INSULIN RESISTANCE AND SALT SENSITIVITY IN THE RAT MODEL OF HYPERTENSION

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PROMOTER: Professor L.I. Somova

DATE SUBMITTED: January 2000

DECLARATION

The Registrar (Academic)
University of Durban-Westville

Dear Sir,

I, Mahendra Lala Channa

Registration Number: 7508116
hereby declare that the dissertation/thesis entitled

Insulin Resistance and Salt Sensitivity in the Rat Model of Hypertension

is the result of my own investigation and research and that it has not been submitted in part or in full for any other degree or to any other university. Where use was made of the work of others, it is duly acknowledged in the text.

M L Channa
ML CHANNA

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INTRODUCTION

Epidemiologic and clinical studies over the last decade have identified individuals with increased plasma insulin concentrations in association with other disorders including hypertension, obesity, dyslipidemia and hyperglycemia. Hyperuricemia and renal dysfunction are sometimes included with these symptoms. (Modan *et al*, 1985; Haffner *et al*, 1988; Reaven, 1995).

Insulin resistance with compensatory hyperinsulinemia has been proposed as the common causative mechanism that links these conditions into a common syndrome referred to as the insulin resistance syndrome (Fuh *et al*, 1987; Reaven, 1988; De Fronzo and Ferrannini, 1991).

Ferranini *et al* (1987) suggested that insulin resistance is associated with essential hypertension and is independant of other factors. A number of epidemiologic studies have also demonstrated the presence of hyperinsulinemia both in the fasting and post-prandial state in normal weight individuals with essential hypertension (Modan *et al*, 1985; Pollare *et al*, 1990; Reaven, 1991(a). These findings are consistent with one of the three following possibilities:

1. Insulin resistance-hyperinsulinemia precedes the onset of high blood pressure and contributes to its pathogenesis.
2. Hypertension precedes the onset of insulin resistance and contributes to its pathogenesis through metabolic and/or hemodynamic alterations.

3. Insulin resistance and hypertension are inherited as separate traits that develop in a parallel but independant manner.

The use of a genetic model of hypertension can help to further elucidate the relationship between insulin resistance and hypertension. In fact, if hypertension or some related aspect is responsible for the development of insulin resistance, one should be able to demonstrate impaired insulin-mediated glucose metabolism in animal models of hypertension.

The reported results on genetic models of hypertension have been contradictory. Some authors reported that spontaneously hypertensive rats are insulin resistant and hyperinsulinemic compared to their control Wistar Kyoto rats (Mondon and Reaven, 1988; Reaven, 1991). Other authors could not support these findings in spontaneously hypertensive rats or in Milan genetic hypertensive rats (Buchanan *et al*, 1992; Frontoni *et al*, 1992). The only results reported thus far in the Dahl genetic model of hypertension were based on serum glucose and insulin determination in response to an oral glucose load (Kotchen *et al*, 1991) or in vitro on glucose transport in isolated adipocytes (Reaven *et al*, 1991).

These important findings however, are based on surrogate measurements of insulin action and not on currently accepted in vivo standard methods.

OBJECTIVES

The objectives of the present study were:

- To establish to what extent features of the insulin resistance syndrome establish themselves in the Dahl salt sensitive rat on feeding a normal/ high salt diet.

- To evaluate in vivo glucose metabolism and insulin sensitivity in the Dahl genetic salt-sensitive model of hypertension by using the euglycemic clamp technique, the best available standard method.

- To determine if there is an association between hypertension, salt-sensitivity and insulin sensitivity in the same model.

LITERATURE SURVEY

1.1 Essential Hypertension

Essential hypertension is a condition in which blood pressure levels are continuously elevated above predetermined cut off points and for which no established endocrine or renovascular cause has been identified. The cut off points for mild moderate and severe hypertension have been defined by the increases in atherosclerotic cardiovascular disease risk found in various community studies (Modan and Halkin, 1991). Hereafter hypertension will refer to essential hypertension.

Given the central role of hypertension in the pathogenesis of both coronary heart disease and stroke, the World Health Organization has expressed concern over the "second wave" epidemic of cardiovascular disease that is now sweeping through the developing countries and the former socialist republics (Guidelines Subcommittee, WHO, 1999).

Haemodynamic studies have shown two distinct hypertensive profiles. The first, termed neurogenic or borderline hypertension is characterized by increased blood volume and cardiac output with total peripheral resistance in the normal or slightly elevated range. The other called nonneurogenic or established hypertension has normal cardiac output, contracted blood volume and increased total peripheral resistance. It has been postulated that neurogenic hypertension evolves into the more severe nonneurogenic type but it is also possible that the two patterns represent two types of hypertension

with different etiologies. The neurogenic type is more prevalent and is associated with atherogenic risk factors like hyperinsulinemia, insulin resistance, dyslipidemia and obesity (Modan and Halkin, 1991).

1.1.1 Elements of blood pressure regulation

Arterial blood pressure can be defined as the product of cardiac output times total peripheral resistance. It is continuously monitored by baroreceptors in the carotid sinus and aortic arch which transmit their information to the central nervous system. The vasomotor centre in turn regulates cardiac output, peripheral vascular resistance and the efferent sympathetic tone. In addition as a slower response, renal renin secretion is increased via the activation of ß-adrenergic receptors in the kidney induced by local noradrenaline release. Renin release, via angiotensin I (Ang I) leads to the formation of Ang II which increases thirst and salt appetite. In high physiological concentrations (50-100 *pmol/l)*, Ang II directly increases peripheral vascular resistance and indirectly increases neurotransmitter release by a presynaptic action at adrenergic nerve endings. Another important action of Ang II is to stimulate the release of aldosterone which stimulates reabsorption of sodium and water in the kidneys.

Atrial natriuretic factor released by the cardiac atria in response to an increase in wall tension and heart rate is a further important component of blood pressure regulation. This hormone, by a number of actions decreases arterial blood pressure and also inhibits aldosterone secretion.

Noradrenaline, Ang II and aldosterone all increase sodium and water reabsorption in the kidney leading to an expansion in extracellular volume and a rise in blood pressure. The kidney responds by an increase in salt and water output following an increase in arterial pressure that is independant of neuronal or humoral factors. This pressure diuresis shows that arterial pressure, besides its role in organ perfusion, also supports the body's fluid balances (Osswald and Mühlbauer, 1995). In recent years, researchers have established the importance of endothelial vasoactive paracrine factors namely nitric oxide, prostacyclin and the endothelins on arterial vascular tone. Ultimately, a stable arterial pressure will depend on collective interactions between the endothelial paracrine pathway, cardiovascular and renal factors. (Shah, 1996).

1.2. Salt Sensitivity

A number of investigations have implicated sodium intake in the pathogenesis of hypertension (Tobian, 1995). Epidemiological studies show that in a given population, some individuals exhibit an increase in arterial blood pressure in response to excess dietary salt. These are referred to as the salt-sensitive group. The group that remains free of the pressor effect of salt is called the salt-resistant or non-salt sensitive group. Similarly in hypertensives, the salt sensitives will experience a significant rise in blood pressure when switching from a low-salt to a high-salt diet compared to the salt resistant hypertensives who will experience a minimal change in blood

pressure (Tobian, 1995). Salt sensitive hypertension is estimated to occur in 60% of the hypertensive population and is more prevalent in Black Africans than in Whites (Luft *et al*, 1991). Currently, the underlying mechanisms of salt sensitivity are not well understood. Hyperinsulinemia, insulin resistance, altered renal haemodynamics, alterations in cellular sodium and calcium metabolism, low dietary mineral intake and elevated activities of $Na^+/K^+/2Cl^-$ erythrocyte cotransport have been suggested to contribute to salt-sensitivity in susceptible individuals (Lluch *et al*, 1996).

1.3. Insulin Resistance

Insulin is a phylogenetically ancient hormone that has a variety of effects on various cell types. Its actions on glucose, lipid and protein metabolism are essential for life. Although insulin is central for intermediary metabolism, its chief control is exerted over the glucose system. For any amount of insulin secreted by the pancreas, the biological response of a given effector is dependant on its insulin sensitivity. Insulin resistance is customarily referred to in terms of the effects of a given amount of insulin on glucose uptake, that is, its effects on glucose metabolism only and not in reference to its other functions (Ferrannini and Mari, 1998). Insulin resistance can be defined as a state where there is hyperinsulinemic response to glucose administration accompanied by impaired or decreased glucose utilization (Sharma *et al*, 1991; Ferrannini and Mari, 1998). At its simplest, elevated plasma insulin

at the same time as normal fasting blood glucose level or a rise in postprandial insulin levels may reflect insulin resistance.

