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Abnormal glucose tolerance and insulin resistance in treated patients with essential hypertension

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ABNORMAL GLUCOSE TOLERANCE AND INSULIN RESISTANCE IN TREATED PATIENTS WITH ESSENTIAL HYPERTENSION

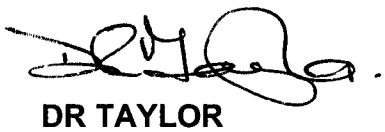
Diane Rosemary Taylor

A dissertation submitted to the Faculty of Health Sciences of the University of the Witwatersrand, Johannesburg, in fulfillment of the requirements for the Degree of Master of Science in Medicine.

Johannesburg 2009

DECLARATION

This dissertation is my own work. It is being submitted for the degree of Master of Science in Medicine at the University of the Witwatersrand, Johannesburg. No part of it has been presented for any degree or examination at any other University.



DR TAYLOR

ABSTRACT

Among patients with mild hypertension, differences in the risk of cardiovascular disease are determined not only by the level of blood pressure, but also by the presence or levels of other risk factors.

The association between elevated insulin concentrations and various metabolic and physiological abnormalities, including hypertension, has been highlighted by several studies. Previous research has suggested that antihypertensive agents, in particular thiazide diuretics, may promote the development of Type 2 diabetes mellitus. However, the results of these studies have been inconsistent.

The purpose of this study was threefold. Firstly we wanted to determine a practical cost effective test to assess glucose tolerance in a Hypertensive Clinic. Secondly, we wanted to compare target organ damage and to determine changes in the metabolic profile of elderly white hypertensive patients treated with low-dose thiazides and, thirdly, we wanted to assess pancreatic β-cell secretary function, platelet cation concentration and membrane ATPase activity in insulin resistant patients with essential hypertension.

Our results showed that a standard 75 g oral glucose tolerance test with a single 2 hour capillary glucose concentration estimated on a reflectance meter was more

accurate than a random glucose, fasting glucose, glycated haemoglobin or urine dipstick in determining glucose tolerance.

A very high incidence of abnormal glucose tolerance, dyslipidaemia and obesity was found in this cohort of elderly hypertensives. Dyslipidaemia was closely related to the use of thiazides, albeit in low doses, whilst the high incidence of abnormal glucose tolerance was related to factors other than thiazides.

Our findings have supported the strong link between hypertension, insulin resistance, hyperinsulinaemia and regional adiposity. The changes in intracellular cation activity reflected both the diabetic and hypertensive states respectively.

This data confirms the hypothesis that abnormal cation exchange, hypertension and insulin resistance coexist.

The work described in this thesis has been published in the South African Medical Journal (1997) and the Cardiovascular Journal Africa (2008). (See References).

Acknowledgements

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CHAPTER 1.

1.1 Introduction

Hypertension is the most common cardiovascular disorder affecting about 20% of the adult population. Data shows that approximately 135 million people suffer from diabetes mellitus worldwide and the World Health Organisation (WHO) (1999) predicts that this number will rise to almost 300 million by the year 2025. This more than twofold rise is projected to occur because of population ageing, unhealthy diets, obesity and sedentary lifestyle. The association between elevated insulin concentrations and various metabolic and physiological abnormalities, including hypertension, glucose intolerance, dyslipidaemia, hyperuricaemia and obesity has been highlighted. by several epidemiological and clinical studies (Haffner *et al*, 1992; Pollare *et al*, 1990; Reaven, 1988; de Fronzo, 1990; Hanson *et al*, 2002; Routolo *et al*, 2002). These disorders, which collectively comprise the insulin resistance syndrome, are associated with an increased risk of coronary artery disease in many populations (Saad *et al*, 1991; Alberti *et al*, 2005; Reaven, 2004; McLoughlin *et al*, 2004). Haffner and co-workers (1998) showed that patients with Type 2 diabetes mellitus who have not had a myocardial infarction, have a risk of infarction similar to that among non-diabetic patients who have had a prior myocardial infarction and they suggest that cardiovascular risk factors in diabetic patients should be treated as aggressively as in non-diabetic patients who have had a myocardial infarction. The UK Prospective Diabetes Study group have shown that tight blood pressure control in patients with hypertension and Type 2 diabetes achieves a clinically important reduction in the risk of deaths related to

diabetes (UK Prospective Diabetes Study, 1998). However, the metabolic abnormalities may be aggravated by some classes of antihypertensive therapy. Long term thiazide administration has been shown to induce both hyperlipidaemia and glucose intolerance in susceptible individuals by various mechanisms, including a decrease in tissue sensitivity to insulin (Murphy *et al*, 1982; Samuelsson *et al*, 1994; Taylor *et al*, 1997; Taylor *et al*, 2006; Elliott *et al*, 2007), and a subnormal release of insulin from the pancreatic beta-cells, partially as a result of hypokalaemia (Heldman *et al*, 1983). Patients with primary hypertension have generally been found to have higher plasma glucose and insulin responses to an oral glucose challenge than do matched normotensive subjects. Insulin resistance, as measured by the euglycaemic clamp technique, has definitely been established in both obese and non-obese hypertensive subjects as well (Wing *et al*, 1994; Mankiardi *et al*, 1986; Reaven *et al*, 1996). However, it remains uncertain whether hypertension is causally linked to hyperinsulinaemia and insulin resistance or if these metabolic changes are primarily attributed to the adverse effects of the antihypertensive drugs utilised.

The 1999 World Health Organisation/International Society of Hypertension Guidelines for the Management of Hypertension (Guidelines Subcommittee, 1999) recommend the use of any of the six main classes of antihypertensive drugs as initial therapy, while the guidelines from the United Kingdom, New Zealand, Canada and the United States of America favour the use of low dose diuretics or ß-blockers as first line therapy (Swales, 1993) and remain the drugs of choice in 1999. Patient data from the HOT study (Hypertension Optimal Treatment) have

led investigators to believe that the improvement in cardiovascular mortality in the United States may be the result of an aggressive approach to antihypertensive therapy. In patients with diabetes mellitus there was a 51% reduction in major cardiovascular events in the subgroup <80 mmHg diastolic blood pressure compared with the subgroup <90 mmHg. The cardiovascular events observed in the HOT study during treatment initiated with a calcium antagonist were much lower than those observed in previous prospective trials with diuretic or ß-blocker initiated treatment, probably because of the deliberately pronounced lowering of blood pressure in this study (Hansson *et al*, 1998).

The 1997 6th report of the Joint National Committee on prevention, detection, evaluation and treatment of high blood pressure (JNC VI), states that most patients with established hypertension do not make sufficient lifestyle changes, do not take medication or do not take enough medication to achieve control. The goal of prevention and management of hypertension is to reduce morbidity and mortality by the least intrusive means possible. This is currently accomplished by achieving and maintaining systolic blood pressure below 140 mmHg and diastolic blood pressure below 90 mmHg, 130/85 mmHg in diabetes mellitus and lower if tolerated, while controlling other modifiable risk factors for cardiovascular disease. The cost of therapy should be an important consideration when selecting antihypertensive therapy. Long acting formulations that provide 24 hour efficacy are preferred to short acting agents. Combination drug therapy as first line, providing there are no specific indications for another drug, is a low dose diuretic

plus a ß-blocker; this is because numerous randomized controlled trials have shown a reduction in morbidity and mortality with these agents.

The Hypertension Society of Southern Africa Guidelines for the management of hypertension at primary care level in 2000 and 2006 recommended low dose thiazide as first line therapy. This has been the policy at the Johannesburg Hospital Hypertension Clinic since 1983.

1.2 Hypertension

Hypertension is the persistent elevation of arterial blood pressure. Kaplan (1998) gave an operational definition as the level of blood pressure at which the benefits (minus the risks and costs) of action exceed the risks and costs (minus the benefits) of inaction.

Elevated blood pressures are pressures that are abnormally high for the population. The relationship between arterial pressure and morbidity is a direct one – the higher the blood pressure, the worse the prognosis. Thus, the relationship between arterial pressure and the incidence of coronary heart disease, severity of changes in the retinal arteries, size of the heart and frequency of cerebral haemorrhage is linear for both diastolic and systolic pressure. It has long been known that arterial pressure is influenced by environmental factors and that blood pressure is raised by anger, fear, pain, cold and exercise. There is also evidence that it falls during sleep.

In order to reduce the risk of cardiovascular disease and associated morbidity and mortality, both the World Health Organisation (WHO, 2003), International Society of Hypertension Guidelines Subcommittee and the 7th report of the Joint National Committee on prevention, detection, evaluation and treatment of high blood pressure (JNC 7; 2003) define hypertension as a systolic pressure of 140 mmHg or greater and/or diastolic blood pressure of 90 mmHg or greater. Although the awareness of hypertension has increased over the last 20 years, the incidence of end stage renal disease and prevalence of heart failure have increased. The new guidelines for adults over the age of 18 years emphasize that the decision to lower the elevated pressure in a particular patient is not based on the level of blood pressure alone but on assessment of the total cardiovascular risk in that individual.

The WHO has used the terms Grade 1, 2 and 3 rather than the terms Stages 1 and 2, used by the JNC 7. The South African Hypertension Guideline 2006 has based its recommendations on the European Society of Hypertension/European Society of Cardiology Guidelines, but the pressures in each category are identical.

1.2.1 Classification of hypertension

Hypertension should be categorized on the basis of both severity and cause to facilitate diagnosis and therapy (Kaplan, 1998). There are two major types of hypertension, essential (primary) and secondary:

a) Essential hypertension is established by exclusion of identifiable secondary causes and accounts for 90 to 95% of all adult cases.

b) Secondary hypertension is where the cause is known and the condition is sometimes reversible.

Table 1: Classification of blood pressure in adults18 years and older

S A Guidelines	BP (mmHg)	
Category	**Systolic**	**Diastolic**
Normal	120-129	80-84
High-normal	130-139	85-89
Stage 1 (mild hypertension)	140-159	90-99
Stage 11 (moderate hypertension)	160-179	100-109
Stage 111 (severe hypertension)	> 180	> 110

The criteria shown in Table 1 are for individuals who are not taking antihypertensive medication and who have no acute illness. This classification is based on an average of two or more blood pressure readings taken at two or more visits after initial screening. When systolic and diastolic blood pressures fall into different categories, the higher category should be selected to classify the individual's blood pressure status. Optimal blood pressure with respect to cardiovascular risk is less than 120/80 mmHg; the higher the blood pressure, the greater the risk to the individual.

1.2.2 Risk stratification

The risk of cardiovascular disease in patients with hypertension is determined not only by the level of blood pressure, but also by the presence or absence of target organ damage or other risk factors. Factors influencing prognosis are shown in Table 2. Framingham study data indicates that a high risk of cardiovascular sequelae of hypertension is concentrated in those with dyslipidaemia, glucose intolerance, cigarette smoking, or left ventricular hypertrophy. About 40% of coronary events in men and 68% in women are attributable to the presence of two or more risk factors (Kannel, 2002; Gorton *et al*, 2004; Grundy *et al*, 2005). Five categories of absolute cardiovascular disease risk have been defined: normal, high normal, Stage 1, Stage 2 and Stage 3. Within each range the risk of an individual will be determined by the severity and number of risk factors present. When treating blood pressure it is essential that the patients' added cardiovascular risk be assessed accordingly.

Patients with Stage 3 (SBP $\geq$ 180 mmHg and/or DBP $\geq$ 110 mmHg may fall into one of three categories which determine the urgency of their treatment.

- Asymptomatic severe hypertension with or without evidence of target-organ damage or associated clinical conditions.
- Hypertension urgency. This level of hypertension is symptomatic, usually with severe headache, shortness of breath and oedema.
- Hypertension emergency. A hypertension emergency exists when acute elevation of blood pressure is associated with acute and ongoing organ

damage to the kidney, brain, heart, eyes (Grade 3 or 4 retinopathy) or vascular system.

Table 2: Major risk factors, target organ damage and associated clinical conditions

Major Risk Factors	Target-Organ Damage	Associated Clinical Conditions
• Levels of systolic and diastolic BP • Smoking • Dyslipidaemia Total cholesterol > 6.5 mmol/L LDL > 4 mmol/L HDL men < 1 women < 1.2 mmol/L • Diabetes mellitus Men > 55 years Women > 65 years • Family history of early onset of cardiovascular disease Men aged < 55 years Women aged < 65 Years • Waist circumference – abdominal obesity. Men ≥ 102 cm Women ≥ 88 cm The exceptions are South Asians and Chinese: Men > 90 cm Women > 80 cm	• Left ventricular hypertrophy: Based pm ECG • Microalbuminuria: Albumin/creatinine ratio 3.30 mg/mmol • Slightly elevated creatinine Men 115-133 µmol/L Women 107-124 µmol/L	• Coronary heart disease • Heart failure • Chronic kidney disease: Albumin/creatinine ratio > 30 mg/mmol • Stroke or transient ischaemic attack • Peripheral arterial disease • Advanced retinopathy haemorrhages or exudates Papilloedemia
Based on the European Society of Hypertension/European Society of Cardiology Guidelines		

1.2.3 Classification by degree

Malignant hypertension occurs at any age but is more frequently found in middle age than in the elderly. It is characterised by neuroretinopathy and by a rapid decline in renal function if left untreated. The pressure is usually very high. Unless the arterial pressure is reduced at an early stage, the condition is fatal within a few years. Causes of death include renal failure, left ventricular failure or cerebral haemorrhage. The classic pathology is fibrinoid arteriolar necrosis. Benign hypertension is usually found in older subjects in whom the pressures are not so severe. The individuals' condition remains relatively stable for years and death, if related to hypertension, is usually due to cardiac failure or stroke. The course of benign hypertension is often complicated by accelerated atherosclerosis resulting in coronary artery disease, cerebrovascular disease and peripheral vascular disease.

1.2.4 Pathogenesis of hypertension

The heart pumps a small quantity of blood with each beat. The systolic pressure is the highest point during the pressure cycle and the diastolic pressure is the lowest. The arterial pulse pressure is the pressure difference between the systolic and diastolic pressures. Stroke volume output of the heart and total peripheral resistance along with correct action of the large arteries are important factors affecting the pulse pressure in the arterial system. Correct arterial performance is the major factor in isolated systolic hypertension.

Mean blood pressure is an average of the arterial pressure taken during a complete pressure pulse cycle. The mean pressure determines the average rate at which blood will flow through the systemic vessels (Pickering, 1986). Mean arterial pressure = cardiac output (CO) x total peripheral resistance TPR. The pressure to move blood through the circulatory bed is provided by the pumping action of the heart. Each of these primary determinants is, in turn, determined by the interaction of the complex series of factors (Figure 1). In the early stages of hypertension, cardiac output is raised and peripheral resistance is normal. Blood pressure rises progressively with age. After the age of 50yrs, women have a higher systolic pressure than men. In women, the systolic blood pressure rises to approximately 160mm Hg between 70-75 yrs, it then decreases over the following decade to 150mm Hg, after which there is no difference between the sexes. Diastolic blood pressure rises with age until the age of 50-60yrs. After this, it tends to level off or even decrease slightly, with advanced age. However, the rise with age is not inevitable and is not seen in truly unsophisticated ethnic groups (Franklin, 1997). Over the years, peripheral resistance rises and cardiac output returns to normal and may fall below normal as cardiac complications arise (Figure 2). Increased total peripheral resistance is the haemodynamic feature of established essential hypertension (Figure 3). The walls of the small resistance arterioles contain smooth muscle cells which respond both to hormonal influences and to neural input through the autonomic nervous system. The contraction of vascular smooth muscle is thought to be determined by their intracellular free calcium content. Prolonged arteriolar vasoconstriction and increased pressure lead to the development of structural changes within the vessels such as

atherosclerosis. In the very large vessels, atherosclerosis is characterized by infiltration of cholesterol and appearance of foam cells in lesions of the walls of the arteries. These distort the vessels and make them rigid and cause below optimum performance. These changes may involve all three major components of the cell wall: endothelial cells, smooth muscle cells and fibroblasts. Hypertensive vessels are characterised by a thickened media, a reduced lumen diameter and increased matrix resulting in an increase in wall to lumen ratio. Narrowing of the lumen leads to an increase in peripheral resistance and a decrease in fitness of the vessel (Pickering, 1986).

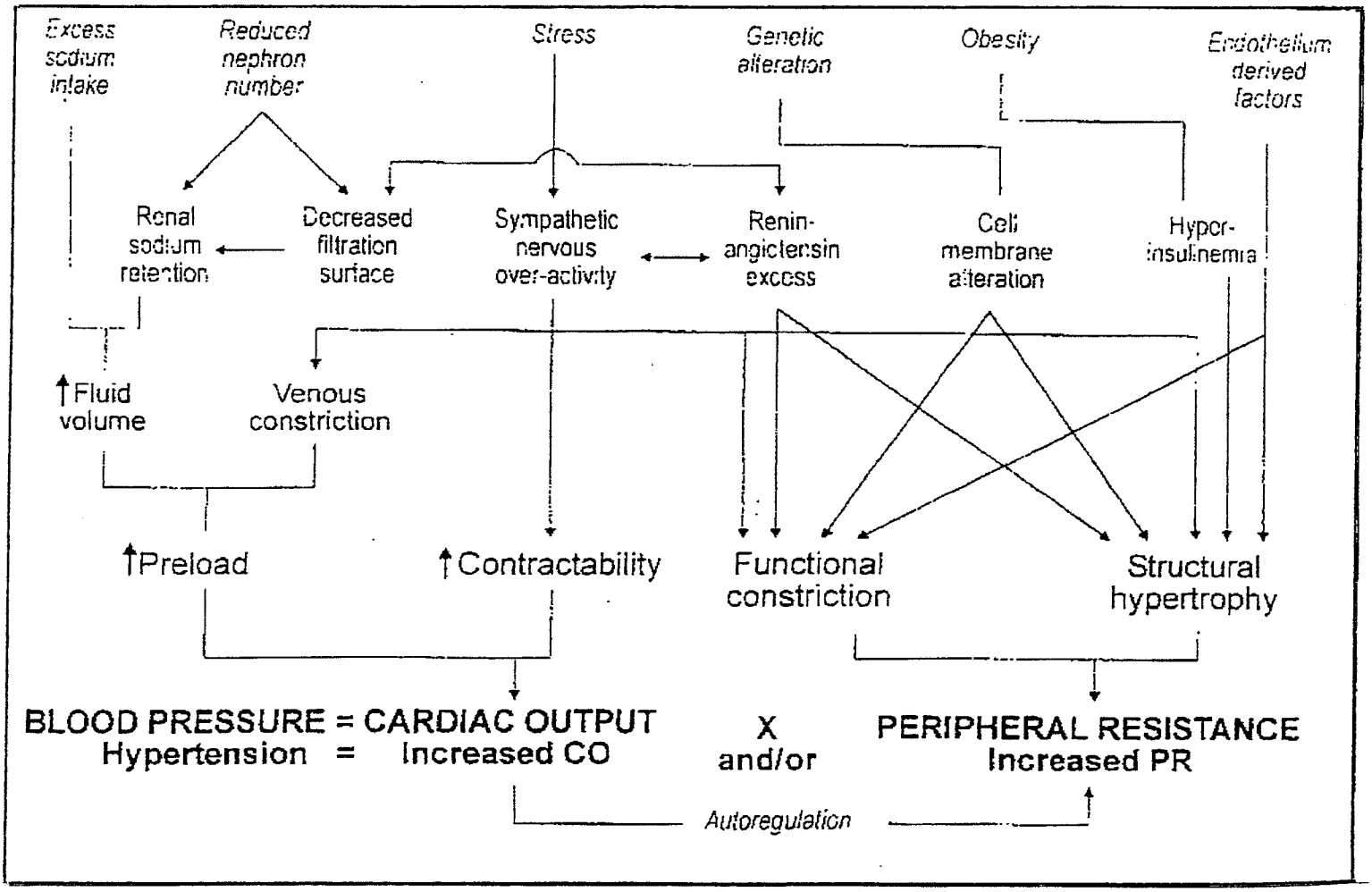


Figure 1: Primary hypertension pathogenesis (Kaplan, 1998).

