Aspects of the interrelation between hypertension and insulin resistance: A preliminary study.

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A thesis submitted in partial fulfillment of the requirements for the degree of Magister Scientiae, in the Department of Medical Bioscience, University of the Western Cape.

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Keywords

Glucose tolerance test

High-fat diet

Hypertension

Insulin resistance

Lipid profile

QUICKI

Sprague Dawley rat

Vascular reactivity

Visceral fat

α- adrenergic response

Abstract

Aspects of the interrelation between hypertension and insulin resistance: A preliminary study.

Background: It is well known that some genetic factors and dietary factors, such as excessive salt intake and excessive caloric intake (resulting in obesity) are risk factors for hypertension. Fifty percent of all hypertensive patients are also insulin resistant. Both hypertension and insulin resistance are again risk factors for other cardiovascular diseases such as atherosclerosis and heart failure. The nature of the association between hypertension and insulin resistance has not been clearly elucidated. Spontaneously hypertensive rats are the ideal models to study the aspects of the relationships between hypertension and insulin resistance. Models of high-fat feeding induce obesity, hypertension and insulin resistance and are thus used extensively to study hypertension because these models closely mimic some of the renal and cardiovascular changes found in human hypertensive patients. The present study was initiated to evaluate if insulin resistance will develop within 6 weeks in a model of high-fat diet induced hypertension and if so, to determine whether captopril will affect the presence of insulin resistance. This model should in future be used to study vascular reactivity to phenylephrine (PHE), acetylcholine (ACH) and sodium nitroprusside (SNP) in hypertensive animals in the absence or presence of insulin resistance and in normotensive insulin resistant animals.

Methods: In a series of experiments, rats were divided into four groups that received different treatments: (i) laboratory pellets, (ii) high-fat diet, (iii) high-fat diet plus captopril and (iv) high-fat diet plus vehicle. Body weight was measured weekly for 6 weeks. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured every week during the 6-weeks feeding period by the tail cuff method using a two channel computerized non-invasive system from Kent Scientific Corporation, USA. Intraperitonealy glucose tolerance tests (IPGTTs) were performed at week 3 and week 6. After 6 weeks, and after an overnight fast, the plasma lipid profile was determined using a portable Cardiochek^{TM} blood test system. Fasting plasma insulin was determined using an immunoenzymatic assay for the *in vitro* quantitative measurement of rat insulin (INS) in serum and plasma. Insulin sensitivity was estimated by the quantitative insulin sensitivity check index (QUICKI) using the fasting plasma insulin and fasting glucose levels. After week 6 on the high-fat diet, thoracic aortae from the control and high-fat fed (HFD) animals were excised and vascular response to PHE, ACH and SNP were assessed in intact and denuded endothelium.

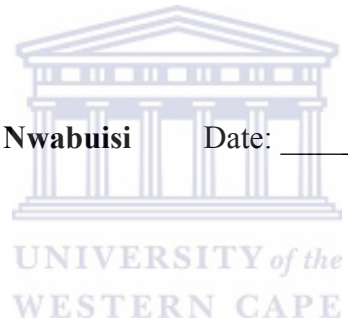
Result: High-fat feeding did not cause a significant increase in body weight. High-fat feeding significantly increased systolic blood pressure from 125±2.1 mmHg in control animals to 155±5.9 mmHg in the HFD group ($P < 0.05$) and 158±5.6 mmHg in the HFDV group ($P < 0.05$). Diastolic blood pressure was increased from 86±2.8 mmHg in the control group to 117±2.5 mmHg in the HFD group ($P < 0.05$) and 113±3.4 mmHg in the HFDV group ($P < 0.05$). Visceral fat was increased from 0.8±0.1g in the control group to 3.1±0.6 g in the HFD group and 3.8±0.6 g in the HFDV group. IPGTTs

performed at weeks 3 and 6 respectively did not differ significantly from the control group as evidenced from the AUC's at weeks 3 and 6 respectively. High-fat feeding had no significant effects on blood cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) values or and fasting plasma insulin levels. The KCl induced contraction in both aortic rings with intact and denuded endothelium did not differ significantly between the control and HFD groups (P = 0.4 and 0.8) respectively. The contraction mediated by KCl in aortic rings with intact and denuded endothelium from the control or HFD groups also did not differ significantly (control: intact vs denuded, P = 0.2; HFD: intact vs denuded, P = 1). Dose response-curves (1-10 μM) to PHE indicated slightly stronger contractions in the high-fat fed animals at submaximal doses tested. The maximum contraction achieved was however the same (94±19% and 99±2.6% relative to KCl induced contraction, in the control and HFD group respectively, $P<0.05$). Relaxation responses to ACH and SNP represent preliminary data.

Conclusion: These data suggest that 6 weeks of high-fat feeding induces hypertension but does not produce obesity, dyslipidemia and insulin resistance. However, this model may be useful in studying vascular reactivity in hypertension in the absence of insulin resistance.

DECLARATION

I declare that *Aspect of the interrelation between hypertension and insulin resistance: A preliminary study* is my own work, that it has not been submitted for any degree or examination in any other university and that all the Sources I have used or quoted have been indicated and acknowledged by complete references.



Full name: **Osuafor, Godswill Nwabuisi** Date: _____

Signed: _____

DEDICATION

In the hours of weakness, I was strong; in the midst of scarcity, I was in abundance. To God be the Glory who made it so. Thanks greatly to my brother JC Osuafor for his steady support through the years, and to Tania Bowers you people are coworkers in making this mission a reality.

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CONTENTS

Page

LIST OF FIGURES

LIST OF TABLES

LIST OF ABREVIATIONS

ACH == Acetylcholine

AGT == Angiotensinogen

AMPK == Adenosine monophosphate activated protein kinase.

AT1R == Angiotensin II type 1 receptor

ATM == Adipose tissue mass

AUC == Area under the curve

BP == Blood pressure

CONT == Control

DASH diet == Dietary approaches to stop hypertension

EC_{50} == Concentration of agonist that provokes a response halfway between the baseline and maximum response.

E_{max} == Maximum contraction

eNOS == endothelial nitric oxide synthase

ETBR == Endothelin-B Receptor

FFAs == Free fatty acids

GLUT2 == Glucose transporter 2

GLUT4 == Glucose transporter 4

HDL –C == High-density lipoprotein cholesterol

HFD == High-fat diet

HFDC == High-fat diet captopril

HFDV == High-fat diet vehicle

IGT == Impaired glucose tolerance

IL-6 == Interleukin 6

IPGTT == Intraperitoneal glucose tolerance test

IQR == Interquartile range

IR == Insulin resistance

IRS == Insulin receptor substrate

IRS-1 == Insulin receptor substrate-1

LDL –C == Low-density lipoprotein cholesterol

LFD == Low fat diet

L-NAME == NG –nitro-L-arginine methyl ester

MCP-1 == Monocyte chemoattractant protein-1

MDA == Malondialdehyde

NADPH == Nicotinamide adenine dinucleotide phosphate

NEFA == Nonesterified fatty acid

PAI-1 == Plasminogen activator inhibitor-1

PHE == Phenylephrine

PI3K == Phosphoinositide 3' kinase

PPARγ == Peroxisome proliferator-activated receptor-gamma

QTLs == Quantitative trait loci

QUICKI == Quantitative insulin sensitivity check index

RAAS == Renin angiotensin aldosterone system

RAS == Renin angiotensin system

RBP4 == Retinol binding protein 4

ROS == Reactive oxygen species

SD == Sprague–Dawley

SHR == Spontaneous hypertensive rats

SNP == Sodium nitroprusside

TGF- β == Transforming growth factor beta

TNF-α == Tumor necrosis factor alpa

TXA2 == Thromboxane 2

VHFD == Very high-fat diet

VPR == Volume pressure recording

WHO == World health organization

CHAPTER ONE

INTRODUCTION

The incidence of cardiovascular diseases has been increasing globally for the past few decades. The incidence of hypertension amongst South African blacks has been on the increase as well. Some sub-Saharan African countries still show clear urban/rural differences in the prevalence of hypertension, but this difference no longer exist in the South African context (Steyn, 2005). The prevalence rates in the rural areas have increased to the levels found in the cities (Mollentze *et al.,* 1995). Connor *et al.,* (2005) in their survey reported the prevalence of hypertension at 59% among the black South Africans. In addition, the Demographic and Health Survey (SADHS) projected that more South Africans will die from heart-related conditions than from Aids by the year 2010 (Department of Health, 2002).

Hypertension is a risk factor for heart attacks, stroke, left ventricular hypertrophy, renal disease, and blindness (Steyn, 2005). This necessitates stringent measures in order to control this disease. The presence of uncontrolled hypertension together with additional risk factors of other lifestyle diseases may result in complications, leading to damage of different organs in all population groups in South Africa.

Hypertension correlates with age, waist:hip ratio and smoking (Steyn, 2005). Van Rooyen *et al.,* (2000) also reported an association between hypertension and malnutrition

in terms of high intakes of saturated fat, animal protein, sodium and vitamins A and B6. While nutrition and lack of physical activity may be the driving factors, genetic predisposition contributes to the etiology of hypertension among black South Africans. Seedat, (1996) reported that black people have an abnormal transport mechanism of sodium and a low renin activity.

Fifty percent of all hypertensive patients have been reported to be insulin resistant (Ginsberg, 2000). In addition, hypertension and insulin resistance have been observed in metabolic syndrome, obesity, pregnancy, infections or severe illness, stress and in the excessive use of steroids (http://www.medicinenet.com/insulin_resistance/article.htm 10/10/2008). Factors that predispose to one also contribute to the other. These two conditions are disease states that can be provoked by genetic factors (Rotimi *et al.,* 1999) and/or life style. It is reported that a western lifestyle contributes to the pathogenesis of hypertension and insulin resistance across Africa (Basciano *et al.,* 2005).

Previous studies where hypertension and insulin resistance were observed after high-fat feeding focused on obesity (Dobrian *et al.,* 2000) and type II diabetes (Srinivasan *et al.,* 2005). From the polygenic perspective, it is clear that the monogenic models of hypertension and insulin resistance cannot represent the human disease overtly (Buettner *et al.,* 2006). Furthermore, models of these disorders generated by pharmacologic measures, such as the dexamethasone mouse model of hypertension (Carlos *et al.,* 2003), alloxan and streptozotocin rat models of insulin resistance and diabetes (Lenzen, 2008; Shafrir *et al.,* 2003) are unphysiologic in many respects (Buettner *et al.,* 2006).

There is sufficient evidence that endothelial dysfunction is implicated in the development of hypertension and insulin resistance. Recent studies have shown increased vascular responses to vasoconstrictor agents (Katakam *et al.,* 2000; Ghatta and Ramarao, 2004) in insulin resistance rodents. It has also been shown that hypertension is associated with increased contractility to potassium chloride (Viswanad *et al.,* 2006), angiotensin II (Ghatta and Ramarao, 2004; Viswanad *et al.,* 2006) and phenylephrine (Ghatta *et al.,* 2005). Furthermore, relaxation due to acetylcholine (Ghatta and Ramarao, 2004; Viswanad *et al.,* 2006) and sodium nitroprusside (Viswanad *et al.,* 2006) are attenuated. It is however not clear whether the vascular dysfunction exists in insulin resistant individuals in the absence of hypertension and vice verse

1.1 The purpose of the study

Understanding the link between insulin resistance and hypertension may reveal a novel strategy for the management of cardiovascular diseases. The aim of this project was to set up a model to elucidate the interrelations between hypertension and insulin resistance.

The objectives included:

- To develop a model for hypertension by high-fat feeding.
- To determine whether the animals fed the high-fat diet will become insulin resistant after six week.
- To set up an experimental system to evaluate vascular responses in a model of diet induced hypertension. This model should in future be used to study vascular reactivity in hypertensive animals in the absence or presence of insulin resistance and in normotensive insulin resistant animals.

CHAPTER TWO

LITERATURE REVIEW

2.1 Hypertension

Hypertension is a sustained increase in arterial blood pressure that is usually indicated by a systolic and diastolic blood pressure of 140 mmHg and 90 mmHg respectively, or above in human. Hypertension is classified as either primary (essential) or secondary hypertension. Primary hypertension is a hypertensive state in which no particular medical cause is established. Secondary hypertension, on the other hand, is a hypertensive state caused by an underlying disease condition such as kidney disease, pheochromocytoma, paraganglioma or genetic mutation. The rest of this discussion will focus on primary hypertension. Hypertension in humans is a well known major risk factor for cardiovascular diseases. High blood pressure is known as a silent killer because the condition may be life threatening by the time the symptoms appear.

It is estimated that a quarter of the world's adult population is hypertensive, and this number is projected to increase to about 30% by 2025 (Kearney *et al.,* 2005). More than 6 million South Africans suffer from high blood pressure. Obesity is considered a serious health problem because it is an important factor that triggers essential hypertension (Hall, 2003). The Framingham Heart Study suggests that hypertension can be attributed to obesity in 78% of men and 65% in women hypertensive patients (Kannel, 2000). Hall *et al.,* (2002) reported that approximately 97 million people in the United States are obese.

Ridgway *et al.*, (2004) reported that about $122.9 billion is spent annually in the United States on hypertension and other obesity-related health issues, such as heart disease, type II diabetes, and stroke.

2.1.1 Risk factors that can induce hypertension.

There is no specific medically known cause of essential hypertension. There are however, quite a number of risk factors that can predispose an individual to hypertension. They are intake of diets high in fats, carbohydrates, salt and low intake of calcium, potassium and magnesium. Smoking, little or no exercise, stress, insulin resistance, age and a hereditary component are also risk factors.

2.1.2 Causes of hypertension

It has been postulated that obesity can cause abnormalities in renal function such as increased renal sodium reabsorption and an impaired pressure natriuresis relation which results in hypertension (Hall, 2003). On the contrary, leptin deficiency as in the ob/ob mouse, leads to decreased arterial pressure, despite severe obesity (Mark *et al.,* 1999). This suggests that increased leptin levels may be a potential cause of blood pressure elevation. However, the mechanisms that link obesity with high blood pressure and altered renal function have not been fully elucidated. For example, Zucker rats have decreased plasma renin activity (PRA) as opposed to the high plasma renin activity observed in humans (Tuck *et al.,* 1981). It has been suggested by previous studies that the diet-induced obese animal models appear to be the most relevant with regard to human obesity. Models of obese rat (Carroll *et al.,* 2006) fed a high-fat diet, are used extensively

to study obesity related hypertension because they closely mimic some of the cardio-renal changes found in obese humans.

Studies suggest that obesity impairs renal-pressure natriuresis as the result of increased tubular sodium reabsorption (Hall, 2003; Strazzullo *et al.,* 2003). These abnormalities of renal function may be partly dependent on the activation of the renin-angiotensin-aldosterone system (RAAS). Some of the major characteristics associated with obesity related hypertension in humans are the activation of the RAAS, (Hall, 1994) high levels of circulating leptin, (Hirose *et al.,* 1998) reduced growth hormone (GH) concentration, (Kopelman *et al.,* 1985) and an activation of the sympathetic nervous system (Tuck, 1992). High Angiotensin II levels due to the conversion of angiotensin I to Angiotensin II by angiotensin converting enzymes (ACE) from pulmonary and renal endothelium surfaces, has been shown to interfere with phosphoinositide 3-kinase (PI3K) activation in vascular smooth muscle cells (Folli *et al.,* 1997). Angiotensin II is part of the RAAS and causes constriction of blood vessels with the concomitant rise in blood pressure. It stimulates the release of aldosterone from the adrenal cortex, which subsequently cause sodium retention in the distal nephron with resultant increase in blood pressure. Figure 2.1 shows the possible mechanisms by which angiotensin in its active form, angiotensin, II can cause an increase in blood pressure.

Studies on regulatory factors that participate in the pathogenesis of hypertension in spontaneously hypertensive rats (SHR) have shown that in the presence of normal plasma renin activity (PRA), angiotensin II (Ang II) plays a key role in the pathogenesis

of the increased blood pressure (Reckelholf and Romero, 2003; Reckelholf et al., 2000). Angiotensin II specifically induces an increase in blood pressure via oxidative stress and endothelin I. Obesity related hypertension is further often associated with dyslipidaemia, indicating low levels of HDL-C-cholesterol (Glueck *et al.*, 1980) and higher levels of triglycerides (Van Itallie *et al.*, 1985). In addition, hyperlipidaemia associated with hypertension may induce glomerulosclerosis in the kidney and eventually alter kidney function (Grone *et al.*, 1993).