For most hormones, regulation is provided mainly by changes in their secretory rates and under normal conditions their degradation is constant at a value typical for each hormone. Insulin is exceptional in that its action can also be regulated through target tissue sensitivity to its action. The coordination between insulin availability (ß-cell function) and insulin need (tissue responsiveness) maintains plasma glucose within a narrow range. The feedback operates in the obese and elderly where insulin secretion rises to accommodate decreased tissue sensitivity and in physically trained individuals in whom insulin secretion falls as tissue sensitivity to the hormone increases (Berne and Levy, 1993). Insulin resistance is a widespread phenomenon both in physiological and in pathophysiological states. Puberty and pregnancy are associated with selective insulin resistance where an increase in insulin secretion facilitates amino acid uptake for tissue growth, while diabetes, obesity and hypertension are disease states associated with insulin resistance.

Insulin mediated glucose uptake is therefore determined by tissue sensitivity to insulin and by the rate of glucose and insulin delivery via blood flow. The degree of insulin resistance will be determined by the relative contribution of each (Ferrannini and Mari, 1998).

1.3.1 Haemodynamic Hypothesis of Insulin Resistance

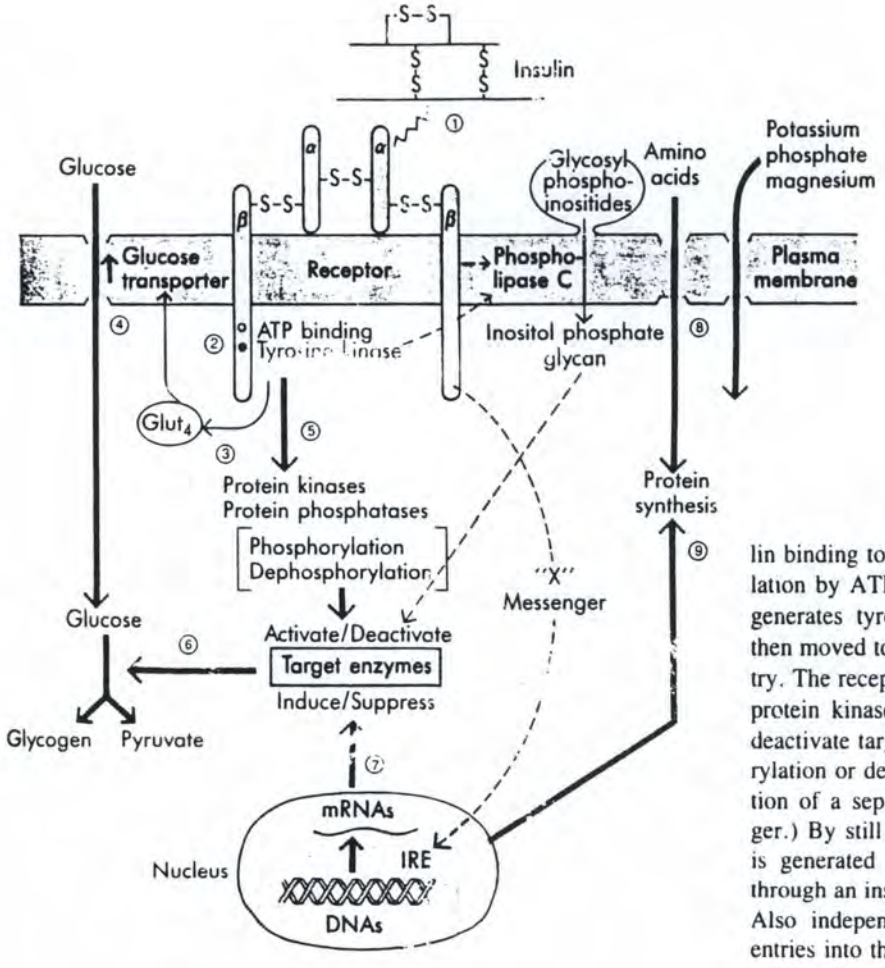
Julius *et al* (1991) proposed the haemodynamic hypothesis of insulin resistance in skeletal muscles. It was suggested that structural vascular changes due to an increased proportion of fast (Type II) versus slow (Type I) muscle fibres and a decreased number of capillaries would decrease skeletal muscle blood supply. The subsequent reduced nutritional flow would limit the diffusion of insulin and substrates from the intravascular space to the target cell surface, thereby causing insulin resistance in skeletal muscles. Since skeletal muscle constitute 30% to 40% of the body mass, insulin resistance here is a major determinant of whole body insulin resistance.

1.3.2 The Insulin Receptor and Insulin Resistance

The insulin receptor is a transmembrane glycoprotein found in the plasma membrane of many cell types. The initial step in insulin action is the binding of insulin to the extracellular subunit of the insulin receptor (Figure 1). The transmembrane and cytoplasmic ß subunit is a tyrosine-specific protein kinase that is activated after insulin binding and undergoes autophosphorylation. Subsequently, the activated insulin receptor kinase phosphorylates in cascade fashion intracellular substrates which ultimately leads to the metabolic effect of the hormone. This includes the movement of glucose transporters that facilitate glucose entry and the activation or deactivation of target enzymes

of glucose metabolism via phosphorylated protein kinases and phosphatases (Berne and Levey, 1993).

In addition to altered tissue sensitivity or reduced blood flow, decreased insulin sensitivity may occur for a number of reasons, including defects in insulin binding caused by reduced receptor number or affinity, defects in signal transduction involving receptor autophosphorylation and tyrosine kinase activity or postreceptor defects at the level of substrates of phosphorylation or effector molecules such as glucose transporters (Sechi *et al*, 1996).



Mechanisms of insulin action on cells. Insulin binding to α-subunit of its receptor causes autophosphorylation by ATP of an intracellular β-subunit receptor site that generates tyrosine kinase activity. Glucose transporters are then moved to the plasma membrane and facilitate glucose entry. The receptor tyrosine kinase is presumed to phosphorylate protein kinases and phosphatases, which in turn activate or deactivate target enzymes of glucose metabolism by phosphorylation or dephosphorylation. (This may be aided by generation of a separate inositol phosphate glycan second messenger.) By still undefined mechanisms, a transcription factor(s) is generated that stimulates or represses gene transcription through an insulin response element (IRE) in DNA molecules. Also independently potassium, magnesium, and phosphate entries into the cell are facilitated.

Figure 1: Mechanism of insulin action on cells (from Berne and Levy, 1993)

1.3.3 Altered target cell calcium ion (Ca^{2+}) concentration as a cause of Insulin Resistance

Genetically induced elevations in intracellular Ca^{2+} can result in insulin resistance in skeletal muscle and adipose tissue. Increased intracellular Ca^{2+} play a role in insulin signal transduction but elevations beyond an optimal range results in a loss of signal transduction. Elevated Ca^{2+} may contribute to insulin resistance by inhibiting the dephosphorylation of the insulin sensitive glucose transporter (Glut 4), and possibly other insulin-sensitive substrates, and thereby decreasing its activity. At this stage it is not clear to what extent increased intracellular Ca^{2+} contributes to insulin resistance (Zemel, 1995).

1.4. Reaven's Hypothesis and the Insulin Resistance Syndrome

Since the initial report 33 years ago of a disproportionately high plasma insulin concentration in hypertensive patients in response to glucose ingestion, many recent studies have also shown that hypertensive individuals are insulin resistant and hyperinsulinemic (Modan *et al*, 1985; Ferrannini *et al*, 1987; Reaven, 1988; De Fronzo and Ferrannini, 1991). Reaven (1988) hypothesised that resistance to the glucoregulatory effects of insulin and hyperinsulinemia may play a role in the development of a constellation of abnormalities (glucose intolerance, obesity, dyslipidemia and hypertension) known as the insulin resistance syndrome, the metabolic syndrome or

syndrome x. Because of its relevance to the present study, features of the hypothesis will be elaborated below.

1.4.1 Pathophysiology of Insulin Resistance

There are large variations in the ability of insulin to stimulate glucose uptake among individuals with normal glucose tolerance. Insulin resistance is not a common feature only in those who are glucose intolerant or with non-insulin-dependant diabetes mellitus (NIDDM). It is estimated that 25% of the apparently healthy population have the same unspecified defect in insulin action as those who are glucose intolerant or with NIDDM (Reaven, 1995).

