0.002)	=0.06	−0.04 (−0.12 to 0.04)	=0.33
Model with CRP				
FSmid versus				
BMI	−0.06 (−0.13 to 0.01)	=0.13	−0.10 (−0.18 to −0.01)	<0.02
log CRP	−0.03 (−0.10 to 0.04)	=0.42	−0.01 (−0.10 to 0.08)	=0.87
SBP	−0.001 (−0.07 to 0.07)	=0.86	−0.08 (−0.16 to 0.005)	=0.06
log HOMA-IR	−0.07 (−0.14 to 0.004)	=0.06	−0.03 (−0.12 to 0.06)	=0.43

BMI, body mass index; SBP, office systolic blood pressure; HOMA-IR, homeostasis model of insulin resistance.
[a] For CRP n = 712 for all participants and 529 for untreated participants.
[b] Additional factors included in the stepwise regression models include age, sex, regular smoking, regular alcohol intake and treatment for hypertension (in all).



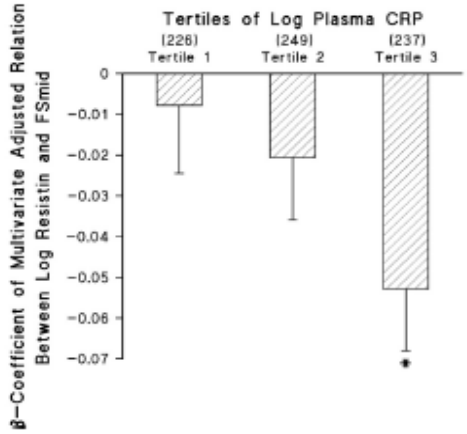


Fig. 2. Multivariate adjusted relations between log circulating resistin concentrations and left ventricular midwall fractional shortening (FSmid) across tertiles of C-reactive protein concentrations (CRP). A comparison of the strength of the relations (partial r) and the magnitude of the effect of resistin on FSmid (β-coefficient) are shown. Adjustments are for age, sex, body mass index, office systolic blood pressure, treatment for hypertension, diabetes mellitus or an HbA1c > 6.1%, regular smoking, and regular alcohol intake. *p < 0.05 vs tertile 1; †p = 0.05 vs tertile 2.

hypertrophy is a well-recognized predictor of outcomes, in large community-based studies, a lack of independent relationship between circulating resistin concentrations and LVM has been reported [18,19]. However, these studies are confounded by a high prevalence of participants receiving antihypertensive therapy [18,19], and a lack or contemporaneous blood and echocardiographic assessment [18]. In the present study we show independent relations between resistin concentrations and LVMI, LVM_{inappr} and LVH in a community sample with a high prevalence of hypertension, but with a low prevalence of antihypertensive therapy, and these findings were reproduced in untreated participants. The present results are therefore the first to suggest that increases in LVM may be one pathway that explains the ability of circulating resistin concentrations to predict human heart failure.

Prior community-based studies that have failed to show independent associations between circulating resistin concentrations and LVM [18,19] have not assessed whether these relations occur with LVM beyond that predicted by loading conditions (stroke work) (LVM_{inappr}). In this regard, in the present study relations between resistin concentrations and LVM tended to be stronger for LVM_{inappr} than LVMI. As LVM_{inappr} and reductions in LVM_{inappr}, rather than LVMI or reductions in LVMI are associated with LV ejection fraction [22], it is possible that the transition to heart failure predicted by circulating resistin is mediated in-part by increases in LVM_{inappr} and consequently decreases in systolic function. Indeed in the present study circulating resistin concentrations were independently and inversely associated with FSmid, an index of myocardial systolic function. However, these relations did not translate into decreases in ejection fraction. Whether resistin produces myocardial systolic dysfunction and subsequently compensatory LVH thus maintaining systolic chamber function, or increases in LVM and hence myocardial systolic dysfunction, is uncertain. Further prospective studies are warranted to evaluate whether circulating resistin predicts the development of a reduced ejection fraction and consequently heart failure.

The inverse relations between circulating resistin concentrations and myocardial systolic function noted in the present study are in keeping with large, community-based studies which have similarly reported on inverse relations between circulating resistin concentrations and LV systolic function [18,19]. However, in these prior studies these relations showed either only trend effects ($p = 0.04$) despite a large study sample ($n = 2615$) [18], or were not adjusted for confounders such as BP and diabetes mellitus [19]. In both studies, a high proportion of participants was receiving antihypertensive therapy [18,19]. In contrast, in the present study we show more convincing relations between circulating resistin concentrations and myocardial systolic function ($p < 0.005$) which were pronounced in participants not receiving antihypertensive therapy ($p < 0.0005$) and these relations were independent of multiple confounders including brachial BP and diabetes mellitus. In addition we show an association between myocardial systolic function and an interaction between circulating resistin and CRP concentrations, independent of the independent terms, an effect which translated into incremental relations between resistin concentrations and myocardial function across tertiles of CRP concentrations. Hence, the present study provides more convincing evidence for a resistin effect on myocardial systolic function in the human heart, and suggests that these effects may depend on the overall level of inflammation that exists.