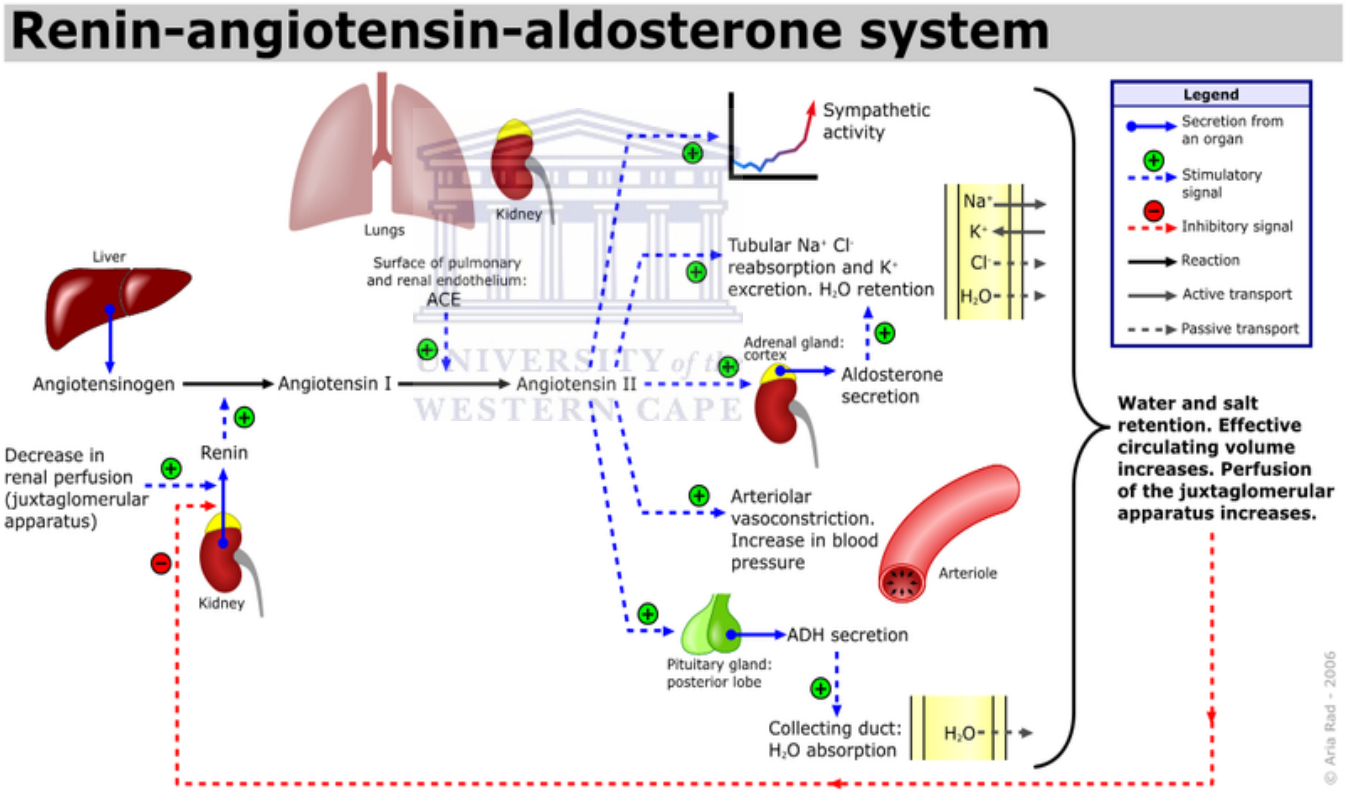


Figure 2.1: The renin-angiotensin system (RAS) or the renin-angiotensin-aldosterone system (RAAS) is a hormone system that regulates blood pressure and water balance. When blood pressure is low, the kidneys secrete renin. Renin stimulates the production of angiotensin. Angiotensin II causes blood vessels to constrict resulting in increased blood pressure. Angiotensin I is converted to angiotensin II by the enzyme angiotensin –converting enzyme (ACE). ACE is the target for inactivation by angiotensin –converting enzyme inhibitor drugs; which reduces the production of angiotensin II. Angiotensin II causes increase in blood pressure by stimulating sympathetic activity, tubular sodium retension, and absorbtion, water retention, potassium excretion, aldosterone secretions antidiuretic hormone secretion and arteriolar vasoconstriction. http://en.wikipedia.org/wiki/Renin-angiotensin-aldosterone_system. 12/02/09.

Oxidative stress has been implicated in the pathophysiology of hypercholesterolemia, diabetes, and hypertension (Steinberg *et al*., 2002; Cai *et al*., 2000). Involvement of oxidation has been reported in animal models of spontaneous hypertension (Wu and Jourlink, 2002), renovascular hypertension (Lerman *et al.,* 2001), the deoxycorticosterone acetate-salt model (Trolliet *et al.,* 2001), and obesity-related hypertension (Dobrian *et al.,* 2001). There is evidence that high doses (Laursen *et al.,* 1997) or subpressor doses (Reckelhoff *et al.,* 2000) of angiotensin II induce oxidative stress. There are also data that indicate increased oxidative stress in human essential hypertension (Sagar *et al.,* 1992; Russo *et al.,* 1998) as well as in obese hypertensive patients, (Van Gaal *et al.,* 1995) which may contribute to the development of atherosclerosis or other cardiovascular diseases. Data from obesity prone rats shows a 2 fold increase in plasma renin activity, which indirectly suggests high circulating levels of angiotensin II in obese hypertensive animals (Dobrian *et al.,* 2001). Oxidative stress has been postulated to be a common link that underlies both obesity and hypertension. Increased levels of circulating renin, and possibly angiotensin II, have been suggested to be associated with oxidative stress. It is not clear whether oxidative stress is a consequence of obesity induced hypertension, but the possibility that it mediates the effect of angiotensin II on blood pressure has been reported (Dobrian *et al.,* 2001).

Several Quantitative trait loci (QTLs) for hypertension have been mapped (Hilbert *et al.,* 1991; Jacob *et al.,* 1991; Pravenec *et al.,* 1995) in spontaneous hypertensive rats (SHR) and stroke-prone SHR, but the identity of the underlying genes remains unknown (Aitman *et al.,* 1999). QTLs linkage for hypertension, hypertriglyceridaemia, reduced

high density lipoprotein (HDL) phospholipids and the metabolic defects in adipocytes map to a single region close to the telomere of chromosome 4 (Aitman *et al.,* 1997; Pravenec *et al.,* 1995; Bottger *et al.,* 1996; Kovacs, 1996).

Based on recent progress of research on adipocytes, visceral obesity plays a critical role in the development of insulin resistance. It is suggested that angiotensinogen, one of the adipokines such as TNF- α and nonesterified fatty acid (NEFA), produced by visceral fat, might contributed to the development of insulin resistance, in the muscle and adipose tissues (Rahmoni *et al.,* 2004). While adipocytes produce adipokines such as angiotensinogen, TNF-α, NEFA, resistin and leptin that have the potential to decrease insulin sensitivitity (Houstis *et al.,* 2006; Lee et al., 2009; fig.2.2), it also secrete adiponectin and adrenomedullin (AM) which increase insulin sensitivity (Fujita, 2007). It is plausible that imbalance in the production of these adipokines may lead to insulin resistance.

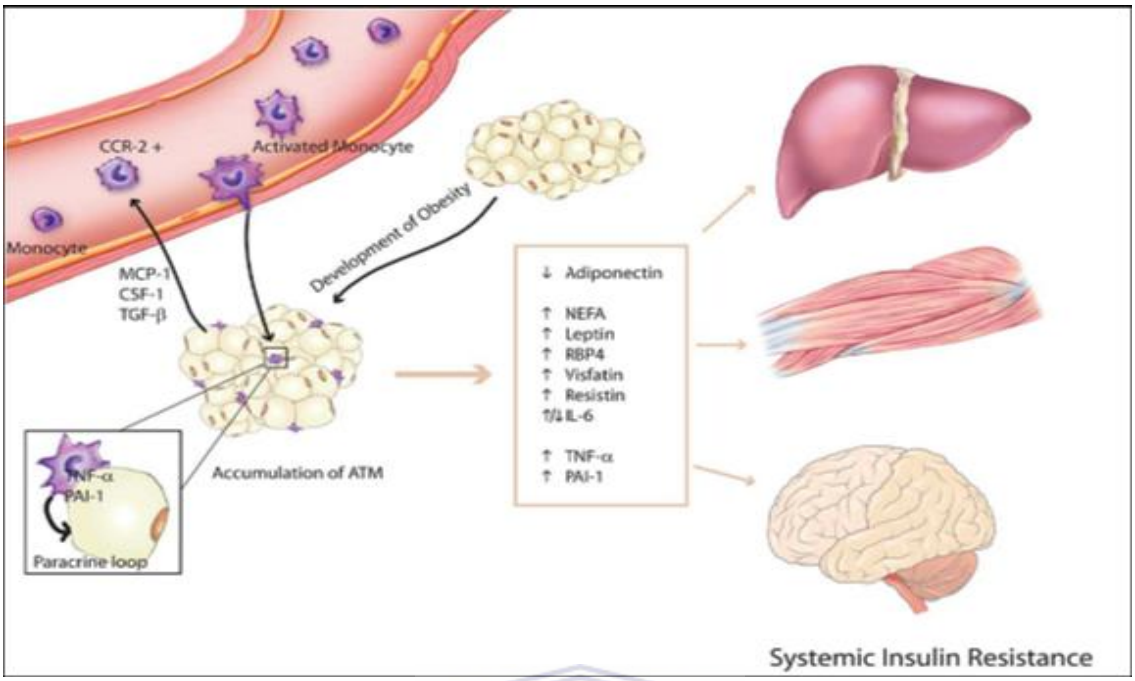


Figure 2.2: The inflammatory hypothesis that under obese state, fat may induce insulin resistance. Some of the molecules secreted by adipose tissue are pro-inflammatory cytokines. The most important adipocytokines is adiponectin which is involved in systemic signaling (Lee et al., 2009)

There are several evidence that cardiovascular disease states such as hypertension, coronary artery disease, and myocardial infarction are strongly associated with endothelial nitric oxide synthase (eNOS) gene polymorphism (Miyamoto *et al.,* 1998; Hingorani *et al.,* 1999; Shimasaki *et al.,* 1998; Cai *et al.,* 1999; Wang *et al.,* 1996; Shoji *et al.,* 2000) and impaired nitric oxide (NO) synthesis (Cai *et al.,* 1999; Wang *et al.,* 1996). Furthermore, the impaired NO synthesis, under some conditions is directly related to the polymorphism (Ohtoshi *et al.,* 2002; Philip *et al.,* 1999) which could predispose to insulin resistance (Sartori *et al.,* 1999; Scherrer *et al.,* 1999). There is also evidence that partial deletion of the eNOS gene does not primarily alter insulin sensitivity or blood pressure in mice. Cook *et al.,* (2004) in a recent study suggested, that partial eNOS

deficiency facilitates the development of insulin resistance and arterial hypertension when challenged with nutritional stress, providing further evidence for the importance of this gene in linking metabolic and cardiovascular disease. In humans, fat intake may induce endothelial dysfunction (Steinberg *et al.,* 1997) due to the impairment of vascular nitric oxide synthesis. A NO production defect was associated with the development of arterial hypertension (Cook *et al.,* 2004). One of the pioneering studies shows that the inhibition of NO synthesis induced by administering of Nω-monomethyl-L-arginine of NǴ–nitro-L-arginine methyl ester (L-NAME) produced a notable vasoconstriction (Baylis *et al.,* 1990; Romero *et al.,* 1992; Vallance *et al.,* 1989), sodium retension (Lahera *et al.,* 1990; Lehera *et al.,* 1991) with concomitantly sustained increased mean arterial pressure (MAP) (Baylis *et al.,* 1990; Lahera *et al.,* 1990; Romero *et al.,* 1992; Vallance *et al.,* 1989). In summary, a decrease in NO with concomitant elevation of blood pressure due to pathological conditions in human was assumed to be linked to endothelial dysfunction (Lehera *et al.,* 1991). The specific metabolic alterations involved in this process are poorly understood (Reckelhoff, 2003).

In the mid-1960s, Welborn and co-workers (1966) observed that hypertension was commonly associated with hyperinsulinaemia. It is known that hyperinsulinaemia can result in increased reabsorption of sodium and water by kidney tubular cells due to insulin action and increased sympathetic activity (Defronzo *et al.,* 1991; Williams 1994; Hall, 1994). This can be associated with a volume-dependent hypertension (Ginsberg, 2000). However, it is still not clear how often volume-dependent hypertension is present in insulin resistant individuals and patients with type II diabetes (Ginsberg, 2000). This

was the basis for the speculation that increased insulin may contribute to the elevated blood pressure (Christlieb *et al.,* 1985; Tuck, 1992). This speculation has been substantiated by additional evidence correlating hyperinsulinaemia with hypertension (Hwang *et al.,* 1987; Sowers *et al.,* 1991). Acute infusion studies suggested that insulin might elevate blood pressure through renal, neural, and/or secondary humoral mechanisms (DeFronzo, 1981; Kirchner, 1988; Morgan *et al.,* 1993). It has also been reported that insulin levels predicted blood pressure elevation in healthy children (Taittonen *et al.,* 1996).

Another cause for hypertension in the insulin resistant patient is over activity of the sympathetic nervous system (Landsberg, 1999). Much evidence suggests that the sympathetic system is over reactive in obese and insulin resistant individuals, but it has not been shown that this is a primary defect in these individuals (Ginsberg, 2000). It has been reported that insulin, in association with increased sympathetic activity, can trigger renal sodium re-absorption (DeFronzo *et al.,* 1991; Williams *et al.,* 1994; Hall, 1994) leading to volume expansion. Thus, it is somewhat difficult to account for insulin resistance in obese patients with overreactive sympathetic nervous system without hypertension (Ginsberg, 2000). An alternative hypothesis to explain the pathogenesis of obesity induced hypertension is that chronic central nervous system–induced sympathetic activation links insulin resistance and hypertension (Rocchini *et al.,* 2004). The sympathetic nervous system function is strongly influenced by dietary intake, which may be associated with high intakes of fat, salt and fructose. Fasting or caloric deprivation reduces sympathetic activity whereas overfeeding stimulates sympathetic activity (Young

et al., 1982). Leptin, a hormone that is secreted from adipocytes in response to food intake, is also known to activate the sympathetic nervous system (Kuo *et al.,* 2003). Leptin, acts at the level of the hypothalamus to increase blood pressure via its central sympatho-excitatory effects (Grassi, 2004; Rahmouni *et al.,* 2005).

Data from human and experimental animal models of hypertension provide evidence that alterations in the sympathetic control of heart rate, cardiac output, peripheral vascular resistance and renal sodium handling may integrally or independently, promote the development and progression of the hypertensive state (Amerena *et al.,* 1998). However, there are studies that oppose the relevance of sympathetic overdrive in hypertension. For instance, microneurographic studies show that sympathetic overdrive is observed in patients without high blood pressure (Grassi *et al.,* 2005), which suggests independence of hypertension. It is important to note that adrenergic overdrive is not reported in secondary forms of hypertension like renovascular hypertension or in hyperaldosteronism (Grassi *et al.,* 1998). The magnitude of the sympathetic activation is reported to be intensified when the hypertensive state is complicated by cardiac hypertrophy (Greenwood *et al.,* 2001; Schlaich *et al.,* 1992). Futhermore, obesity in the absence of blood pressure elevation, shows features of adrenergic activation, like increased resting heart rate values and elevated plasma norepinephrine values (Young *et al.,* 1982).

There is consistent evidence that microalbuminuria, is an aspect of metabolic syndrome implicated in hypertension (Ferrannini *et al.,* 1987; Chen *et al.,* 1998). Indeed prospective studies stated that elevated systolic blood pressure is a significant determinant of

microalbuminuria development (Metcalf *et al.,* 1997). People with microalbuminuria that have not developed diabetes manifested multiple cardiovascular disease risk factors, like hypertension, dyslipidaemia (characterized by low HDL-C and elevated triglyceride) and high plasma levels of insulin (Mykkanen *et al.,* 1994), suggesting that microalbuminuria is an important component of the cardiovascular metabolic syndrome.

There is a correlation between hypertension and impaired glucose tolerance (IGT) in diabetic patients (Reaven *et al.,* 1990; Haffner *et al.,* 1992). Systolic blood pressure has shown a strong correlation with both fasting plasma glucose (FPG) and glucose levels measured 2 hours post an oral glucose ingestion (Reaven *et al.,* 1990). The risk of hypertension is higher in lean men with impaired fasting glucose compared to those with normal fasting glucose (Suematsu *et al.,* 1999). The reason for the association between hypertension and hyperglycaemia is still controversial because, it is not clear whether the high levels of blood glucose alone is responsible for the progressive development of hypertension or whether additional factors such as insulin resistance, dyslipidemia and obesity are involved (Invitti, 2003).

2.1.3 Treatment of hypertension

(a) Lifestyle modification (nonpharmacologic treatment)

The immediate treatment of mild to moderate hypertension starts with a change of lifestyle, weight reduction and regular aerobic exercise. Exercise improves blood flow, enhances reduction of resting heart rate and blood pressure. Nonpharmacological treatment includes a decrease in dietary sugar and salt intakes, and discontinuation of

tobacco use and alcohol consumption. An increase in calcium and potassium intakes, and DASH (dietary approaches to stop hypertension), a diet which is rich in fruits and vegetables and low fat or fat-free dairy foods are beneficial in managing hypertension. The supplementation of antioxidants, particularly in the form of fresh fruit and vegetables, reduces blood pressure (Ceriello, 2008). In most cases lifestyle modification takes precedence over medication unless hypertension is severe. However lifestyle changes are still recommended even concomitantly with drug therapy.

(b) Pharmacologic treatment

There are five main classes of antihypertensives for treating hypertension. Each of these classes has its own merits, disadvantages and salient properties that influence the choice for a particular patient. They act via different or related mechanisms to lower blood pressure.

These classes are:

- Diuretics
- Beta-blockers
- Calcium channel blockers
- Angiotensin converting enzyme inhibitors (ACEI)
- Alpha1-blockers

The goal of treating hypertension is to reduce the incidence of hypertensive complications such as coronary heart disease and stroke. An ideal drug should achieve these ends, as well as simply lowering blood pressure.