1.4.2 Hyperinsulinemia and the Pathogenesis of Insulin Resistance

In insulin resistance, the level to which glucose tolerance will deteriorate depends on the magnitude of loss of in vivo peripheral insulin action and the ability of the pancreatic ß cells to compensate for this defect. In an effort to maintain plasma glucose levels between 4,5 mmol/ℓ to 7,8mmol/ℓ, the pancreatic cells will try to secrete whatever amount of insulin is required to maintain the ambient plasma glucose level. The more resistant the individual, the greater will be the compensatory hyperinsulinemia. If the ß cell feedback cannot sustain the compensatory "philanthropic" effort, plasma glucose concentrations will begin to rise, leading to a series of events that

culminate in prediabetes and finally NIDDM. Insulin resistant individuals who have normal or near normal glucose tolerance are hyperinsulinemic compared to insulin sensitive controls. It is the increased ß cell secretion that permits them to overcome the defect in insulin action. This "homeostatic victory" is accompanied by a state of chronic hyperinsulinemia which can "hardly be called benign." It is the pathophysiological consequences of insulin resistance and/or chronic hyperinsulinemia that may manifest itself as the insulin resistance syndrome after a period of several years (Reaven, 1995). The clinical implication of this syndrome is that any abnormality can be due to insulin resistance itself or to the chronic effects of the compensatory hyperinsulinemia.

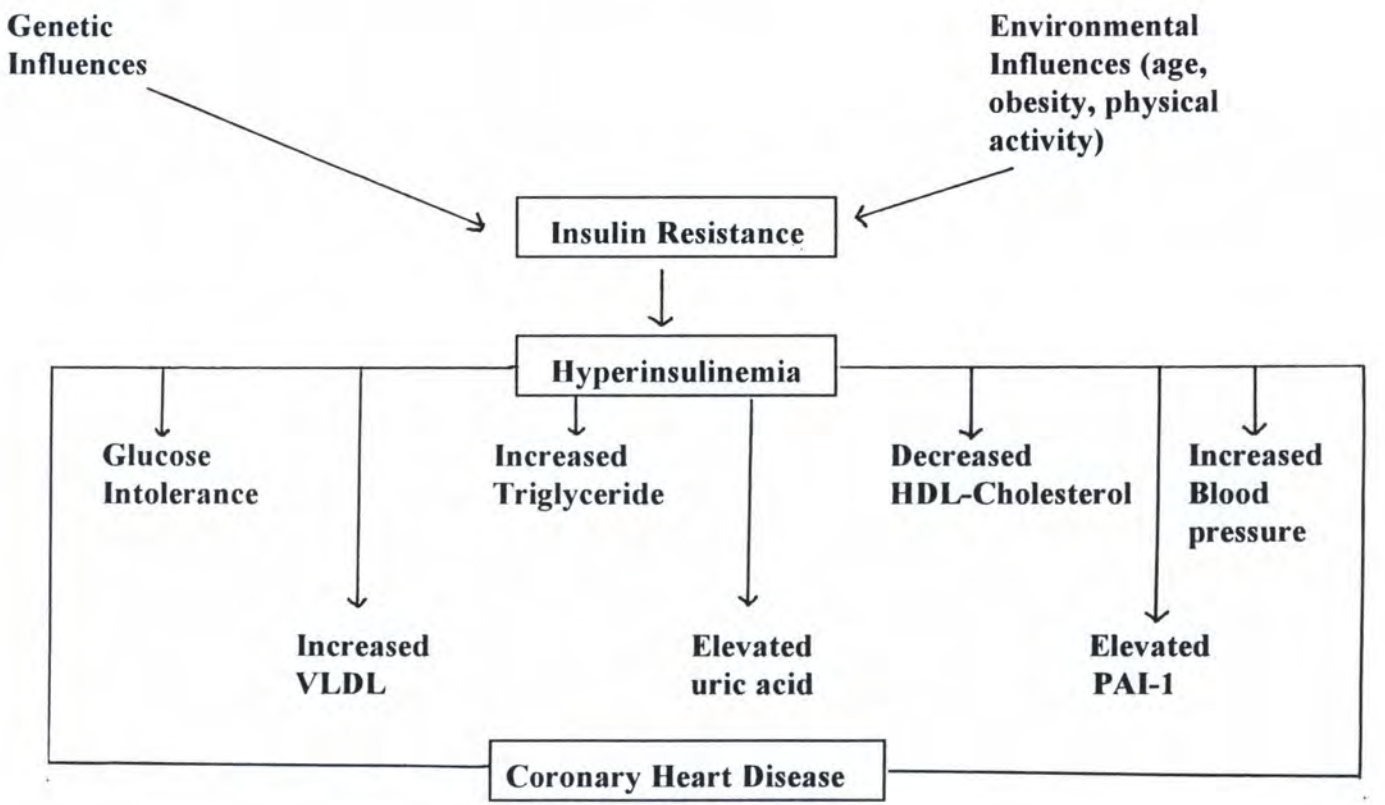


Figure 2. Proposed relationships in the Insulin Resistance Syndrome. PAI-1, plasminogen activator inhibitor; VLDL, very low density lipoprotein; HDL, high density lipoprotein (from Reaven, 1995)

1.4.3 Characteristics of the Insulin Resistance Syndrome

In Figure 2 Reavens "mechanistic" hypothesis (Brown *et al*, 1997) is presented. It is a unified approach which attempts to explain the clustering of atherogenic risk factors which arise from a primary metabolic disturbance featuring insulin resistance and hyperinsulinemia. Insulin plays a central role in lipoprotein metabolism that is not affected by resistance to insulin mediated glucose disposal. The mechanism by which insulin resistance leads to dyslipidemia has not been completely characterized. There is good evidence to suggest that two of the ways in which hyperinsulinemia elevates triglycerides are through enhanced hepatic very low density-lipoprotein (VLDL) synthesis and through decreased VLDL clearance. An increased rate of degradation of apoprotein AI (the major lipoprotein in high-density lipoprotein (HDL) cholesterol) is believed to be a major cause for the reduced level of HDL cholesterol observed in many insulin resistant subjects. Although low density lipoprotein (LDL) cholesterol levels may not be significantly elevated in individuals with the insulin resistance syndrome, recent studies have shown that there may be disturbances in LDL composition. A significant association has been shown between plasma insulin concentration and the presence of relatively smaller more dense LDL particles in individuals with insulin resistance. The small dense LDL might result from the altered VLDL metabolism already discussed (Howard, 1993).

Elevated plasminogen-activator-inhibitor 1 (PAI-1) is also a feature of this cluster and is associated with deficient fibrinolysis. PAI-1 is closely

associated with plasma triglyceride concentration but a direct relationship with insulin resistance still has to be defined (Reaven, 1995). Nevertheless, this feature has unfavourable effects on the blood coagulation and fibrinolytic system and predisposes the insulin resistant individual to thrombosis and atherosclerosis.

Elevated uric acid levels are also a feature in patients displaying features of the insulin resistance syndrome. This increase is believed to arise from a shift in glucose metabolism to the pentose monophosphate shunt which leads to increased purine synthesis and increased uric acid or from decreased renal excretion of urate (Modan and Halkin, 1991; Puig and Ruilope, 1999).

The proposed role of insulin resistance and hyperinsulinemia in the pathogenesis of hypertension is discussed separately below.

1.5. Insulin Resistance and Hyperinsulinemia in the Regulation of Blood Pressure

Almost 16-40% nonobese hypertensive patients appear to be insulin resistant and hyperinsulinemic and over 50% of insulin resistant hypertensives display the cluster of cardiovascular risk factors of the insulin resistance syndrome. Although the association between insulin resistance and hypertension may vary among different population, it is generally believed that insulin resistance has an important role in the pathogenesis of hypertension in the majority of populations (Lithell, 1995; Lind *et al*, 1995; Kotchen, 1996).

Reaven's hypothesis proposes a common causative mechanism that links hypertension pathologically with disordered carbohydrate and lipoprotein metabolism. It may also explain why lowering of blood pressure alone does not necessarily decrease the risk of coronary heart disease. It must also be noted that the relationship between insulin resistance, hyperinsulinemia and hypertension exists even in the absence of overt glucose intolerance and obesity (Ferrannini *et al*, 1987). Hypertension has often been described as an insulin resistant state and it has been suggested that insulin resistance may somehow be related to the fundamental determinants of blood pressure. Figure 3 illustrates the multifactorial relationship between insulin resistance and hypertension.