As insulin resistance has previously been demonstrated to be associated with LVM [26,27] and the development of heart failure [2], and resistin promotes myocardial insulin resistance [9], an important consideration of the present study is whether relations between circulating resistin concentrations and LVM or dysfunction are mediated through insulin resistance. In this regard, in the present study HOMA-IR was independently associated with increases in LVMI and LVM_{inappr}, but not FSmid. Relationships between circulating resistin concentrations and LVM, LVH or FSmid were nevertheless independent of HOMA-IR. Hence, it is possible that resistin versus LVM or LVH and resistin versus FSmid relations are explained through effects other than insulin resistance. In this regard, resistin overexpression in the rat heart promotes myocardial oxidative stress, fibrosis and apoptosis [8].

The limitations of the present study include the cross-sectional design which precludes us from drawing causal inferences. Second, TDI measures of diastolic function were available in 60% of participants with echocardiograms, albeit that the characteristics of this group were similar to those without TDI measurements. Thus, we cannot be certain that circulating resistin concentrations are not associated with E/e′. Third, our study sample was exclusively of African ancestry. Whether similar findings occur in other ethnic groups requires further consideration.

In conclusion, in the present study conducted in a large community sample with a high prevalence of obesity and where antihypertensive therapy was limited, we show that circulating resistin concentrations are independently associated with LVM, LVH and systolic function beyond adiposity indices, diabetes mellitus, BP, insulin resistance and general inflammation. The present study therefore supports the view that resistin may mediate the transition to heart failure in-part through increases in LVM and myocardial systolic dysfunction.

Sources of funding

This study was supported by the Medical Research Council of South Africa, the University Research Council of the University of the Witwatersrand, the National Research Foundation of South Africa (grant numbers: 73713, 77423, 81026, 87768, 90527) (Women in Research and the Thuthuka Program), the Circulatory Disorders Research Trust, and the Carnegie Programme.

Conflict of interest

None of the authors have any conflicts of interest to declare.

Acknowledgments

This study would not have been possible without the voluntary collaboration of the participants and the excellent technical assistance of Mthuthuzeli Kiviet, Nomonde Molebatsi, and Nkele Maseko.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijcard.2015.05.184.

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Original Article

Circulating resistin concentrations are independently associated with aortic pulse wave velocity in a community sample

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Aims: The role of the adipokine, resistin in mediating increases in aortic stiffness is uncertain. We aimed to determine independent relations between circulating resistin concentrations and aortic pulse wave velocity (PWV) and wave reflection in a community-based sample with a high prevalence of untreated hypertension and obesity.

Methods: Plasma resistin, adiponectin, and C-reactive protein concentrations (ELISA); carotid-femoral (aortic) PWV and the aortic reflected wave index (applanation tonometry and SphygmoCor software) were determined in 683 randomly selected participants of African ancestry from SOWETO, South Africa who had never received antihypertensive therapy.

Results: Resistin concentrations were not independently associated with office or 24-h ($n=492$) blood pressure (BP). In a stepwise regression model with BMI included in the model, age ($P<0.0001$), mean arterial pressure ($P<0.0001$), plasma resistin concentrations ($P<0.005$), female sex ($P=0.01$), and creatinine concentrations ($P<0.01$) contributed independently to variations in PWV. Independent relations between resistin concentrations and PWV persisted with further adjustments for C-reactive protein concentrations ($P<0.005$), and the homeostasis model of insulin resistance ($P<0.02$). Similar relations were noted with waist circumference rather than BMI in the model. Resistin concentrations were not independently associated with aortic reflected wave index or aortic BP.

Conclusion: Resistin is independently and directly associated with aortic stiffness and these effects occur beyond BP, insulin resistance, and general inflammation.

Keywords: aortic pulse wave velocity, aortic wave reflection, obesity, resistin

Abbreviations: BP, blood pressure; CRP, C-reactive protein; HOMA-IR, homeostasis model of insulin resistance; PP, pulse pressure; PWV, aortic (carotid-femoral) pulse wave velocity; SBPc, central aortic SBP

INTRODUCTION

The steady increase in the global prevalence of obesity [1] and its associated cardiometabolic risk factors is a critical obstacle to the effective prevention of cardiovascular disease. Although a half to three-quarters of obesity-related cardiovascular disease may be attributed to conventional risk factors, the remaining one-quarter to a half remains unaccounted for [2,3]. More recently, several inflammatory substances released from adipocytes (adipocytokines or adipokines) have been suggested to contribute toward the adverse effects of obesity on the cardiovascular system. However, at present the contribution of resistin, a circulating adipokine, which in humans is predominantly expressed in monocytes and macrophages, and which is responsive to inflammatory stimuli [4–6], is uncertain.