2.1.4 Vascular reactivity in hypertension

An increase in peripheral resistance has been postulated to be due to an increase in alpha-adrenergic mediated vasoconstriction, a decrease in beta-adrenergic vasodepressor activities or both (Field and Soltis 1985). These changes have been suggested to be due to the inability of the vascular smooth muscle cell to handle calcium. In addition, it has been reported that changes in vascular adrenergic responsiveness as well as maximal potassium chloride induced contraction in the SHR are due to increases in blood pressure (Field & Soltis 1985). They also suggested that the decrease in beta-adrenergic responsiveness may be a specific defect in the beta-adrenergic system rather than the defect in vascular smooth muscle cells to sequester calcium.

Several factors have been hypothesised to be responsible for the increase in peripheral vascular resistance in spontaneously hypertensive rats. These include an increase in sympathetic nervous system activities (Nilsson & Folkozo, 1982), alteration in blood vessel structures (Folkow *et al.,* 1973), and an increase in vascular smooth muscle reactivity (Field *et al.,* 1972; Bohr, 1974). There is evidence that the increase in responsiveness of vascular smooth muscle of SHR to norepinephrine is due to an increase in the permeability of calcium in the cell membrane (Noon *et al.,* 1978). A recent report attributes altered vascular reactivity in the thoracic aorta of rats fed dietary fat to oxidative stress (Viswanad *et al.,* 2006). It has been hypothesized that the altered vascular reactivity in SHR is an intrinsic defect of the vascular smooth muscle cells independent of blood pressure. In addition, prehypertensive SHR treated with reserpine to prevent the

increase in blood pressure exhibited altered vascular reactivity (Cheng and Shibata, 1981).

There is sufficient evidence of increased vascular responses to vasoconstrictor agents in insulin resistance rodents. An increase in blood pressure has been associated with increased contractility to phenylephrine (Smith *et al.,* 2006) as well as attenuated relaxation induced by acetylcholine and isoproterenol (Paula et al., 2006). In addition, vascular studies have suggested that hypertension developed in high-fat diet fed rats may be linked to enhanced vasoreactivity to various spasmogenic and antispasmogenic agents (Ghatta *et al.,* 2005).

2.2 Insulin resistance

Insulin resistance, in classic terms is the inability of insulin to stimulate glucose uptake in insulin sensitive peripheral tissues. The gold standard for assessment of insulin resistance in medical research is the hyperinsulinemic euglycemic clamp which measures the amount of glucose necessary to compensate for an increased insulin level without causing hypoglycemia. This technique is rarely used in clinics because it is cumbersome. Alternative techniques for determining insulin sensitivity which correlate very well with the hyperinsulinemic euglycemic clamp have evolved. The first was the Homeostatic Model Assessment (HOMA). The most recent method is the quantitative insulin sensitivity check index (QUICKI). These techniques apply both fasting insulin and glucose levels to calculate insulin resistance. According to the WHO guidelines, after 2 hours of a glucose tolerance test a glycaemic level less than 7.8 mmol/l is considered

normal, a glycaemia of between 7.8 mmol/l to 11.0 mmol/l is considered as Impaired Glucose Tolerance (IGT) and a glycaemia of greater than or equal to 11.1 mmol/l is considered Diabetes Mellitus.

The definition in the previous paragraph is different from the clinical syndrome known as the insulin resistance syndrome which integrates the additional factors such as insulin, lipid, protein metabolism, endothelial function and gene expression (Deedwania, 1998; Opara and Levine, 1997). The clinical and laboratory abnormalities that represent this syndrome consist of Type II diabetes mellitus, central obesity, dyslipidaemia (increased triglycerides, decreased HDL, and increased small dense LDL), hypertension, increased prothrombotic and antifibrinolytic factors (i.e. hypercoagulatability) and a predilectation for heart disease (Cefalu, 2001). Insulin resistance as a fundamental aspect of the etiology of type II diabetes was first suggested by Prof. Wilhelm Falta and was published in Vienna in 1931(Falta and Boller, 1931). This suggestion was confirmed in 1936 by Sir Harold Percival Himsworth of the University Collage hospital Medical Center in London (Himsworth, 1936). Insulin resistance implicating a wide array of other pathophysiologic sequel including hypertension, hyperlipidaemia, atherosclerosis (i.e., the metabolic syndrome, or syndrome X), and polycystic ovarian disease was introduced by Reaven (Reaven, 1988).

Succinctly, insulin resistance is a clinical state in which a normal or elevated insulin level produces an impaired biological response. In addition, there are a number of other conditions associated with insulin resistance which present specific clinical

manifestations such as polycystic ovarian syndrome, pregnancy or glucocorticoids therapy (Hunter *et al.,* 1998), haemochromatosis, hypercortisolism and a sedentary lifestyle which can lead to obesity. These conditions may include some or none of the features of the insulin resistance syndrome (Hunter *et al.,* 1998). In summary, insulin resistance can be generally viewed as a molecular and genetic abnormality involving defective insulin signaling and glucose transport into cells (Ginsberg, 2000).

2.2.1 Causes of insulin resistance

In most cases of insulin resistance, the causes are not known. However, there is strong evidence of inherited traits based on the rates of insulin resistance and type II diabetes among the close relatives of type II diabetic patients. Substantial evidence exists that insulin resistance is related to intake of a high carbohydrate diet (Parillo *et al.,* 1992, Grundy and Unger, 1992). In addition, a recent study shows that glucosamine may cause insulin resistance (Jenkins *et al.,* 2007). Glucosamine has been reported to increase flux through the hexosamine pathway, causing insulin resistance and disturbances similar to diabetic glucose toxicity (Bailey and Turner, 2004). In addition, insulin resistance induced by glucosamine could not be reversed by three agents (metformin, peroxovanadium and d-pinnitol) known to enhance or partially mimic the effects of insulin (Bailey and Turner, 2004). Oral glucosamine is used in the treatment of osteoarthritis.

Based on recent research on adipocytes, visceral obesity also plays a critical role in the development of insulin resistance (Fujita, 2007). At the molecular level, causes of insulin

resistance have been identified as an excessive phosphorylation of serine residues of the insulin receptor, mutations in the insulin receptor gene or insulin receptor substrate-1 (IRS-1), a cellular adenosine depletion, a deficiency in peroxisome proliferator-activated receptor gamma (PPAR-gamma) and a defect at the glucose transport level (Dunaif *et al.,* 1997). It has been postulated, based on the evidence from previous studies that the insulin resistance syndrome is caused by excessive accumulation of fat in intra-abdominal adipocytes (Bjorntorp, 1990; Després *et al.,* 1989; Kissebah, 1991). Some other studies reported that muscle triglyceride content is increased in insulin resistant humans and rats; this observation precipitated the alternative hypothesis that increased muscle triglyceride content is responsible for the insulin resistance (Jacob *et al.,* 1999; Koyama *et al.,* 1997). It is documented in humans, that the triglyceride content of muscle correlates directly with insulin resistance, and the fatty acid composition of muscle phospholipids influences insulin insensitivity (Borkman *et al.,* 1993).

Insulin resistance in obesity and type II diabetes is manifested by decreased insulin-stimulated glucose transport and metabolism in adipocytes and skeletal muscle and by impaired suppression of hepatic glucose output (Reaven, 1995). The initial molecular signal for insulin action shown in figure 2.2, involves activation of the insulin receptor tyrosine kinase, which results in phosphorylation of insulin receptor substrates (IRSs) on multiple tyrosine residues (Kahn and Flier, 2000). These phosphotyrosine residues act as docking sites for many SH2 domain–containing proteins, including the p85 regulatory subunit of phosphoinositide 3' kinase (PI3K) (Kahn, and Flier, 2000). It is suggested that the binding of the p110 catalytic subunit of PI3K to p85 activates the lipid kinase that

promotes glucose transport (White, 1998). The serine phosphorylation cascade initiated by PI3-kinase involves activation of PI3K-dependent serine/threonine kinases (PDK), and, in turn, Akt and results in the translocation of intracellular GLUT4 to the cell surface. It is the increased amount of GLUT4 on the cell plasma membrane that results in an increased rate of glucose transport into the cell.

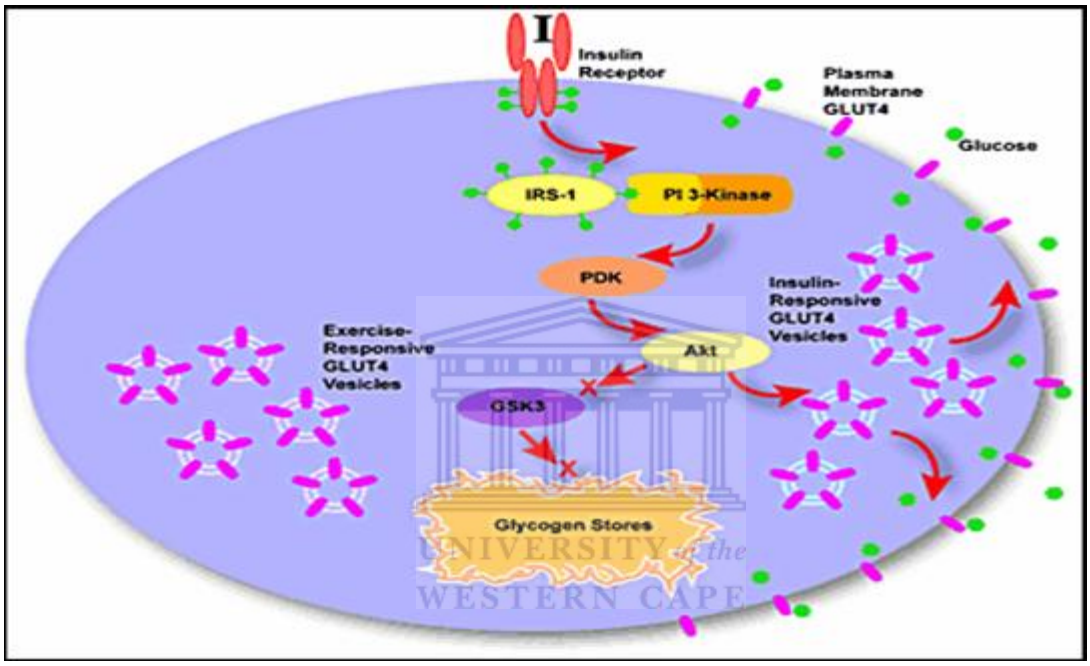


Figure 2.3: Insulin signaling pathways involved in stimulating glucose transport. Insulin binding to the IR results in phosphorylation of tyrosine residue on the receptor and substrates such as IRS-1. Docking of the regulatory subunit of PI3-kinase to phosphostyrosine residue of IR-1 activates its serine/threonine kinaseactivity and the phosphorylation cascade involving PDKs and Akt. These steps are necessary for the recruitment of intracellular pools of insulin-responsive glucose transport to the plasma membrane. www.endotext.org/.../diabetes_14/diabetes 14.html 7/1/09.

The glucose transporter (GLUT4) is down regulated in the adipocytes of all forms of obesity and diabetes, thus it is the main factor contributing to the impaired insulin action. On the contrary, in the skeletal muscle of obese and diabetic humans, GLUT4 expression is normal (reviewed in Shepherd *et al.,* 1999) and defective glucose transport appears to be due to impaired translocation, docking, or fusion of GLUT4-containing vesicles with

the plasma membrane (Zierath *et al.,* 1997; Hansen *et al.,* 1998). It is further suggested that the insulin action defect in muscle may involve impaired activation of PI3K, possibly due to elevations in protein kinase C theta (PKCθ) (Griffin *et al.,*1999), or acquired loss of PI3K activation in muscle as a result of a high-fat diet (Zierath *et al.,* 1997). PKC theta inhibits insulin receptor substrate (IRS) activation and thus prevents glucose uptake in response to insulin action.

The spontaneously hypertensive rat (SHR) has been proposed as a model of the insulin resistance syndromes because it develops insulin resistance, hypertriglyceridaemia, abdominal obesity and hypertension (Iritani *et al.,* 1977; Rao *et al.,* 1993; Aitman *et al.,* 1997). In SHR adipose tissue, defective insulin action is accompanied by a defect in catecholamine-mediated lipolysis (Reaven *et al.,* 1989), an additional feature of insulin resistance syndromes in humans (Reynisdottir *et al.,* 1994; Reynisdottir *et al.,* 1995; Bougneres *et al.,* 1997). It is suggested that the mechanism for defective insulin action in SHR is in part attributable to QTLs on other chromosomes apart from chromosome-4 (Aitman *et al.,* 1997). However, chromosome 4-encoded insulin resistance might be due to either primary effects through the peroxisome proliferator-activated receptor-gamma (PPARγ) pathway, or secondary to the disturbance in cellular fatty acid metabolism (Aitman *et al.,* 1999).

There is evidence that TNF-α signaling impairs insulin signaling, in part through serine phosphorylation of IRS-1 (Peraldi *et al.,* 1999; Hotamisligil, 1999), and can thus reduce

GLUT4 gene expression, so a plausible cellular basis for TNF-α as a mediator of insulin resistance has been established (Kahn and Flier, 2000).

Hyperinsulinaemia per se can cause insulin resistance by down regulating insulin receptors and desensitizing post receptor pathways, as was confirmed by overexpression of insulin in livers of otherwise normal transgenic mice (Patti *et al.,* 1996). It is reported that exposure of cells to insulin decreases GLUT4 receptors on cell membranes (Flore-Riveros, 1993). This leads to a greater need for insulin leading to fewer glucose receptors. This condition can be reversed in muscle tissues by exercise (MacLean, 2002), but can transpire to insulin resistance if neglected. Current evidence suggests that insulin resistance is associated with deficiency of leptin. Severe insulin resistance is a well known feature of deficiency of leptin or its receptor in the diabetic or obese mouse strains, and these models were among the first to be investigated for the pathogenesis of insulin resistance in the early 1970s (Kahn and Flier, 2000).

It has also been hypothesized that the sympathetic overactivity occurring in metabolic syndrome is dependent on the hyperinsulinaemia and the related insulin resistance state characterizing the disease (Landsberg, 1996). This hypothesis is on the basis that the acute systemic administration of insulin provokes sympathetic stimulation without affecting glucose levels in experimental animals as well as in humans (Landsberg, 1996; Egan, 2003; Scherrer *et al.,* 1997). It was reported that hyperinsulinaemia act centrally to enhance the activity of the sympathetic nervous system (Reaven *et al.,* 1996; Moan *et al.,* 1995). However, there are reports that the sympathetic-insulin crosstalks are not straight

forward. Thus, whether the sympathetic activation induces the insulin resistance state or poses as an epiphenomenon of the metabolic alteration is still unknown (Landsberg, 1996; Egan, 2003; Jamerson *et al.,* 1993).

2.2.2 Treatment of insulin resistance

The treatment of insulin resistance invariably, as in the case of hypertension, involves both pharmacological and non-pharmacological interventions. The primary treatments for insulin resistance are non-pharmacological interventions in the form of exercise and weight loss. Switching to a low–glycemic or low carbohydrate diet may attenuate insulin resistance in some individuals (Sebely *et al.,* 2008). Some polyunsaturated fatty acids such as omega-3 may promote or enhance insulin sensitivity (Gadja *et al.,* 2007).

There are three main classes of antihyperglycemic drugs for treating insulin resistance. They act via different or related mechanisms to lower blood glucose levels. These classes are:

- **Biguanides-**primarily suppress hepatic glucose production and intestinal glucose absorption; activates AMP-activated protein kinase (AMPK), a liver enzyme that is vital in insulin signalling, eg. metformin.
- **Thiazolidinediones**-decrease insulin resistance by activating peroxisome proliferator-activated receptors gamma (PPARγ). Examples are rosiglitazone, pioglitazone and troglitazone
- **Sulfonylureas**-act by increasing insulin release from the beta cells in the pancreas. Examples are acetohexamide, tolbutamide and chlorpropamide.

The drugs used for the treatment of insulin resistance presently are those approved for type II diabetes such as metformin, glyburide and thiazolidinediones. Although metformin is commonly prescribed, the Diabetes Prevention Program shows that exercise and diet were approximately twice as effective as metformin in reducing the risk of type II diabetes development (Knowler *et al.,* 2002).

2.2.3 Hypertension and insulin resistance

Hypertension and insulin resistance have been documented as two main phenomena occurring in parallel in both human (Reaven, 1991) and in rodents (Bhanot and McNeil, 1996). It is reported that among patients with hypertension, insulin resistance is present in all of those who are obese and in about one half who are not obese (Kaplan, 2000). Although hypertension and insulin resistance may probably share some features, the relationship between hypertension and insulin resistance are better discussed separately. Epidemiological studies indicate that insulin resistance and arterial hypertension are related (Lucas *et al.,* 1985), suggesting the possibility of a common underlying mechanism. From the clinical point of view, and experimental observations, it was suggested that insulin resistance in association with metabolic impairments are directly related to the development of hypertension (Reaven, 1991; Reaven *et al.,* 1996). Although high blood pressure and high insulin levels have been shown to be associated, independently of weight or body mass index, the link between obesity and both insulin resistance and hypertension further complicates the relationship between insulin resistance and hypertension (Ginsberg, 2000). Higher fasting and postprandial insulin levels have been observed in untreated essential hypertensive patients than normotensive

subjects irrespective of body mass; indicating a direct correlation between plasma insulin concentrations and blood pressure levels exists (Ferrannini *et al.,* 1987; Shen *et al.,* 1988). Also, in genetic models of hypertension such as the Dahl salt sensitive hypertensive rat (Kotchen *et al.,* 1991), the spontaneously hypertensive rat (Reaven *et al.,* 1991), and the Zucker obese hypertensive rat strain (Standley *et al.,* 1993), insulin resistance and hyperinsulinaemia exist.