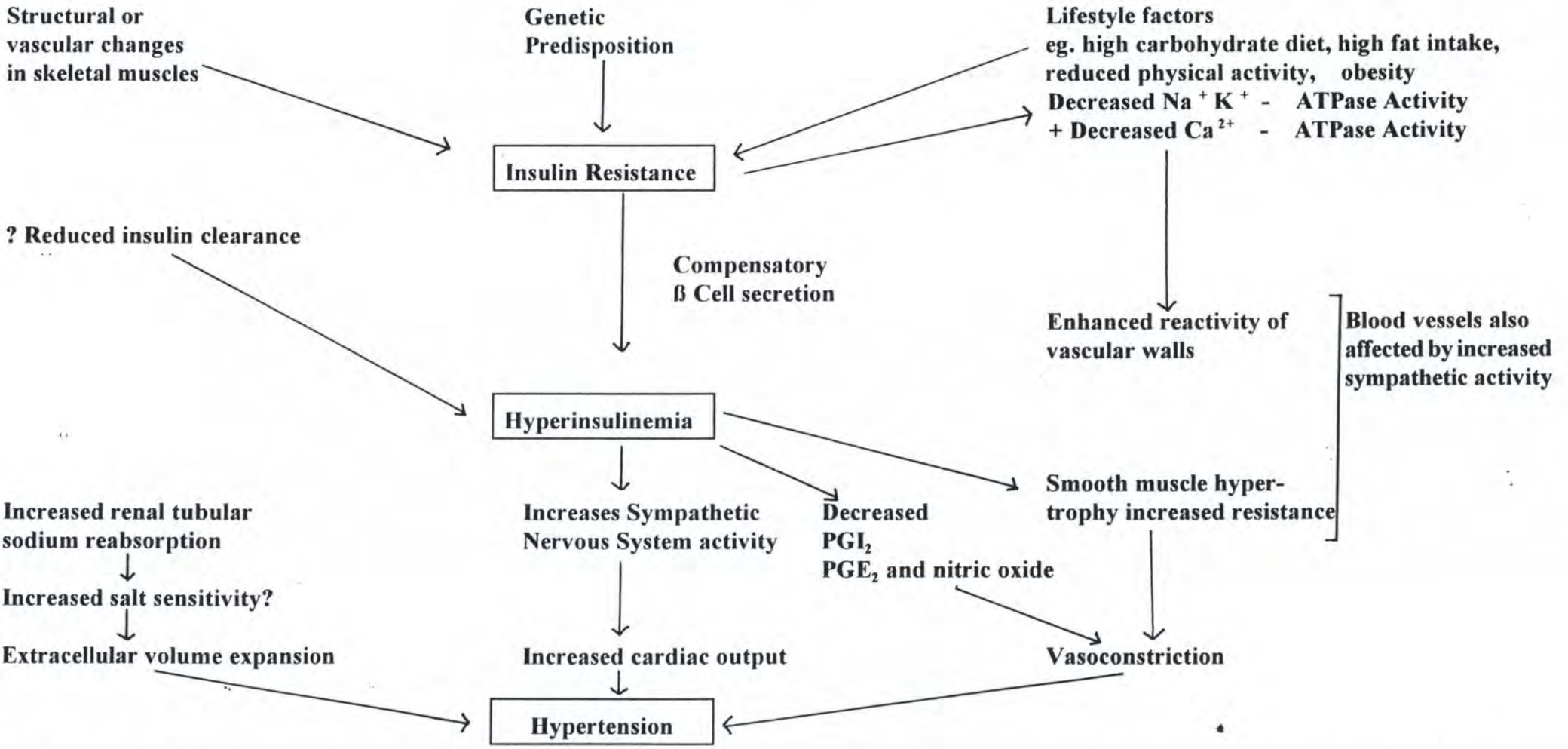


Figure 3. The pathogenesis of insulin resistance, hyperinsulinemia and hypertension (adapted from Edelson and Sowers, 1993). PGI_2, Prostaglandin I_2, PGE_2 , Prostaglandin E_2

1.6 Insulin Resistance and the role of insulin in hypertension

With reference to Figure 3, the etiology of hyperinsulinemia in hypertension is thought to be the consequence of a compensatory increase in pancreatic ß cell secretion as a response to decreased peripheral (skeletal muscle) insulin mediated glucose utilization. Hyperinsulinemia could also be enhanced due to reduced metabolic insulin clearance rate which could result in insulin remaining in the circulation for a longer period of time (Lender *et al*, 1997).

The consequences by which insulin resistance/ hyperinsulinemia could result in hypertension are discussed below.

1.6.1 Enhanced Renal Sodium Retention

Insulin resistance and or acute hyperinsulinemia can result in chronic sodium retention. Insulin can enhance renal sodium uptake both directly through stimulating Na^+K^+-ATPase activity in the proximal tubules and indirectly through stimulating the sympathetic nervous system, augmenting angiotensin II mediated aldosterone production and by altering the secretion of atrial natriuretic peptide (Edelson and Sowers, 1993). It has been also suggested that an elevated glomerular filtration fraction associated with insulin resistance may also favour sodium and water retention by the kidneys (Dengel *et al*, 1996). Because of the Na^+ retaining effects of both insulin and insulin resistance, these states may be critical for the pathogenesis of salt sensitivity in essential hypertension. In support of this possibility,

studies performed on both normotensive and hypertensive subjects have demonstrated an association between salt sensitivity and insulin resistance (Sharma *et al*, 1991; Donovan *et al*, 1993; Zavaroni *et al*, 1995; Galetti *et al*, 1997).

1.6.2 Activation of the Sympathetic Nervous System

Acute hyperinsulinemia can activate the sympathetic nervous system. This causes the release and elevation of noradrenaline and other vasoconstrictor hormones, increased cardiac output due to sympathetic inotrophic and chronotrophic effects on the heart and an increase in renal and splanchnic blood flow (Modan and Halkin, 1991). This combined with or independant of renal effects exerts a strong pressor effect on the cardiovascular system.

1.6.3 Changes in vascular structure and reactivity

Insulin and insulin-like growth factors are mitogens capable of stimulating smooth muscle proliferation. Hyperinsulinemia could result in vascular smooth muscle hypertrophy, narrowing of the lumen of resistance vessels and ultimately the development of hypertension. Insulin could also directly enhance vascular reactivity to both noradrenaline and angiotensin II (Modan and Halkin, 1991).

1.6.4 Modulation of Transmembrane Electrolyte Pumps and Ion Transport

Insulin is known to have a significant effect on several transmembrane ion-exchange systems including Na^+, K^+ - ATPase, Ca^{2+}, Mg^{2+} - ATPase and Na^+/ H^+ exchanger. Hyperinsulinemia may be responsible for intracellular cation imbalances in vascular tissues. One of the well-described cellular actions of insulin is the stimulation of Na^+, K^+ - ATPase activity. Based on the exchange of three intracellular potassium ions with two extracellular sodium ions, stimulation of Na^+,K^+- ATPase will result in hyperpolarization of the cell membrane. In vascular smooth muscle cells this will trigger the closure of voltage dependant Ca^{2+} channels resulting in decreased intracellular Ca^{2+} concentration. This will lead to relaxation of the smooth muscle cells and hence vasodilation of the vascular bed. Na^+ K^+ - ATPase is also located in endothelial cells in which hyperpolarization will lead to a Ca^{2+} influx because of the increased electrogenic driving force (calcium channels in endothelial cells are voltage independent). The increase in cytosolic Ca^{2+} concentration will stimulate endothelial synthesis and release of nitric oxide. Insulin mediated vasodilation therefore occurs via the Na^+ K^+ - ATPase pump and also via nitric oxide dependent vasodilation. In insulin resistant tissues these actions of insulin may be blunted leading to an absence of insulin induced vasodilation, increased vascular tone and increased reactivity of vascular smooth muscles all contributing to an increase in blood pressure (Weidmann *et al* 1995; Tack *et al*, 1996). Accordingly, vascular smooth muscle insulin resistance rather than hyperinsulinemia may result in hypertension. Further, on one hand it appears that insulin resistance in skeletal muscle and adipose tissue may occur due to increased intracellular

Ca^{2+} whereas on the other hand insulin resistance causes disordered regulation of vascular smooth muscle intracellular Ca^{2+} and increases in blood pressure (Zemel, 1995).