Although some studies suggest that circulating resistin concentrations predict the development of cardiovascular events [7–10], these findings are not supported by all studies [11,12]. A fundamental mechanism that may explain relations between circulating resistin concentrations and vascular disease has only recently begun to emerge. Importantly, few studies have evaluated relations between resistin concentrations and large artery function, including aortic (carotid-femoral) pulse wave velocity (PWV) and reflected waves, both of which predict cardiovascular outcomes beyond conventional risk factors [13–17]. One small ($n=271$) case–control study suggests that circulating resistin concentrations are associated with increases in brachial-ankle PWV [18]. In contrast, in a large ($n=749$) cross-sectional study conducted in a sample with a high prevalence of antihypertensive treatment, circulating resistin concentrations were inversely related to aortic PWV [19], and in a small study ($n=141$) conducted in treated hypertensives, circulating resistin concentrations failed to predict changes

Journal of Hypertension 2016, 34:274–281

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Received 3 July 2015 **Revised** 24 August 2015 **Accepted** 12 October 2015

J Hypertens 34:274–281 Copyright © 2016 Wolters Kluwer Health, Inc. All rights reserved.

DOI:10.1097/HJH.0000000000000792

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in PWV over 24 months of follow-up [20]. The presence of antihypertensive treatment [18–20], small study samples [18,20], and the use of brachial-ankle PWV measurements [18], which assess stiffness of large and medium-sized arteries, are likely to have confounded the results of previous studies [18–20]. Therefore, in the present study we aimed to assess whether independent relations between circulating resistin concentrations and aortic PWV or wave reflection occur in a relatively large community-based sample with prevalent obesity and never-treated hypertension.

METHODS

Study group

The present study was conducted according to the principles outlined in the Helsinki declaration. The Committee for Research on Human Subjects of the University of the Witwatersrand approved the protocol (approval numbers: M02-04-72, renewed as M07-04-69 and M12-04-108). Participants gave informed, written consent. The study design has previously been described [21,22]. Nuclear families of black African descent with siblings older than 16 years were randomly recruited from the South West Township (SOWETO) of Johannesburg, South Africa. Of the 1211 participants recruited, 1041 participants had aortic PWV measurements of which 683 were not receiving treatment for hypertension. 492 of the sample had 24-h ambulatory blood pressure (BP) measurements that met with prespecified quality control criteria (longer than 20 h and more than 10 and five readings for the computation of day and night means, respectively).

Clinical, demographic, and anthropometric measurements

Demographic and clinical data were obtained using a standardized questionnaire [21,22]. Regular tobacco use was defined as daily cigarette smoking and regular alcohol consumption as either one beer a day, a bottle of wine (750 ml), a week or 250 ml of spirits a week. Height, weight, and waist circumference were measured using standard approaches and participants were identified as being overweight if their BMI was $\geq$25 kg/m^2 and obese if their BMI was $\geq$30 kg/m^2. Central obesity was defined as an enlarged waist circumference ($\geq$88 cm in women and $\geq$102 cm in men). Laboratory blood tests of renal function, liver function, blood glucose, lipid profiles, hematological parameters, and percentage glycated hemoglobin (Roche Diagnostics, Mannheim, Germany) were performed. Fasting plasma insulin concentrations were determined from an insulin immulite, solid phase, two-site chemiluminescent immunometric assay (Diagnostic Products Corporation, Los Angeles, California, USA), and insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) using the formula [insulin (μU/ml) $\times$ glucose (mmol/l)]/22.5. Diabetes mellitus or abnormal blood glucose control was defined as the use of insulin or oral hypoglycemic agents or a glycated hemoglobin value greater than 6.5%, respectively.

Blood pressure

Nurse-derived conventional BP was measured after 10 min of rest in the seated position as previously described [20,21] within a half hour of obtaining blood samples and in the opposite arm to that subjected to venesection. These measurements were performed to an accuracy of 2 mmHg by a trained nurse using a mercury sphygmomanometer and an appropriate-sized cuff according to the European Society of Hypertension guidelines [23]. Five consecutive BP readings were obtained 30–60 s apart. The average of the five readings was taken as the BP. Only 0.29% of visits had fewer than the planned BP recordings. The frequency of identical consecutive recordings was 0.44% for SBP and 1.46% for DBP. No BP values were recorded as an odd number. Hypertension was diagnosed as a conventional BP $\geq$ 140/90 mmHg.