Studies have suggested a link between hyperinsulinaemia, increased sympathetic nervous system activity, and obesity-related hypertension (Rocchini *et al.,* 1999; Kriger *et al.,* 1988). Insulin resistance, i.e., resistance to insulin's ability to stimulate glucose uptake, has been speculated to be the common metabolic abnormality shared by these three conditions. This hypothesis is supported by reports that document a relation between insulin resistance and hypertension (Pollare *et al.,* 1990; Shen *et al.,* 1988) in the absence of obesity and/or of diabetes mellitus (Ferrannini, 1987; Capaldo *et al.,* 1991).

Studies (Pasquali *et al.,* 2002; Chang *et al.,* 1983) demonstrated that hyperinsulinaemia and insulin resistance are common features of a large number of patients affected by polycystic ovary syndrome (PCOS). In addition to hyperinsulinaemia and insulin resistance, altered first-phase insulin secretion, impaired glucose tolerance, dyslipidaemia, hypertension and impaired fibrinolysis have also been described in PCOS (Talbott *et al.,* 1995; Dunaif *et al.,* 1997). The origin of insulin resistance in PCOS, which in recent years has become established as a feature of this syndrome, is still a matter of debate.

Mechanisms for the development of hypertension in insulin resistance cum hyperinsulinaemia include activation of the sympathetic nervous system, renal sodium retention, altered transmembrane cation transport, growth-promoting effects of vascular smooth muscle cells, and vascular hyperreactivity (Hunter *et al.,* 1998; Reaven *et al.,* 1996). Keen *et al.,* (1996) reported that insulin-induced hypertension in rats requires a normal ability of the rat to synthesize thromboxane. It has been hypothesized that hyperinsulinaemia/insulin resistance may cause an increase in blood pressure stimulating increase in the activity of endothelium-derived vasoconstrictors, such as thromboxane (TXA2) (Galipeau *et al.,* 2001). Further evidence suggested that vascular smooth muscle cell [Ca2+]/[Mg2+] ratio is increased in insulin resistance states, and this promotes insulin resistance and hypertension (McFarlane *et al.*,2001).

Despite the myriads evidence showing a correlation between insulin resistance and hypertension, a number of experimental observations suggest that the relation between insulin resistance and obesity induced hypertension is not so straightforward (Ferrannini *et al.,* 1990; Hall *et al.,* 1990). Some epidemiologic studies do not portray a correlation between plasma insulin levels and systolic blood pressure (Muller *et al.,* 1993). High insulin levels alone seam insufficient to substantially raise blood pressure. For instance, chronic hyperinsulinaemia (by infusion) does not induce hypertension in dogs, even in the presence of a high salt intake, obesity, or reduced renal mass (Hall, 1994; Hall *et al.,* 1995). Invariably, humans with insulinoma do not become hypertensive and their blood presssure does not fall after successful surgery (O'Brien *et al.,* 1993). Two weeks

administering of insulin to obese hypertensive subjects who were insulin resistant had a small blood pressure lowering effect (Heise *et al.,* 1998).

It is further suggested that if hyperinsulinaemia goes hand in hand with insulin resistance, and insulin resistance is linked to hypertension, it is expected that hypertensives would have higher insulin levels than normotensives (Cubeddu, and Hoffmann, 2002). Therefore, insulin concentrations may be correlated with blood pressure (BP) levels (Cubeddu, and Hoffmann, 2002). However the results are inconsistent. Rocchini *et al.,* (2004) concluded in their study that obesity induced hypertension and obesity induced insulin resistance are not directly related. In addition, insulin resistance is mediated through the central and or peripheral alpha-2-adrenoceptors, whereas hypertension is mediated through α-1- and or ß-adrenoceptors (Rocchini et. al., 2004). These contradictory findings suggest that, if insulin is important in the pathogenesis of hypertension, then inter-patient variability coupled with additional factors may be playing significant roles (Moan *et al.,* 1995).

The relationship between insulin and hypertension seen in essential hypertension does not occur with secondary hypertension (Reaven *et al.,* 1991; Sech *et al.,* 1992). Accordingly, insulin resistance and hyperinsulinaemia are not consequences of hypertension, but, instead, a genetic predistribution may contribute to both disorders (McFarlane *et al.,* 2001). It is therefore a possibility that there could be a genetic susceptibility to the development of hypertension or to the effects of insulin on blood pressure (Kaplan, 2000).

In summary, the relationship between hypertension and insulin resistance is well documented. However, the correlation between blood pressure and plasma insulin levels has been demonstrated to be inconsistent and apparently weak (Cefalu, 2007). There is little evidence that chronic hyperinsulinemia causes blood pressure elevations in humans (Cefalu, 2007; Hall *et al.,* 1999). It has been shown in animal and human studies that both acute and chronic hyperinsulinemia lasting for several weeks did not cause a hypertensive shift of pressure natriuesis or increased arterial pressure (Hall *et al.,* 1995; Hall,1993). Furthermore, insulin does not potentiate the blood pressure or kidney effects of norepinephrine or angiotensin II (Hall, 1993 and Hall *et al.,* 1995). Available studies do not suggest that chronic elevated insulin levels in obesity can account for induced increases in blood pressure (Cefalu, 2007). Evidence show that most work on insulin resistance did focus on its role in the pathophysiology of type II diabetes mellitus. Unfortunately, the type of detailed mechanistic information describing the link between insulin resistance and dyslipidaemia is not available for the link between hypertension and insulin resistance (Ginsberg, 2000).

The findings in gene-modified mouse models were paralleled by the observation that a strain of the spontaneously hypertensive rat (SHR) had mutations in CD36 (also known as FAT, as it encodes fatty acid translocase), that appeared to be associated with insulin resistance (Aitman *et al.,* 1999). However, Gotoda *et al.,* (1999) reported that the original SHR line, which is insulin resistant, has no defects in its CD36 gene. This is an irrefutable appeal for further developments in this area of investigation. Insulin sensitivity varies largely between normotensive and hypertensive patients; some hypertensives and

normotensives have a similar degree of insulin resistance and not all hypertensives are insulin resistant (Cubeddu, and Hoffmann, 2002). Insulin resistant hypertensives had BP levels comparable to that of non-insulin resistant hypertensives; suggesting that insulin resistance may not contribute to the BP levels of the hypertensive population.

The link between endothelial dysfunction and the hypertension of the insulin resistance syndrome is enticing because of the possibility that defective vasodilatation actually produces insulin resistance (Ginsberg, 2000). Abnormalities in vasodilatation and blood flow have been suggested to provide a link between hypertension and insulin resistance (Ginsberg, 2000). Understanding the link between insulin resistance and hypertension may reveal a novel strategy for the management of cardiovascular diseases. It is important to reiterate, however, that the association between insulin resistance and hypertension is not as strong as between insulin resistance and dyslipidaemia; only about 50% of hypertensive subjects are insulin resistant (Ginsberg, 2000).

2.3 Roles of the diet in hypertension and insulin resistance

Nutrition is a lifestyle element that can be regimented, and that can directly influence health; therefore, preventative nutrition and weight control should become the prime focus of consumers and prepared-food providers (Cummings *et al.,* 1988). The westernization of diets, with an increase in availability of high calorie foods certainly contributes to the epidemic of metabolic syndrome (Basciano *et al.,* 2005).

Every component in the dietary regimen has its own benefits and demerits. In most cases the metabolic abnormalities may be triggered by either the genetic predisposition or overindulgence. The components of a constituted diet can pose diverse effects which may be beneficial or detrimental to health. For instance, Fields and Lewis, (1999) reported that a combination of high-fat diets with fructose resulted in increased circulating triacylglycerol, while fructose with copper deficiency resulted in a significant increase in blood cholesterol. However, hyperlipidaemia did not occur when starch was combined with fructose (Fields and Lewis, 1999).

2.3.1 High-fat diet

In 1959, Masek & Fabry gave the nutritional description of a high-fat diet that would induce obesity. Dietary fat and its relation to obesity has been a controversial issue for several years because in the United States, the intake of fat appears to be declining, whereas the prevalence of obesity rises (Heini *et al.,* 1997). There is no hard and fast rule to define the term high-fat diet. Conventionally, low-fat diets (LFD) have about 10% of the calories coming from fat, high-fat diets (HFD) have about 30-50% of calories coming from fat and very high-fat diets (VHFD) contain greater than 50% fat (Gadja *et al.,* 2007).

Several studies have indicated that a high-fat diet increases body fat, with a substantial increase in serum leptin levels (Ahren *et al.,* 1997; Bahcece *et al.,* 1999). This increase in leptin could be one of the mechanisms by which dietary fat induces high blood pressure.

Some studies suggest that rats and mice fed a high-fat diet have increased visceral fat accumulation, whole body and muscle insulin resistance, and hyperinsulinaemia within 4 weeks (Grundleger *et al.,* 1982; Zierath *et al.,* 1997). On the contrary, Kim *et al.,* (2000) did not observe either significant effect on weight gain or fasting plasma glucose concentration after 4 weeks on a high-fat diet. Previous studies suggest that high-fat diets cannot cause hyperinsulinaemia, but most definitely will decrease glucose metabolism in peripheral tissues (Kraegen *et al.,* 1986). There is evidence that a high-fat diet does not cause muscle insulin resistance unless energy intake is sufficiently high to result in increased visceral fat accumulation (Kim *et al.,* 2000). Evidence exists that high-fat diets increase the hepatic triglyceride concentration with concomitant reduction of very low density lipoprotein (VLDL) secretion by 50% (Kalopissis *et al.,* 1979). In addition, excess triglyceride due to decreased synthesis of VLDL in hepatic cells by high-fat diet intervention is suggested to form ketone bodies and carbon dioxide via an oxidative pathway (Mooney *et al.,* 1981). Ordinarily, high-fat diet induced increase in muscle triglyceride content plays a less important role in causing muscle insulin resistance than does the increase in visceral fat (Kim *et al.,* 2000). The degree of insulin resistance in insulin-sensitive tissues is at least in part dependent upon how much fat is deposited in these tissues (Hannele, 2003). While there is disparity as to whether an increase in visceral fat or muscle triglyceride causes muscle insulin resistance, there is interesting evidence that muscle insulin resistance induced by a high-fat diet is mediated by the diet per se rather than by visceral fat accumulation (Barnard *et al.,* 1998).

There are a number of the mechanisms by which high-fat diet induce insulin resistance. High-fat diet reduces the number of insulin receptors and decreases the activity of the glucose transport system and the intercellular metabolism of glucose (Olefsky and Saekow, 1978). Further evidence shows that a high-fat diet decreases the activity of the intracellular enzymes implicated in fatty acid synthesis and suppresses their intracellular capacity to utilize glucose, which results in a blunted glucose response to insulin action (Lavau *et al.,* 1979). A high-fat diet has been reported to decrease GLUT2 and glucokinase mRNA concentration in pancreatic β-cells, thus reducing the rate of glucose entering pancreatic cells and invariably poor insulin secretion (Kim *et al.,* 1995).

There is evidence that mice fed high-fat diets have shown reduced insulin-mediated glucose metabolism in muscle and adipose tissues (Han *et al.,* 1997; Hansen *et al.,* 1998; Tremblay *et al.,* 2001). It is of major importance that chronic high-fat feeding trigged a substantial reduction in GLUT4 expression in both adipose and skeletal muscles (Sevilla *et al.,* 1997; Kahn, 1994). High-fats diet increases the levels of malondialdehyde (MDA) in serum, liver, aorta and kidney of Sprague Dawley rats (Dobrian *et al.,* 2001). In mice a high-fat diet provoked increased thiobarbituric acid-reactive species in cerebral, renal and hepatic tissues as well as elevated serum glucose level, suggesting oxidative stress in various tissues (Vanderlei *et al.,* 2003).

There is evidence that visceral obesity is a strong risk factor for the co-mobidity of insulin resistance/hyperinsulinaemia, dyslipidaemia, type II diabetes, hypertension, coagulation abnormalities and premature cardiovascular diseases (Tchernof et.al., 1996;

Banerji *et al.,* 1997). Although most rodents tend to become obese on high-fat diets and very high-fat diets, there can be variable responses in glucose tolerance, insulin resistance (IR), triglycerides and other parameters depending on the strain and gender (Levin *et al.,* 1997, Rossmeisl *et al.,* 2003). It has been suggested that among various animal models, Sprague–Dawley (SD) rats reveal accurately the mechanisms that are applicable to polygenic animal obesity, as one-half of SD rats seam to develop obesity when fed diets moderately high in energy and fat (Lauterio *et al.,* 1994). A number of mechanisms have been postulated for the differences in response to dietary fat, which include differential sensitivities to neurotransmitters, to the intestinal peptides, enterostatin, and to individual fatty acids (Bray *et al.,* 2002).

There is evidence that the duration of feeding the animals with a high-fat diet correlates with the reversibility of obesity. When animals gain weight by feeding on a high-fat diet up to 18 weeks, their body weight will return to the control level on switching to low-fat diet (Hill *et al.,* 1992). When the feeding interval on high-fat diet exceeds 30 weeks, body weights do not return to control levels despite reduction in dietary fat (Bray *et al.,* 2002). Jang *et al.,* (2003) suggests that the discrepancy between dietary fat type and body-fat accumulation in many studies may be partly due to the genetic background of experimental animals.

There is evidence that the sympathetic overdrive plays some role in human obesity which somehow depend on the specific pattern of fat distribution. For instance, microneurographic study result shows that the degree of sympathetic activation and the

magnitude of the insulin resistance are much greater in patients with visceral body fat deposits than in those with peripheral distribution of the adipose tissue (Graasi *et al.,* 2004).

The age of the rodents also contribute to the induction of metabolic abnormalities following the diet intervention. The mode of high-fat diet induced metabolic syndrome may take different dimensions with respect to age. In the studies of high-fat diets by Dobrian *et al.,* (2000) and Smith *et al.,* (2006), using adult and young male Sprague-Dawley rats respectively, hypertension and insulin resistance were induced in the animal models of both studies. The interesting thing in the above studies using adult and young rats is the rate at which hypertension occurs in the rats fed the high-fat diets. In the adult rats 50% became hypertensive (Dobrian *et al.,* 2000), whereas only three out of thirty-eight young rats did not develop hypertension (Smith *et al.,* 2006). This difference in the rates in which hypertension develops suggests that a physiological change occurs before the rats reach adulthood, which either predisposes or protects them from obesity (Smith *et al.,* 2006), or hypertension. Another interesting observation from the above-mentioned models of high-fat diets is the response of the renin-angiotensin-aldosterone system (RAAS). In the adult rats, the RAAS was activated whereas in the young rats it was not. The absence of elevated plasma renin and aldosterone suggest that the sympathetic nervous system may not be activated in young rats (Smith *et al.,* 2006). In the young rats, the high-fat diet induced an increase in reactivity to phenylephrine, blood pressure, blood, glucose levels, plasma insulin, visceral fat, heart size, and oxidative stress at an early age without the activation of the RAAS (Smith *et al.,* 2006). Zhou *et al.,* (2005) have shown

that young female rats fed a high-fat diet do not develop hypertension; however, when treated with 5α-dihydrotestosterone and the high-fat diet, the mean arterial pressure is increased. However, there are reports that female and male rats raised on a high-fat, refined-carbohydrate (HFS) diet developed hypertension and endothelial dysfunction (Barnard *et al.,* 1998; Reil *et al.,* 1999). The above studies suggest that high-fat diet intervention at early age could lead to the development of a series of metabolic abnormalities.

It has been reported that high-fat diets cause the down regulation of cytochrome P450 CYP4A and CYP2C23 in renal tubules. These proteins are responsible for the formation of renal eicosanoids; hydroxyeicosatetraenoic acids (HETEs) and epoxyeicosatrienoic acids (EETs). Evidence shows that the biological actions of these metabolites are associated with the regulation of renal function and blood pressure in many animal models of hypertension (McGiff *et al.,* 1999; Imig, 2000; Roman, 2002). It is suggested that the change in tubular 20-HETE synthesis is responsible for resetting renal function and the regulation of blood pressure in hypertensive animal models and in human hypertension (Laffer *et al.,* 2003). In the renal vasculature, 20-HETE causes vasoconstriction, whereas EETs cause vasodilatation of renal arterioles (Hardwick, 1991; Omata *et al.,* 1992; Ma *et al.,* 1993). Wang *et al.,* (2003) reported decreased expression levels of CYP4A and CYP2C23 in the renal microsomes of rats fed high-fat diets.

It has been shown that arachidonic acid metabolites play an important role in the inhibition of ion transport along the nephron (McGiff *et al.,* 1999; Roman, 2002). The

down regulation of the synthesis of these metabolites in the tubular sites due to a high-fat diet may produce an increase of sodium reabsorption and sodium retention because 20-HETE and EET are well known to inhibit sodium transport from the lumen of the proximal tubule, the thick ascending limb of the loop of Henle (TALH) and from the collecting duct into peritubular fluid (Schwartzman *et al.,* 1985; Wang *et al.,* 1995; Sakairi *et al.,* 1995). This down regulation of the synthesis of these metabolites causing the augmentation of ion reabsorption in the kidneys may result in the elevation of blood pressure in rats on a high-fat diet (Wang *et al.,* 2003). It is noteworthy that not all the rats on a high-fat diet for 10 weeks became hypertensive (Wang *et al.,* 2003), suggesting resistance to the down regulation of CYP4A and CYP2C23 in renal tubules by high-fat diet.