1.6.5 Effects of Sympathetic Activity on Skeletal Muscle Insulin Resistance

While the haemodynamic hypothesis is used to explain the cause of insulin resistance in skeletal muscle, sympathetic hyperactivity due to hyperinsulinemia itself could cause haemodynamic and structural changes in peripheral vessels thereby initiating or further aggravating insulin resistance. There is therefore also a possibility of insulin resistance being secondary to abnormal sympathetic and vascular mechanisms of skeletal muscles in hypertension.

Increased sympathetic activation by itself could decrease blood flow and initiate changes in cellular uptake of glucose in skeletal muscle. Increased adrenergic tone is known to be associated with the development of hypertrophy of vessel walls and possible vascular rarefaction. A decrease in nutritional supply to skeletal muscle due to reduced vascular function and/or a decrease in the number of capillaries could therefore contribute to the decreased glucose uptake seen in hypertension.

Skeletal muscles consists of slow twitch red fibres (Type 1) which contain myoglobin, have a high oxidative - low glycolytic capacity, a high insulin

binding, a high basal glucose uptake, a high insulin sensitivity and a rich capillary supply. The fast twitch white (Type II) consist of a group 11-b that have opposite characteristics to the slow twitch, that is a high glycolytic - low oxidative capacity and a low capillary density.

The group IIa have an intermediate speed of contraction, metabolic characteristics and capillary density when compared to the other two groups. Prolonged sympathetic stimulation can cause a transition in the ratio of slow to fast twitch fibres as seen in hypertension (Julius *et al*, 1991).

1.6.6 Vasculopathy - The Proximal Pathogenic Event in Hypertension

Vasculopathy, a dysfunction in the form of inappropriate vasoconstriction and structural alterations like arterial narrowing and stiffness due to abnormal cell proliferation and atherosclerosis is seen both in hypertension and NIDDM. Insulin appears to be one of a mosaic of factors contributing to this vasculopathy either by direct action on blood vessels themselves or in part through the associated metabolic abnormalities like hyperglycemia, hypertension and dyslipidemia (Weidmann *et al*, 1995). It has been suggested that in certain populations, insulin resistance may be an independent cause of atherosclerosis and both may share common roots (Taegtmeyer, 1996).

1.7 Summary - Insulin Resistance, Hyperinsulinemia and Hypertension

Insulin resistance and hyperinsulinemia have been correlated with hypertension, dyslipidemia and glucose intolerance which appear as a constellation of cardiovascular risk factors. Hypertension has been termed as an insulin resistant state with the skeletal muscles being identified as the major site of insulin resistance (Reaven, 1995).

Hyperinsulinemia has effects on tissue that are not usually considered as insulin targets (Sechi, 1999). Effects like activation of renal sodium reaborption, increased sympathetic stimulation, modulation of transmembrane cation transport and the promotion of vascular hyper-reactivity are all hypothetical contributions to the pro-hypertensive actions of insulin (Sechi, 1999).

Evidence also points to the role of insulin as a hypotensive hormone since it stimulates blood flow and decreases vascular resistance in skeletal muscle mediated in part by an endothelium dependant mechanism. Hypertension may therefore result due to an imbalance between the pressor and depressor action of insulin.

Because of sodium retaining effects of insulin resistance with the associated hyperinsulinemia may be critical for the pathogenesis of salt sensitivity in hypertension. The underlying mechanisms responsible for the coupling of insulin resistance/ hyperinsulinemia to the pathogenesis of hypertension still have to be understood (Ferrari *et al* 1991; Axelrod, 1991).

1.8. Current Perspectives

Since none of the proposed mechanisms in the pathogenesis of hypertension have been established beyond doubt, it is necessary to provide evidence for and against the proposed relationships.

Evidence in support: In the general population, blood pressure tends to correlate positively with plasma insulin levels. Young, overweight borderline hypertensive subjects tend to have higher fasting plasma insulin levels. Impaired insulin sensitivity as well as hyperinsulinemia was noted in some essential hypertensive patients even in the absence of obesity or diabetes mellitus (Ferrari *et al*, 1991). Insulin has been shown in short term studies to have multiple effects on the kidney, the sympathetic nervous system and cardiovascular system and sustained infusion could lead to hypertension (Weidmann *et al*, 1995).

Evidence Against: The evidence marshalled against the role of hyperinsulinemia and insulin resistance in blood pressure regulation is that acute hyperinsulinemia in humans leads to vasodilation and a lowering of blood pressure rather than vasoconstriction. Chronic insulin infusion in dogs does not change blood pressure (Reaven, 1995). The acute effects of insulin on Na^+ resabsorption may not be sufficient for chronic elevation of blood pressure or for the maintenance of increased sympathetic nervous system activity. Lastly, not all hypertensives are insulin resistant and not all insulin resistant subjects are hypertensive.

1.8.1 Selective Insulin Resistance

Insulin promotes both blood flow and glucose extraction in insulin sensitive tissues. This vasodilatory response promotes insulins metabolic action by improving target tissue perfusion. Insulin resistance refers to a decreased insulin stimulated glucose disposal. Selective insulin resistance implies that while peripheral (muscle) tissue may become insensitive to insulins glucose lowering effects and there is resistance to insulin mediated skeletal muscle vasodilation, there will still be a preservation of renal and sympathetic nervous system sensitivity to insulin where the hormones action will still be unopposed (Heise *et al*, 1998). Excessive insulin induced effects may also possibly occur in tissues like the kidney where sensitivity to insulin is preserved (Sechi, 1999).

This explains why insulin action on skeletal muscle vasodilation is blunted during insulin resistance. There is evidence to indicate that in obese and non-obese hypertensives insulin resistance is selective (predominantly involving glucose metabolism although amino acid and fatty acid metabolism can also be involved), tissue specific (predominantly affecting skeletal muscle, although liver, adipocytes and leucocytes may also be affected) and pathway specific (only glycogen synthesis is affected; however during diabetic ketoacidosis all anabolic pathways are resistant to insulin effects). Therefore in any individual or animal, the degree to which insulin resistance is tissue and pathway specific may determine whether hypertension will develop or not (Roccini, 1995).

1.8.2 Alternatives to Reaven's Hypothesis

While the association between insulin resistance, hyperinsulinemia and hypertension is established but not totally explained, it is also theoretically possible that insulin resistance is secondary to structural changes in skeletal muscle caused by hypertension. Recent studies have also shown the blood pressure elevation could be mediated by insulin resistance rather than by hyperinsulinemia and that hypertriglyceridemia could also be causes of insulin resistance (Ferrannini *et al* 1997; Heise *et al* 1998). However, evidence against insulin resistance and hyperinsulinemia being the consequence of hypertension is based on observations that the relationship between insulin and hypertension occurs both in humans and animals in primary hypertension and does not seem to occur during secondary hypertension (Sechi, 1999).

1.9 Hypotheses relevant to the present study

On the basis of available information, the frequent occurrence of insulin resistance and hypertension may reflect three distinct possibilities.

1. Insulin resistance and/or hyperinsulinemia, whatever the underlying mechanisms and time of appearance may cause and aggravate hypertension.
2. Insulin resistance may occur as a secondary change during the development of hypertension and may have no primary effect on the pathogenesis of hypertension.

3. Insulin resistance and hypertension are inherited as separate states, are causally unrelated and may develop in parallel but independent manner.

It must also be emphasised that blood pressure is controlled by an "orchestra" of neurohumoral, endocrine, metabolic and renal factors amongst which insulin may be but one "instrument" (Ferrari *et al*, 1991). Hyperinsulinemia, in contrast to catecholamines and angiotensin II does not acutely modify blood pressure. It may rather be representative of a "slow" pressor factor which compliments many other important mechanisms in the pathogenetic mosaic of hypertension (Weidmann *et al*, 1995).