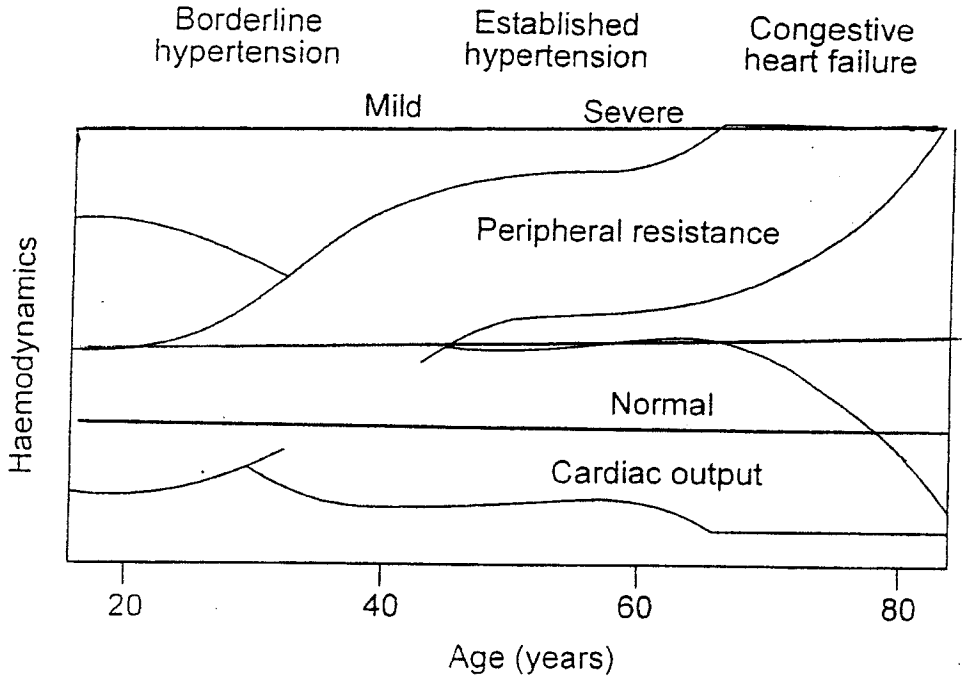


Figure 2: Changes in cardiac output and total peripheral resistance as hypertension progresses. R Touyz, personal communication.

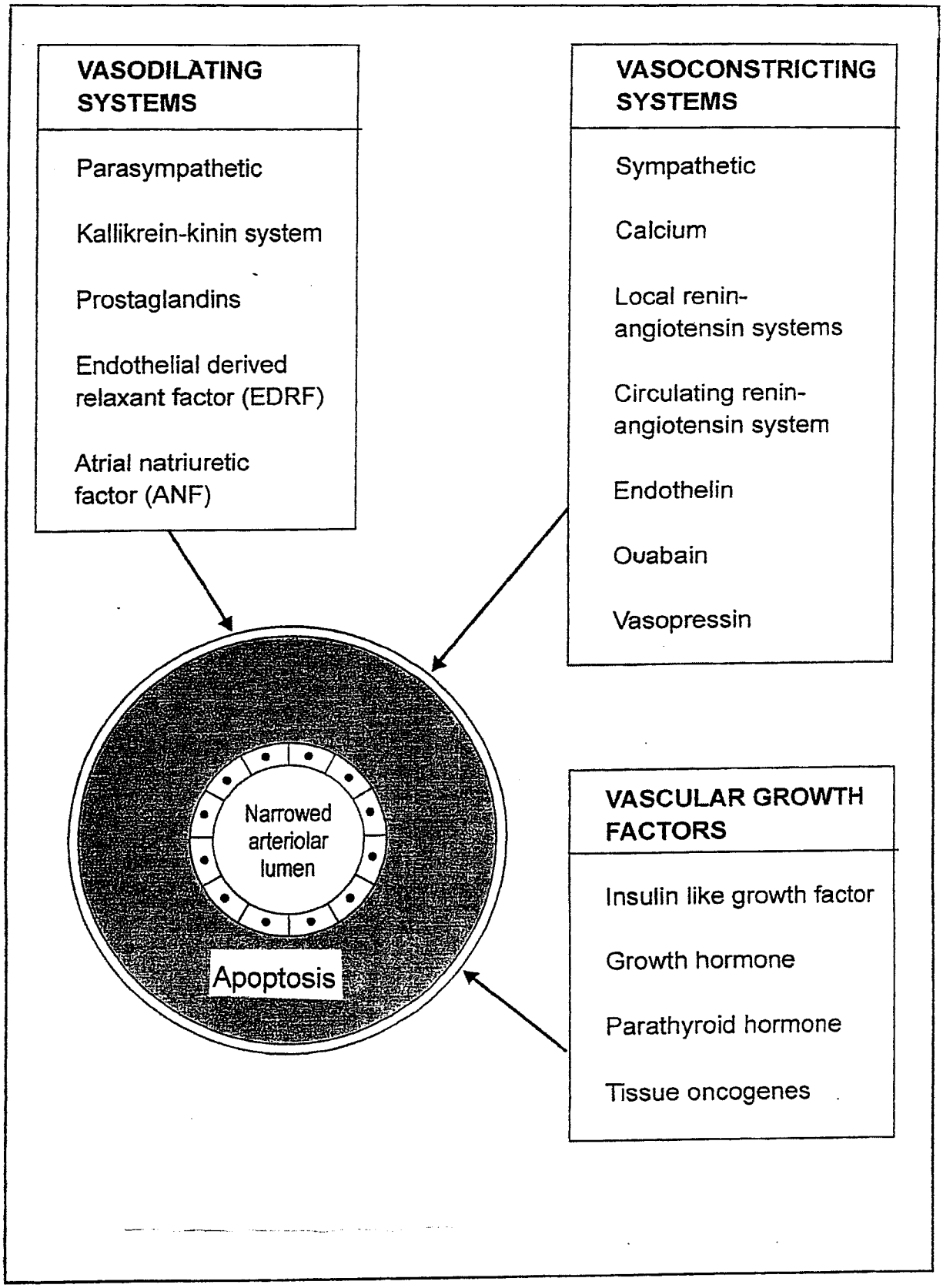


Figure 3: The control of peripheral arteriolar resistance.

1.2.5 The autonomic nervous system

There are other factors together with the autonomic nervous system plays an important role in short term variations of blood pressure in response to stress, exercise and changes in posture. Hypertension is a disease of the regulatory mechanisms which are responsible for maintaining normal pressure. These are: -

- nervous mechanisms that regulate arterial pressure by controlling the degree of arteriolar constriction;
- a capillary fluid shift mechanism which regulates arterial pressure by altering the blood volume;
- a kidney excretory mechanism which also regulates arterial pressure by altering the blood volume; and
- hormonal mechanisms that regulate either the blood volume or the degree of arteriolar constriction.

1.2.6 Sympathetic nervous system effects on the circulation

The vasomotor centre controls the circulation mainly through the sympathetic nervous system. Nerve impulses are transmitted down the spinal cord into the sympathetic chains and thence to the heart and blood vessels. These impulses increase the rate of the heartbeat and its strength of contraction which increase the arterial pressure. They also cause vasoconstriction of the blood vessels which increases the total peripheral resistance and, therefore, increases the arterial pressure. The parasympathetic system decreases the arterial pressure by decreasing the number of impulses transmitted to the blood vessels, allowing the

arterioles to dilate and reduce the pressure. When the vasomotor centre becomes ischaemic the arterial pressure rises. The baroreceptors in the arch of the aorta and the internal carotids detect the degree of stretch in the walls of the arteries by the pressure. When activated by a rise in blood pressure or central venous pressure, the baroreceptors normally reduce heart rate and lower blood pressure by vagal stimulation and sympathetic inhibition. With hypertension, the baroreceptors become less sensitive to the increased pressure. It has been suggested that sympathetic activation, whilst contributing to blood pressure elevation, can also have the adverse consequence of undesirable metabolic effects in skeletal muscle by impairing glucose delivery to muscle, causing insulin resistance and hyperinsulinaemia (Beck-Nielson, 1992) and in the liver by retarding postprandial clearing of lipids, contributing to hyperlipidaemia (Esler, 1990).

Figure 4 illustrates the renin-angiotensin-aldosterone system (RAAS) which plays a central role in the regulation of vasomotor tone, sodium retention and blood pressure. Renin is an enzyme secreted from the juxtaglomerular cells of the renal cortex, in response to hypovolaemia, hypotension and sympathetic activation. Renin cleaves the small peptide, angiotensin I, from angiotensinogen which is synthesized in the liver. Angiotensin I is then enzymatically transformed by angiotensin converting enzyme (ACE) in the pulmonary circulation and other vascular beds to angiotensin II. Angiotensin II is a potent vasoconstrictor which exerts its action by a direct effect on the brain and indirectly by increasing peripheral neurotransmission. In addition, angiotensin II promotes the activity of noradrenaline and is an important stimulus for the synthesis of aldosterone by the

adrenal cortex. Both aldosterone and angiotensin II regulate sodium and water balance.

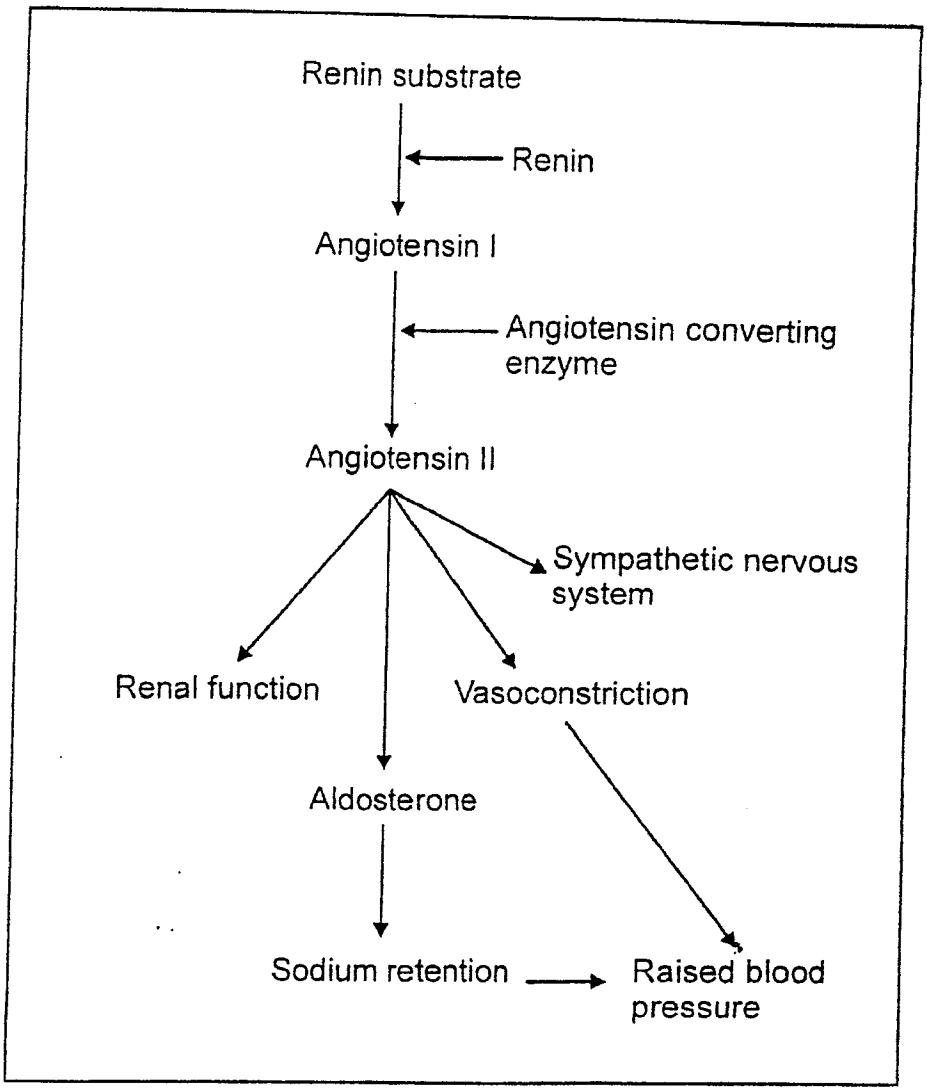


Figure 4: The rennin-angiotensin-aldosterone system (Beevers and MacGregor, 1987).

Angiotensin II is produced by a circulating, as well as a tissue rennin-angiotensin system; these systems are both up-regulated in cardiovascular disease. Endothelial cells, smooth muscle cells and plaque-laden macrophages express ACE, which may lead to increased production of angiotensin II at the tissue level (MacGregor, 1983). Recent studies indicate that components of rennin-angiotensin system (RAS) are expressed and produced by adipose tissue. Abdominally obese hypertensive subjects with the cardiovascular risk cluster exhibit a more active RAS since the local release of RAS components could influence adipose tissue perfusion and hormonal exposure, over activity of this system might play a role in the predisposition to insulin-resistant lipolysis (Hennes, 1996).

1.2.7 Sodium transport

There are at least three pumps that regulate sodium transport across cell membranes (Figure 5). The sodium-sodium counter transport pump, the sodium-potassium co-transport pump, and the ouabain-sensitive sodium potassium ATPase pump. These transport systems maintain concentrations of sodium, potassium, calcium and other ions across cell membranes. Intracellular sodium content of red and white cells is raised in many patients with essential hypertension. Blaustein's hypothesis (Figure 6), suggests that raised intracellular sodium can facilitate a rise in intracellular calcium which, in turn, could cause increased excitability of smooth muscle cells which would lead to constriction of peripheral arteriolar smooth muscle. Abnormalities of the physical properties of

the membrane or of one or more of the transport systems have been implicated in the pathogenesis of hypertension.

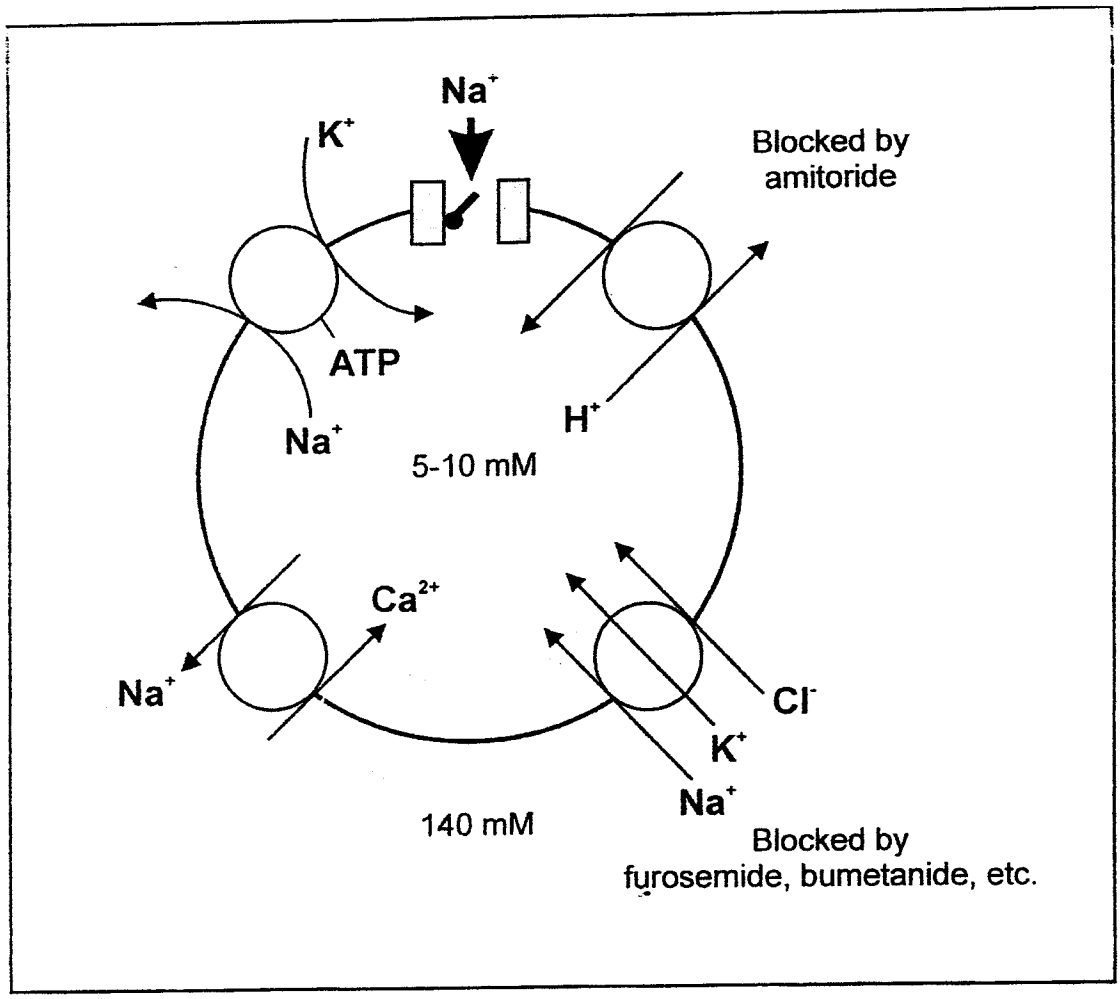


Figure 5: Sodium transport systems. *Clockwise* from the top, these include the sodium channel, Na^+/H^+ antiport exchange, Na-K contransport, Na^+-Ca^{2+} exchange and the Na^+-K^+ ATPase pump (Lazdunski, 1988).