Ambulatory 24-h, day and night BP was determined using SpaceLabs monitors (model 90207; Spacelabs, Redmond, Washington, USA) as previously described [22]. The size of the cuff was the same as that used for conventional BP measurements. Monitors were programmed to measure 24-h BP at 15-min intervals from 0600 to 2200 h and at 30-min intervals from 2200 to 0600 h. Intraindividual means of the ambulatory measurements were weighted by the time interval between successive readings [22]. The average ($\pm$SD) number of BP readings obtained was 60.8 $\pm$ 12.2 (range = 24–81) for the 24-h period.

Central aortic hemodynamics

Central aortic hemodynamics were determined as previously described [21,22]. After participants had rested for 15 min in the supine position, arterial waveforms at the radial (dominant arm), carotid and femoral artery pulses were recorded by applanation tonometry. Pressure waveforms were recorded during an 8-s period using a high-fidelity SPC-301 micromanometer (Millar Instrument, Inc., Houston, Texas, USA) interfaced with a computer employing SphygmoCor, version 9.0 software (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia). Aortic PWV was determined from sequential waveform measurements at carotid and femoral sites as previously described [22]. The time delay in the pulse waves between the carotid and femoral sites was determined using an electrocardiograph-derived R wave as a fiducial point. Pulse transit time was taken as the average of 10 consecutive beats. The distance which the pulse wave travels was determined as the difference between the distance from the femoral sampling site to the suprasternal notch, and the distance from the carotid sampling site to the suprasternal notch. Aortic PWV was calculated as the ratio of the distance to the transit time (m/sec). To derive an approximate standard aortic PWV ('converted standard PWV') for comparative purposes, we multiplied travel distance derived from the subtracted distance by the ratio (50.7 : 45.5 = 1.114) of mean reference distance/mean tape measure distance using the 'subtraction' approach given in Table 1 of the recent consensus document [24]. However, as advised by the consensus document [24] to perform our primary analysis we did not employ formulae published to convert subtracted distance to direct distance as these formulae introduce additional errors.

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TABLE 1. Characteristics of study sample having never received antihypertensive therapy

Number (% women)	683 (58.1)
Age (years)	37.8 ± 16.4
BMI (kg/m^2)	27.1 ± 6.7
% Overweight/obese	24.5/30.8
% Regular tobacco[a]	18.7
% Regular alcohol[a]	23.1
% DM or HbA1c > 6.5%	6.3
% Hypertension	27.2
Total cholesterol (mmol/l)	4.41 ± 1.08
LDL cholesterol (mmol/l)	2.47 ± 0.90
Creatinine (μmol/l)	73.6 ± 15.8
Office SBP/DBP (mmHg)	125 ± 20/82 ± 12
24-h SBP/DBP (mmHg)(n = 492)	116 ± 14/72 ± 10
Aortic PWV (m/s)	5.79 ± 2.21
Converted standard aortic PWV (m/s)[b]	6.45 ± 2.47
Aortic pulse pressure (mmHg)	33 ± 14
Aortic SBP (mmHg)	116 ± 22
Aortic forward wave pressure (mmHg)	22.8 ± 8.3
Aortic backward wave pressure (mmHg)	15.7 ± 7.5
Aortic reflected wave index (%)	69 ± 20
Adiponectin (mg/l)	8.4 (range = 0.08–36.6)
Resistin (ng/ml)	10.5 (range = 1.4–82.5)
C-reactive protein (mg/l)	2.86 (range = 0.04–53.8)
HOMA-IR	1.58 (range = 0.21–42.0)

DM, diabetes mellitus; HbA1c, glycated hemoglobin; HOMA-IR, homeostasis model of insulin resistance.
[a]See 'Methods' for definitions.
[b]Calculated from 1.114 × subtracted wave travel distance (see methods for explanation).

To determine aortic wave reflection and aortic BP the pulse wave obtained from the radial tonometer recordings was calibrated by manual measurement (auscultation) of brachial BP taken immediately before the recordings. The radial pressure waveform was converted into a central (aortic) waveform using a validated generalized transfer function incorporated in SphymoCor software. The aortic backward (reflected) and the aortic forward wave pressures were determined from the aortic pulse wave using SphygmoCor software which separates the aortic waveform using a triangular flow wave [25]. Aortic reflected wave index was calculated as aortic backward (reflected) wave pressure/aortic forward wave pressure × 100, as previously described [16]. Central aortic SBP was derived from the aortic waveform and central aortic pulse pressure was calculated from the difference between central aortic SBP and DBP.

Inflammatory markers and adipokines

Blood samples were centrifuged and immediately stored at −80°C. Plasma concentrations of human resistin, adiponectin, and high-sensitivity (range 0.01–50 ng/ml) C-reactive protein (CRP) concentrations were measured using enzyme-linked immunosorbent assays (Quantikine; R&D Systems Inc., Minneapolis, MN, USA). The resistin assay had a lower detection limit of 0.026 ng/ml and intra-assay and inter-assay coefficients of variation ranging from 3.8 to 5.3% and 7.8 to 9.2% respectively. The adiponectin assay had a lower detection limit of 0.246 ng/ml and intra-assay and inter-assay coefficients of variation ranging from 2.5 to 4.7% and 5.8 to 6.9% respectively. The CRP assay had a mean lower detection limit of 0.010 ng/ml and intra-assay and inter-assay coefficients of variation ranging from 3.8 to 8.3% and 6.0 to 7.0% respectively. CRP concentrations could not be determined on 15 participants whose concentrations were below the lower level of detection.