1.10 Animal Studies

Experimental hypertensive rats are a widely used model in the investigation of the pathogenesis of human hypertension. There is evidence that resistance to insulin-induced glucose disposal and hyperinsulinemia exists in rat genetic models of hypertension such as the Zucker obese, the spontaneously hypertensive rat, the Milan hypertensive rat and the Dahl salt-sensitive rat (Mondon and Reaven 1988; Reaven 1991; Kotchen *et al*, 1991; Reaven *et al*,1991; Buchanan *et al*,1992; Frontoni *et al* 1992). In genetically non-hypertensive rats, continuous high carbohydrate feeding results in hypertension that is also correlated to insulin resistance (Hwang *et al*, 1987; Reaven and Ho, 1991). Other studies have demonstrated that long term insulin administration in rats also leads to increased blood pressure

(Brands *et al* 1991; Meehan *et al* 1994). The insulin induced rise in blood pressure is reversed with the termination of insulin infusion indicating the specific effects of hyperinsulinemia (Hsieh *et al*, 1993). These observations provide direct support for the role of insulin resistance/hyperinsulinemia in the pathogenesis of hypertension in rats but like human studies, the precise mechanisms are not clearly established.

Because insulin induced hypertension is absent in dogs, it is suggested that there may be important species - specific differences in the haemodynamic response to insulin (Brands *et al*, 1997).

Insulin induced hypertension in rats shows two main features that are different from humans. Firstly, insulin induced hypertension is not associated with marked sodium retention. There is a shift in the pressure naturesis relation and the rats are in sodium balance at an elevated blood pressure. Total peripheral resistance rather than cardiac output is increased suggesting that there is an activation of a vasoconstrictor mechanism. In addition contrary to human studies it has been reported that insulin induced hypertension in rats is not sensitive to changes in sodium uptake (Hall *et al*, 1992; Brands *et al*, 1997).

1.10.1 The Salt Sensitive Dahl Rat - A Brief History

Human essential hypertension is a classic example of a complex multifactorial and polygenic disease. Rat and mouse models of hypertension have been

developed by selective breeding over many generations. Their major advantages include complete genetic homogeneity, large numbers of offspring, the ability to produce large genetic crosses and to provide tight control over environmental influences (Dominiczak *et al*, 1988). In the 1960's Dr Lewis K Dahl and associates developed from the Sprague Dawley line two strains of rats that were either susceptible or resistant to the hypertensive effects of a high salt (8% NaCI) diet. In the 1970s John Rapp began systematic brother-sister matings of Dahl rats and in 1985 he described the development of fully inbred strains of Dahl Salt-Sensitive and Dahl Salt-Resistant Rats. These were homozygous at 100% of all genetic loci, thus fixing the characteristics of the strain. These were termed the Dahl (SS/Jr) and Dahl (SR/Jr) respectively. Rapp provided these rats to Harlan Sprague Dawley, Inc (HSD Indianapolis, USA) in 1986 and since then multiple investigators have been provided with inbred Dahl/Rapp salt sensitive (SS/Jr Hsd) or salt resistant (SR/ Jr Hsd) animals for physiological, biochemical and genetic studies of salt-sensitive hypertension. The inbred Dahl/Rapp salt sensitive rat, hereafter referred to as the DSS rat, represent one of the best characterized and most widely studied models of low renin salt-sensitive hypertension (Lezin *et al*, 1994).

1.10.2 Characteristics of the Dahl Salt-Sensitive Rat

When the DSS rat is fed a high salt diet (8% NaCI), mean arterial pressure typically increases by 20 mm Hg within 24 hours and continues to rise to about 170 mm Hg or higher around two weeks. The initial rise in arterial

pressure appears to be triggered by sodium retention. The animal gains about 7% in body weight and plasma volume and cardiac output increases significantly. Later, the cardiac output returns towards control values and the hypertension is maintained by increased peripheral resistance (Wilson *et al*, 1998). In similarly treated Dahl salt resistant (DSR) rats, blood pressure remains in the normotensive range and the animals respond mildly if not at all to salt changes in the diet.

The age at which a high salt diet is started partly determines the magnitude of the blood pressure response in DSS rats. When given a high salt diet at weaning (21-23 days of age) rats reach a mean blood pressure of 200 mm Hg within 6 weeks. If salt feeding is delayed until 3 months of age, the hypertension develops less rapidly and mean blood pressure reaches about 185 mm Hg by 16-20 weeks. DSS rats do not become hypertensive when placed only on a high salt diet. In fact, on a normal rat diet that contains 1% NaCl, DSS rats become markedly hypertensive but it takes a longer for the hypertension to develop. On low salt diets (0,3 to 0,4% NaCl) DSS rats often have pressures 15-20 mm Hg higher than DRS rats. Thus the absolute amount of Na^+ in the diet is an important determinant of final blood pressure (Rapp, 1982).

In DSS rats the rate of hypertension development is directly related to the daily salt intake. A 4% NaCl diet brings about 30% rise in blood pressure by the 11th week of feeding while in the same period the 8% NaCl diet induces a 70% rise in blood pressure (Dahl *et al*, 1968). This allows the DSS rat to serve as a model of hypertension which can be studied both in its acute and

chronic forms. DSS rats possess a genetic substratum in the form of a polygenic trait for the development of hypertension. The environmental factor in the form of a high salt diet in turn accelerates and accentuates the production of the phenotype.

1.10.3 Mechanism of Salt-Sensitive Hypertension in Dahl Rats

The mechanism for the rise in blood pressure in the DSS rat has not yet been fully established and has been debated since the strain was first introduced. Renal, central nervous system mechanisms and various hormonal systems have been suggested to be responsible for the rise in blood pressure. Current literature attributes sodium induced hypertension to be due to genetic renal alterations that precede the development of hypertension. Under normal homeostatic conditions, when arterial pressure rises, the pressure-natriuretic mechanism operates as an infinite gain system and adjusts blood volume and cardiac output through increased urinary sodium and water excretion until arterial pressure returns to normal. Various studies on DSS rats have indicated that this pressure-natriuretic response is impaired or reset to a higher renal perfusion pressure, leading to sustained hypertension (Alvarez-Guerra *et al*, 1998). DSS rats require a higher perfusion on pressure to achieve the same sodium excretion as a DSR rat. The resetting is largely due to a marked increase in Na^+ and Cl^- reaborption in the thick ascending limb of the loop of Henle and a deficiency in the production of 20-hydroxyeicosa - 5,8,11,14-tetranoic acid (20 - HETE) which is a Na^+, K^+, $2Cl^-$ co-transporter inhibitor in this area of the nephron (Wilson *et al*, 1998). The kidney as a

source of one or more factors that induce hypertension in the Dahl and other genetically hypertensive rats is shown in studies demonstrating that hypertension can be transferred by renal transplantation from SHR rats to normotensive Wistar-Kyoto rats. Correspondingly, transplanting kidneys from normotensive to genetically hypertensive rats leads to a decrease in the hypertension of the recipients (Osswald *et al*, 1995).

Neural mechanisms and humoral factors also contribute to the development of salt induced hypertension in Dahl S rats. A high salt intake can enhance sympathetic excitation and attenuate sympathetic inhibition. Arterial and cardiopulmonary baroreflex control of efferent sympathetic nerve activity are reported to be impaired in DSS rats. It is thought that increased ouabain-like activity in several brain areas during high salt intake could contribute to the development of hypertension (Huang and Leenen, 1998).

With regards to humoral factors, the endogenous nitrovasodilator or L-arginine: nitric oxide pathway plays an important role in the regulation of blood pressure and responses to changes in dietary salt. It has been shown that during salt overload, renal nitric oxide synthesis is impaired in the DSS rat thereby contributing to the hypertension via renal effects and via effects on vascular tone. Similarly, the vascular endothelin vasopressor system has also been implicated. During a high salt diet, vascular endothelin-1 (ET-1) levels are thought to increase, leading to potent local vasoconstriction and smooth muscle proliferation in blood vessels (Barton *et al*, 1998).