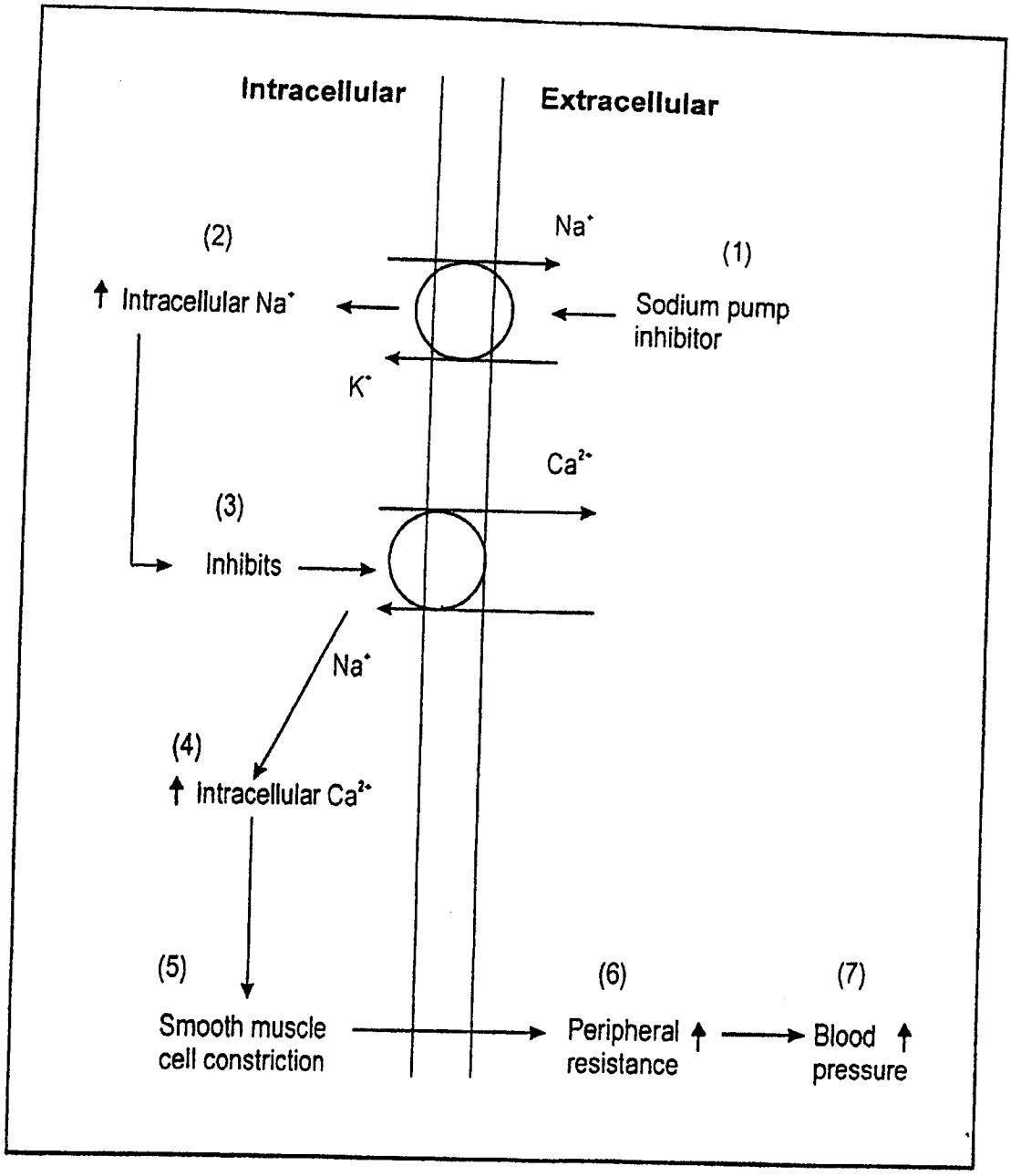


Figure 6: Blaustein's hypothesis (Kaplan, 1998).

1.2.8 The Na^+-K^+-ATPase pump

The Na^+-K^+-ATPase sodium pump regulates the rate of sodium entry into cells. The activity of the pump is stimulated by an increase of sodium on the inside or of potassium on the outside of the cell membrane and is inhibited by cardiac glycosides. Failure of this pump results in increased myocardial contractility and vasoconstriction and enhances responsiveness of blood vessels to vaso-active agents.

1.2.9 Genetic factors

It is unclear to what extent either genetic or environmental factors determine the development and/or the severity of hypertension, but almost certainly both play a major role. The complexity of blood pressure regulation suggests that there is substantial genetic heterogeneity. Individuals with the same blood pressure level do not necessarily have the same genotype at relevant loci, nor do individuals with the same genotype at particular loci necessarily have the same blood pressure level. Despite successes in identifying genes for rare Mendelian forms of hypertension, progress towards identifying and characterizing genes contributing to hypertension in the general population has been much more modest (Turner, 2000).

1.2.10 Environmental factors

Body fat and blood pressure are positively correlated. This relationship is not restricted to the obese but is continuous throughout the entire range of fat distribution. Most studies have documented a stepwise increase in blood pressure

with progressively higher levels of body weight. A two to six fold increase in the risk of developing hypertension is associated with being overweight (Whelton *et al*, 2002).

There is also a close relationship between the incidence of hypertension and salt intake. Sodium restriction to less than 100 mmol/24 hrs has been found to be beneficial in hypertension and may reduce the need for drug therapy. Potassium supplementation has a small blood pressure lowering effect, but the effects of calcium and magnesium supplementation on blood pressure are not conclusive (JNC 7, 2003). Vegetarians have a lower blood pressure and less of the age related increase compared with meat eaters. A high intake of dietary fibre and fish oils and increasing the polyunsaturated to saturated fat ratio may also lower blood pressure.

Epidemiological studies have shown a direct relationship between alcohol consumption and blood pressure. The effect of alcohol on blood pressure is seen in both sexes and in all races. The effect is age dependent and appears to be independent of, but additive to, obesity and the use of oral contraceptives (Whelton *et al*, 2002).

Blood pressure may rise sharply after one or two cigarettes. However, despite this acute effect, epidemiological studies show no long term correlation between blood pressure and cigarette smoking. Cigarette smoking does, however, increase the risk of CHD and ischaemic stroke at all ages (WHO/ISH Management of

Hypertension, 2003). Regular physical activity has a protective effect against blood pressure elevation and reduces the risk of CHD. The investigation of environmental stress and high blood pressure is confounded by other social factors including poverty, dietary fat, alcohol and cigarette smoking. However, high blood pressure is the disease of high pressure societies and many studies have shown higher pressures in urban migrants than in populations of rural origin (Figure 7).

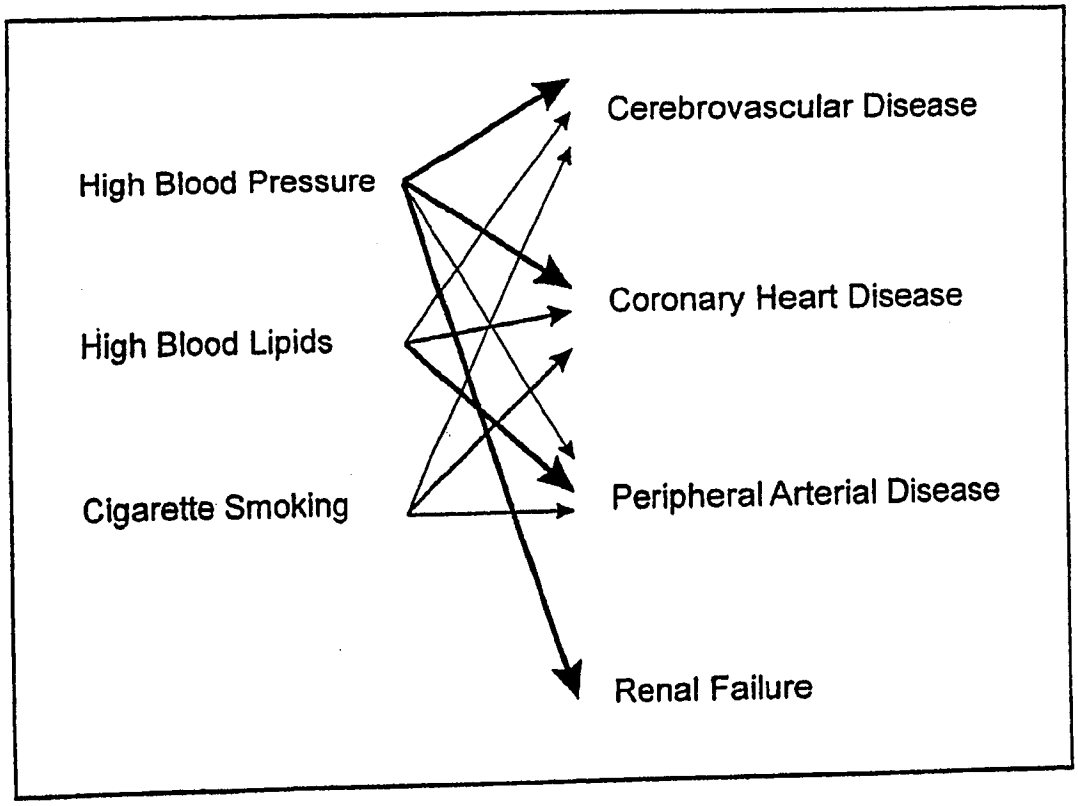


Figure 7: The three major modifiable cardiovascular risk factors and their disease manifestations (line thickness denotes strength of association (Beevers and MacGregor, 1987).

Major complications of hypertension include cardiac, renal, cerebral and aortic disease. A persistently elevated blood pressure results in an increased workload in the heart causing left ventricular hypertrophy. Left ventricular hypertrophy has been identified as the most powerful risk factor for future cardiovascular events, including myocardial infarction, congestive heart failure and sudden death (Amery & de Schaepdryver, 1973). Hypertensive heart disease can present as cardiac failure or myocardial infarction.

Hypertensive encephalopathy is a hypertensive emergency, presenting with headaches, altered levels of consciousness and fits, and is due to dilated cerebral arterioles with over perfusion of the brain and cerebral oedema. High blood pressure is one of the most important risk factors for both ischaemic and haemorrhagic stroke. Even modest blood pressure reduction in hypertensive people (5-6 mmHg) could reduce 50% of the stroke events worldwide (WHO Report, 2003). Large vessel, especially aortic aneurysms are more common in hypertensives that also have an increased risk of developing aortic dissection. Renal complications of hypertension include arteriolar nephrosclerosis, fibrinoid necrosis, renal insufficiency and acute or chronic renal failure. Peripheral vascular disease is complicated by stenosis and aneurysms. The most common clinical presentation resulting from arterial stenosis is intermittent claudication.

1.3 DIABETES MELLITUS

Diabetes mellitus is a group of metabolic diseases characterised by hyperglycaemia, resulting from defects in insulin secretion, insulin action or both (Figure 8).

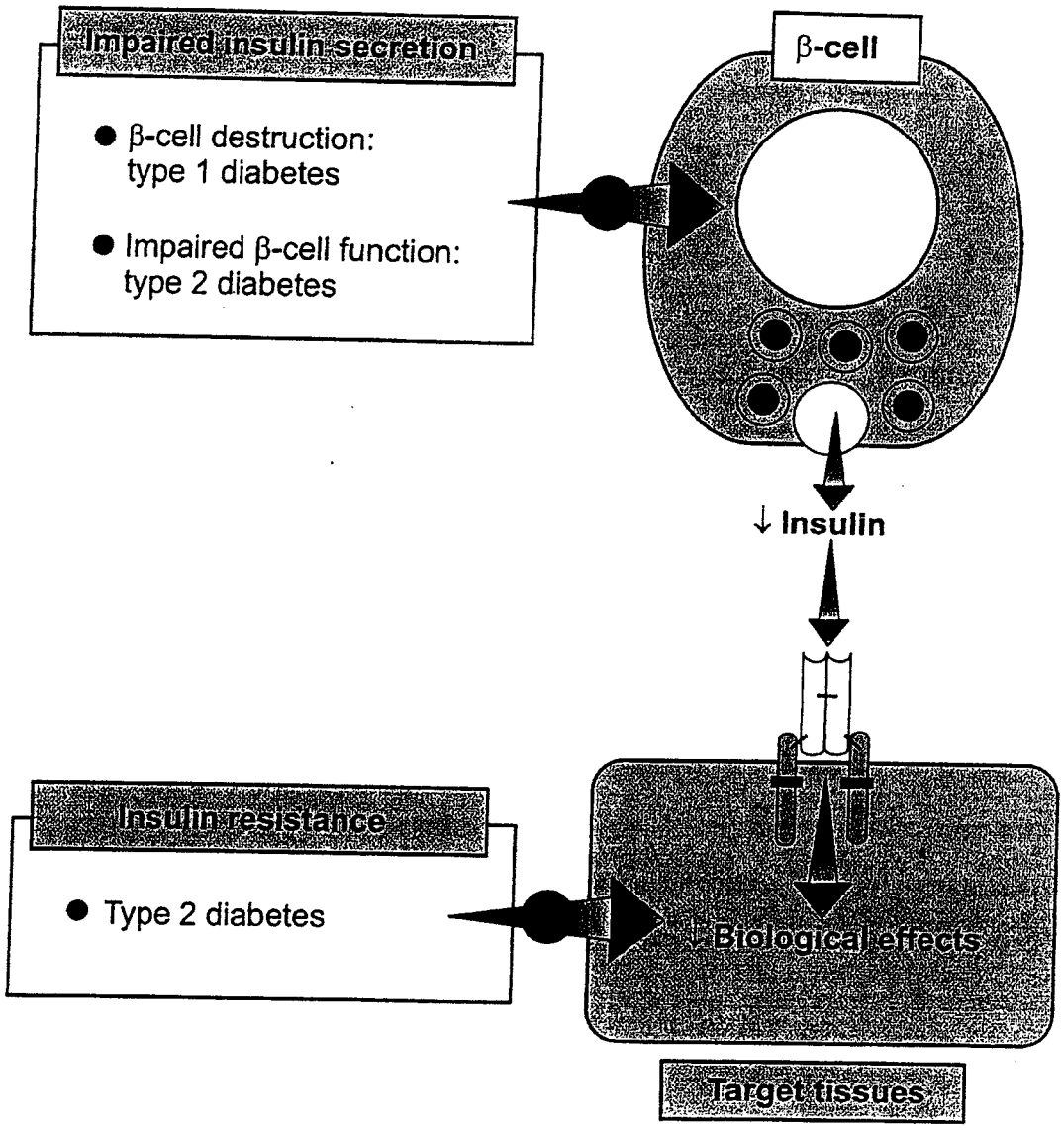


Figure 8: Defects in islet β-cell and in insulin action in Type I and Type II Diabetes (Williams and Pickup, 1999)

1.3.1 Aetiological classification of diabetes

Four main aetiological categories of diabetes have been identified.

- Type I diabetes results from a cell-mediated autoimmune destruction of the ß-cell which is variable in degree. The destruction is rapid in children and infants and slower in young adults. Some patients, particularly children and adolescents, present with ketoacidosis as the first manifestation of the disease. There is little or no insulin secretion as manifested by low or undetectable levels of plasma C-peptide and these individuals are dependent on insulin for survival.
- Type 2 diabetes is the most common form of diabetes and results from an imbalance between insulin sensitivity and insulin secretion. The earliest detectable abnormality in Type 2 diabetes is impairment in the body's ability to respond to insulin. Glucose tolerance remains normal as the pancreas is able to appropriately augment its secretion of insulin to offset the insulin resistance. With time however, the ß-cell fails to maintain its high rate of insulin secretion and the relative insulinopaenia leads to the development of impaired glucose tolerance and eventually overt diabetes mellitus. Insulin resistance in Type 2 diabetes involves both hepatic and peripheral muscle tissue. In the post absorptive state, hepatic glucose output is normal or increased, despite the presence of fasting hyperinsulinaemia, whereas the efficiency of tissue glucose uptake is reduced (De Fronzo *et al*, 1992). The United Kingdom Prospective Diabetes Study (UKPDS, 1998) has shown that Type 2 diabetes mellitus is a progressive disorder that can be treated

initially with oral agents. However, many patients will need insulin therapy to achieve target glycaemic levels.

- Maturity Onset Diabetes in the Young (MODY) is a variant of non insulin dependent diabetes mellitus (NIDDM). It is defined as hyperglycaemia diagnosed before the age of 25 years and treatable for at least five years without insulin in patients who do not have markers of Insulin Dependant Diabetes Mellitus (IDDM). MODY is generally inherited as an autosomal dominant gene and is more common in black races.
- Other types of diabetes mellitus are associated with well-defined conditions such as chronic pancreatitis, endocrine disorders and drug administration (Table 3).

Table 3: Some other causes of diabetes

Gestational Diabetes

Diabetes due to pancreatic disease

- Chronic or recurrent pancreatitis
- Haemochromatosis

Diabetes due to other endocrine disease

- Cushing's syndrome
- Acromegaly
- Phaeochromocytoma
- Glucagonoma

Diabetes due to drugs and chemicals

- Glucocorticoids and corticotrophin
- Diuretics
- B-blockers

Diabetes due to abnormalities of insulin or its receptor

- Insulinopathies
- Insulin receptor defects
- Circulating anti-receptor antibodies

Diabetes associated with genetic syndromes

- Wolfram syndrome
- Lipoatrophic diabetes
- Cystic fibrosis

1.3.2 Criteria for the diagnosis of diabetes mellitus

The following are the recommendations of the World Health Organisation (WHO) (2006).

- Normal glucose regulation (NGR) criteria should be <6.1 mmol/l plus 2hr plasma glucose <7.8 mmol/l.

- The American Diabetes Association (ADA) 2003 recommends that fasting plasma glucose be lowered to <5.6 mmol/l (Table 4).

The WHO recommendations for normal glucose regulation are based on measuring both fasting and 2hr post-load glucose concentrations. They also recommend that a standardized 75g oral glucose tolerance test (OGTT) should be performed in the absence of overt hyperglycaemia.