Data analysis

Database management and statistical analyses were performed with SAS software, version 9.3 (SAS Institute Inc., Cary, North Carolina, USA). Continuous data are reported as mean ± SD or mean (range). Unadjusted means and proportions were compared by the large-sample z-test and the χ^2-statistic, respectively. As HOMA-IR was positively skewed (skewness = 4.39, kurtosis = 26.05; Shapiro-Wilk's statistic = 0.56, $P < 0.0001$) HOMA-IR was log transformed which resulted in an improved distribution (skewness = 0.44, kurtosis = −0.29; Shapiro–Wilk's statistic = 0.97). Similarly, as resistin concentrations were positively skewed (skewness = 2.97, kurtosis = 18.78; Shapiro–Wilk's statistic = 0.80, $P < 0.0001$) resistin concentrations were log transformed which resulted in an improved distribution (skewness = 0.07, kurtosis = 0.77; Shapiro–Wilk's statistic = 0.99). Moreover, as adiponectin concentrations were positively skewed (skewness = 1.18, kurtosis = 1.72; Shapiro–Wilk's statistic = 0.92, $P < 0.0001$) adiponectin concentrations were similarly log transformed which resulted in an improved distribution (skewness = −0.68, kurtosis = 0.61; Shapiro–Wilk's statistic = 0.97). Bivariate correlations for continuous data were assessed from Pearson's correlation coefficients. Independent relations were assessed from multivariate linear regression analysis with appropriate adjustors (see legends of tables). As aortic functional changes not only contribute toward increases in PP and SBP, but increases in PP and SBP are risk factors for aortic functional changes, in separate models adjustments for clinic and 24-h PP and SBP were made when assessing independent relations between circulating adiponectin or resistin concentrations and aortic function. Probability values were further adjusted for non-independence of family members using the method of maximum likelihood as implemented by the mixed procedure as defined in the SAS package.

RESULTS

Participant characteristics

Table 1 shows the characteristics of the participants in the study sample. More women than men participated and a high prevalence of hypertension (27%) and obesity (30.8%) was noted. Of the regular smokers, only four participants smoked one cigarette/day. None of the sample was receiving lipid lowering therapy (they are not available at a primary care level in the public healthcare sector of South Africa).

Relationships between adiposity indices and circulating adipokine concentrations

On bivariate analysis, BMI and waist circumference were directly associated with resistin and CRP concentrations as well as HOMA-IR, but inversely associated with adiponectin concentrations ($P < 0.0001$). However, with adjustments for

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TABLE 2. Relationships between plasma resistin concentrations and carotid-femoral (aortic) pulse wave velocity in participants having never received antihypertensive therapy

Log resistin versus log aortic PWV			
Adjustments	**Partial *r* (95% CI)**	***P* value**	**Model r^2**
	($n=683$)		
Unadjusted	0.17 (0.09 to 0.24)	<0.0001	0.027
[a]	0.10 (0.03 to 0.17)	<0.01	0.396
[a] + office MAP	0.11 (0.04 to 0.19)	<0.005	0.451
[a] + office PP	0.09 (0.02 to 0.17)	<0.02	0.432
[a] + office SBP	0.11 (0.04 to 0.19)	<0.005	0.453
	With 24-h BP ($n=492$)		
Unadjusted	0.18 (0.10 to 0.27)	<0.0001	0.033
[a]	0.11 (0.02 to 0.19)	<0.02	0.407
[a] + 24-h PP	0.10 (0.01 to 0.19)	<0.05	0.411
[a] + 24-h SBP	0.11 (0.02 to 0.20)	<0.02	0.432
	Women ($n=397$)		
Unadjusted	0.16 (0.07 to 0.26)	0.001	0.027
[a]	0.12 (0.02 to 0.22)	<0.02	0.417
[a] + office MAP	0.12 (0.02 to 0.21)	<0.05	0.487
[a] + office PP	0.12 (0.02 to 0.21)	<0.05	0.446
	Men ($n=286$)		
Unadjusted	0.18 (0.06 to 0.28)	<0.005	0.031
[a]	0.07 (−0.04 to 0.19)	0.21	0.388
[a] + office MAP	0.10 (−0.01 to 0.22)	0.09	0.431
[a] + office PP	0.06 (−0.05 to 0.18)	0.296	0.432

CI, confidence interval; MAP, mean arterial pressure; office, brachial blood pressure; PP, pulse pressure.
[a]Adjustments are for BP as indicated in separate models, age, sex, pulse rate, body mass index, regular smoking, regular alcohol intake, diabetes mellitus or an HbA1c > 6.5%, LDL cholesterol, and creatinine concentrations. Probability values are further adjusted for nonindependence of family members.

confounders although adiposity indices remained associated with adiponectin and CRP concentrations and HOMA-IR, no independent relations were noted with resistin concentrations (Table S1, http://links.lww.com/HJH/A555).