1.10.4 Components of the Insulin resistance syndrome in Dahl Rats

Currently, the DSS rat is one of the best characterized and most widely studied model of genetic salt sensitive hypertension and is considered a good counterpart to human salt sensitive hypertension. There are various reports on insulin resistance occurring in genetic rat models of hypertension. Resistance to insulin induced glucose disposal has been reported in Zucker obese, DSS, Spontaneously Hypertensive rat (SHR) and the Milan hypertensive rat (Brands *et al*, 1991; Lembo *et al*, 1995; Kotchen, 1996). Exogenous insulin infusion has been reported to accelerate and aggravate the development of hypertension in the DSS rat (Tomiyama *et al*, 1992). When the euglycemic clamp technique was used to measure insulin sensitivity in the SHR and Milan hypertensive rat, hepatic and peripheral insulin sensitivity were found to be normal for both animal models (Frontoni *et al*, 1992). Similarly, the reports on Dahl rats being insulin resistant were based on surrogate measurements of insulin action like serum glucose and insulin determination in response to an oral glucose load or an in vitro method like glucose transport in isolated adipocytes (Kotchen *et al*, 1991; Reaven *et al*, 1991). Apart from this, there have been no attempts to assessing the Dahl rat as a model for the insulin resistance syndrome as fashioned on the study by Vrána *et al* (1993). In this study inbred rat strains including the Hereditary hypertriglyceridemic (Htg), Brown Norway (BN), the SHR and the polydactyl-luxate syndrome carrying PD strain of Wistar origin were assessed for hypertriglyceridemia, impaired glucose tolerance, hyperinsulinemia, insulin resistance and hypertension. The Htg strain presented all the above symptoms of the insulin resistance syndrome. The

PD strain was normotensive while presenting all other symptoms. The SHR strain only showed the expected hypertension and only a mild increase in triglycerides and no changes in glucose tolerance. The shortcoming of the study was again that serum insulin and a oral glucose tolerance test rather than the euglycemic clamp technique was utilized used to assess insulin sensitivity.

1.10.5 Molecular Mechanism of Insulin Resistance in Animal Models

At receptor level, decreased sensitivity to insulin may occur due to defects in insulin binding, reduced receptor numbers, defects in signal transduction or modifications in the post receptor cascade (Selchi *et al*, 1996).

The number of insulin receptors in target tissues is regulated by several different hormones and physiological conditions. A major factor that determines both insulin receptor number and gene expression is the ambient insulin concentration (homologous regulation). In addition to insulin, other endocrine and metabolic factors such as glucagon, growth hormone, glucocorticoid and ketone concentrations may affect the insulin receptor number (heterologous regulation) (Kahn, 1983).

Thus far only a few studies have addressed the molecular mechanisms of insulin resistance. Mondon *et al* (1989) found no difference in insulin receptor number or affinity in skeletal muscles of both SHR and Wistar Kyoto (WKY) rats. Another study demonstrated decreased

autophosphorylation of the insulin receptor and decreased phosphorylation of insulin receptor substrate IRS-I in liver and muscle of SHR compared with WKY rats (Kahn and Saad, 1992). Overall, these and other studies indicate the possible role of reduced insulin receptor kinase activity and reduced substrate phosphorylation in skeletal muscle and liver play a role in the insulin resistance of SHR (Setchi, 1999).

1.10.6 Insulin Receptors, Salt Sensitivity and Insulin Resistance in Rat Models of Hypertension

Sechi *et al* (1994) demonstrated an inverse relationship between dietary sodium intake and renal insulin receptor number and mRNA levels in Sprague-Dawley rats fed on low (0,07%), normal (0,3%) and high (7,5%) NaCl diets. This dose dependant relationship between salt intake and renal insulin receptor downregulation is mediated at the level of gene expression. Further, this observation suggested the existence of a feedback mechanism that limits insulin-induced sodium retention when extracellular fluid volume is expanded (Figure 4).

In another study with SHR rats, which in addition to being hypertensive, are also insulin resistant (Sechi *et al*, 1996) demonstrated that renal insulin receptor density and mRNA levels in the SHR do not differ from those of the WKY when the rats are fed a low salt diet. A high salt diet decreases the receptor number and mRNA in WKY rats but not in the SHR. This is indicative of the SHR rats having lost the suggested feedback mechanism

that limits insulin induced sodium retention and further sodium retention may be implicated in the development and maintenance of hypertension in this model.

In Dahl rats, Sechi *et al*, (1993) observed no difference in tissue distribution, number and affinity of skeletal muscle and kidney insulin receptors in DSS and DSR rats fed different salt intakes. Binding parameters, hepatic, muscular and renal insulin receptor in mRNA were also comparable in both groups at different salt intakes (Setchi *et al*, 1997). Therefore in Dahl rats, hypertension and the reported insulin resistance is associated with normal insulin receptor binding and gene expression in peripheral tissues and the decreased sensitivity may therefore be due to a post receptor defect and insulin-mediated sodium retention also appears to have no role in the salt sensitivity of the Dahl rat.

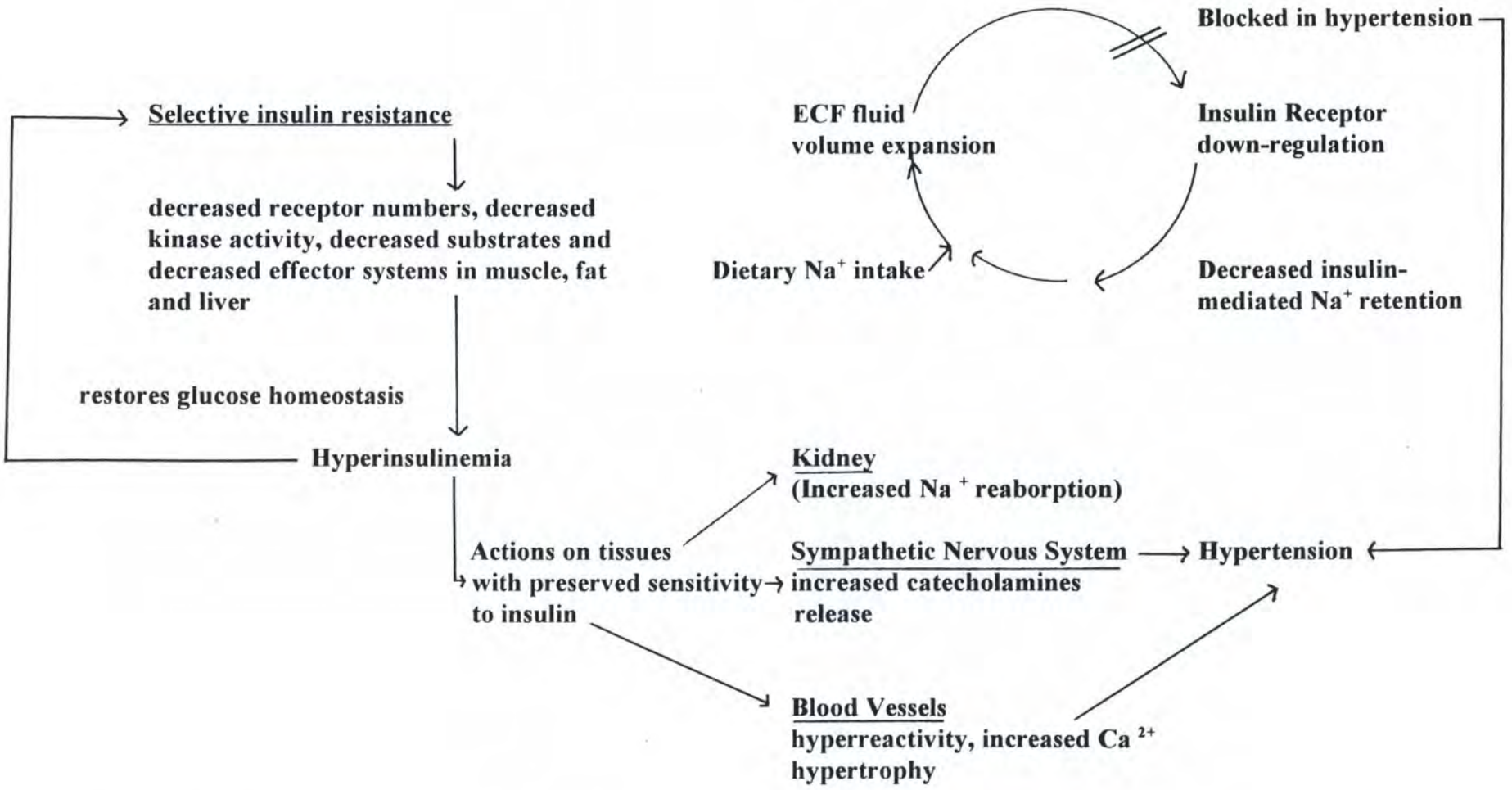


Figure 4: Representation of how insulin resistance might be linked to salt-sensitivity and hypertension (from Sechi, 1999)

1.11 Major Questions to be addressed by Study

From the above review on Dahl rats, major questions that have to be addressed by this study are clarified below.

1. To what extent do the features of the insulin resistance syndrome establish themselves in the DSS rat model of genetic hypertension when fed a normal/ high salt diet?