Table 4: Criteria used for normal glucose metabolism classification from WHO (2006) and ADA (1997 and 2003)*

Category	Source	Classification Criteria mmol/l
Normal glucose regulation (NGR)	WHO ADA (1997) ADA (2003)	FPG<6.1 + 2h PG < 7.8 FPG < 6.1 FPG < 5.6
Impaired fasting glucose (IFG)	WHO ADA (1997) ADA (2003)	FPG ≥ 6.1 and < 7.0 + 2h PG< 7.8 FPG ≥ 6.1 and < 7.0 FPG ≥ 5.6 and < 7.0
Impaired glucose tolerance (IGT)	WHO	FPG < 7.0 + 2h PG ≥ 7.8 and < 11.1
Impaired glucose homeostasis (IGH)	WHO	IFG or IGT
Diabetes Mellitus (DM)	WHO ADA (1997) ADA (2003)	FPG ≥ 7.0 or 2h PG ≥ 11 FPG ≥ 7.0 FPG ≥ 7.0

*FPG = fasting plasma glucose

**Adapted from ESC Guidelines, 2007.

1.3.3 Major risk factors for Type 2 Diabetes

- Family history of diabetes
- Obesity (> 20% over ideal body weight)
- Ethnic origin
- Age > 40 years plus any of the first 3 shown above
- Previously identified impaired glucose tolerance
- In pregnant women, a history of gestational diabetes or delivery of babies > 4.5 kg
- Hypertension
- Significant hyperlipidaemia
- Increased percentage of body fat distributed predominantly in the abdomen

Type 2 diabetes frequently goes undiagnosed for many years because the hyperglycaemia develops gradually and in the early stages may not be severe enough to produce symptoms. However, these patients are at risk of developing both macrovascular and microvascular complications. Diabetes is a common disease in older people with almost 50% of Type 2 diabetic patients being over 60 years of age (Morley, 1998). Diabetes may be difficult to diagnose in the elderly because of its often atypical asymptomatic presentation. Polyuria or polydipsia are not present in many elderly diabetics because the glomerular filtration rate and thirst threshold decline with age, while the renal threshold for glycosuria increases.

Ketoacidosis seldom occurs spontaneously and is only seen in association with the stress of other illnesses such as infection. Symptoms in the elderly diabetic are usually non-specific, such as weakness, fatigue and weight loss or frequent minor infections. They may also present with neurologic findings such as cognitive impairment, acute confusion or depression (Figure 9).

1.3.4 Complications

The microvascular complications of diabetes include retinopathy, neuropathy and nephropathy. The lesions of retinopathy can be grouped into those associated with background retinopathy which is characterized by capillary dilatation, capillary occlusion, micro-aneurysms, blot haemorrhages and hard exudates. Pre-proliferative retinopathy in which there are 'cotton-wool' spots indicating retinal ischaemia and venous and arterial abnormalities. Proliferative retinopathy is when new vessels arise in the periphery and/or on the optic disc causing visual complications by vitreous haemorrhage and subsequent retraction and detachment of the retina. The presence of retinopathy increases with the duration of diabetes but retinopathy can be present in the first few years of diabetes. Diabetic neuropathy is classified as either reversible or established. Neuropathy may develop acutely during periods of poor glycaemic control and often tends to resolve when glycaemic control is improved. Diabetic nephropathy is defined by persistent albuminuria, declining glomerular filtration rate and rising blood pressure. Albuminuria in diabetic nephropathy is due to glomerular capillary damage and reflects generalized damage to the microcirculation and large vessels.

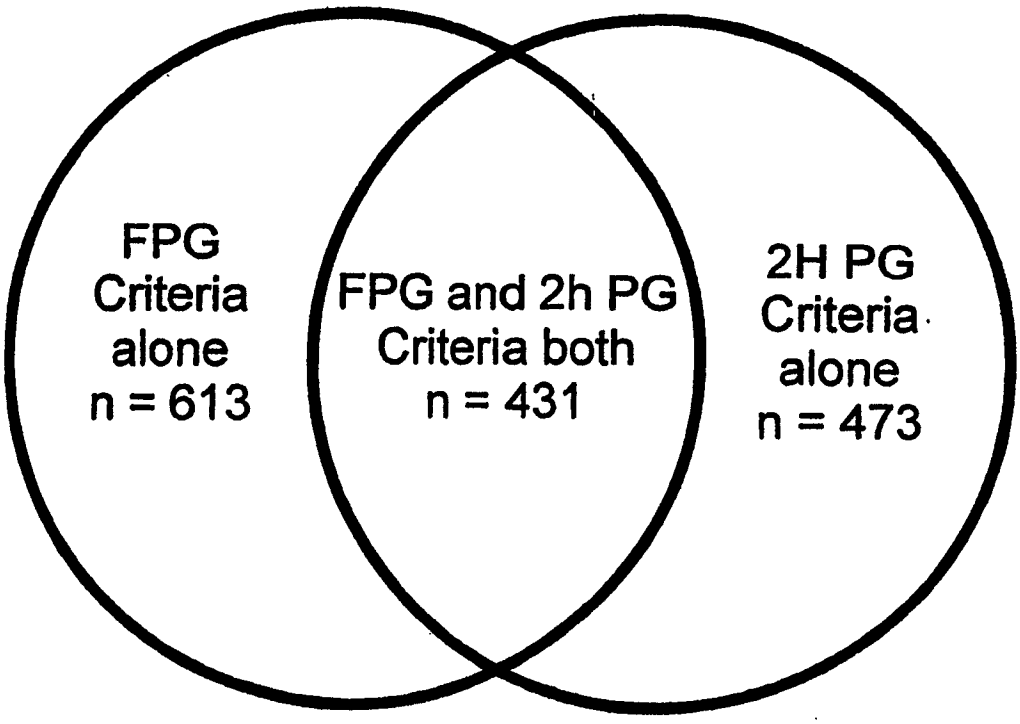


Figure 9: Fasting and post-load glucose levels identify different individuals with asymptomatic diabetes (Decode Study Group, 1998).

Autonomic neuropathy may evolve through defects in thermoregulation and sweating in the legs, followed by impotence and bladder dysfunction to cardiovascular reflex abnormalities, postural hypotension and reduced awareness of hypoglycaemia. Microvascular disease is an important cause of morbidity in diabetes, but diseases of the larger arteries and the heart are responsible for well over half the deaths in diabetic patients. Macrovascular disease or atherosclerosis accounts for up to 80% of all diabetic mortality (Franco et al, 2007).

1.3.5 Heredity

A familial tendency exists in both Type 1 and Type 2 diabetes. However, genetic factors are probably more important in those who develop diabetes after the age of 40 years (Panz *et al*, 1997). The mother has been found to be a more important conduit for inheritance of diabetes than the father. Glucose tolerant offspring of two Type 2 parents were found to have hyperinsulinaemia and manifest all the metabolic abnormalities that characterise the fully established state with the exception of insulin deficiency (Gulli, 1992).

1.3.6 Impaired glucose tolerance (IGT)

The term impaired glucose tolerance refers to a metabolic stage intermediate between normal blood glucose homeostasis and frank diabetes. The degree of hyperglycaemia may change over time depending on the extent of the disease process thus causing impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) without reaching the criteria for the diagnosis of diabetes mellitus (DM). The severity of the metabolic abnormality can progress, regress or stay the

same. Many individuals with IGT are euglycaemic and may have normal or near normal glycated haemoglobin levels. Hyperglycaemia will only manifest itself when challenged with the oral glucose load used in the standard OGTT.

IGT is a risk factor for future diabetes and cardiovascular disease and is associated with the insulin resistance syndrome which includes insulin resistance, compensatory hyperinsulinaemia to maintain glucose homeostasis, obesity (especially abdominal or visceral obesity), dyslipidaemia of the high triglyceride and/or low HDL cholesterol type and hypertension (Alberti *et al*, 2005).

1.3.7 Testing for diabetes in undiagnosed asymptomatic individuals

There is epidemiological evidence that retinopathy begins to develop at least 7 years before the clinical diagnosis of Type 2 diabetes (UKPDS, 1998). Undiagnosed diabetes is a serious condition because hyperglycaemia in Type 2 diabetes causes microvascular diabetes and may cause, or contribute to, macrovascular disease. Thus, early detection and, consequently, early treatment might well reduce the burden of Type 2 diabetes and its complications. The Expert Committee on diabetes mellitus have suggested criteria for screening, which includes individuals over the age 45 years and hypertensives (>140/90 mmHg). (Table 5 shows the full list).

Table 5: Recommended criteria for screening asymptomatic individuals

(a) Testing for diabetes should be considered in all individuals aged 45 years and over and, if normal, it should be repeated at 3 yearly intervals.

(b) Testing should be considered at a younger age or be carried out more frequently in individuals who:

- are obese (BMI > 27 kg/m^2)
- have first degree relatives with diabetes
- are members of a high-risk ethnic population
- have delivered a baby weighing >4.5 kg or have been diagnosed with gestational Diabetes Mellitus
- are hypertensive (BP > 140/90 mmHg)
- have an LDL cholesterol <0.9 mmol/l and/or a triglyceride level =2.5 mmol/l
- if have had impaired glucose tolerance or fasting glucose previously

1.3.8 Predicted increase in prevalence of Diabetes Mellitus

Many non-communicable diseases are predicted to increase dramatically during the 21st Century. The prevalence of diabetes mellitus world wide is predicted to double from 124 million people in 1997 to 220 million by the year 2010 (Amos,1997). Regions with the greatest potential increase are Africa and Asia. These are also the areas with the greatest potential increase in HIV infection and AIDS according to the United Nations Population Division (UNPD, 1999). Panz and

Joffe (1999) assessed the impact of HIV/AIDS on the projected prevalence of Type 2 diabetes for South Africa in the year 2010 by using the population data from the United Nations Population Division (1999) and the estimate in the increase of diabetes mellitus by Amos (1997). The highest age specific prevalence of HIV infection is forecast for those aged 20-34 years and the increase estimated for Type 2 diabetes will probably occur mainly in those aged 50-59. When the effect of HIV/AIDS on population growth is taken into account, the reduction of 30% is substantial and represents over 100 000 fewer cases of Type 2 diabetes by the year 2010 in South Africa alone. These predictions highlight the need to adjust for HIV/AIDS when projecting the increased prevalence of chronic disease and health budgets. Diabetes is an expensive disease, both for the patient and the health care provider, placing a substantial financial burden on health care resources.

1.3.9 The cost of Diabetes

The emotional and social impact of diabetes and the demands of therapy may cause significant psychosocial dysfunction in patients and their families. To accept their new state of health implies that the patients must mourn their previous state of health. The emotional reactions connected with the mourning process can be shock, fear, anxiety, anger, protest, sadness, hope. When patients finally accept their condition, they feel more relaxed.

With the onset of diabetes some people may feel restricted in terms of their professional choices and are faced with the dilemma of whether or not to inform employers or potential employers of their condition. For example, they may not get

the job; they could possibly be transferred to another department; they could lose the job altogether or they could be considered an invalid.

Diabetes affects families in different ways. Certain people may try to conceal their diabetes from close relatives or, alternatively, gain advantages as a result of the illness. The patient's partner may not accept the condition or could assume the role of guardian controlling and forbidding certain things. Diabetes modifies the self image.

In general, people are ignorant of the disease of diabetes and have adverse reactions to the patient's state as a result of this lack of knowledge. This can lead to feelings of isolation and general withdrawal from social life by the patient who may fear becoming hypoglycaemic in public. The restraints caused by lifestyle changes make the patient feel different from other people and embarrassed, especially when using medications, e.g. glycaemic products and injections.

Even if the majority of medical costs are covered by the state or insurance companies, there are still many items which are not reimbursed; for instance hi-tech equipment or equipment such as crutches or wheelchairs. There is also the cost of extra visits to the hospital (Lacroix, 1995). It is essential that administrators and those who pay for health care understand the benefits of early preventative strategies in the management of chronic conditions such as diabetes. Only then will resources be allocated realistically (Table 6).

Table 6: The cost of diabetes

The Cost of Diabetes	
Health	**Psychosocial**
Heart Disease	Psychological
Kidney Failure	Professional
Blindness	Family Life
Neuropathy	Social
Peripheral vascular disease	Financial
Foot lesions	
Gangrene	
Amputation	
Impotence	
Problem pregnancy	

1.4 INSULIN

1.4.1 Molecular structure

Insulin is a protein consisting of 51 amino acids contained within two peptide chains. An 'A' chain with 21 amino acids and a 'B' chain with 30 amino acids. The chains are connected by two disulfide bridges (Figure 10).

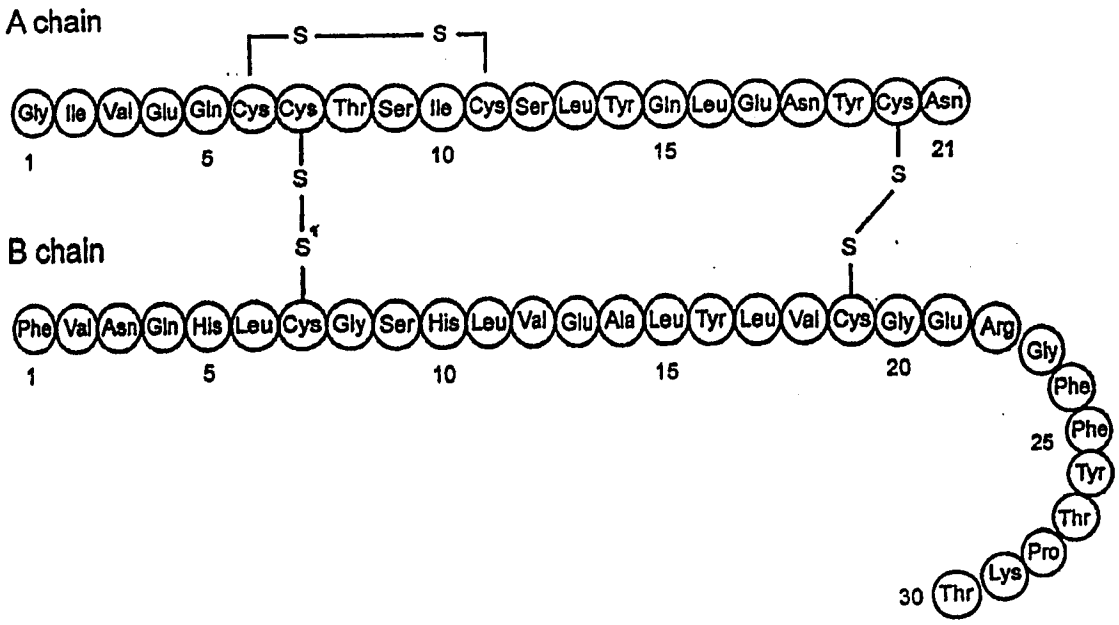


Figure 10: Molecular structure of insulin (Williams and Pickup, 1999).

The human pancreas secretes the equivalent of 40 - 50 units of insulin each day in normal adults. The basal concentration of insulin in the blood of fasting humans averages 10 μU/ml and seldom rises above 100 μU/ml after a standard meal. There is an increase in peripheral insulin concentration beginning 8-10 minutes after ingestion of food and reaching peak concentration in peripheral blood by 30-45 minutes. This is followed by a rapid decline in postprandial plasma glucose concentration which returns to baseline values within 90-120 minutes.

Pro-insulin is the single chain precursor of insulin. Cleavage occurs on the ß-chain and peptide chain at position 32/33 and a second cleavage then takes place at position 65/66 on the 'A' chain. The responsible enzymes are two separate endopeptides with trypsin-like specificity: Endopeptidase I cleaves at 32/33 and endopeptidase II at the 65/66 position. Carboxypeptidase then removes two arginine molecules ARG 31/32 and LYS ARG 64/65 creating another two intermediates. Split forms 32/33 and 65/66 are rapidly converted to the des diamino forms. These forms are further converted to insulin and C peptide which are secreted in equimolar quantities from the ß-cell into circulation. Pro-insulin and split pro-insulin contain the structures of both insulin and C peptide (Figure 11).

In normal glucose tolerance, pro-insulin like molecules account for only 10-20% of the immunologically active insulin-like molecules in the plasma but, in diabetes, they are present in higher concentrations. Their biological activity is much lower than that of insulin and their measurement as insulin may lead to overestimations of true serum insulin concentrations (Temple *et al*, 1992).

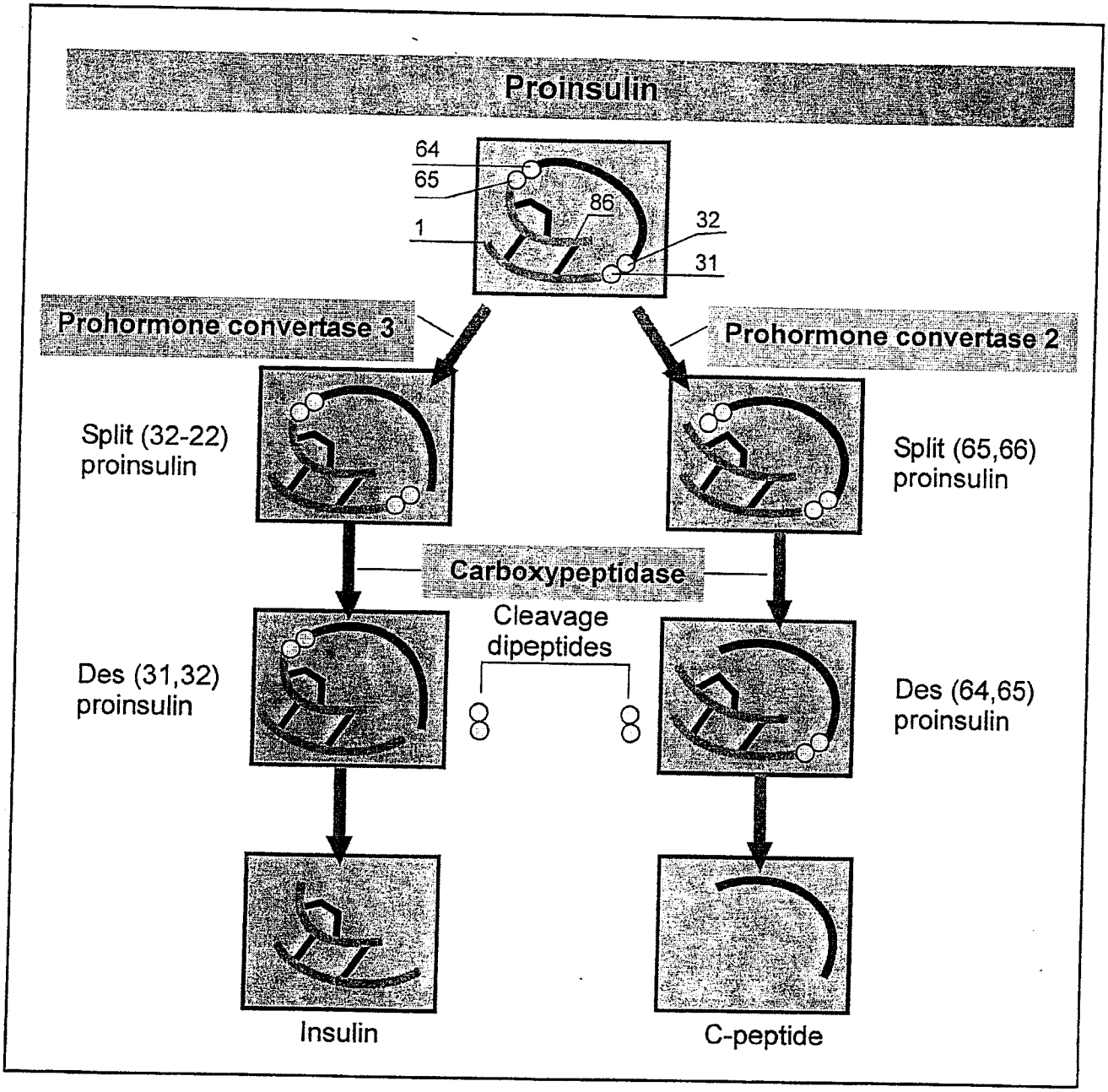


Figure 11: Insulin biosynthesis and processing (Williams and Pickup, 1999).