Relationships between circulating adipokine concentrations and metabolic factors and C-reactive protein

In multivariate adjusted models adiponectin concentrations were inversely associated with HOMA-IR and triglyceride concentrations and directly related to HDL cholesterol concentrations (Table S2, http://links.lww.com/HJH/A555). In contrast, in multivariate adjusted models, resistin concentrations were directly associated with CRP and creatinine concentrations, but unrelated to other metabolic factors including lipid concentrations (Table S2, http://links.lww.com/HJH/A555).

Associations between adipokines and blood pressure

Although office, 24-h and aortic BP were correlated with circulating adiponectin, resistin, or CRP concentrations ($P < 0.0001$ for all), with adjustments for confounders, only modest associations were noted between adiponectin concentrations and clinic and aortic SBP (Table S3, http://links.lww.com/HJH/A555).

Associations of plasma adipokine concentrations with aortic (carotid-femoral) pulse wave velocity

On bivariate analysis and in multivariate adjusted models including adjustments for brachial office or 24-h mean arterial pressure (distending pressures), PP, or SBP in separate models, plasma resistin (Table 2), but not adiponectin (Table S4, http://links.lww.com/HJH/A555) concentrations were associated with aortic PWV. These relationships were independent of either BMI or waist circumference (Tables 2 and 3). These relationships were noted in women, but failed to achieve significance in men (Table 2). In stepwise regression models, plasma resistin concentrations were third only to age and mean arterial pressure and similar to creatinine and female sex in the impact (standardized β-coefficient) of the relations with PWV (Table 3).

Associations of plasma adipokine concentrations with aortic reflected wave and aortic blood pressure

In multivariate adjusted models neither plasma resistin (Table 4), nor adiponectin (data not shown) concentrations were independently associated with aortic reflected wave index or aortic pulse pressure.

Associations of plasma resistin concentrations with aortic (carotid-femoral) pulse wave velocity are independent of C-reactive protein and homeostasis model of insulin resistance

In bivariate models, CRP concentrations [Pearson's $r = 0.22$, confidence interval = 0.14–0.29, $P < 0.0001$], but not HOMA-IR ($P = 0.50$) were correlated with aortic PWV. However, with adjustments for confounders, CRP concentrations failed to associate with PWV (partial $r = 0.01$, confidence interval = −0.07–0.08, $P = 0.83$). With multivariate adjustments including CRP concentrations and HOMA-IR in

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TABLE 3. Factors independently associated with carotid-femoral (aortic) pulse wave velocity in 683 participants having never received antihypertensive therapy

	With BMI in model		With waist circumference in model	
Log PWV versus	β-coefficient[a] (±SEM)	*P* value	β-coefficient[a] (±SEM)	*P* value
Age	0.47 ± 0.04	<0.0001	0.47 ± 0.04	<0.0001
Mean arterial pressure	0.28 ± 0.03	<0.0001	0.28 ± 0.03	<0.0001
Log resistin	0.09 ± 0.03	<0.005	0.08 ± 0.03	<0.01
BMI	−0.01 ± 0.04	0.83	–	–
Waist circumference	–	–	−0.01 ± 0.03	0.74
Female sex	0.10 ± 0.04	0.01	0.11 ± 0.04	<0.01
DM or HbA1c > 6.5%	0.01 ± 0.03	0.91	−0.001 ± 0.03	0.98
Regular smoking	0.01 ± 0.03	0.63	0.01 ± 0.03	0.66
Regular alcohol	−0.07 ± 0.03	<0.05	−0.08 ± 0.03	<0.02
Pulse rate	−0.03 ± 0.03	0.30	−0.03 ± 0.03	0.31
LDL cholesterol	−0.10 ± 0.03	<0.005	−0.10 ± 0.03	<0.002
Creatinine	0.10 ± 0.03	<0.01	0.10 ± 0.04	<0.01

DM, diabetes mellitus; HbA1c, glycated hemoglobin.
[a]Indicates standardized β-coefficient.