There are substantial evidence that suggests that the intake of saturated fats are implicated in the development of obesity and insulin resistance, whereas polyunsaturated fats (PUFAS) are not (Pan *et al.,* 1994, Storlien *et al.,* 2000). This is most likely because of the difficulty in mobilising saturated fats by lipolytic stimuli (Mougios *et al.,* 1995, Raclot *et al.,* 1997). On the other hand PUFAs are easily used for energy production after ingestion (Leyton *et al.,* 1987). Furthermore, saturated fats reduces metabolic rates and decreases beta-adrenoreceptor binding when integrated into cell membranes (Matsuo and Suzuki, 1997) whereas n-6 PUFAs presence in diets increases beta-adrenoreceptor affinity (Nicolas *et al.,* 1991). Some studies have shown inconsistent detrimental effects of high-fat diets on insulin sensitivity over a broad range of dietary fat content, including several randomised studies using the hyperinsulinaemic glucose clamp technique or

frequently sampled intravenous glucose tolerance test (fsIVGTT) to quantify insulin sensitivity (Borkman *et al.,* 1991; Garg *et al.,* 1992).

It has been shown that high-fat diets cause enhanced vasoconstriction to Ang II (Ghatta and Ramarao, 2004), phenylephrine (Ghatta *et al.,* 2005) and impaired acetylcholine mediated vasodilatation (Viswanad *et al.,* 2006). Arterial vascular abnormalities have also been reported in animal models of obesity (Dobrian et al, 2000) and in the prediabetic insulin resistance state (Viswanad *et al.,* 2006) due to defects in arterial contractility mediated by the endothelium. Obesity induced by long–term dietary fat is known to be associated with endothelial dysfunction. The Possible causes of endothelial dysfunction in obesity may in parts depend on increased levels of nonesterified fatty acids (NEFA), total cholesterol and triglycerides. There are reports that short-term feeding of fatty diets induces endothelium–dependent and independent arterial dysfunction (Naderali and Williams, 2001), suggesting independence of obesity. Human and animals fed with diets high in fat and cholesterol for over 16 weeks develop endothelial-dependent and independent vascular dysfunction (Dobrian et al, 2000). Vascular dysfunction has been reported in human and animal hypertension, insulin resistance, raised triglycerides and NEFA levels (Lewis *et al.,* 1999; Steinberg *et al.,* 1997).

2.3.2 Fructose

Diets high in fructose contribute to the metabolic disturbance in animal models resulting in weight gain, hyperlipidaemia (Kasim-Karakas *et al.,* 1996), and hypertension (Hwang *et al.,* 1987). Studies involving commonly consumed fruit juices showed that natural fructose can alter lipid and protein oxidation biomarkers in the blood, and mediate

oxidative stress responses *in vivo* (Breinholt *et al.,* 2003). The long-term negative effects can include changes in digestion, absorption, plasma hormone levels, appetite, and hepatic metabolism, leading to development of insulin resistance, diabetes, obesity, and inevitably cardiovascular disease (Basciano *et al.,* 2005). It is conceivable that hyperinsulinaemia or insulin resistance may underlie development of hypertension in the fructose-fed animal models.

Evidence has been gathered that cardiovascular interactions of altered sex hormone profiles and high levels of insulin may aggravate hypertension with a concomitant increased risk of cardiovascular mortality in both men and women (McFarlane *et al.,* 2001). Furthermore, studies using 24-hour ambulatory BP monitoring have shown higher BP in men than in women at similar ages (Wiinberg *et al.,* 1995). Sex hormones play a vital role in high fructose diet induced hyperinsulinaemia/insulin resistance and hypertension. Estrogen suppresses high fructose diet induced insulin resistance and high blood pressure in female mice. On the other hand, androgens are necessary for the development of hypertension in animals fed a high fructose diet (Dongzhe Song *et al.,* 2004). Several investigations have demonstrated that male rats have higher BP than females of the same age group. This has been shown in SHR (Chen *et al.,* 1991; Reckelhoff *et al.,* 1999) Dahl salt-sensitive rats, (Rowland *et al.,* 1992) deoxycorticosterone-salt hypertensive rats, (Ouchi *et al.,* 1987) and in New Zealand genetically hypertensive rats (Ashton *et al.,* 1991). In fructose fed hypertensive rats (FHR), male rats had significant hypertension and hyperinsulinaemia after 9 weeks of a high-fructose diet whereas female rats did not (Galipeau *et al.,* 2002). Dongzhe Song *et*

al., (2004) concluded that these findings strongly suggest that androgens may also play a key role in the development of fructose-induced hypertension.

Some other studies reported that hyperinsulinaemia is associated with fructose induced hypertension in rats in the same manner as in humans (Lucas *et al.,* 1985; Modan *et al.,* 1985). Some studies did not report changes in blood pressure of rats fed high fructose but rather insulin resistance (Kotchen *et al.,* 1997; Johnson *et al.,* 1993). Bezerra *et al.,* (2001) reported that there was no significant difference in blood pressure levels between the high-fructose and control groups, indicating that the ingestion of fructose alone was not sufficient to cause an increase in arterial blood pressure in this model. They envisaged that insulin resistance is a risk factor for the development of hypertension in this model rather than hyperinsulinaemia, because there were no changes in the serum insulin concentration. Furthermore, a high-fructose diet alone does not induce hyperinsulinaemia and hypertension when the sodium/potassium ratio is normal (Bezerra *et al.,* 2000). D'Angelo *et al.,* (2005) in their studies, while endorsing the previous reports documenting the metabolic abnormalities produced by fructose feeding, insinuated that a high-fructose diet does not elevate blood pressure in a common strain of normotensive rats. They did not rule out the possibility that insulin resistance does not cause or contribute to hypertension in all conditions, but rather suggested that the degree of the metabolic dysfunction in rats fed a high fructose diet is not sufficient to produce an effect on blood pressure.

There is emerging evidence that fructose induction of hypertension also depends on the integrity of the renin angiotensin system. There is evidence implicating Ang II

dependence for the increases in BP, (Navarro-Cid *et al.,* 1995) left ventricular weight, (Kobayashi *et al.,* 1993) and plasma insulin, (Navarro-Cid *et al.,* 1995) in rats fed diets high in simple sugar content. The work of Verma *et al.,* (1994) in metformin treated, fructose-fed rats provides additional support for the hypothesis that fructose-induced hypertension is insulin mediated. It is important to reiterate that high sugar diets also have been reported to raise BP without changing insulin, (Preuss *et al.,* 1992; Johnson *et al.,* 1993; Hulman *et al.,* 1994) to raise insulin without changing BP (Kobayashi *et al.,* 1993) and to have no effect on either variable, when arterial pressure was measured 24 hours daily throughout the study (Brands *et al.,* 1994) or after 23 weeks of feeding (Van der Schaaf *et al.,* 1995). Further evidence for differences in fructose feeding versus insulin-induced hypertension is that fructose feeding was reported to raise plasma Ang II levels in a study in which insulin levels increased but BP did not. (Kobayashi *et al.,* 1993). For instance, the AT1 receptor antagonist losartan and an angiotensin-converting enzyme inhibitor attenuated the magnitude of the blood pressure elevation and improved insulin sensitivity in fructose-fed rats (Navarro-Cid *et al.,* 1995; Kobayashi *et al.,* 1993).

The role of sodium/potassium balance or lard is very crucial for understanding fructose induced hypertension. For instance, a previous study reported an increase in blood pressure only in the changed sodium/potassium ratio diet groups (Bezerra *et al.,* 2000). A high fructose diet suppressed potassium channel function (Erdös *et al.,* 2004). Matsui *et al.,* (1997) observed significant higher systolic blood pressure in the high fructose lard

group compared to the high fructose and control groups. There are suggestions that other factors such as strain, sex, age at the start of the diet, and additions to the fructose diet regimen such as salt, fat, or trace elements may render animals more susceptible to developing fructose-induced hypertension (D'Angelo *et al.,* 2005).

It is well documented that fructose ingestion in humans result in observable increased rates of de novo lipogenesis (Schwarz *et al.,* 1995) whereas eucaloric glucose ingestion does not increase de novo lipogenesis (Hellerstein *et al.,* 1996). Fructose being more lipogenic than glucose, the intake can worsen health conditions with preexisting hyperlipidaemia, insulin resistance or type II diabetes. It is noteworthy that Hellerstein *et al.,* (1996) observed a little de novo lipogenesis from glucose under eucaloric conditions in humans. However, 3-5 fold increases in fractional de novo lipogenesis from fructose above fasting conditions have been documented (Schwarz *et al.,* 1995) in both lean and obese individuals. In addition, nearly 30% of circulating triacylglycerol palmitate originated from de novo lipogenesis due to fructose ingestion (Schwarz *et al.,* 1995). It is however documented that substituting fructose or xylitol for sucrose did not influence plasma cholesterol or triacylglycerol concentrations in a study carried out on 127 healthy individual for 2 years (Huttunen *et al.,* 1976). Importantly, fructose does not provoke the production of insulin and leptin, the two hormones involved in the long term regulation of energy homeostasis. Thus, the decrease in insulin response to meals and leptin production associated with chronic consumption of diets high in fructose may have deleterious long term effects on the regulation of energy intake and body adiposity (Elliot *et al.,* 2002). Apart from the known effects of dietary fat, dietary fructose has been shown to produce

weight gain and induce insulin resistance, hyperlipidaemia and hypertension in experimental animals. It is conceivable that increased consumption of fructose could contribute to weight gain and its associated metabolic disturbances in human (Elliot *et al.,* 2002).

2.3.3 Cholesterol

It has been hypothesized that abnormally high cholesterol levels (hypercholesterolemia), or higher concentrations of LDL-C and lower concentrations of functional HDL-C are strongly associated with cardiovascular disease because these promote atheroma development in arteries (atherosclerosis) (http://en.wikipedia.org/wiki/cholesterol 9/10/08). This disease process culminates in myocardial infarction (heart attack), stroke and peripheral vascular disease. An increase in dietary fat, dietary cholesterol and blood cholesterol levels have been reported to be linked with an increased risk of cancers of the colon, pancreas and prostate (Byers *et al.*, 2002).

Cholesterol is required to build and maintain cell membranes. Some research indicates that cholesterol may act as an antioxidant (Smith, 1991). It is not known if exogenous cholesterol affects these conditions separately or via a common link. However, studies suggest that high and low levels of HDL-C and LDL-C respectively have cardioprotective effects (Smith, 1991). These conditions are associated with low plasma insulin levels (Smith, 1991) which may enhance glucose uptake and improve plasma lipids and lipoproteins profile. Evidently, enhanced insulin sensitivity is associated with higher HDL cholesterol and lower triglyceride concentrations.

The rat is an atherosclerosis-resistant species. Unlike humans and similar to mice, rats do not have plasma cholesteryl ester transfer protein (CETP), and high density lipoprotein (HDL) is the major carrier of plasma cholesterol (Moghadasian, 2002). Rats are generally hypo-responsive to dietary cholesterol; thus, hyperlipidemia and atherogenesis may only be induced in rats by high cholesterol/high-fat diets containing cholic acid and thiouracil (Joris *et al.,* 1983). The mechanism of action of cholic acid is two fold: an increase in cholesterol absorption and a concomitant suppression of cholesterol -7- α-hydroxlyase activity that results in decreased cholesterol excretion (Moghadasian, 2002). Thiouracil induces clinical hypothyroidism with a consequent decreased low density lipoprotein (LDL)-receptor activity and hypercholesterolemia. Several strains of rats with heritable hyperlipidemia, some of these associated with atherogenesis, have been described (Russell *et al.,* 1993).

2.3.4 Protein

There are recent reports that the source of protein contributes to the insulin resistance state in rats fed high-fat diets. It has been shown that high-fat diets prepared with protein derived from cod, as compared to soy protein or casein, do not lead to insulin resistance (Lavigne *et al.,* 1999; Storlien *et al.,* 2000). Further mechanistic investigations showed that the cod protein improves GLUT4 translocation to skeletal muscle T-tubules, but not to the plasma membrane (Tremblay *et al.,* 2001). The T-tubule GLUT4 protein correlates with insulin stimulated glucose transport, and is most interesting in terms of the possibility that a specific protein might be critical in skeletal muscle insulin-stimulated

glucose transport. The effect might be due to a specific protein which has a gene-specific effect at the intestinal level, a protein which escapes full digestion, a molecule which is co-extracted with protein, or indeed a particular amino acid pattern unique to cod. In this regard, the observation that L-glutamine supplementation of a high-fat diet has beneficial effects on glycaemia and insulinaemia in mice may be relevant (Opara *et al.,* 1996).

2.3.5 Salt sensitive hypertension and insulin resistance

The arterial pressure of some human hypertensive patients is very sensitive to changes in sodium intake, and they have been classified as "salt-sensitive", but the cause of the salt-sensitivity is not known. It is generally accepted that high salt intake is one of the major causes of human hypertension and cardiovascular damage. Animal studies have reported ROS overproduction in salt-sensitive hypertension (Ono *et al.,* 1997; Huang and Leenen 1998; Huang *et al.,* 2001; Miyajima and Bunag, 1987). Salt loading increases production of reactive oxygen species (ROS) in a salt-sensitive hypertension animal model (Fujita *et al.,* 2005). ROS overproduction has several harmful effects, such as insulin resistance, peripheral and central sympathetic over-activity, an enhanced oxidized low-density lipoprotein (LDL) receptor-1and lectin-like oxidized LDL receptor (LOX-1) expression. (Ando and Fujita, 2004). Another study connects salt intake with oxidative stress and nephrosclerosis in Dahl-sensitive hypertensive rats (Trolliet *et al.,* 2001). There are convincing evidence that an intimate relationship exists between salt sensitive hypertension and insulin resistance in obese hypertensive patients (Suzuki *et al.,* 2000; Sharma *et al.,* 1993; Galleti *et al.,* 1997).

Augmentations of sympathetic nerve activity (SNA) in the loading of Dahl-salt sensitive rats but not Dahl salt resistance has been reported (Huang and Leenen, 1998). It has also been reported that salt loading induced augumentation of NADPH oxidase activity in the cardiac tissue of Dahl-salt sensitive rats (Matsui *et al.,* 2006). Recently it was reported that, in DSS rats, salt-induced hypertension may result from central sympathetic activation because of NADPH oxidase–induced ROS production in the brain (Fujita *et al.,* 2007). Several factors in the brain have been demonstrated to play important roles in the sympathetic activation in salt-induced arterial pressure elevation (Fujita *et al.,* 2007). The central renin-angiotensin system might stimulate SNA and mediate salt-induced hypertension (Huang and Leenen, 1998; Kim-Mitsuyama *et al.,* 2005; Ito *et al.,* 2003). It has been shown that while insulin resistance is observed in the muscle and adipose tissues, it is not seen in the kidney or the sympathetic nervous system. Insulin can increase sodium reabsorption in the proximal tubules and stimulate sympathetic tone. Hyperinsulinaemia increases blood pressure by inducing salt retention and central sympathetic overactivity (Fujita, 2007).

One important contributor to hypertension in salt-sensitive animal models and humans seems to be endothelial dysfunction, in particular the altered vascular reactivity due to impairment in nitric oxide (NO) production (Luscher *et al.,* 1987; Nishida *et al.,* 1998). Recent studies have indicated that nitric oxide (NO) production in salt-sensitive essential hypertensives is decreased. However, little is known about the importance of NO in salt-sensitive hypertension and specifically the relative importance of the various isoforms of nitric oxide synthase (NOS) in the kidney in causing salt-sensitivity.

There are several evidence that salt-dependent hypertension is linked with increase production of vascular endothelin (ET-1) in most animal studies (Schiffrin, 1999). Furthermore, preclinical data suggested that the ET-1 system is primarily activated in severe BP elevation of deoxycorticosterone acetate-salt, Dahl salt sensitive and stroke prone spontaneously hypertensive rats (Neeraj *et al.,* 2008). In addition, pharmacological or genetic inhibition of Endothelin-B Receptor (ETBR) activity results in a severe form of hypertension that depends on salt intake (Gariepy *et al.,* 2000; Pollock and Pollock, 2001).

2.4 Scope of the study

In my study, I will set up a model of hypertension by high-fat feeding and determine whether these animals fed the high-fat diet will become insulin resistant after six weeks. An experimental system will also be set up to evaluate vascular responses in a model of diet induced hypertension. This model could in future be used to study vascular reactivity in hypertensive animals in the absence or presence of insulin resistance and in normotensive insulin resistant animals.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Chemicals and Drugs

All the chemicals used in the study were of standard grade. Fructose, potassium chloride, sodium bicarbonate, calcium chloride, di-hydrogen potassium phosphate, magnesium sulphate, and glucose were obtained from Kimix Laboratories, South Africa. Casein, cholesterol, phenylephrine and acetylcholine were obtained from Sigma-Aldrich, Germany. Captopril (Novartis, Sandoz (PTY) LTD) and sodium pentobarbitone (Kyron Laboratories (PTY) LTD) were obtained from a local pharmacy. Strawberry jelly (Pioneer foods (PTY) LTD), Gelatin (Davis gelatin Industries (PTY) LTD), and Cooking fat (HUDSON & KNIGHT) were obtained in a local supermarket. Carbogen (95% Oxygen, 5% Carbon dioxide) were obtained from Afrox (Pty Ltd).