1.4.2 Insulin resistance

Insulin resistance has been defined as a state in which normal amounts of insulin produce a subnormal biological response (Krentz, 1997). Insulin resistance can be both inherited and/or acquired.

Impairment of intrinsic tyrosine kinase domain activity in the insulin receptor has been reported in patients with Type 2 diabetes and also in non-obese normoglycaemic patients with insulin resistance (Krentz, 1997). Polymorphisms have also been detected in South African non-diabetic black and white subjects (Panz *et al*, 1996).

Tissue insulin resistance and defective ß-cell function in Type 2 diabetes may be aggravated by overactivity of the hexosamine pathway as a result of raised blood glucose concentrations. In addition, raised plasma concentrations of non-esterified fatty acids antagonise the uptake and oxidation of glucose in skeletal muscle through the glucose-fatty acid cycle; gluconeogenesis is accelerated exacerbating the hyperglycaemia and raised fatty acid concentration and may also contribute to hypertriglyceridaemia (Krentz, 1997).

The association between elevated insulin concentrations and various metabolic and physiological abnormalities, including hypertension, dyslipidaemia and glucose intolerance, has been highlighted by several epidemiological and clinical studies. These disorders are all characterised by a state of insulin resistance (Davies *et al*, 1993; O'Rahilly *et al*, 1994; Opie, 2007) (Figure 12).

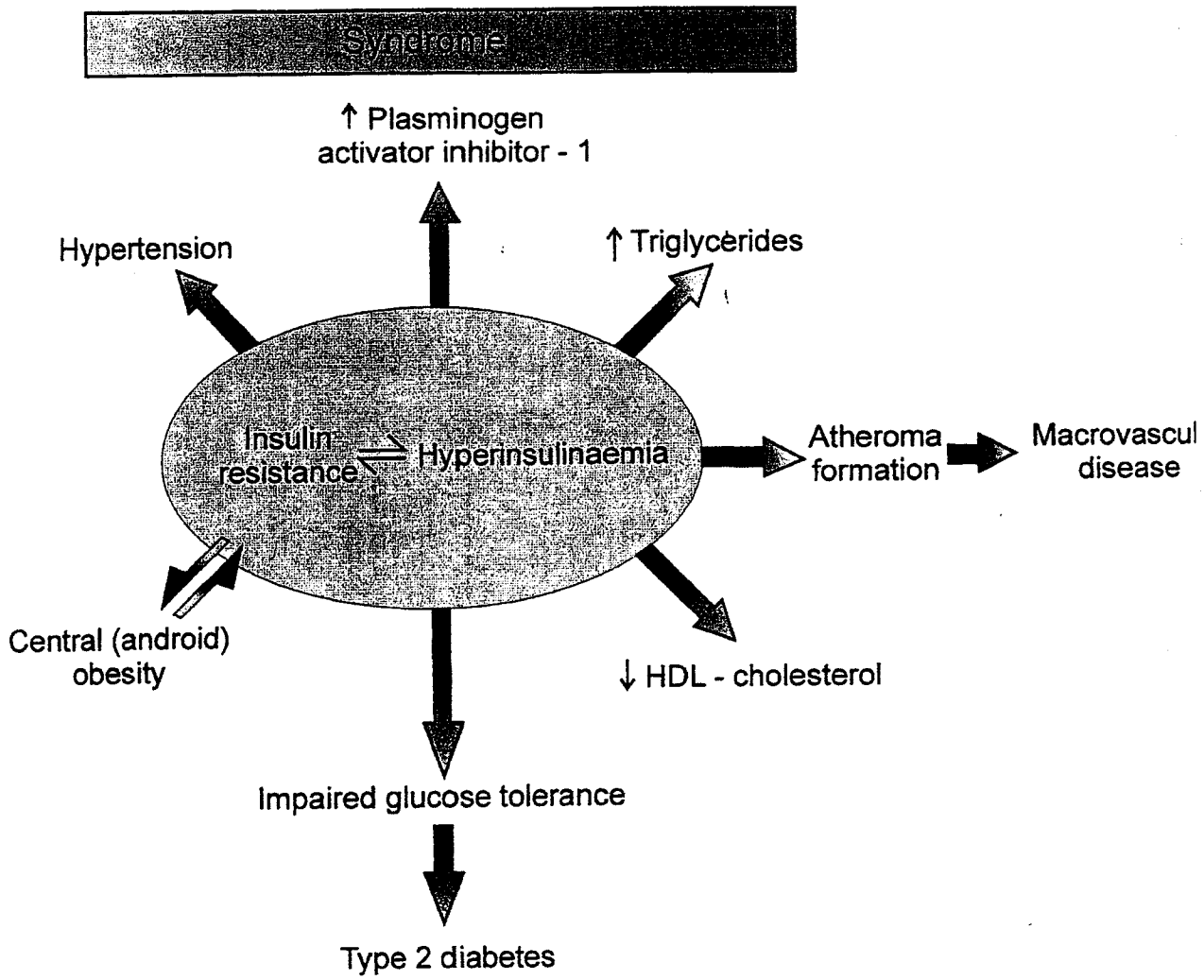


Figure 12: The insulin resistance syndrome (Williams and Pickup, 1999).

1.4.3 The role of insulin in the pathogenesis of hypertension

Insulin can raise blood pressure by:-

- Increasing sodium retention. Insulin has an antinatriuretic effect on the kidneys. This results in an increase in extracellular volume which produces volume dependent hypertension.
- Insulin can stimulate the activity of the sympathetic nervous system. An increased concentration of noradrenalin leads to an increased heart rate, vasoconstriction, sodium retention and elevated blood pressure. The mechanisms by which insulin stimulates the sympathetic nervous system are unknown.
- Insulin also affects the transport of cation ions across the cell membrane. These include sodium, potassium, calcium and magnesium. An increased sodium concentration in the endothelial cell can cause swelling and thus reduce the vascular lumen.

Manicardi and co-workers (1986) reported hyperinsulinaemia in obese patients with hypertension and a strong positive relationship between plasma insulin concentration and blood pressure. This study also revealed a high incidence of impaired glucose tolerance in hypertensive subjects with a plasma insulin response that was three fold greater than in the control group of normotensive subjects. Insulin resistance has also been established in non-obese hypertensive subjects. Thus, irrespective of weight, hypertension is associated with hyperinsulinaemia and insulin resistance (Pollare *et al*, 1990; Ferrannini, 1992; McLoughlin *et al*, 2004) (Figure 13).

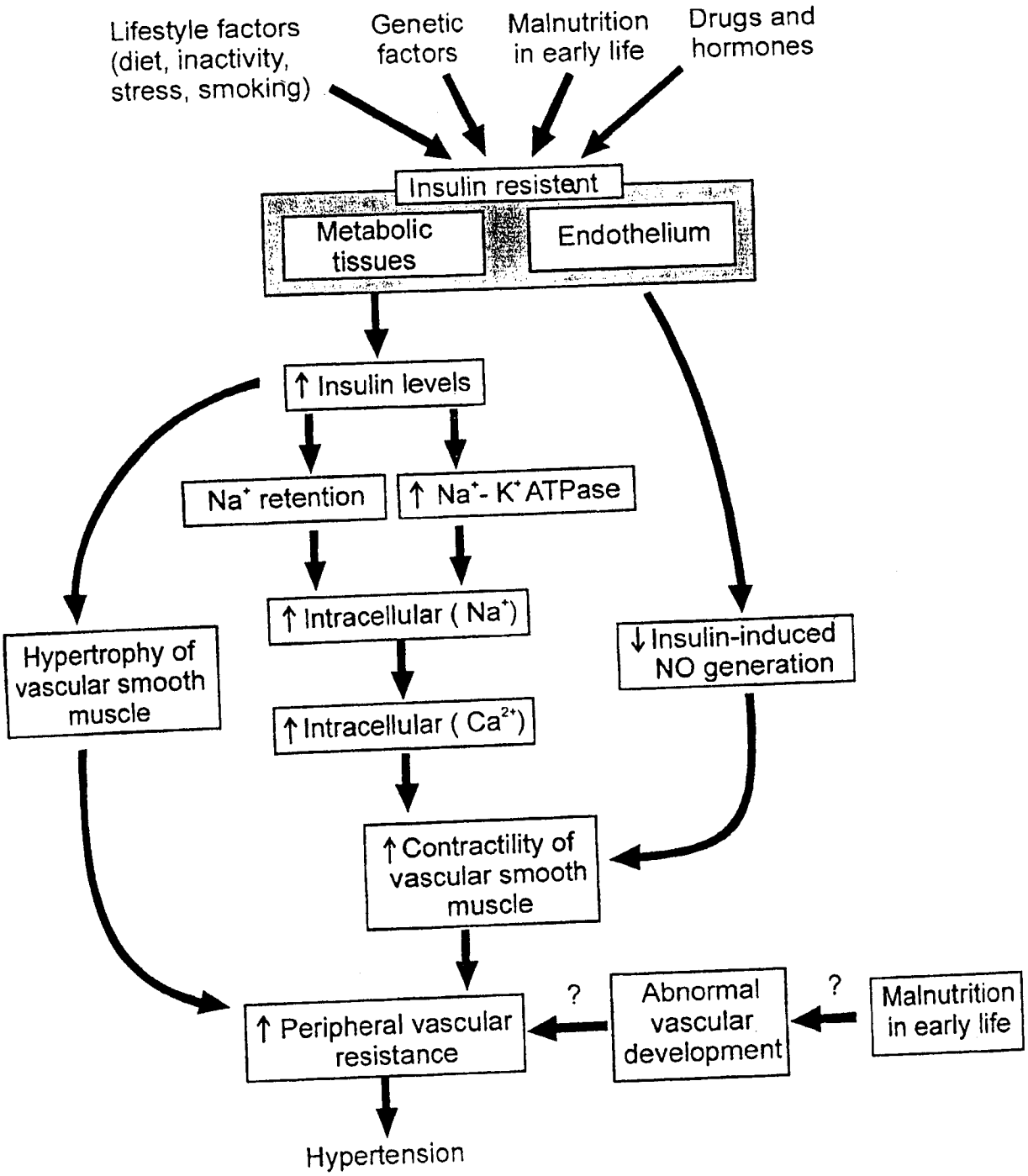


Figure 13: Possible basis of hypertension in patients with insulin resistance (Williams and Pickup, 1999).

It has been suggested that there is an association between cation metabolism and the regulation of blood pressure (Resnick, 1992, 1993). Individuals with untreated essential hypertension had consistently lower levels of intracellular free magnesium than normotensive control subjects (Resnick *et al*, 1984). Studies have also demonstrated that basal plasma and erythrocyte magnesium levels were significantly lower in untreated hyperinsulinaemic (Type 2) diabetic subjects, than in the control non-diabetic group (Fox *et al*, 2001).

Oral magnesium supplementation was given to patients who had received diuretics for 1 – 17 years for hypertension or congestive heart failure and who were shown to have significantly reduced muscle concentrations of magnesium. Magnesium, potassium and sodium potassium pumps were normalised in muscle after 26 weeks in most cases (Dorup *et al*, 1993).

Potassium supplementation in black patients with mild to moderate essential hypertension caused a large rise in urine potassium, a moderate increase in plasma potassium and had the added benefit of a small fall in blood pressure (Matlou *et al*, 1986).

Essential hypertension is characterised by high levels of free intracellular calcium and suppressed free magnesium (Resnick, 1992). Hyperinsulinaemic Type 2 patients have also demonstrated low erythrocyte magnesium levels, thus magnesium and potassium concentrations in muscle may be reduced during long term diuretic treatment (Dorup *et al*, 1993). Thiazides may induce deterioration of

glucose tolerance after prolonged treatment by inhibition of pancreatic insulin release and the blockade of peripheral glucose utilisation(Amery *et al*, 1978; Mason *et al*, 2005; Mancia *et al*, 2006; Opie *et al*, 2004). See Section 1.6. The insulin resistance in essential hypertension has been found to be predominantly located in skeletal muscle (Beck-Nielsen *et al*, 1992).

1.5 METABOLIC SYNDROME

There are currently several definitions for the metabolic syndrome. The most recent has been issued by the International Federation of Diabetes (Table 7). The pathogenesis of the metabolic syndrome and its components are complex, however, central obesity and insulin resistance are important causative factors. Abdominal circumference is the clinical screening factor for the metabolic syndrome and is associated with much more metabolic risk than body mass index.

Alone, each component of the cluster conveys increased cardiovascular and macrovascular disease risk but, as a combination, they become much more powerful risk factors. The metabolic syndrome can be present for up to 10 years before detection of the glycaemic disorders. This is important in relation to the aetiology of hyperglycaemia and associated CVD risk, and the potential to prevent CVD and its morbidity and mortality in persons with glucose intolerance (McLoughlin *et al*, 2004; Gorton *et al*, 2004). The metabolic syndrome with normal glucose tolerance identifies subjects with a very high risk of future diabetes. Thus, vigorous early management of all aspects of the syndrome may have a significant impact on the prevention of both diabetes and cardiovascular disease (Alberti *et al*, 1998).

Table 7: International Diabetes Federation: Metabolic Syndrome Definition.

Central obesity (defined as waist circumference ≥ 94 cm for Europid men and ≥ 80 cm for Europid women, **plus** any two of the following four factors:

- Raised TG levels ≥ 1.7 mmol/L or specific treatment for this lipid abnormality.
- Reduced HDL cholesterol < 1.03 mmol/l in males and < 1.29 mmol/l in females or specific treatment for this lipid abnormality.
- Raised blood pressure:

  Systolic ≥ 130 mmHg or

  Diastolic ≥ 85 mmHg or

  previously diagnosed hypertension
- Raised fasting plasma glucose ≥ 5.6 mmol/L or

  previously diagnosed Type 2 diabetes

1.6 THIAZIDE DIURETICS

Thiazide diuretics act by inhibiting sodium and chloride co-transport across the luminal membrane of the proximal segment of the distal convoluted tubule. After a degree of sodium and water loss has occurred, humoral and intra-renal counter-regulatory mechanisms block the effect on the kidney so that, within a few days, no additional loss of sodium occurs but total body sodium is maintained at a slightly lower level for as long as the diuretic is taken. This causes a fall in the extracellular fluid volume and a decrease in plasma and blood volume. With the loss of sodium and water, there is a rise in rennin release from the kidney leading to the formation of angiotensin II which is a vasoconstrictor and mediator of adrenocortical aldosterone secretion.

Thiazide diuretics have a dosage plateau, above which enhanced blood pressure reduction is rare and adverse metabolic effects worsen. Thiazides are the class of diuretics used to treat the majority of patients with essential hypertension.

Diuretic therapy is considered to be well tolerated and its complications offset by the beneficial effects of lowering elevated blood pressure. However, from the onset, metabolic disturbances were noted with diuretic therapy. The European Working Party on Hypertension in the Elderly (EWPHE) observed that diuretic therapy impaired glucose tolerance within two years in elderly patients (Skarfors *et al*, 1991). The Antihypertensive treatment and Lipid Profile In a north of Sweden Efficacy evaluation (ALPINE) study showed that, after 12 months of treatment, the

blood glucose and serum insulin concentrations increased, in both fasting and 2 hour glucose oral test samples in those patients in the diuretic group (Lindholm *et al*, 2003). A 10 year follow up of men in Uppsala supports the view that antihypertensive drugs can precipitate diabetes in predisposed men and there is evidence from the San Antonio Heart Study that thiazide diuretics and β-blockers can increase the risk of a patient developing type 2 diabetes (Morales *et al*, 1993). The Atherosclerosis Risk in Communities (ARIC) study showed that after a follow up of 6 years, hypertensive patients treated with ß-blockers had significantly higher risk of becoming diabetic, compared with those treated with thiazide diuretics (Gress *et al*, 2000). The Losartin Intervention for Endpoint reduction in hypertension (LIFE) study also showed a greater risk of developing diabetes in hypertensive patients treated with ß-blockers (Dalhof *et al*, 2002). These antihypertensive drugs appear to double the risk of a hypertensive patient developing Type 2 diabetes, irrespective of the baseline insulin sensitivity. These data indicate that the benefits of blood pressure reduction may be offset by the metabolic side effects of these drugs which should be avoided when selecting therapy for hypertensive patients who are obese or who have impaired glucose tolerance.

The reason for the widespread use of diuretics in essential hypertension is their proven antihypertensive efficacy and capability to reduce the incidence of stroke, congestive heart failure, progressive hypertension and renal failure. They have the ability to potentiate the effect of other agents by overcoming the reactive fluid

retention seen with some other agents (Knauf, 1993; Savage *et al*, 1998). Due to the fact that most of the potential adverse effects of diuretics are dose dependent the "low dose principle" has been recommended. Nevertheless, according to Knauf (1993), low doses of hydrochlorothiazide (e.g. 12.5 mg) or chlorthalidone (25 mg), lower blood pressure in fewer than 50% of hypertensive patients. However, as Milne (1997) points out, in South Africa, given our economic situation and the fact that most hypertensives are black with a low incidence of coronary artery disease, there is reasonable consensus that low dose diuretics should be the first line of therapy unless there are clinical contraindications, and this is endorsed by the South African Medical Association Hypertension Guideline Committee (Seedat, 2006).

1.7 HYPERLIPIDAEMIA

Hyperlipidaemia is commonly associated with hypertension, both of which are known to be important cardiovascular risk factors. However, when these two risk factors occur together, there is a markedly increased risk of coronary events. Dyslipidaemia is defined as a clinically significant alteration in circulating lipids and lipoproteins predisposing to coronary heart disease and related disorders. In practice the most important and common dyslipidaemia is hypercholesterolaemia with an elevated low density lipoprotein (LDL) cholesterol level that is the major risk factor for cardiovascular disease.

An estimated 50% of all diabetics, whether Type 1 or Type 2 are dyslipidaemic. Both triglyceride and cholesterol abnormalities occur. Dyslipidaemia in poorly controlled Type 1 patients presents as elevated triglyceride and, to a lesser extent, cholesterol levels. However, in well controlled patients, the levels of total cholesterol, triglycerides and LDL cholesterol are similar to those of normo-glycaemic individuals.