TABLE 4. Relationships between plasma resistin concentrations and aortic reflected wave index or aortic pulse pressure in participants having never received antihypertensive therapy

Adjustments	Partial *r* (95% CI)	*P* value	Model r^2
	Log resistin versus aortic reflected wave index ($n = 683$)		
Unadjusted	−0.03 (−0.11 to 0.04)	0.38	0.001
[a]	−0.06 (−0.14 to 0.01)	0.09	0.419
[a] + office MAP	−0.06 (−0.14 to 0.01)	0.11	0.438
	Log resistin versus aortic pulse pressure ($n = 683$)		
Unadjusted	0.07 (−0.002 to 0.15)	0.06	0.005
[a]	0.03 (−0.04 to 0.11)	0.41	0.362
[a] + office MAP	0.04 (−0.03 to 0.12)	0.24	0.478

CI, confidence interval; MAP, mean arterial pressure.
[a]Adjustments are for age, MAP as indicated, sex, pulse rate, body mass index, regular smoking, regular alcohol intake, diabetes mellitus or an HbA1c > 6.5%, LDL cholesterol and creatinine concentrations. Probability values are further adjusted for nonindependence of family members.

the models, circulating resistin concentrations remained independently associated with aortic PWV (Table 5).

DISCUSSION

The main findings of the present study are as follows: in a relatively large randomly selected community sample of black African ancestry with a high prevalence of obesity and untreated hypertension, circulating resistin, but not adiponectin or CRP concentrations were associated with aortic PWV beyond adiposity indexes, BP, HOMA-IR, general inflammation (as indexed by CRP concentrations) and additional confounders. However, neither adiponectin, nor resistin concentrations were independently associated with aortic BP or aortic backward waves.

Although circulating resistin concentrations have been noted to predict cardiovascular events beyond conventional risk factors [7–10], the mechanisms that account for this effect are unclear. In the present study, we show that circulating resistin concentrations are directly associated

TABLE 5. Relationships between plasma resistin concentrations and carotid-femoral (aortic) pulse wave velocity independent of C-reactive protein concentrations or the homeostasis model of insulin resistance in participants having never received antihypertensive therapy

	Log resistin versus log PWV			
Adjustments	*n*	Partial *r* (95% CI)	*P* value	Model r^2
Adjusted for CRP				
[a] + office MAP + log CRP	668	0.12 (0.04 to 0.19)	<0.005	0.449
[a] + office SBP + log CRP	668	0.11 (0.04 to 0.19)	<0.005	0.450
[a] + 24-h SBP + log CRP	481	0.11 (0.02 to 0.20)	<0.02	0.434
Adjusted for HOMA-IR				
[a] + office MAP + HOMA-IR	683	0.10 (0.02 to 0.18)	<0.02	0.650
[a] + office SBP + HOMA-IR	683	0.10 (0.02 to 0.17)	<0.02	0.466
[a] + 24-h SBP + HOMA-IR	492	0.10 (0.01 to 0.19)	<0.05	0.441

CI, confidence interval; MAP, mean arterial pressure; HOMA-IR, homeostasis model of insulin resistance; CRP, C-reactive protein.
[a]Adjustments are for age, BP as indicated in separate models, sex, pulse rate, body mass index, regular smoking, regular alcohol intake, diabetes mellitus or an HbA1c > 6.5%, LDL cholesterol, and creatinine concentrations. Probability values are further adjusted for nonindependence of family members.

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with an increased aortic PWV independent of adiposity indices, concentrations of the inflammatory marker CRP, insulin resistance as indexed by HOMA-IR, and conventional cardiovascular risk factors. These data are in sharp contrast to a similarly large ($n = 749$) cross-sectional study conducted in a sample with a high prevalence of antihypertensive treatment (which will decrease PWV), where circulating resistin concentrations were inversely related to aortic PWV [19], and in a small longitudinal study ($n = 141$) conducted in treated hypertensives, where circulating resistin concentrations failed to predict changes in PWV over 24-months of follow-up [20]. Only one prior case-control study conducted in a small study sample ($n = 271$) suggests that circulating resistin concentrations are directly associated with increases in PWV [18]. However, that study [18] assessed brachial-ankle PWV which is determined by a composite of elastic and muscular artery stiffness. Hence, the present study provides the first clear evidence that circulating resistin concentrations are independently and directly associated with aortic stiffness and these relations are not explained by obesity, insulin resistance or increases in general inflammation. As aortic (carotid-femoral) pulse wave velocity (PWV) predicts cardiovascular outcomes beyond conventional risk factors [13,14], the present study suggests a possible mechanism that may explain the ability of circulating resistin concentrations to predict cardiovascular events beyond conventional risk factors [7–10].

In the present study, relations between resistin concentrations and aortic PWV were independent of several BP values including office and 24-h brachial mean arterial pressure, SBP and PP. However, adjustments for brachial PP tended to reduce the strength of the relation. In this regard, caution should be exercised in interpreting these results as increases in aortic PWV occur as a consequence of increases in PP, and aortic PWV also determines PP. Hence, resistin versus PWV relations are likely to have been overadjusted for with PP included in the model, thus artificially diminishing the strength of the relation.