2. Is the DSS rat really insulin resistant when the "gold standard" for the measurement of insulin sensitivity is applied?

3. Is insulin resistance related to blood pressure and salt intake in this model of genetic hypertension?

2. METHODS AND MATERIALS

2.1. Introduction and Overview

In order to attain the objectives put forth in the literature survey, the study design spans both the molecular/ cellular and integrative levels of physiological function. In the first phase of the study, termed Experiment 1, biochemical parameters relating to the insulin resistance syndrome were determined in DSS and DSR rats on low and high sodium diets. The same parameters also measured in 2 additional matched 3 week old weanling DSS and DSR groups.

In the second phase of the study, termed Experiment 2, the euglycemic glucose clamp technique was utilized to study in vivo glucose metabolism and insulin sensitivity in Wistar rats and in DSS and DSR rats fed normal and high salt diets. All experiments were approved by the Ethics Committee of the University of Durban-Westville.

Experiment 1: Table 1: Animals, groups and diets for Experiment 1

Animal Groups (males)	DSS	DRS	DSS	DRS	WDSS	WDSR
Numbers per group	8	8	8	8	8	8
Diet (for 2 months)	Low salt	Low salt	High salt	High salt	Weanlings	Weanlings

WDSS - Dahl Salt Sensitive weanlings, WDSR - Dahl Salt Resistant Weanlings

1. Blood pressure measurements were made weekly for duration of diet.
2. Urinary Na^+ / K^+ ratio, blood glucose, plasma insulin, plasma uric acid and a blood lipid profile were determined for all groups.
3. Skeletal muscle insulin receptors were extracted and purified.
4. Receptor insulin associated tyrosine kinase activity assayed for all groups.

Experiment 2: Table 2: Animals, groups and diets for Experiment 2

Animal Groups (males and females)	Wistar	DSR	DSS	DSR	DSS
Number per group	12 males	6 males 6 females	6 males 6 females	3 males 3 females	3 males 3 females
Diet	normal	normal	normal	salt loaded	salt loaded

1. Determination of glucose metabolism and insulin sensitivity using euglycemic clamp for all groups

Experiment 1:

2.2.1 Determination of Biochemical Parameters related to the Insulin Resistance Syndrome

2.2.1 Animals and Diets

Three weeks old male DSS and DSR rats were used. The parent colony were obtained from Harlan Sprague Dawley Inc (Indianapolis, USA). Eight rats from each group were fed a standard rat diet (60% carbohydrate; 22% fat and 18% protein) with low sodium (0,2% NaCl) and another 8 rats from each group were fed the same diet but with high sodium (4% NaCl). A 4% NaCl diet rather than a 8% diet was used to moderate blood pressure changes and avoid the development of renal damage that occurs with a 8% NaCl diet (Campbell *et al*, 1996). The feeding regimen for the high and low salt groups lasted 8 weeks with both food and distilled water available ad libitum. The 4 groups were housed 4 per cage at a constant temperature and exposed to a 12 hour light/dark cycle.

2.2.2 Blood Pressure Measurement

Body mass, systolic and diastolic blood pressure with the heart rate were measured weekly between 9h00 and 12h00 for all animals with a widely used non-invasive computerized tail cuff method. (IITC, Model 31, CA, USA). Before monitoring, rats were trained for 5 consecutive days with daily recording to minimize stress reactions. With measurement procedures

being non-invasive, the apparatus used was technically convenient for long term animal studies.

2.2.2.1 Operation Principle

The system employed an automatic scanner, a pump, a tail cuff with a photoelectric sensor and an amplifier, to measure and count the heart pulses in the animal's tail. The principle of operation is related to the Riva-Rocci method used in humans. Conscious, unanaesthetised animals were placed in a perspex restrainer and the pump automatically inflated the tail cuff placed at the base of the rat tail. This results in arterial blood supply to the tail be occluded. The cuff was then deflated and the reappearance of pulsations, as detected by the photoelectric sensor, was taken as the systolic blood pressure. As the pressure continued to fall, the computer stored the value of each high pulse point which was accepted as a mean pressure if there was no higher pulse within the next two seconds. The diastolic pressure for the recording was computed using the equation: diastolic = (3 mean - systolic)/2.

The test results were displayed as data plots and summaries on the computer screen (Figure 5). The test information has 2 tracings - one showing the pressure applied to the sensing cuff and the other shows the pulse detected by the sensor. The systolic, mean and calculated diastolic blood pressures as well as the heart rate are also displayed.

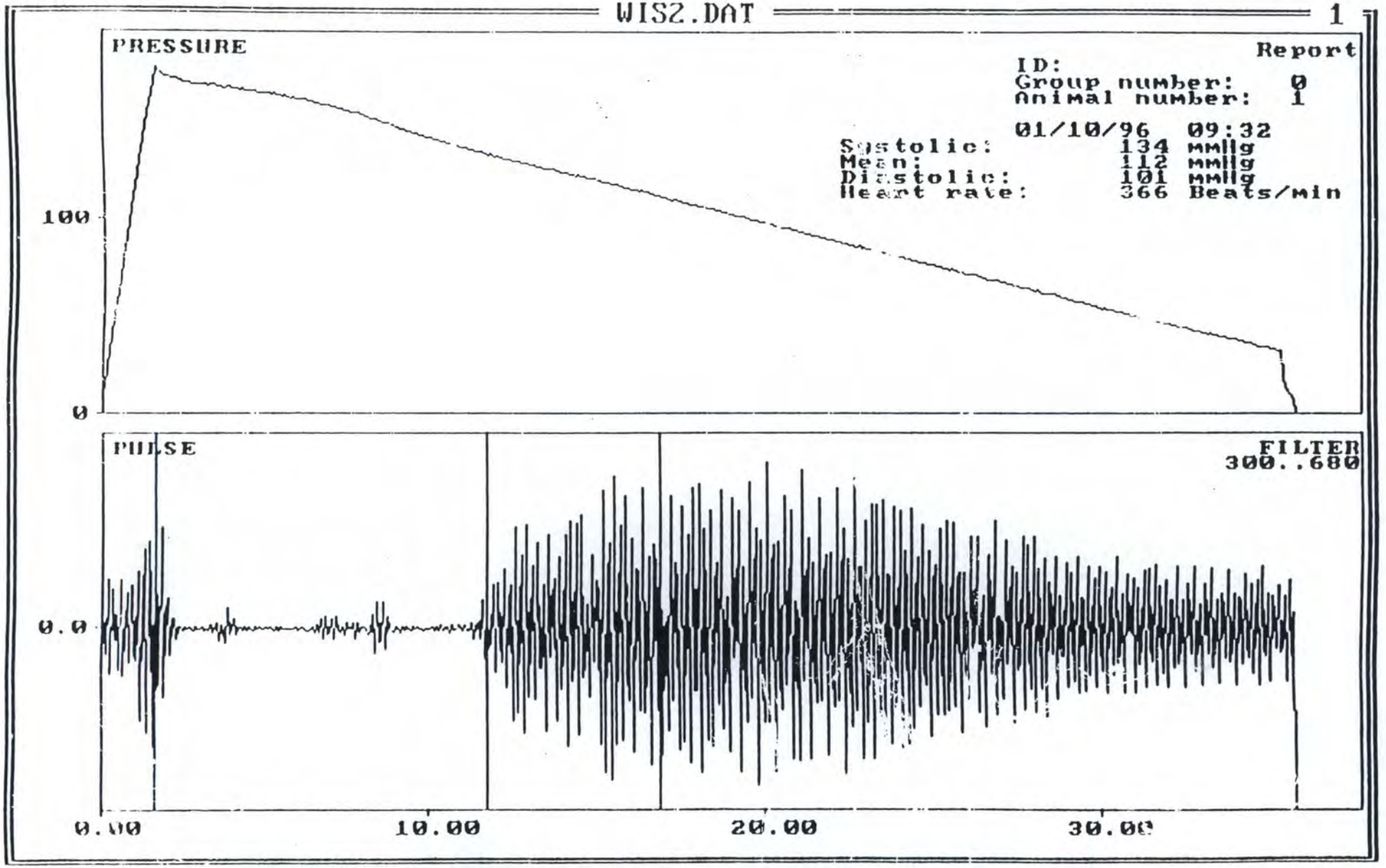


FIGURE 5: A typical rat tail pulse recording using the IITC blood pressure monitor.