The most frequently encountered disturbances in Type 2 diabetes are an increase in triglyceride concentrations and a reduction in HDL cholesterol. These changes tend to be more marked in women than in men. LDL cholesterol tends to be mildly elevated. LDL particles tend to be smaller and denser which may increase their atherogenicity. The small dense particles make it easier to penetrate the arterial

walls and bind to proteoglycans which result in them being trapped within the arterial wall (Routolo *et al*, Brunzell, 2007).

Most patients developing coronary heart disease have plasma cholesterol concentrations exceeding 5 mmol/l with LDL cholesterol of 3 mmol/l. The Joint British Societies (JBS2) Guidelines on Prevention of Cardiovascular Disease recommend plasma cholesterol =4 mmol/l with LDL cholesterol =2mmol/l in patients with pre-existing atherosclerotic disease or diabetes (Wood *et al*, 2005).

1.8 THE AIM OF THIS DISSERTATION

Patients with essential hypertension often manifest abnormal glucose tolerance including impaired glucose tolerance (IGT) and diabetes mellitus (DM) and a number of defined relationships between hypertension and abnormal glucose regulation have been documented. Firstly, as a component of the Metabolic Syndrome, essential hypertension is independently associated with increased insulin resistance akin to a reduction in the measured insulin-mediated glucose disposal rate evident in patients with Type 2 diabetes (Ferannini, 1987; Shen, 1988). Secondly, the frequent association of insulin resistance and hyperinsulinaemia in patients with hypertension suggests that these metabolic changes may influence blood pressure control by several mechanisms including enhanced sodium retention, sympathetic activation and also endothelial dysfunction (Kahn, 2005; Landsberg, 2001).

Thirdly, it has been proposed that a common mechanism is able to link abnormal blood pressure control and insulin resistance/hyperinsulinaemia and ion transport (Eckel, 2005; Resnick, 1993).

Lastly, it is also possible that antihypertensive drugs including the use of thiazide therapy combined with cardioselective beta blockers may contribute to abnormal glucose tolerance in hypertensive subjects (Gress, 2000). The aim of this study was to identify some of the important clinical factors that may contribute to the abnormal glucose tolerance evident in hypertensive patients. We were specifically

interested in documenting the measured changes in insulin resistance, endogenous beta cell processing/secretion of insulin and platelet cation concentration and membrane ATPase activity in a cohort of thiazide-treated hypertensive patients with prospectively defined normal glucose tolerance, impaired glucose tolerance and hitherto undiagnosed and untreated type 2 diabetes mellitus.

CHAPTER 2.

The work described in this dissertation was conducted in the Department of Medicine, University of the Witwatersrand, South Africa. Approval for the studies was obtained from the Committee for Research on Human Studies (Protocol N° 930537). Informed consent was obtained from all subjects according to the guidelines laid down by the above committee.

2.1 Study Subjects

Caucasian patients attending the Johannesburg Hospital hypertension clinic were selected at random. Criteria for inclusion in the studies were as follows:

- Attendance at the hypertension clinic for more than 5 years;
- Treatment with diuretic anti-hypertensive medication for more than 2 years;
- Aged between 40 and 80 years at commencement of study.

2.2 Study design

The study was conducted in four parts.

Part 1: Twenty two people were recruited for this part of the study in which we wished to determine a practical, cost effective and accurate test to assess glucose tolerance which could be carried out in both primary and tertiary clinics.

Part 2: Having established from part 1 that a much higher than expected number of patients had abnormal glucose tolerance, the subjects were re-tested. We then compared target organ damage and determined changes in the metabolic profile of these elderly hypertensive patients all of whom were receiving

thiazide diuretic medication. The 22 subjects from part 1 plus a further 36 subjects took part in this arm.

Part 3: A sub-group (14) of impaired glucose tolerant patients from Part 2 were re-tested 10 months after withdrawal of low dose thiazide diuretic medication.

Part 4: We tested the hypothesis that a common mechanism is able to link abnormal blood pressure control and insulin resistance/hyperinsulinaemia and ion transport. Thirty nine of the above patients took part in this arm.

2.3 Methods

The methods were the same for all 4 arms of the study.

- Participants completed a questionnaire pertaining to medication and medical history (as well as family medical history).

- Blood pressure was calculated with a standard, calibrated mercury sphygmomanometer. Diastolic pressure was recorded at the fifth Korotkov sound. Blood pressure was measured on the right arm of a seated patient after 5 minutes rest. A mean of 5 separate measurements was calculated for each patient.

- Anthropomorphic measurements were recorded as body mass index (BMI = weight(kgs)/height $(cms)^2$). Waist:hip ratio (WHR) was measured as the

circumference of the waist midway between the superior iliac crest and the 12th rib and of the hips at the level of the greater trochanter.

- A resting 12 lead electrocardiogram (ECG) for left ventricular hypertrophy (LVH) defined as a score of >5 on the Romhilt and Estes point score system was done.

- Random non-fasting glucose was calculated for each person from readings extracted from clinic records over a 5 year period. Early morning urine was assessed for micro-albumin.

- An immunoturbidometric test was used for the quantitative determination of human albumin in urine (Boehringer Mannheim, Germany). Microalbumin was assessed by the albumin:creatinine ration (A:Cr). Microalbuminuria is defined as a A:Cr >3.7mg/mmol.

- A glucose oxidase reaction strip (Aims Glucostix) was used for measuring glucose in capillary blood from a finger prick. Results were recorded on a reflectance metre (Aims Glucometer 2, Model 5529).

- A standard oral glucose test (75g glucose monohydrate dissolved in 250ml water) was performed on participants who fasted from 22h00 the previous evening. A Teflon™ catheter was inserted into an arm vein to eliminate

repeated venepuncture and appropriate samples of venous blood for each arm of the study were withdrawn.

The following laboratory measurements were done:

- Plasma glucose was measured by a standard glucose oxidase method (Synchon CX3 analyser, Beckmann Instruments, Galway, Ireland);
- Glycated haemoglobin was measured using a two channel method (Tina-Quant, Roche Diagnostics). Haemoglobin A_1C was measured on a Hitachi 717 analyser.
- Serum insulin was measured by radioimmunoassay (Medginex Diagnostics, SA, Fleuris, Belgium);
- Intact pro-insulin and 31,32 des-pro-insulin concentration was measured using an in house, specific and sensitive two-site, immunoradiometric assay based on the method of Sobey (1989);
- Serum sodium, potassium, uric acid and lipids were measured using standard methods;
- Serum creatinine was measured by the Jaffe method (Boehringer Mannheim, Mannheim, Germany);
- Total platelet cation content, platelet isolation and membrane preparation as well as ATPase activity for magnesium, calcium, sodium and potassium were performed using previously described methods (Touyz, 1991 and 1993);

- Total platelet calcium and magnesium were measured by atomic absorption spectrophotometry (Varian Techtron AA 175 series with a Mg^{++} or Ca^{++} hollow cathode lamp);
- The magnitude of insulin resistance was calculated using the Homeostasis Model Assessment (HOMA) parameters (Matthews, 1985).

Statistical analysis was performed using a standard computer package (Epistat). Student's t test was used for comparisons between groups. Analysis of variance was determined for comparison within groups and the chi-square test for trend was performed. Significant relationships were assessed by Spearman's Rank Correlation Coefficient. Statistical significance was taken as $P < 0.05$. Results are given as mean $\pm$ SEM.

CHAPTER 3.

3.1 Results of Part 1

Based on the 2 hr venous glucose concentrations, 4 (18%) of the patients had previously undiagnosed diabetes mellitus (DM), 6 (27%) impaired glucose tolerance (IGT) and 12 (55%) had normal glucose tolerance (NG). Table 8 presents the characteristics of the 22 study subjects. The weight and BMI were similar in all three groups, although the patients with diabetes had greater waist to hip ratio (0.94±0.07) in comparison to the normal glucose group (0.86±0.04) and impaired glucose tolerance group (0.81±004). This was not, however, statistically significant.

Table 8: Characteristics of 22 subjects (Part 1)

Demographics (Mean ± SEM)			
	Normoglycaemic	**Impaired Glucose Tolerance**	**Diabetes**
n =	12	6	4
Sex male : female	2 : 10	1 : 5	2 : 2
Age (yrs)	66.50 ± 3.28	63.00 ± 3.92	67.00 ± 5.89
Weight (Kg)	68.82 ± 3.78	72.90 ± 4.65	71.05 ± 10.54
B.M.I.	26.67 ± 1.02	28.95 ± 1.67	27.85 ± 1.95
W.H.R.	0.86 ± 0.04	0.81 ± 0.04	0.94 ± 0.07
Blood pressure			
Systolic mmHg	149.33 ± 5.2	155.00 ± 5.1	153.25 ± 8.7
Diastolic	83.25 ± 1.9	92.17 ± 2.5	87.25 ± 2.3
Hypertension (yrs)	16.25 ± 2.42	17.17 ± 3.16	17.25 ± 3.30
Duration of clinic follow-up (yrs)	9.75 ± 1.08	6.50 ± 1.50	11.00 ± 3.19

The glycated haemoglobin, random venous glucose and fasting, 1 and 2 hour capillary and venous plasma glucose measurements are represented in Figure 14. Glycated haemoglobin was in the normal range for all three groups, as was the random glucose. The mean fasting venous glucose increased progressively in the three groups (NG = 5.16±0.14 mmol/l, IGT = 5.45±0.30 mmol/l and DM 5.85±0.27 mmol/l), although the differences were not statistically significant. However, the two hour venous glucose measurements were significantly different in the three subgroups, (NG = 6.06±0.41 mmol/l, IGT 8.58±0.43 mmol/l, and DM 12.60±1.80 mmol/l; p = <0.001). There was a weak correlation between the venous and capillary glucose measurements in the fasting state (Figure 15). At one hour, the separation between the three sub-groups was evident (Figure 16), and there was an excellent correlation between the two hour venous and capillary glucose measurements for all three groups (NG r = 0.9 p = 0.002; IGT r = 0.8 p = 0.01; and DM r = 0.8 p = 0.01). Figure 17 shows the coefficient of correlation on the two hour glucose concentrations (r = 0.97; p = 0.0)1. Glycosuria measured by dipstix was only documented in one patient (IGT) in the two years prior to the study.

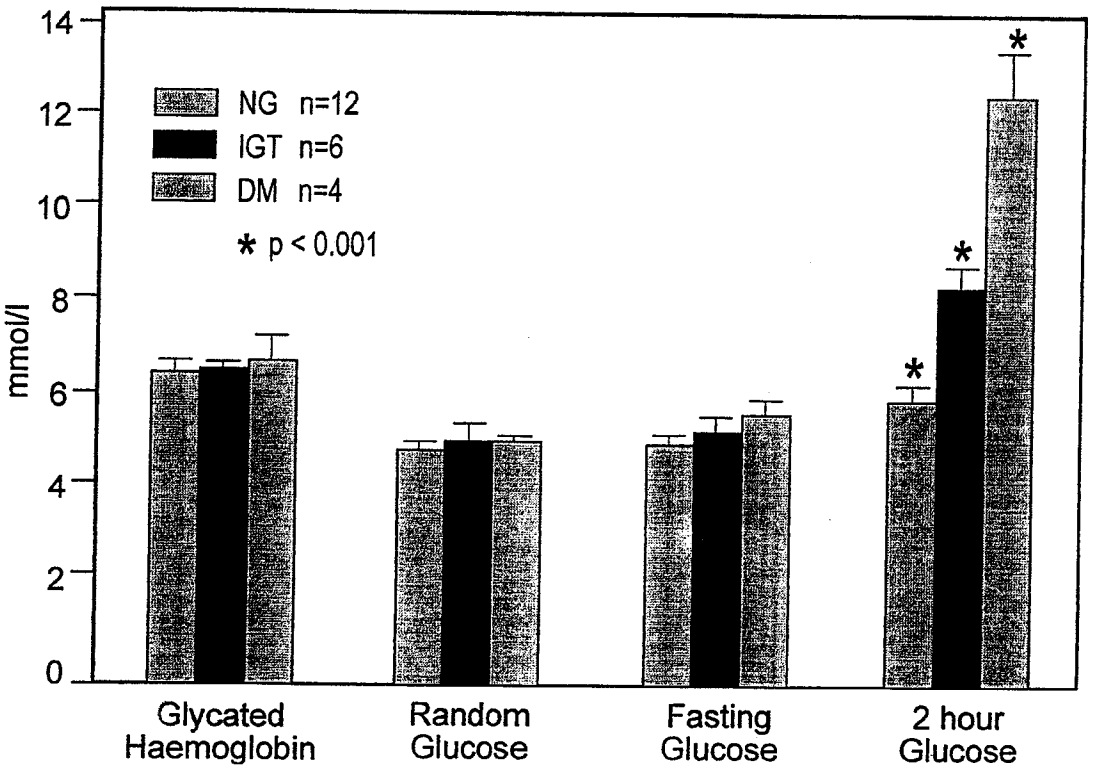


Figure 14: Glucose measurements (Mean ± SEM)