3.1.2 Instruments and Equipment

Multi-Channel, Computerized, Non-Invasive Blood Pressure System for rat version 6.v25 (Kent scientific corporation)

Cardiochek™ (Polymer Technology system, Inc.Indianapolis, USA)

Lipid panels (Polymer Technology system)

Glucose meter (Ascensia)

Glucose strip (Bayer health care)

Tissue baths

Heater with circulator pump (Haake)

Isometric force transducer (Harvard apparatus)

Powerlab 4/25T (AD instruments)

Chart 6.0 software (AD instruments)

3.2 ANIMAL PREPARATION

Male Sprague Dawley rats were obtained from the University of Kwazulu Natal South Africa, and were housed in the Medical Bioscience Department, University of the Western Cape, in a temperature controlled room (23±1˚C) with a 12-hour light and dark cycle. Light hours were from 07h00 to 19h00 and hours of darkness from 19h00 to 07h00. Animals received food and water ad libitum during a 2 week acclimatization period. In addition, rats were adapted to the procedure of blood pressure measurement during the 2 weeks of acclimatization.

3.3 EXPERIMENTAL PROTOCOL

3.3.1 High-fat Diet Model

After a 2 week acclimatization period, forty male Sprague Dawley rats of approximately equal weights (163–169g) were randomly placed into 4 groups for 6 weeks. The control group was fed normal laboratory pellets. The high-fat diet (HFD) group was given a high-fat diet prepared in-house. The captopril (HFDC) group was given captopril (12.5mg/day) through a vehicle (gelatine) and the high-fat diet. A fourth group which served as the captopril control received the high-fat diet and the vehicle (HFDV). The composition of the diet is shown in table 3.1. Body weight and blood pressure were measured weekly for 6 weeks. An intraperitoneal glucose tolerance test (IPGTT) was performed at the end of

week 3 and week 6. After 6 weeks on the high fat diet, the animals were fasted overnight. The next day, the animals were anesthetized, the lipid profile was determined, plasma was collected for insulin determination and the heart and visceral fat were excised and weighed. Four animals from the control and the HFD groups were anesthetized with sodium pentobarbitone (60 mg/kg) and the thoracic aorta was dissected for the vascular reactivity experiment.

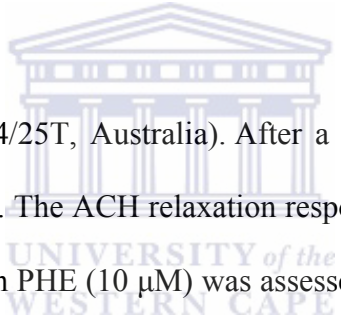
Table 3.1. **The compositions of the diet prepared in-house are**

Content	**Quantity (g)**
Cooking fat	400
Fructose	100
Casein	100
Cholesterol	10
Rat pellets (Chow)	390

3.3.2 Vascular response protocols

The thoracic aorta was cut into rings 3 mm in length. The aortic ring was mounted between a pair of stainless steel hooks in a water-jacketed organ bath containing 10 ml of Krebs–Henseleit solution (KHS) saturated with 95% O_2 and 5% CO_2 and maintained at 37±1°C with a heater pump (Haake) and reservoir system (figure 3.1). The muscle was stretched to a resting tension of 1g and allowed to equilibrate for 30 minutes. The KHS was changed every 15 minutes.

After a 30 minutes equilibration period, the resting tension was reset to 1 g, if necesary. Aortic rings were initially exposed to 80 mM KCl to obtain the maximal KCl-induced response using an isometric force transducer (Harvard apparatus) connected to a data



acquisition system (Powerlab 4/25T, Australia). After a washout, the aortic rings were precontracted with 10 μM PHE. The ACH relaxation response (3 μM–1000 μM) in aortic rings previously contracted with PHE (10 μM) was assessed. After a washout, increasing concentrations of PHE (1 μM –10 μM) was applied, and dose responses to this contractile agent were obtained. In other aortic rings, the endothelium was mechanically removed by rubbing the aorta over a thin wire. The absence of endothelium was confirmed by the inability of 200 μM acetylcholine to produce relaxation in the PHE precontracted tissue. Concentration dependent relaxation responses to sodium nitroprusside (3 μM -1000 μM) were obtained for the denuded aortic rings. After a washout period, increasing concentrations of PHE (1 μM –10 μM) were applied to the denuded aortic rings and the dose responses to this contractile agent were obtained. All aortic rings were exposed to 80 mM KCl at the end of the experiment to confirm that the rings were able to maintain the initial KCl response.

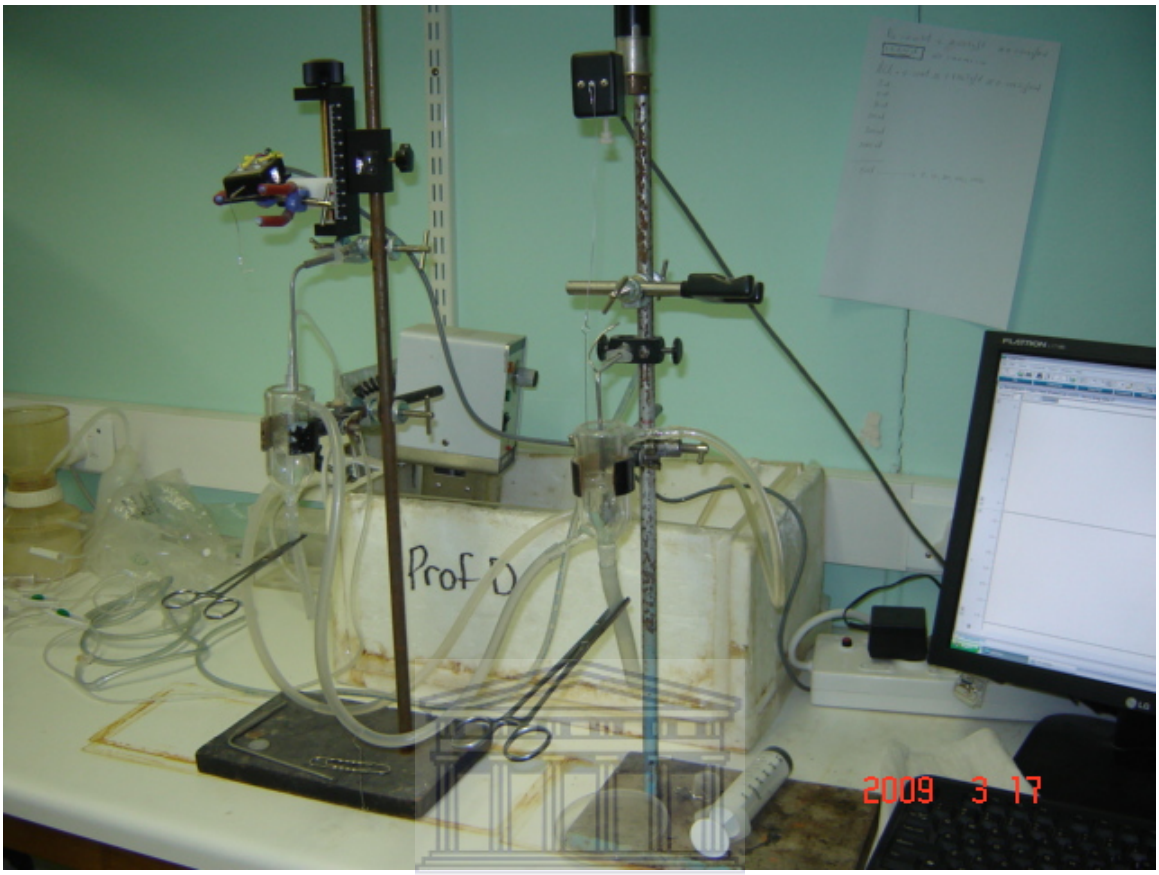


Figure 3.1: Experimental set up used to measure vascular responses

3.4 PARAMETERS MEASURED

3.4.1 Blood pressure determination

The blood pressure was measured by the tail cuff method using a two channel computerized non-invasive system from Kent Scientific Corporation, USA. This system (figure 3.2) uses a volume pressure method to determine blood pressure. Animals were allowed to walk into the rodent holder, which was placed on a heating pad while maintaining the ambient temperature at 30˚C. Animals were allowed to settle for 15 minutes. The nose cone was adjusted to limit the animal's movement. The occlusion cuff was placed proximally on the tail of the animal and allowed to fit loosely for free

movement of the tail. A VPR cuff was placed distally behind the occlusion cuff. After 15 minutes, the averages of 5 pressure readings were recorded for each measurement.

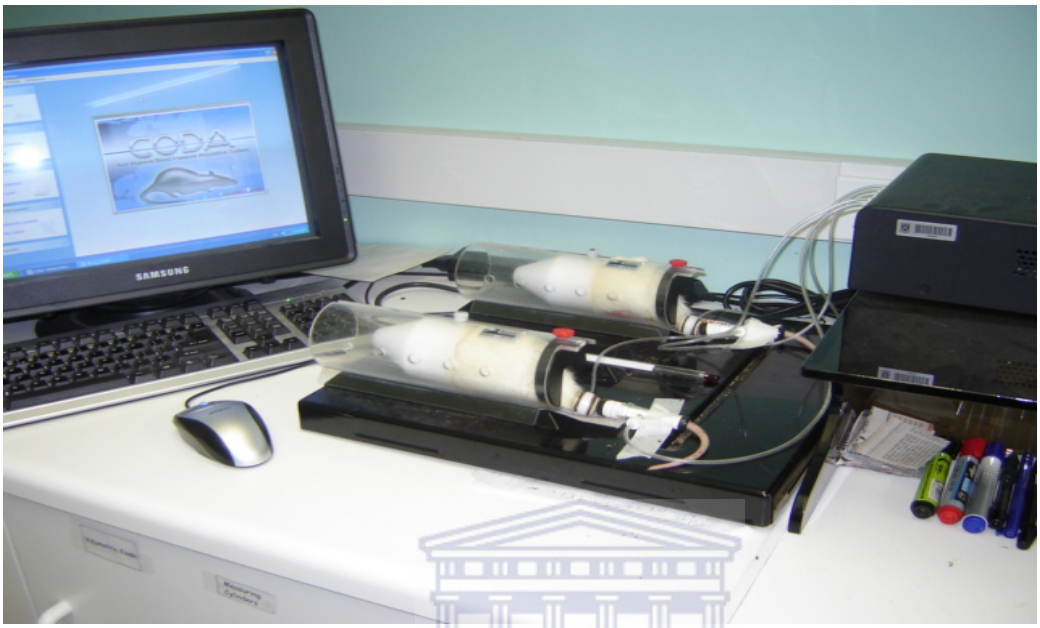


Figure 3.2: Non-Invasive Blood Pressure system showing the rats in rodent holders on a heating stage to increase blood flow to the tail.

3.4.2 Intraperitoneal glucose tolerance test (IPGTT)

At the end of weeks 3 and 6 of treatment, intraperitoneal glucose tolerance tests were performed on all the rats using a hand-held Ascentia ELITE blood glucose meter.

After an overnight fast, animals were anaesthetized with sodium pentobarbital (50 mg/kg i.p) and the fasting blood glucose level was determined (at time zero) from a small drop of blood collected by snipping the tail. Rats were then injected with 2 g/kg glucose. The blood glucose levels were monitored at 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes post injection.

3.4.3 Lipid profile

The following lipid parameters were measured after week 6: total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) in the whole blood. The lipid profile was assessed using a portable Cardiochek^{TM} blood test system. The animals fasted overnight and were anaesthetized with sodium pentobarbital (50 mg/kg i.p). The tails were snipped and 40 µl of blood was collected in a capillary tube and deposited immediate on the lipid panel sensor that was fitted in the Cardiochek^{TM}.

3.4.4 Determination of insulin

Insulin was measured in the plasma using a commercially available (DRG Diagnostics, Germany) immunoenzymatic assay for the *in vitro* quantitative measurement of rat insulin (INS) in serum and plasma. At the end of week 6, the animals fasted overnight and were anaesthetized with sodium pentobarbital (50 mg/kg i.p). The trunk blood was collected immediately and put into eppendoff tubes and kept on ice, before being centrifuged for 10 minutes, (5000 rpm). Plasma was stored at -20˚C for the insulin determination using a Rat Insulin Elisa, according to the manufacturer's instructions.

The required volume of the Enzyme Conjugate was prepared by mixing 50 µl Enzyme Conjugate (11X dilution) with 500 µl Enzyme Conjugate buffer (1:10) for each strip.

The Wash Buffer (21X dilution) was diluted in redistilled water (1:20) and mixed properly. The standards, unknowns and the Enzyme Conjugate were added to anti-insulin wells and incubated on a shaker for 2 hours at room temperature. The reaction volume was aspirated and 350 µl wash buffer was added to each well and aspirated 5 times. The

plate was inverted and tapped firmly against absorbent paper. The substrate, 200 µl TMB, was added to the standards and the unknown and incubated. After 15 minutes incubation, 50 µl of the STOP solution was added to each of the standards and the unknowns. The plate was then placed on the shaker for 5 seconds to ensure mixing of substrate and STOP solution. The absorbance was read at 450 nm. Insulin (pmol/l) = (OD + 0.049)/0.474). Insulin sensitivity was estimated by a quantitative insulin sensitivity check index (QUICKI) using the fasting plasma insulin and fasting glucose as 1/ [log (fasting insulin (mU/l)) + log (fasting glucose (mmol))] (Wallace *et al.,* 2004).

3.5 DATA ANALYSIS

All data are reported as means and SEM or median and interquatile range (IQR). Statistical differences among groups were analyzed by analyses of variance (ANOVA) or the Kruskal-Wallis test followed by Newman-Keul multiple comparison test or Dunn's multiple comparison test respectively. $P< 0.05$ was considered significantly different. The Mann Whitney test was used to assess the differences in vascular responses between the control and HFD group. Data analysis was done using GraphPad Prism version 3.1 for Windows (GraphPad Software, San Diego, CA, USA).

3.6 ETHICAL CONSIDERATIONS

All experimental procedures were conducted after ethical clearance was obtained from the University of the Western Cape Senate Research Committee.

CHAPTER FOUR

RESULTS

4.1 Effect of high-fat feeding on body mass, heart: body weight ratio and visceral fat weights.

The initial mean body weights of the five groups were approximately the same before the dietary intervention as shown in table 4.1. The increase in BW amongst the groups did not differ over the 6 weeks period (P = 0.09) (figure 4.1; table 4.1). The HFD and HFDV groups however, have more visceral fat compared to the control group (P < 0.05 and 0.01 respectively). There were no differences in the heart weight:body weight (HW:BW) ratio (P > 0.05).

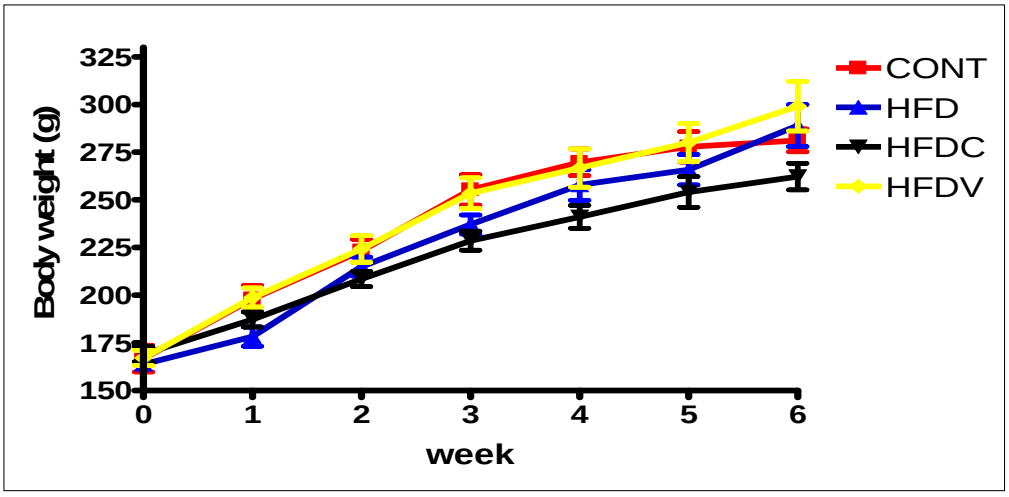


Fig.4.1: Effect of high fat diet on body weight.
Values are expressed as the mean ± SE. N = 10 rats

Table 4.1 Effect of high-fat feeding on body weight, visceral fat and heart weight:body weight ratio.

	CONTROL	HFD	HFDC	HFDV
Initial BW (g)	166.9±7.2	163.9±3.9	169.2±4.1	167.2±4.8
N	10	10	10	10
P (Comparison to Control)		> 0.05	> 0.05	> 0.05
Final BW (g)	281.2±6.9	289±11.4	262.2±7.2	299.1±13.1
N	10	10	9	10
P (Comparison to Control)		> 0.05	> 0.05	> 0.05
Visceral fat (g)	0.8±0.1	3.1±0.6*	2.2±0.4	3.8±0.6*
N	6	7	5	7
P (Comparison to Control)		**< 0.05**	> 0.05	**< 0.01**
(HW:BW) *100	0.32±0.03	0.34±0.02	0.33±0.02	0.34±0.01
N	6	7	5	7
P (Comparison to Control)		> 0.05	> 0.05	> 0.05

Values are expressed as the mean ± SE.
*P < 0.05 compared with control. N = number of rats

4.2 Effect of high-fat feeding on SBP, DBP and HR.

The initial baseline of SBP and DBP of the four groups were within the normal range. Figure 4.2 shows that the high-fat diet increased the SBP of the HFD and HFDV groups significantly compared to the control group (P < 0.05 and < 0.01 respectively). The DBP of the HFD and HFDV groups were significantly increased compared to the control group (P < 0.05 and < 0.05 respectively, figure 4.3). Analysis of variance shows that the SBP and DBP of HFD and HFDV groups were significantly increased compared to the control groups from week 3 to week 6. The SBP and DBP of the HFDC group did not differ from that of the control group. However, the SBP and DBP of the HFDC group were

significantly lower compared to the HFD group from week 2 onwards. This shows that captopril completely prevented the increase in SBP and DBP induced by high-fat feeding. The heart rate (table 4.2) did not differ significantly amongst the groups (P = 0.25).