In the present study, the independent relationship between resistin and aortic PWV failed to translate into associations with aortic reflected wave index, aortic pulse pressure, aortic SBP, or 24-h brachial BP. These findings are in accordance with a prior large study reporting on a lack of association of circulating resistin concentrations with brachial PP [26]. Hence, resistin is unlikely to produce cardiovascular damage through an impact on BP. However, increases in aortic PWV may mediate adverse cardiovascular effects through facilitation of transmission of pulsatile energy into end-organ circulatory systems [27]. Moreover, increases in aortic stiffness are associated with a reduced baroreceptor responsiveness, the consequence of which may be an increased vascular stress [28]. Alternatively, increases in PWV may reflect the extent of arteriosclerosis and therefore herald the onset of vascular events.

Explanations for independent relations between circulating resistin concentrations and aortic PWV in the present study, although speculative, require consideration. Resistin promotes endothelial activation by upregulating endothelin-1, adhesion molecules, monocyte chemoattractant factors and pro-inflammatory cytokines [29,30], the possible consequence being enhanced arteriosclerosis. In this regard, the arteriosclerotic process is associated with vascular fibrosis and degeneration of elastic tissue, the possible result being an increase in large artery stiffness. Alternatively, as resistin is expressed in macrophages in atheroma [31] and increases in aortic stiffness may reflect the extent of atherosclerosis, atherosclerotic lesions may be the source of circulating resistin (reverse causality). Importantly, in the present study resistin concentrations were independently associated with CRP and creatinine concentrations. Hence, it may be argued that resistin-PWV relations are attributed to the previously described resistin versus CRP or creatinine relations [32,33]. However, in the present study, resistin-PWV relations were independent of CRP and creatinine concentrations and additional confounders.

The limitations of the present study warrant consideration. As this was a cross-sectional study no conclusions can be drawn regarding cause and effect. Second, the present study was conducted in a group of black African ancestry and hence the present results require validation in alternative ethnic groups. Third, PWV measurements were obtained from pulse transit distance using subtracted distances (difference between the distance from the femoral sampling site to the suprasternal notch, and the distance from the carotid sampling site to the suprasternal notch), prior to the recommendation that a standardized approach of 80% of the direct carotid-femoral distance be used [24]. Hence, our PWV values are likely to differ from standard noninvasive measurements (see converted standard aortic PWV values given in Table 1). Fourth, although independent relations between resistin concentrations and PWV were noted in women, we were not statistically powered to show independent relations between resistin concentrations and PWV in men. Hence, further studies in men are required. Fifth, the calibration of aortic pressure from radial tonometry employs brachial pressure measurements and ignores possible amplification of pressures from the brachial to the radial artery [34]. However, although this would affect relations between resistin concentrations and aortic PP, relations with aortic PWV or aortic reflected wave index, the principle outcomes of the present study, would not have been influenced by this possible error as neither PWV nor aortic reflected wave index depend on errors in calibration. Last, aortic wave separation was performed using a 'triangular' flow waveform rather than a measured or 'physiological' aortic flow waveform, and hence aortic reflected wave index should be considered as an approximate at best.

In conclusion, in the present study, we provide the first evidence derived from a relatively large, randomly selected community-based sample of participants having never been treated with antihypertensive therapy and with a high prevalence of obesity, that independent of confounders, circulating resistin concentrations are independently associated with aortic PWV. These relations persisted with further adjustments for CRP and HOMA-IR. These findings therefore suggest a potential mechanism that may explain relations between circulating resistin concentrations and cardiovascular disease.

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ACKNOWLEDGEMENTS

This study would not have been possible without the voluntary collaboration of the participants and the excellent technical assistance of Mthuthuzeli Kiviet, Nomonde Molebatsi, and Nkele Maseko.

Funding: This study was supported by the Medical Research Council of South Africa, the University Research Council of the University of the Witwatersrand, the National Research Foundation, the Circulatory Disorders Research Trust, and the Carnegie Program.

Conflicts of interest

There are no conflicts of interest.

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Reviewers' Summary Evaluations

Reviewer 1

Authors investigated the association between two adipokines (adiponectin and resistin) and CRP with aortic (carotid-femoral) stiffness and pulse wave reflections in a population sample from nuclear families of black African descent living in the SOWETO township of Johannesburg. The study was well performed using appropriate methods. The study showed that only resistin was an independent predictor of aortic stiffness. These results may contribute to our understanding why some obese patients show an increased aortic stiffness and others do not. The study was performed in black Africans and has to be confirmed in other ethnic populations.

Reviewer 2

Strengths: This paper took advantage of a large population of never treated hypertensive patients and found meaningful relations between resistin levels and aortic stiffness, suggesting that the relation between increased stiffness and obesity results from abnormal hormonal stimulation. Weaknesses are related to the cross-sectional nature of the study.

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