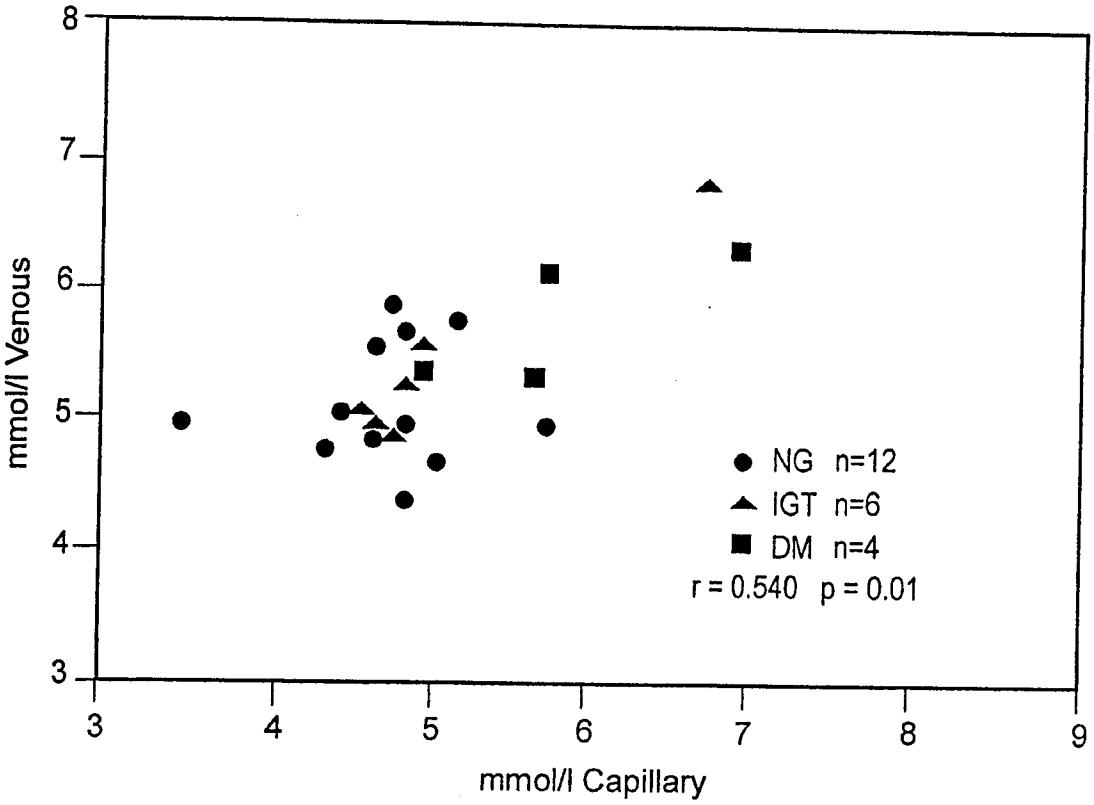


Figure 15: Fasting glucose

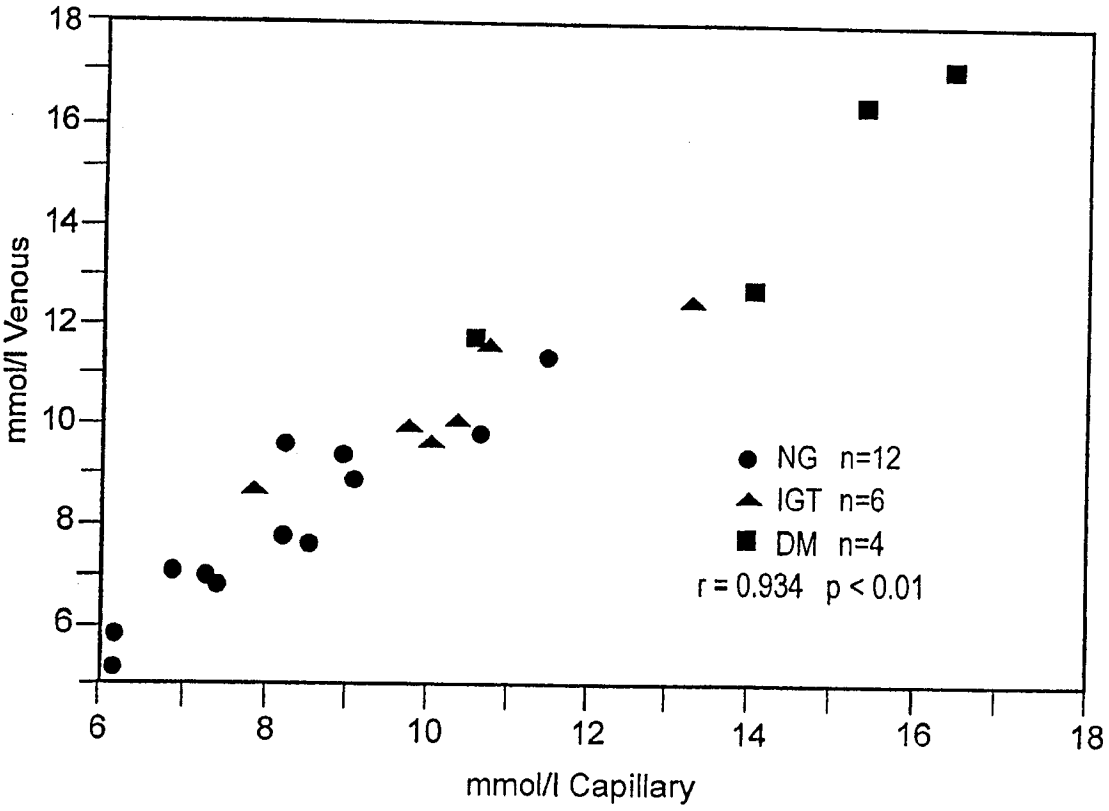


Figure 16: 1 hour glucose

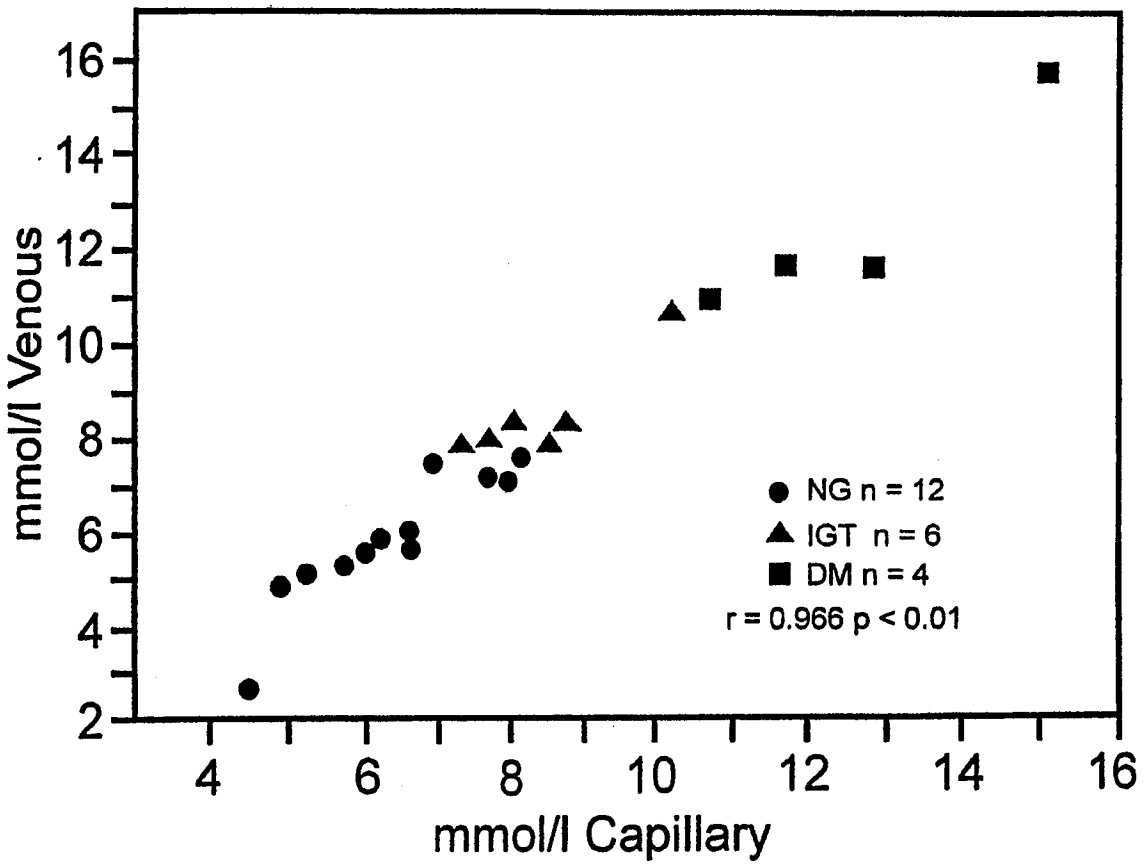


Figure 17: 2 hour glucose

3.2 Results of Part 2:

The mean age of the 58 subjects was 66.4 ± 1.4 years and there were 12 men and 46 women. Table 9 gives the demographic data of these patients and Table 10 shows the biochemical data. They were divided according to their glucose status, based on the 2 hour estimation, into those with NG (<7.8 mmol/l) and those with abnormal glucose (AG) (>7.8 mmol/l). Of the 58 subjects, 24 (41%) had NG and 34 (59%) AG. Diastolic blood pressure was significantly higher in those with AG. The duration of recorded hypertension was also significantly longer in this group (p = 0.01), but there was no difference in the duration of clinic follow up.

Table 9: Demographic data

	NG=24	**AG=34**	**P**
Males	6 (25%)	6 (18%)	NS
Females	18 (73%)	28 (82%)	NS
Age (years)	67.0 ± 2.2	66.1 ± 1.7	NS
SBP (mmHg)	150.0 ± 2.9	154.0 ± 2.3	NS
DBP (mmHg)	84.0 ± 1.7	90.0 ± 0.9	0.003
Duration HT (yrs)	16.2 ± 1.4	22.1 ± 1.6	0.01
Duration follow-up (yrs)	9.8 ± 0.7	10.5 ± 0.6	NS

Table 10: Biochemical data

	NG=24	AG=34	P
Fasting glucose mmol/L	5.0 ± 0.1	5.8.0 ± 0.1	0.001
2 Hours glucose mmol/L	6.1 ± 0.2	9.9 ± 0.4	≤0.001
Fasting insulin mU/L	13.0 ± 1.5	16.4 ± 1.9	NS
Total cholesterol mmol/L	5.4 ± 0.2	6.1 ± 0.2	0.03
Triglycerides mmol/L	1.2 ± 0.1	1.9 ± 0.2	0.007
HDL cholesterol mmol/L	1.5 ± 0.1	1.4 ± 0.1	NS
LDL cholesterol mmol/L	3.3 ± 0.2	3.7 ± 0.2	NS
Uric acid mmol/L	0.40 ± 0.0	0.38 ± 0.0	NS
Potassium mmol/L	3.9 ± 0.1	3.9 ± 0.1	NS

Although there was a highly significant difference in the fasting glucose concentration between the groups (5.0 ± 0.1 mmol/l vs 5.8 ± 0.1 mmol/l, P = 0.001), both levels are within the normal range. It is only on the basis of the 2 hour glucose estimation that a definitive diagnosis of AG can be made (6.1 ± 0.2 mmol/l vs 9.9 ± 0.4 mmol/l, p = 0.001). The fasting insulin was greater in those with AG (16.4 ± 1.9 mU/l) than those with NG (13.0 ± 15 mU/l) but this was not significant. The lipid profile showed significantly higher total fasting cholesterol (p = 0.03) and triglyceride (p = 0.007) concentrations in AG, but the LDL cholesterol and the HDL cholesterol levels were the same. There was no difference in the uric acid or potassium levels.

Table 11 gives the other characteristics of these subjects in terms of possible genetic, environmental and outcome differences. Although not significant, the questionnaire revealed an increased family history of known diabetes mellitus in the AG group compared with the NG (10 (29%) vs 14 (17%), respectively). The incidence of familial hypertension was the same (62% in both groups) as was the family history of cerebrovascular disease. However, there was a significant family history of myocardial infarction in those with AG, 28 (82%) compared to 7 (29%) in the NG group ($P = 0.05$). The AG group tended to be more overweight and demonstrated a higher body mass index and W/H than the NG subjects. NG subjects had a longer duration on both total diuretics and total thiazide use, 103.2 ± 12.7 months and 90.0 ± 10.7 months respectively, compared with 93.4 ± 6.8 months and 81.4 ± 7.0 months for AG subjects. This finding was reversed for total β-blockers and other antihypertensive agent usage. None of the trends in drug usage was significant. There were no differences in the albumin:creatinine ratio (A/CR) nor the incidence of left ventricular hypertrophy between either group.

Table 11: Causes and consequences of the metabolic syndrome

	NG=24	AG=34	P
1. Causes			
(a) Genetic Family History			
Diabetes	4 (17%)	10 (29%)	NS
Hypertension	15 (62%)	21 (62%)	NS
MI	7 (29%)	28 (82%)	0.05
CVA	9 (38%)	9 (26%)	NS
(b) Environmental			
Smoker	2 (8%)	6 (18%)	NS
Weight (kg)	72.4 ± 3.2	73.9 ± 2.9	NS
BMI (Kg/m^2)	27.5 ± 1.1	29.1 ± 1.1	NS
W H ratio	0.87	0.88	NS
Total diuretics (months)	103.2 ± 2.7	93.4 ± 6.8	NS
Total thiazides (months)	90.0 ± 10.7	81.9 ± 7.0	NS
Total ß-blockers (months)	85.5 ± 9.8	92.4 ± 8.3	NS
Total other anti-hypertensives (months)	94.1 ± 19.6	115.7 ± 17.4	NS
2. Consequences			
LV hypertrophy	4 (17%)	4 (12%)	NS
Serum creatinine µmol/l	104 ± 6.3	99.4 ± 4.9	NS
Urinary albumin/ creatinine ratio	4 (25%)	5 (28%)	NS

3.3 Results of Part 3

Having defined the initial metabolic status in 14 abnormal glucose patients (4 men and 10 women), with a mean age of 61.4±4.6y, all participants were re-tested 10 months after withdrawal of low dose thiazide therapy. One patient reverted to normoglycaemia while 12 remained glucose intolerant and one progressed to overt diabetes mellitus. Table 12 compares the biochemical data from the first and second tests in these 14 subjects. There was no significant change in any of the glucose levels or the fasting insulin concentrations. However, total cholesterol, triglycerides and LDL cholesterol were all significantly lower and the HDL cholesterol higher after withdrawal of the thiazides. There was little change in uric acid or potassium concentrations. After thiazide diuretic withdrawal, both systolic and diastolic blood pressure showed a significant rise despite the addition of other antihypertensive agents. Weight remained unchanged, indicating no significant alteration in the diet of these subjects.

Table 12: The effect of stopping low dose thiazide diuretic in 14 patients with abnormal glucose tolerance.

	1st Test	2nd Test	P
Glucose (mmol/l)			
Fasting	5.52 ± 0.20	5.36 ± 0.20	NS
One hour	10.68 ± 0.62	10.76 ± 0.77	NS
Two hour	8.78 ± 0.16	9.24 ± 0.32	NS
Insulin (mU/ml)			
Fasting	17.81 ± 3.90	15.06 ± 2.04	NS
Fasting Biochemistry (mmol/l)			
Cholesterol	6.24 ± 0.27	5.85 ± 0.29	0.03
Triglycerides	1.89 ± 0.27	1.51 ± 0.24	0.0008
HDL	1.23 ± 0.04	1.41 ± 0.07	0.003
LDL	4.16 ± 0.04	3.77 ± 0.25	0.02
Potassium	3.88 ± 0.09	4.06 ± 0.07	NS
Uric acid	0.42 ± 0.02	0.41 ± 0.02	NS
Blood Pressure (mmHg)			
Systolic	150 ± 4.37	160 ± 3.59	0.01
Diastolic	84 ± 3.22	91 ± 2.36	0.003
Weight (kg)	72.7 ± 2.71	71.9 ± 2.90	NS

3.4 Results of Part 4

Table 13 shows the demographic data of the 39 subjects in this arm of the study. Based on the 2 hr post glucose challenge, 7 of the 39 patients were found to have newly diagnosed diabetes mellitus with glucose measurements >11.1 mmol/l. Eighteen patients manifested impaired glucose tolerance with measurements between 7.8 – 11.0 mmol/l. Fourteen subjects were confirmed as having normal glucose tolerance levels (<7.7 mmol/l). These 3 subgroups of thiazide-treated hypertensive patients were compared to a control group (CG) of normotensive subjects (n = 13) with normal glucose tolerance and all of whom had fasting plasma glucose =5.6 mmol/l.

Table 13: Demographic Data

	NG	IGT	DM	Significance
Total patient number	14	18	7	
Men	4	5	1	
Women	10	13	6	
Age (yr)	67.1±2.8	68.2±1.8	62.1±3.6	p=ns
Weight (kg)	71.6±3.9	75.9±4.2	76.8±10.0	p=ns
BMI (kg/m^2)	26.8±1.3	29.1±1.7	28.5±2.6	p=ns
Waist-hip ratio	0.83±.02*	0.87±.02	0.92±03*	p<0.04
SBP (mmHg)	151±4.3	155±3.9	145±5.4	p=ns
DBP (mmHg)	80±1.8	84±3.0	80±2.0	p=ns
Diuretic use (months)	67.9±3.7	64.7±6.5	66.6±13.6	p=ns
ß-blocker use (months)	59.8±8.9	70.1±7.8	63.1±11.3	p=ns
Family history diabetes	5 (36%)	5 (28%)	5 (71%)	p=ns

Within the hypertensive subgroups of patients there were no differences in either the mean total duration of thiazide therapy use (DM = 66.6 ± 13.6 months; IGT = 64.7 ± 6.5 months; NG = 67.9 ± 3.7 months) nor in the mean duration of ß-blocker therapy (DM = 63.1 ± 11.3 months; IGT = 70.1 ± 7.8 months; NG = 59.8 ± 8.9 months). A family history of diabetes involving a first degree relative was documented with the following frequency: DM = 71%; IGT = 28%; NG = 36% without any statistical significance being evident. BMI and waist to hip ratio showed a progressive increase from NG to IGT and DM but significance was only established in the greater waist to hip ratio between the DM and NG subgroups (DM = 0.92 ± 0.03; NG = 0.83 ± 0.02; $p < 0.04$).

A positive correlation was established between the following variables: weight with fasting glucose ($r = 0.39$, $p < 0.05$) and fasting insulin ($r = 0.43$, $p = 0.02$); BMI with fasting glucose ($r = 0.39$, $p < 0.05$) and fasting insulin ($r = 0.52$, $p < 0.01$); waist hip ratio with fasting glucose ($r = 0.61$, $p < 0.01$); waist circumference with fasting glucose ($r = 0.60$, $p < 0.01$) and fasting insulin ($r = 0.66$, $p < 0.01$).

The serum insulin, pro-insulin and 31,32 des-pro-insulin measurements in response to the glucose challenge are shown in Figure 18. In the top panel the NG patients showed a typical, time-related insulin response from 0 hr to 1 hr and 2 hr, respectively with the peak response occurring at 1 hour. In comparison to the NG patients, the IGT group exhibited a mean insulin response that was both increased and delayed such that ongoing hyperinsulinaemia was evident after the 2 hr time point. Significance between these two groups was established at 0 hr and 2 hr,

respectively (NG vs IGT 0 hr = 59.2 ± 4.4 and 121.6 ± 16.0 pmmol/l, $p < 0.01$ and NG vs IGT 2 hr = 372.6 ± 68.0 vs 932.7 ± 15.9 pmmol/l, $p < 0.01$). The DM patients manifested dynamic insulin responses that were intermediate, being greater in magnitude and delayed in time at 2 hr in comparison to the NG – these changes were lower than those present in the IGT group and significance was established at the following time-points: NG vs DM 0 hr 59.2 ± 4.4, 115.2 ± 36, $p = 0.05$; NG vs DM 2 hr 372.6 ± 68.0 vs 764.7 ± 112, $p = 0.05$.

The mean serum pro-insulin response (middle panel) showed a progressive increase in both the fasting and post-stimulated state in the progression from NG to IGT and DM. Significant differences were evident at all time points, 0 hr, 1 hr and 2 hr, respectively between the NG and DM groups. A similar trend was present in the responses of the 31,32 split pro-insulin measurements (lower panel) and significance was established between the NG and DM groups at two time points, 0 hr and 2 hr.

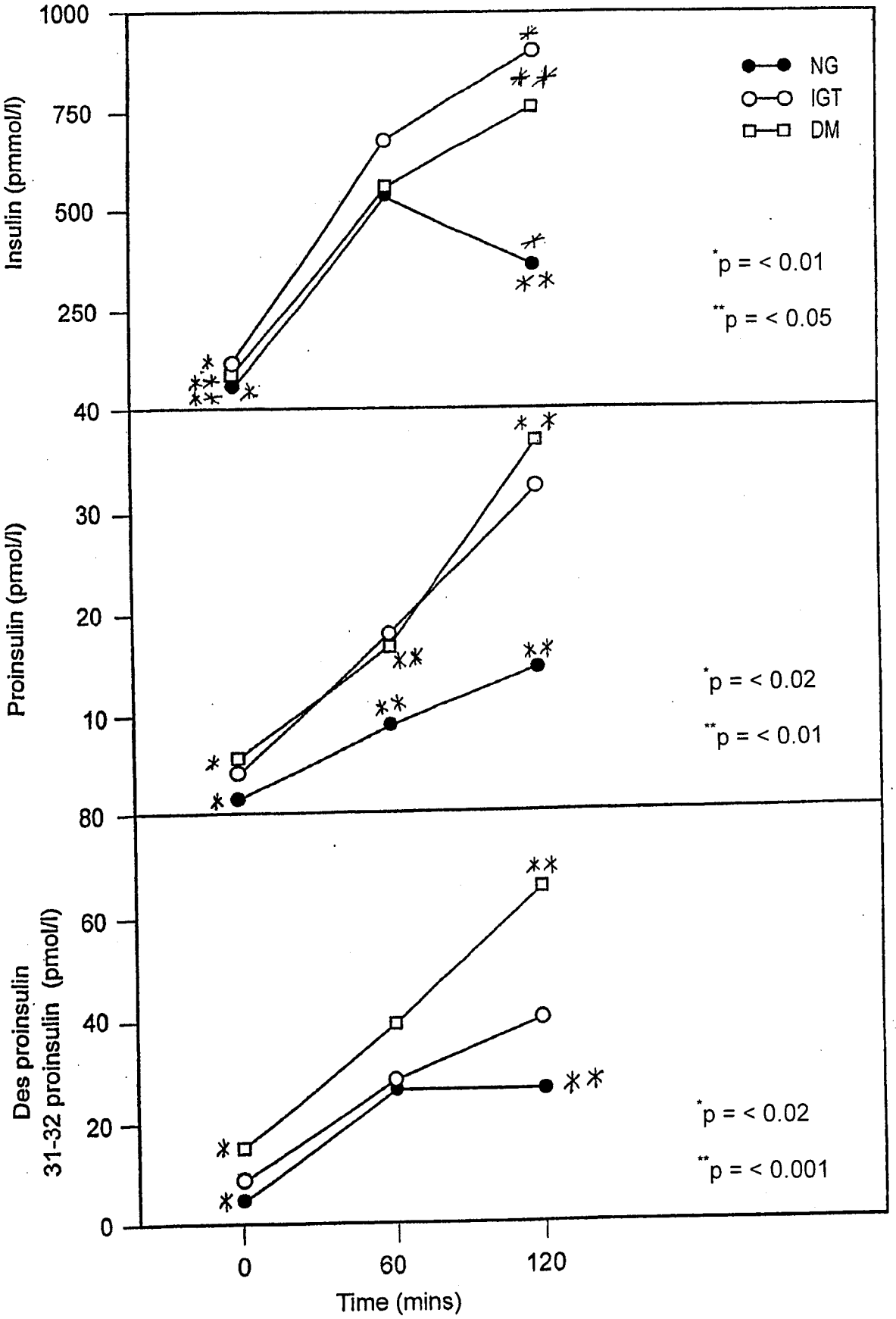


Figure 18: Serum insulin proinsulin and 32-33 proinsulin response to glucose

Based on the measured fasting insulin and glucose concentrations a progressive increase in the calculated degree of insulin resistance was evident in the change from NG (2.27 ± 0.61), IGT (3.92 ± 0.52) and DM (6.79 ± 1.2). Significance was established between the DM IGT and NG group ($p < 0.001$) as well as the DM and IGT group ($p < 0.01$) (Table 14).