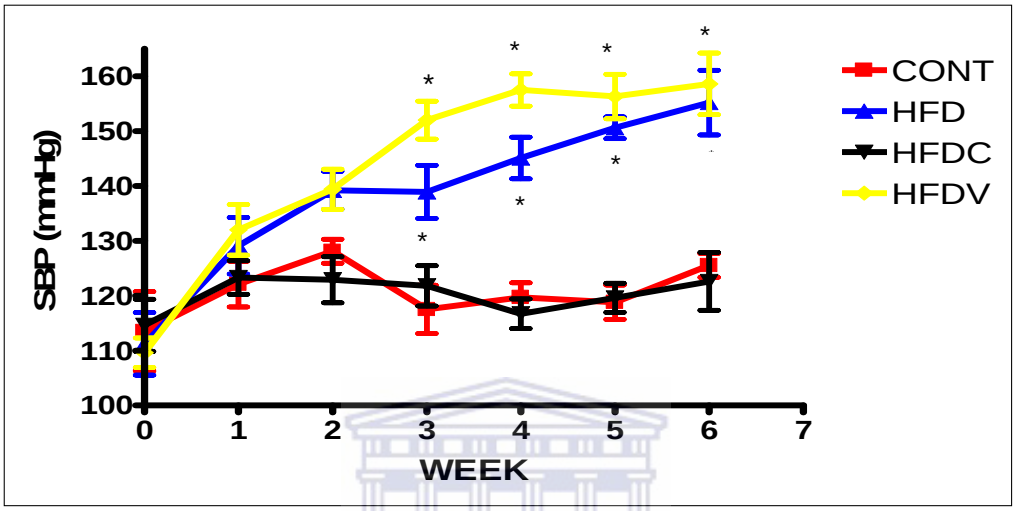


Fig.4.2: Effect of high fat feeding on SBP.
Values are expressed as the mean ± SE.
*P < 0.05 compared with control; N = 5-10 rats

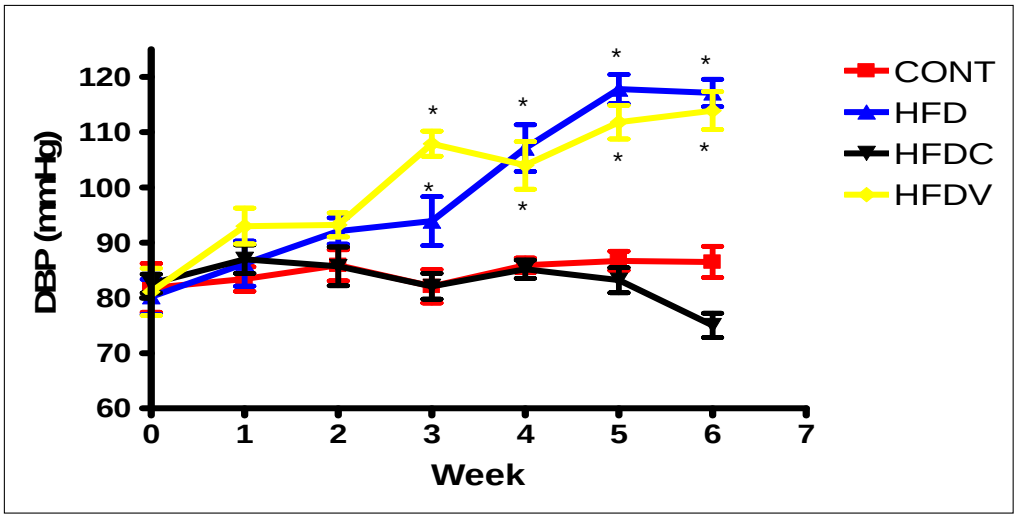


Fig.4.3: Effect of high fat feeding on DBP.
Values are expressed as the mean ± SE.
*P < 0.05 compared with control; N = 5-10 rats

Table 4.2: Effect of high- fat feeding on SBP, DBP and HR

variables	CONTROL	HFD	HFDC	HFDV
Initial SBP (mmHg)	113.5±7.2	111.2±5.7	114.6±4.7	109.6±2.7
N	10	10	10	10
P (comparison to control)		> 0.05	> 0.05	> 0.05
Final SBP (mmHg)	125.5±2.1	155.2±5.9*	122.6±5.3	158.6±5.6*
N	6	7	5	7
P (comparison to control)		**< 0.05**	> 0.05	**< 0.01**
Initial DBP (mmHg)	81.8±4.4	80.2±3.2	82.6±1.7	81.1±4.3
N	10	10	10	10
P (comparison to control)		> 0.05	> 0.05	> 0.05
Final DBP (mmHg)	86.5±2.8	117.1±2.5*	75±2.2	113.9±3.4*
N	6	7	5	7
P =(comparison to control)		**< 0.05**	> 0.05	**< 0.05**
Initial HR (bpm)	490±2.1	503±12.6	478±8.6	497±1.3
N	10	10	10	10
P (comparison to control)		> 0.05	> 0.05	> 0.05
Final HR (bpm)	512.5±14.8	492.2±10.5	494±22.4	554.7±18.5
N	6	7	5	7
P (comparison to control)		> 0.05	> 0.05	> 0.05

Values are expressed as the mean ± SE. *P < 0.05 compared with control; N = number of rats

4.3 Effect of high-fat feeding on IPGTT.

Intraperitoneal glucose tolerance tests (IPGTT) performed at week 3 is shown in figure 4.4. The glucose tolerance at week 3 did not show a significant difference in the high-fat fed groups compared with the control group (P = 0.2). The fasting blood glucose of the HFDC group (4.3±0.1 mmol/l) was statistically higher than the control (3.5±0.2 mmol/l) at time zero (P < 0.05). The HFD group had seemingly high glucose levels at 30, 45 and 60 minutes post glucose administration. Analysis of variance however indicates that the values are not significantly different between the groups (P = 0.3, 0.1, and 0.3 at 30, 45 and 60 minutes respectively). The area under the curves (AUC) of IPGTT's at week 3 (table 4.3) do not differ amongst groups (P = 0.47). IPGTT's performed after week 6, also did not differ amongst the groups (figure 4.5) (P-value = 0.4). Despite the higher glucose levels at some points, the dynamics were similar across the groups in both week 3 and week 6. Comparison of the AUC between week 3 and week 6 for the corresponding groups did not differ significantly (table 4.3).

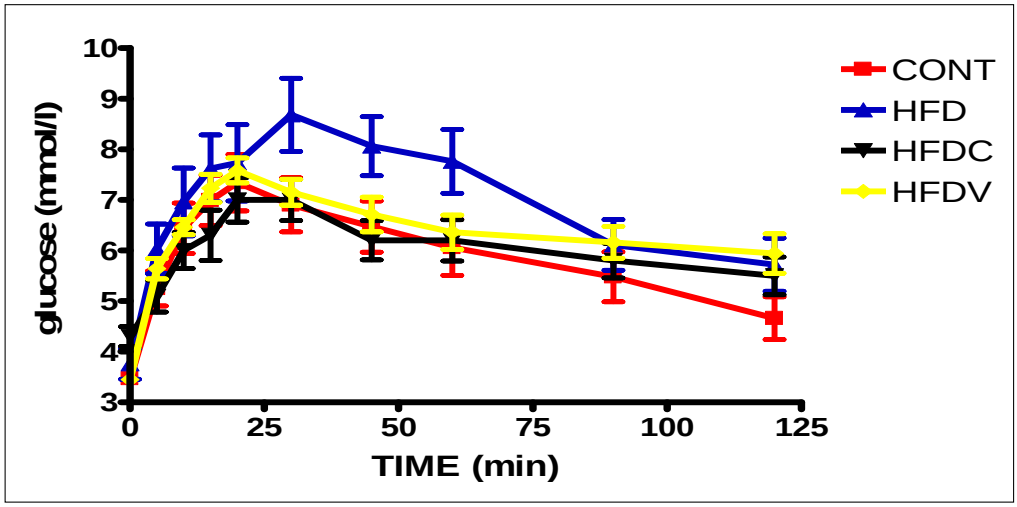


Fig. 4.4: Effect of high fat feeding on IPGTT at week 3.
Values are expressed as the mean ± SE. N = 10 rats

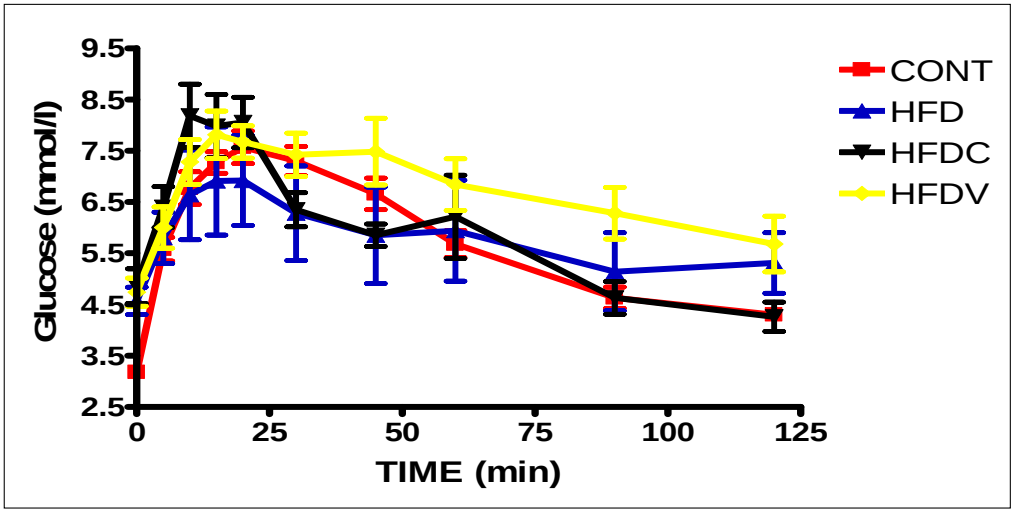


Fig. 4.5: Effect of high fat feeding on IPGTT at week 6.
Values are expressed as the mean ± SE. N = 5-10 rats

Table 4.3 Area under the curve of the IPGTT and comparison between week 3 and week 6

	CONTROL	HFD	HFDC	HFDV
3 weeks	296.1±59	350.9±68	232.5±37	341.0±30
N	10	10	10	10
P (Comparison vs Control)		> 0.05	> 0.05	> 0.05
6 weeks	274.8±36	166.6±79	160.6±27	249.3±66
N	7	7	5	7
P (Comparison vs Control)		> 0.05	> 0.05	> 0.05
P (3 week vs 6 week)	0.7	0.1	0.1	0.1

Values are expressed as the mean ± SE. N = number of rats

4.4 Effect of high-fat feeding on biochemical parameters.

The effect of the high-fat diet on the fasting lipid profile, blood glucose and plasma insulin are shown in (table 4.4). The whole blood cholesterol, blood triglyceride, HDL-C

and LDL-C levels did not differ amongst the groups (P = 0.54, 0.12, 0.1 and 0.28 respectively). The fasting plasma insulin was not significantly different amongst groups (control 128.8±28 vs HFD 146.1±12, HFDC 82.6±29, HFDV 177±37pmol/l., P > 0.05). Despite the higher blood glucose levels in the high-fat fed animals (P<0.002) the QUICKI index did not show significant differences amongst groups (P > 0.05).

Table 4.4: Effect of high- fat feeding on biochemical parameters.

	CONTROL	HFD	HFDC	HFDV
Cholesterol (mmol/l)	2.59±0.0	2.59±0.0	2.68±0.09	2.60±0.01
N	6	6	5	6
P (Comparison to Control)		> 0.05	> 0.05	> 0.05
Triglyceride (mmol/l)	0.67±0.07	0.59±0.01	0.74±0.06	0.57±0
N	6	6	5	6
P (Comparison vs Control)		> 0.05	> 0.05	> 0.05
HDL-C(mmol/l)	0.71±0.11	0.57±0.04	0.77±0.08	0.65±0.09
N	6	6	5	6
P (Comparison to Control)		> 0.05	> 0.05	> 0.05
LDL-C (mmol/l)	1.75±0.11	1.90±0.04	1.76±0.04	1.82±0.09
N	6	6	5	6
P (Comparison to Control)		> 0.05	> 0.05	> 0.05
3wk Fasting Glucose (mmol/l)	3.5±0.1	3.8±0.3	4.3±0.5	3.5±0.5
N	10	10	10	10
P (Comparison to Control)		> 0.05	**< 0.05**	> 0.05
6wk Fasting Glucose (mmol/l)	3.18±0.1	4.57±0.3*	4.86±0.3*	4.74±0.3*
N	6	6	5	6
P (Comparison to Control)		**< 0.001**	**< 0.001**	**< 0.001**
Insulin (pmol/l)	128.6±28	146.1±12	82.6±29	177±37
N	6	6	5	6
P (Comparison to Control)		> 0.05	> 0.05	> 0.05
QUICKI (insulinogenic index)	0.58±0.03	0.51±0.01	0.59±0.03	0.49±0.02
N	6	6	5	6
P (Comparison to Control)		> 0.05	> 0.05	> 0.05

Values are expressed as the mean ± SE. *P < 0.05 compared with control; N = number of rats

4.5 Vascular reactivity

4.5.1 KCl induced contraction

KCl induced aortic ring contraction is shown in table 4.5. The contraction mediated in aortic rings with intact or denuded endothelium did not differ significantly between the control and HFD groups (P = 0.4 and 0.8) respectively. The contraction mediated by KCl in aortic ring with intact and denuded endothelium from the control or HFD groups also did not differ significantly (control: intact vs denuded, P = 0.2; HFD: intact vs denuded, P = 1).

Table 4.5: Contraction induced by 80 mM KCl in intact and denuded endothelium aortic rings.

	CONTROL	HFD
Tension developed in intact aorta ring (g)	1.1±0.3	0.7±0.3
N	3	4
P (control vs HFD)		> 0.05
Tension developed in denuded aorta ring (g)	0.49±0.1	0.47±0.1
N	3	4
P (control vs HFD)		> 0.05

Values are expressed as the mean ± SE. N = number of rats

4.5.2 Phenylephrine induced contraction

The cumulative dose-response curve to phenylephrine (1 μM-10 μM) in KCl precontracted aortic rings with intact endothelium and denuded endothelium are shown in figures 4.6 and 4.7. Contraction increased with increasing doses of PHE. Maximal contraction ($E_{max)}$ induced in aorta intact with endothelium was not significantly higher compared with the corresponding denuded aorta (control Intact endothelium 94±19%

versus denuded endothelium 100±25%; P = 0.8); (HFD Intact endothelium 99±14% versus denuded endothelium 80±13%; P = 0.1).

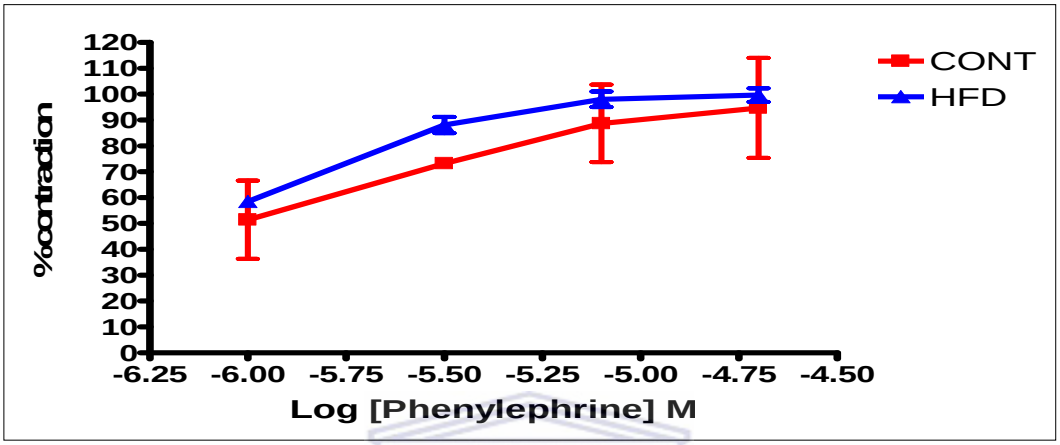


Fig. 4.6: Cumulative dose-response curve to phenylephrine in aortic rings with an intact endothelium.
Responses are expressed as a percentage of the response to 80 mM KCl.
Values are expressed as the median ± IQR for 4 rats.

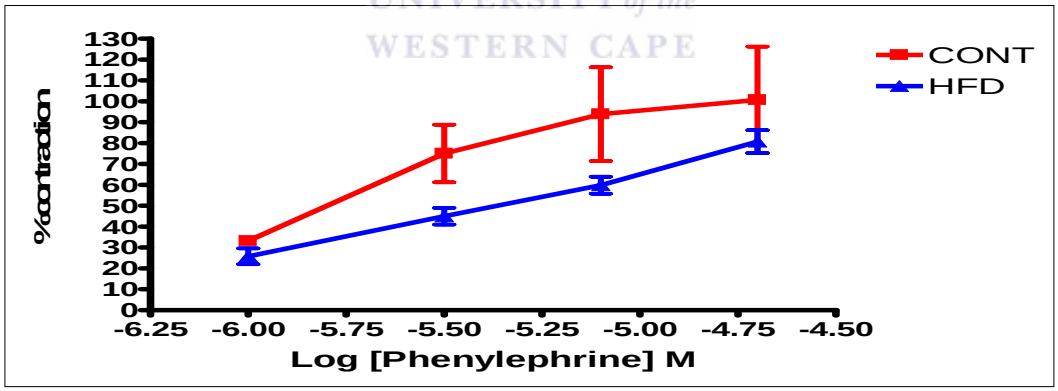


Fig. 4.7: Cumulative dose-response curve to phenylephrine in aortic rings in which the endothelium were denuded.
Concentration responses are expressed as a percentage of the response to 80 mM KCl.
Values are expressed as the median ± IQR for 4 rats

4.5.3 Acetylcholine and sodium nitroprusside-induced relaxation

A cumulative dose-response curve to acetylcholine (3 μM-1000 μM) is shown in figure 4.8. The endothelium dependent relaxation response of aortic rings precontracted with PHE did not differ between the control and the HFD groups. The maximum relaxation

response for the control and HFD groups were 78±15% and 109±13% (P=0.6) respectively. The EC_{50} values for the control and HFD groups were 2.9±2.7 x 10^{-4} M and HFD 5.8 ±7.9 x 10^{-4} M respectively. Considering the fact that the graphs seem to diverge after the dose 143 µM of acetylcholine and the high spread of the data particularly in this region we treat the data with caution and consider it as preliminary data. The cumulative dose-response curves to sodium nitroprusside (3 µM-1000 µM) are shown in figure 4.9. Maximum endothelium-independent relaxation response of aortic rings did not differ significantly between the control and HFD groups (Control 159±37% vs HFD 126±14%, P = 0.9). Interestingly SNP caused almost complete relaxation at the lowest dose in some of the rings in the HFD group. The responses of the rings isolated from the control animals follow a dose-dependent response.

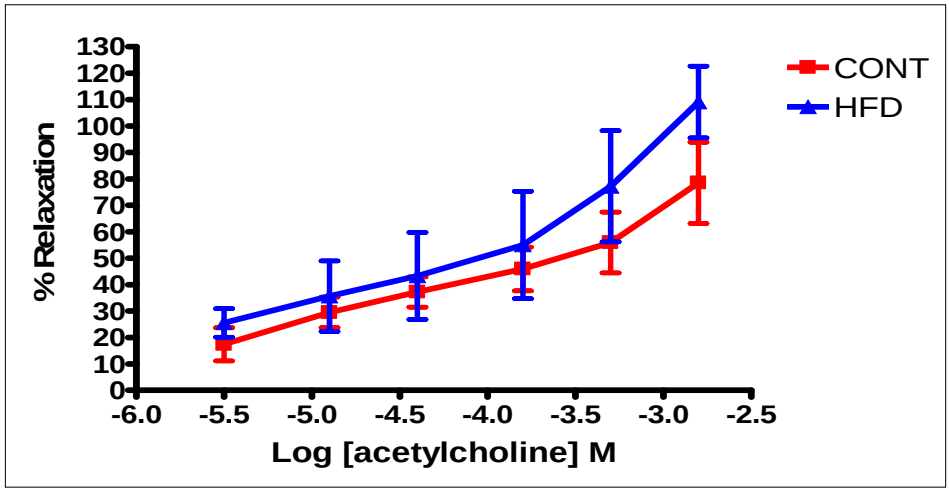


Fig. 4.8: Cumulative dose-response curve to acetylcholine induced relaxation
Relaxation responses are expressed as a percentage of precontraction induced by PHE (10µM). Values are expressed as the median ± IQR for 4 rats.

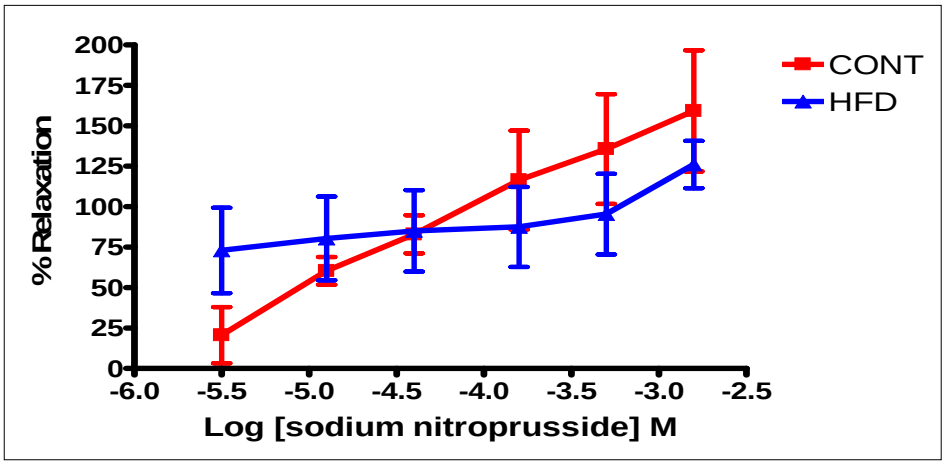


Fig. 4.9: Cumulative dose-response curve to sodium nitroprusside induced relaxation
Relaxation responses are expressed as a percentage of precontraction induced by PHE (10μM). Values are expressed as the median ± IQR for 4 rats.

CHAPTER FIVE

DISCUSSION

Hypertension and insulin resistance are important independent risk factors for cardiovascular disease. However, 50% of all hypertensive patients are also insulin resistant. Hypertension and insulin resistance, or even diabetes, can be induced experimentally by feeding animals a diet high in fat (40% fat) content (Straznicky *et al.,* 1999) or by adding sucrose to the diet (Asghar *et al.,* 2006) or drinking water (Cao *et al.,* 2007; Ribeiro *et al.,* 2005). We set up a model of dietary induced hypertension by feeding rats a high (40%) fat diet containing 10% fructose and cholesterol. The intention is to use the model in the future to study vascular reactivity in hypertensive and normotensive animals in the presence or absence of insulin resistance, and to explore some of the mechanisms which may link hypertension and insulin resistance. This study provides baseline data of certain biochemical, metabolic and vascular properties after six weeks of high-fat feeding.

5.1 High-fat feeding and hypertension

After six weeks of high-fat feeding the rats were hypertensive and the visceral fat mass was increased. The blood lipid profile was not altered, blood glucose was within normal levels and insulin resistance was absent. Interestingly, both hypertension and visceral fat gain occurred in the absence of a significant increase in body weight (table 4.1). This is in

sharp contrast with studies that have shown significant weight gain in rats on a fed high-fat diet for four weeks (Zierath *et al.,* 1997) or 8 weeks (Han *et al.,* 1997; Yoshioka *et al.,* 2000). The lack of significant increased body weight under conditions of high-fat feeding for 6 weeks could be due to enhanced activity of the sympathetic nervous system (Reaven *et al.,* 1996; Moan *et al.,* 1995). The hyperadrenergic state triggers thermogenesis which prevents further weight gain, leading to a sympathetic-induced rise in the systemic blood pressure (Kaplan, 2000). The heart rate of our animals fed with the high-fat diet did not differ from that of control rats (table 4.1). It is thus unlikely that increased sympathetic activity is responsible for the hypertensive state of our HFD group. We can however not exclude the possibility completely.

The results of this study is in agreement with previous studies which show that high-fat feeding results in increased adipose mass (Yoshioka *et al.,* 2000; Dobrian *et al.,* 2000; Huang *et al.,* 2004). As will be discussed, when adipose mass is increased, secretions from adipose tissue such as IL-6, angiotensin II and fatty acids (Lee *et al.,* 2009) can cause both hypertension and insulin resistance. It has been previously reported that various components of the renin-angiotensin system (RAS) are expressed in adipose tissue (Engeli *et al.,* 2000), thus suggesting a possible link between adipose tissue mass and hypertension. In addition, the overexpression of angiotensinogen (AGT) in adipose tissue has been reported to be associated with a higher blood pressure and increased fat mass (Massiera *et al.,* 2001). It therefore follows that the production and secretion of vasoactive precursors, such as angiotensin II, by adipose tissue could be one of the mechanisms by which a high-fat diet increases blood pressure. Furthermore, it has

recently been shown that angiotensin II causes a greater contractile response in thoracic aorta isolated from rats fed a high-fat diet than in aorta from the control animals (Ghatta and Ramarao, 2004; Viswanad *et al.,* 2006). Thus, not only is the vasoconstrictor angiotensin II released by adipose tissue but the vasculature is also more responsive to angiotensin II. Our own results, but not that of Bourgoin *et al.,* (2008), show a similar increased responsiveness to PHE at the submaximal doses tested. Denuding the aorta resulted in weaker PHE responses in the HFD group particularly at doses between 3 mM and 8 mM PHE. This is similar to results obtained by Viswanand *et al.,* (2006) and Ghatta and Ramaro (2004) who showed that the E_{max} induced by angiotensin II was significantly influenced by denudation in control animals but not in animals fed the HFD. Increased contraction to vasoconstrictors in aorta with intact endothelium is due to formation of free radicals and can be reversed by antioxidants (Viswanand *et al.,* 2006). It must however be considered that the highest dose used in their study was 1 mM.

Blood pressure may be increased not only by the production of the vasoconstrictor angiotensin II in adipose tissue and by the possible increased responsiveness to the angiotensin II, but it may also be due to impaired relaxation of the vasculature. Our results show that the EC50 (the concentration required to cause half maximal relaxation), of the aorta isolated from animals on the HFD is twofold higher than that of the control animals, indicating impaired relaxation. Because of the scatter of our data and the small sample size we consider this data as preliminary and are cautious not to make a firm conclusion. Endothelial dependent relaxation, stimulated by acetylcholine (Viswanad *et al.,* 2006) or carbachol (Bourgoin, *et al.,* 2008) were found to be incomplete in aorta

isolated from rats fed the high-fat diet while the aorta isolated from the control animals relaxed completely (100%) in response to acetylcholine or carbacol. Impaired relaxation in animals fed a high-fat diet has been shown to be due to increased endothelin-1 protein content (Bourgoin, *et al.,* 2008), and decreased formation of nitric oxide (Bourgoin *et al.,* 2008, Roberts *et al.,* 2000, Lee *et al.,* 2009) associated with a decrease in the expression of endothelial nitric oxide (eNOS) synthase (Bourgoin *et al.,* 2009). This would be in line with a decreased EC_{50} for acetylcholine rather than a decrease in maximal relaxation. One can then argue that higher doses of acetylcholine may be needed to induce complete relaxation in the HF fed animals. The argument is further supported by the fact that endothelium-independent relaxation is complete as illustrated by denuded aorta (fig. 4.9) and results of others (Viswanad, *et al.,* 2006) and the fact that addition of L-NAME restores complete endothelium dependent relaxation (Bourgoin *et al.,* 2008).

The vasoconstrictory function of angiotensin II is mediated by activation of the angiotensin II receptor 1. Activation of this receptor at the adrenal gland may also cause release of aldosterone which further contributes to a hypertensive state, especially in the presence of increased visceral fat (Roberge, *et al.,* 2007).

Another factor that may have contributed to the increased BP is the pressor effect of the dietary fat. A previous study has reported that even in isocaloric feeding, dietary fat increase blood pressure (Yoshioka et al 2000), which suggests that the hypertension occurred independent of overfeeding.

5.2 High-fat feeding and insulin resistance

A second objective of this study was to determine whether feeding rats our high-fat diet for six weeks will result in the development of insulin resistance. Analysis of the AUC's of IPGTT's and QUICKI showed no difference between groups, indicating that the animals did not become insulin resistant. The HFD also did not induce any change in the lipid profile of the rat. Because the animals did not become insulin resistant we could not determine whether the intervention to prevent hypertension by administration of captopril would also affect the IR status of the animals.

The fact that the animals did not become IR was unexpected. Several studies indicate that feeding rats a diet in which 40% of the calories is derived from lard causes IR together with changes in the plasma lipid profile after 4 weeks (Grundleger *et al.,* 1982; Han *et al.,* 1994; Zierath *et al.,* 1997). Furthermore, feeding rats a normal laboratory chow diet and supplementing the drinking water with 10% fructose (Liang *et al.,* 2007) or combinations of high-fat/high sucrose diets (Bourgoin et al 2008) also resulted in IR. Our result is particularly surprising in view of the increased adipose tissue mass observed (table 4.1). White adipose tissue releases inflammatory cytokines, such as TNF-α and IL-6, together with other adipokine secretions such as leptin, retinol-binding protein 4 and resistin; all of which act in an autocrine and paracrine fashion to play a role in the pathogenesis of IR by modifying key steps in the insulin signaling pathway (Bastard, *et al.,* 2006; Lee *et al.,* 2009; van Gaal *et al.,* 2006). Fatty acids and cytokines released from adipose tissue inhibit PI3K (Lee *et al.,* 2009). This results in decreased recruitment of GLUT4 receptors

to the cell membrane (fig 2.2) causing impaired glucose translocation and insulin resistance.

The result of this study is in agreement with that of Buettner *et al.,* (2006) who also did not observe differences in glycemic level of rats fed a high-fat diet (compared to the control group), but is in conflict with a report that high-fat diet induced hyperglycemia (Ghatta and Ramarao, 2004). The discrepancies in the present study and the ones in which IR was present could also be as a result of differences in the type of fat used, eg. Ghatta and Ramarao (2004) used lard while the present used cooking fat (palm oil). The ratio of unsaturated fatty acid to saturated fatty acids in lard is 1.3 in comparison with the ratio of 1.0 in palm oil (Ong and Goh, 2002). Saturated fats cause obesity and insulin resistance, whereas unsaturated fatty acids do not (Pan *et al.,* 1994, Stolien *et al.,* 2000). Furthermore, high intake of coconut fat does not lead to insulin resistance (Schwab *et al.,* 1995) and chronic consumption of coconut fat does not predispose to obesity (Taylor *et al.,* 1983). This might suggests that IR may not be associated with fats from plant origin. Palm oil also contains tocotrienols (Johannesen, 2005) which has antioxidant properties and which, amongst other, lower serum cholesterol levels and decrease platelet aggregation (Johannesen, 2005). It is possible that the strong antioxidant effects of tocotrienols in palm oil masked the ability to induce IR.

β-cell dysfunction is a primary defect in high-fat feeding (Huang *et al.,* 2004). At three weeks the IPGTT curve of our HFD group is elevated between 25 and 60 minutes (fig 4.4) suggesting that the normalization of the blood glucose level after a glucose load lags

behind that of the other groups. At 6 weeks the glucose handling of the HFD group has apparently improved and the IPGTT curve of this group matched that of the control group more closely. Insulin resistance is accompanied by β-cell adaptation resulting in an increase in β-cell mass and increased insulin secretion (Park *et al.,* 2007) in the wake of simultaneous β-cell apoptosis. Progression to the diabetic state is accompanied by accelerated apoptosis which exceed β-cell proliferation. One is thus tempted to speculate that the improved glucose handling after six weeks indicates a very early stage of β-cell adaptation preventing IR.

IR is associated with an abnormal lipid profile. In the IR state the body uses stored fat as metabolic substrate in preference to glucose, resulting in dyslipidemia. Enhanced lipid breakdown causes an increase in the plasma free fatty acid levels with subsequent changes in lipoprotein composition (van Gaal *et al.,* 2006). Elevated levels of cholesterol (Kushwaha *et al.,* 1991), LDL (Dobrian *et al.,* 2000) and triglyceride (Mooney *et al.,* 1981) have been reported in high-fat feeding. Rats, however, are generally hypo-responsive to dietary cholesterol thus it has been suggested that hyperlipidemia may only be induced in rats by high cholesterol/high-fat diets containing cholic acid and thiouracil (Joris *et al.,* 1983). The normal lipid profile observed in our high-fat fed animals may thus be indicative of the absence of IR or the lack of cholic acid and thiouracil in our diet.

In recent experiments in the laboratory, rats were fed the high-fat diet or normal laboratory pellets for 12 weeks. At the end of the 12 week period the QUICKI between the groups were found to be significant (0.4414 ± 0.02415 and 0.5509 ± 0.02415

respectively, P = 0.0166), indicating that the high-fat fed group were IR. Our six week experimental period was thus too short to induce IR, but prolonging the high-fat feeding period causes IR.

From the literature it is unclear whether hypertension develops prior to IR or vise verse. Studies reported on earlier in this thesis (Randle *et al.,* 1963; Kim *et al.,* 1996; Ghatta and Ramarao, 2004) do not give clarity on this issue since the IR status is reported at one time point only, when the animals are both hypertensive and IR. After six weeks on the high-fat diet our rats were hypertensive but not insulin resistant. One can thus conclude that, under our experimental conditions and dietary intervention, hypertension developed prior to IR.

5.3 Conclusions

After 6 weeks of high-fat (40% fat) feeding our rats were hypertensive and visceral fat mass was increased. Dyslipidemia and insulin resistance were absent. Extending the study period to 12 weeks resulted in development of insulin resistance. We do not make firm conclusion regarding the effect of high-fat feeding on vascular responsiveness because of the small number of animals used in the vascular reactivity experiments. Preliminary results however, indicate possible increase in EC_{50}.

5.4 Future perspectives

It is clear from our laboratory that insulin resistance occurred after 12 weeks on the high-fat diet without cholic acid or thiouracil. However, addition of cholic acid or thiouracil in

the diet should be considered if subsequent studies will last for a shorter period. In further studies on the model secretions from adipose tissue such as angiotensin II, TNF and IL-6 should be measured since they seem to mediate the link between hypertension and IR. In order to know when insulin resistance occurred, insulin levels must be determined on a weekly basis in the same manner as blood pressure. This model should in future be used to study vascular reactivity in hypertensive animals in the absence or presence of insulin resistance and in normotensive insulin resistant animals.

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