Table 14: Calculation of insulin resistance homeostasis model assessment (HOMA) formulation

Group	Number	Mean	SEM	Significance
Normal (NG)	13	2.27*	0.61	P=<0.001*
Impaired (IGT)	15	3.92*#	0.52	P=<0.01#
Diabetic (DM)	7	6.79*#	1.25	

*NG-DM & NG-IGT p = < 0.001; #DM & IGT p = < p = < 0.01

The total platelet sodium, calcium and magnesium concentrations were similar in all the subgroups of hypertensive patients (NG, IGT and DM) and also in the normotensive controls with normal glucose tolerance (CG). In contrast, the mean total platelet potassium concentration in the hypertensive DM group (2.28 ± .05) was significantly reduced in comparison to the hypertensive IGT (2.69 ± .03; p = 0.02), hypertensive NG (2.65 ± 0.4); p = 0.02) and normotensive CG (2.63 ± .02; p < 0.01). Of note this finding was evident in the presence of similar serum potassium concentrations in all subgroups (Table 15).

Table 15: Total intracellular platelet cation concentrations in normotensive subjects with normal glucose tolerance (CG) and hypertensive patients with normal glucose tolerance (NG), impaired glucose tolerance (IGT) and Diabetes Mellitus (DM).

n =	13	14	18	7	
Cation	CG	NG	IGT	DM	Significance
Sodium	1.0 ±.09	0.8 ±.08	0.9 ±.09	0.9±.15	P=ns
Potassium	2.63±.02$^{\#}$	2.65±.04*	2.69±.03*	2.28±.05$^{*\#}$	*p=0.02 $^{\#}$p=0.01
Calcium	0.65±.06	0.59±.09	0.45±.05	0.43±.05	p=ns
Magnesium	0.92±.05	0.99±.12	0.92±.98	1.04±.17	p=ns
(Cation concentration expressed in µmol/l x 10^8 cells) DM – IGT p=0.02; DM – NG p=0.02; DM – CG p=<0.01					

Platelet ATPase activity showed no measured differences in sodium-potassium ATPase and calcium ATPase activity within and between the four subgroups, hypertensive NG, hypertensive IGT, hypertensive DM and normotensive CG. Platelet magnesium ATPase activity, however, showed an increased activity in the control group (non-hypertensive, normoglycaemic) which was weakly significant when compared against two of three hypertensive groups (NG p = 0.02 and IGT p = 0.001 but not DM). Comparison between the control group and the combined hypertensive groups (NG + IGT + DM) confirmed a decrease in magnesium ATPase activity in the hypertensive group 70.64 ± 4.4 versus the normotensive controls 52.86 ± 3.6 (p = 0.01) (Table 16).

Table 16: Platelet ATPase activity in normotensive subjects with normal glucose tolerance (CG) and hypertensive patients with normal glucose tolerance (NG), impaired glucose tolerance (IGT) and Diabetes Mellitus (DM).

ATPase	CG	NG	IGT	DM	Significance
Na-K	7.33±.99	10.15±1.5	10.29±1.73	9.38±2.46	p=ns
Calcium	5.9±.87	5.23±.39	7.18±.96	8.7±1.32	p=ns
Magnesium	70.64±4.4$^{\#*+}$	51.8±6.2$^{\#*+}$	47.28±4.1$^{\#+}$	66.04±8.7^{+}	*p=0.02 $^{\#}$p=0.001 $^{+}$p=0.01
(ATPase activity expressed in nmol Pi/mg protein/minute) CG – NG p=0.02; CG – IGT p=0.001; CG – NG, IGT, DM p=0.01					

CHAPTER 4

4.1 Discussion

We have demonstrated a very high incidence of abnormal glucose tolerance, dyslipidaemia and obesity in a cohort of elderly white patients with primary hypertension and have confirmed the close clustering of the major components of the metabolic syndrome in susceptible individuals. It is therefore important that a practical, accurate, yet inexpensive assessment of glucose tolerance be available for use in both primary and tertiary care settings.

Various studies have examined the use of different methods. Random urinalysis is known to be uniformly inaccurate and unreliable as a means of diagnosing diabetes mellitus. Forrester (1988) and Davies and co-workers (1993) reported a sensitivity of 89% for one hour postprandial glycosuria and suggested that this may be a useful screening test. However, this test is not practical for general clinic use. In our experience, serial, random, non-fasting glucose measurements differentiated poorly between normal and abnormal glucose tolerance. A random glucose measurement is generally misleading as it is largely determined by the nature and time of the last meal. In our 3 groups, there was a clear distinction between mean fasting glucose but the diagnostic threshold of >7.8 mmol/l, or even the new diagnostic threshold of > 6.1mmol (WHO, 2006), was not reached in the diabetic group. This may be partially attributed to the small patient cohort and sensitivity decreasing with age. Forrester and co-workers (1988) reported that the fasting blood glucose (FBG) level alone is an insensitive test for the diagnosis of diabetes

in most populations. They also reported that the fasting glucose level which corresponds to a diabetic cut-off of >11.1 mmol/l at 2 hours is 5.6 mmol/l.

The formal laboratory determined two hour plasma glucose level after a 75 gm glucose load, clearly defined the separation between normoglycaemic, impaired glucose tolerance and diabetes, but, as a primary health care screening test, it may not be practical and would be relatively expensive. We have shown that a standard 75 gm OGTT followed by a single 2 hour capillary glucose measurement, provided a simple, accurate and cost effective way of assessing glucose tolerance. A reflectance meter eliminates errors due to incorrect timing. If a reflectance meter is not available, the strip can be read visually by comparing the colour of the strip reagent pad to the colour on the chart on the container. This method has been shown to give a 96% clinically accurate performance rate (Clarke, 1988).

Although the costs of blood glucose reagent strips are double that of the urine strips, the results are more meaningful. Nevertheless, the accuracy of the test depends on several variables. The patient must have fasted from the previous evening. The oral glucose must be given under supervision to ensure that it is all taken. Accurate timing of the blood sample relative to when the glucose load was consumed and the accuracy of the person conducting the test is also important. It has been suggested that the nature of the initial instruction to the operator on how to use the oxidase strips is also an important variable (Kucler *et al*, 1990). People who have had formal instruction have demonstrated better scores than those who used self instruction. Whether this was due to learning in a group situation or

commitment to the procedure could not be ascertained. The frequency of performing subsequent monitoring at the work site was not associated with significant change in the ability of the person to perform the test and there was no change in performance when the test was repeated 8 months later.

Manifestations of the metabolic syndrome were found in subjects treated with antihypertensive medication, which included hydrochlorothiazide prescribed in a low and "metabolically friendly" dose. Reversal of some of the abnormalities (hyper-triglyceridaemia and hypercholesterolaemia), but not others (impaired glucose tolerance and diabetes mellitus) was evident following withdrawal of the long term thiazide therapy. However, it is of interest that 41% of the patients had no manifestations of the metabolic syndrome despite 10 years of low dose diuretic therapy.

It has been well established that resistance to insulin action, namely a decrease in insulin-stimulated glucose disposal and hyperinsulinaemia, is strongly associated with the components of the insulin resistance syndrome and premature coronary artery disease in many groups (Cuspidi, 2004). Furthermore, the use of thiazide therapy has been shown to affect both insulin action and release adversely and thereby to aggravate insulin resistance, dyslipidaemia and glucose tolerance. In the light of this, the beneficial effects of blood pressure reduction therapy are seemingly negated by the associated metabolic deterioration (Reaven, 1988; De Fronzo, 1990; De Fronzo *et al*, 1991; Haffner *et al*, 1992). Occlusive coronary artery disease therefore remains the most common cause of death in patients with

treated hypertension and, in an attempt to reduce the incidence of this complication, the use of metabolically neutral doses of thiazide therapy has gained widespread acceptance (Swales, 1993).

Our patients had all received low dose thiazide therapy for 10 years. In the subgroup diagnosed with AG (IGT and overt Type 2 diabetes mellitus), significantly higher mean fasting total cholesterol and triglyceride concentrations were measured in comparison with the patients with NG. Withdrawal of thiazide therapy resulted in the lipid concentrations (cholesterol, triglycerides and HDL cholesterol) changing significantly (6%, 20% and 14% respectively) to levels similar to those in the group with NG. These improvements in lipid profile were apparent in the absence of changes in glucose tolerance, fasting insulin, weight or lifestyle and suggest that thiazide therapy, even in low doses, exerts an independent effect on lipid metabolism which may be atherogenic. The incidence of coronary artery disease in black South Africans is significantly lower than in other ethnic groups and this has been attributed to the favourable lipid profile inherent in the black community (Wyndham, 1979; Walker *et al*, 1993). It is of interest that our group has previously documented that young black hypertensive patients receiving thiazide therapy manifest a change in lipid profile similar in magnitude to that evident in our elderly white patients with IGT (Joffe *et al*, 1996). This lends further evidence to the dyslipidaemia potential of low-dose thiazide therapy given to an ethnic group which has previously been relatively protected from coronary artery disease.

The incidence of diabetes mellitus/IGT generally increases with age (Franse *et al*, 2001; Harris *et al*, 1987) and it was therefore not surprising to find this component of the insulin resistance syndrome in our elderly subjects. However, 58% is an extremely high incidence, even at the age of 66 years. We were unable to identify specific inter-group differences which may have contributed to the development of the AG. Certain trends emerged in the group with IGT/diabetes mellitus in comparison to the normal group, although these were only weakly significant, There was a longer duration of hypertension, poorer diastolic blood pressure control and a family history of myocardial infarction. Our sample was relatively small and these trends may have reached greater significance in a larger sample. Also, an insulin level measured after 2 hours during the glucose tolerance test may have been a more accurate index to insulin resistance and insulin sensitivity. Withdrawal of thiazide did not reverse the abnormalities in glucose homeostasis and the incidence of IGT Type II DM remained unchanged. However, the changes seen in the lipid profile imply that thiazides caused a rise in triglycerides and LDL cholesterol and a decrease in HDL cholesterol. It is possible that the thiazides caused a change in the insulin resistance which, in turn, caused the changes in the lipid profile that may have been missed by measuring the insulin only at hour 0 of the glucose tolerance test. The use of concomitant medication e.g. β-blockers, and/or other factors such as an increased body mass index and primary genetic factors, may have contributed to these patients' abnormal tolerance and 'non-reversible' glucose intolerance. The discordant changes in the metabolic parameters measured in our patients with treated hypertension therefore suggest

that, in contrast to dyslipidaemia, AG does not improve 10 months after thiazide withdrawal. Subjects receiving ß-blockers in the United Kingdom Prospective Diabetes Study in 1998 (UKPDS) gained slightly more weight and showed a slightly greater increase in glycated haemoglobin concentrations. However, this did not affect the reduction in clinical endpoints. There were also no consistent trends in the concentrations of triglycerides, total cholesterol or high density lipoprotein cholesterol over the nine years of the study. It is of interest that thiazide diuretics were not used in this study.

An early morning urine sample for albumin:creatinine ratio assessment is a useful and practical screening method for detecting microalbumin in the elderly (Winocour, 1992). Microalbuminuria, an early sign of renal disease that predicts the risk of cardiovascular disease in people with and without diabetes mellitus (Mattock *et al*, 1992) was similar in both our groups. Despite 10 years of follow up in these patients, there was no greater evidence of target organ damage in the group with the metabolic syndrome, at least as measured by left ventricular hypertrophy, microalbuminuria and serum creatinine concentrations. This finding is in keeping with a Swedish study (Samuelsson *et al*, 1996).

UKPDS (1998) demonstrated conclusively the benefits of improved glycaemic control but also highlighted the greater impact of blood pressure control in reducing diabetes related endpoints. Results showed that long term tight blood pressure control in hypertensive patients with Type 2 diabetes mellitus resulted in a significant reduction in all diabetes related endpoints (24%), including diabetes

related deaths (32%). When all macrovascular diseases were combined, including myocardial infarction, sudden death, stroke and peripheral vascular disease, the group assigned to tight blood pressure control had a 34% reduction in risk compared with the group assigned to less tight control. To achieve tight control in this study (mean BP 144/82 mmHg), 29% of patients required three or more antihypertensive treatments. The Hypertension Optimal Treatment (HOT) randomised trial also demonstrated lower cardiovascular events and deaths with tighter blood pressure control in patients with diabetes mellitus (Hansson *et al*, 1998)

Blood pressure control, whether by high or low dose diuretics and ß-blockers, clearly decreases cardiovascular morbidity and mortality, with most benefit shown in the reduction of stroke (JNC VI, 1997). The meta-analysis of randomised, placebo controlled clinical trials in hypertension according to first line treatment strategy shows the considerable benefit obtained from low dose diuretics and ß-blockers on the incidence of stroke as well as benefit in coronary artery disease. The benefit was less impressive in cardiovascular mortality and total mortality. These findings are consistent with the postulated importance of worsening in metabolic control attributed to high dose of these drugs. Coronary artery disease showed improvement with high dose diuretics.

In Part 4 of this study, the overall incidence of abnormal glucose tolerance in our subjects was 64% with the incidence of IGT being 46% and that of diabetes mellitus 18%. The changes in the WHO 1998 recommendation in the diagnostic

value of the fasting plasma glucose levels greater than 7.0 mmol/l made very little difference to this group of patients as only two subjects were affected. In this regard it has been estimated that up to 50% of patients with Type 2 diabetes remain undiagnosed. In addition, as many as a third of patients with primary hypertension will manifest with overt diabetes mellitus in time and our incidence of diabetes is in keeping with these reported ranges (Sowers, 2001; Reaven, 2003; Savage, 2003). Within our patient groups, this high incidence of IGT and DM may be explained by two major factors namely, increased age and weight and, particularly, centripetal weight distribution. The mean age of our cohort was 65.8 years and the background prevalence of Type 2 diabetes is known to be high in many elderly populations. Based on the calculated BMI, our three groups of hypertensive patients were not overtly obese but were somewhat overweight. Nonetheless, even in this setting, a significant difference in the waist to hip ratio was evident between the NG and DM patients, attesting to the known importance of regional adiposity in the genesis of insulin resistance and diabetes (Yusuf, 2005; Kragelund, 2005). We were able to document positive and significant correlations between indices of adiposity, mean fasting glucose and insulin resistance (mean fasting insulin) in our hypertensive patients. Thus, we were able to associate primary hypertension with a high incidence of the metabolic co-morbidities of glucose intolerance, diabetes and increased regional adiposity.

Although the presence of a positive family history of diabetes mellitus showed a progressive increase in prevalence from the NG to DM subjects, we were unable to assign statistical value(s) of significance to this trend; however, our findings do not

negate the importance of a genetic/constitutional risk in the development of diabetes in subjects with hypertension. In this regard, it has been well established that normotensive, non-diabetic, first degree relatives of hypertensive subjects demonstrate insulin resistance at a young age (Raitakari, 1995; Reaven, 1988). We were also unable to document any adverse metabolic/diabetogenic effect(s) attributable to antihypertensive therapy (especially thiazide therapy) that may have contributed to the high incidence of IGT/diabetes in our patients (Sobey, 1989; Taylor *et al*, 1997). As all the patients were uniformly treated with thiazides, we assessed the total duration of drug exposure and found no significant differences in this parameter of drug exposure between the three subgroups of patients. Thus, thiazide therapy, prescribed in low doses, seemingly had little or no impact on promoting glucose intolerance in these elderly and otherwise, "at-risk" patients with hypertension. This is in contrast to other studies (Opie, 2004; Lindholm, 2003; Messerli, 2004) which suggest that the combination of thiazide (given in low daily doses) and ß-blocker therapy (mainly Atenolol™) had an increased diabetogenic effect in comparison to angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARBs) and calcium channel blockers (CCBs).

It has been documented that up to 50% of patients with essential hypertension will manifest insulin resistance or hyperinsulinaemia (Sheu, 1992). In this setting, it also seems likely that the incidence of hypertension is significantly greater in these "at risk" subjects who are insulin resistant than in those who are insulin sensitive (Leahy, 1990). Based on the plasma insulin responses to an oral glucose challenge and also on the HOMA-derived level of insulin resistance, we were able

to identify subsets of hypertensive patients who were either insulin sensitive with normal glucose tolerance (35%), or insulin resistant with IGT or diabetes mellitus (65%). The majority of the latter group manifested pre-diabetes rather than overt diabetes. Thus, serum insulin responses to an oral glucose challenge were significantly different in magnitude and duration in the three groups of hypertensive patients with varying degrees of glucose tolerance/intolerance. In comparison to the patients with normal glucose tolerance (NG), the IGT patients showed an insulin response both increased and delayed and the DM patients a response that was reduced and delayed. The observed trend in the hyper secretion of endogenous insulin was paralleled by an increase in the HOMA calculation of insulin resistance of two-fold and threefold in the progress from NG to IGT and DM, respectively. The pathogenesis of Type 2 diabetes is ascribed to both insulin resistance and pancreatic beta cell dysfunction. This abnormality in insulin secretion is initially manifested as increased or exaggerated secretion as a compensatory response to insulin resistance (stages of IGT and pre-IGT). It subsequently manifests as a progressive decline in insulin secretion as beta cell failure emerges and hyperglycaemia supervenes (DM). These linked stages of hyperinsulinaemia followed by glucose intolerance are thus similar to the pathogenetic abnormalities evident in most patients who develop Type 2 diabetes. Apart from changes in insulin secretion, a noted manifestation of pancreatic dysfunction that has been identified in the pre-diabetic and diabetic state includes the hyper secretion of insulin precursors, specifically pro-insulin and 31, 32 split pro-insulin. It has also been demonstrated that these changes in the secretion of serum insulin precursor peptides occur incrementally as glucose intolerance

deteriorates. We were also able to demonstrate that, in hypertensive patients who manifest with varying degrees of glucose intolerance, a progressive and significant increase in the stimulated serum pro-insulin and 31,32 split pro-insulin concentrations was evident in the progression from normal to IGT and DM. Thus, as beta cell decompensation occurs in insulin resistant hypertensive patients, a manifestation of this "ß cell stress" becomes evident in the concomitant and parallel hyper secretion of insulin precursor peptides.

In our patient cohort, platelet cation activity showed two significant correlations. Diabetes compared to non-diabetes was associated with a reduction in total platelet potassium concentration, while hypertension compared to non-hypertension was associated with reduced platelet membrane magnesium ATPase activity. The pathogenetic relevance of these measured changes is uncertain and our findings need to be interpreted in the context of our small patient cohort and the potential role of antihypertensive therapy and dysglycaemia in modulating cation concentration (Taylor *et al*, 2008). No difference in serum potassium concentration was evident between our patient subgroups but of note was that serum magnesium measurements were not undertaken and our findings may reflect relative magnesium deficiency between the control and treated hypertensive groups. Nonetheless, the regulatory role of intracellular magnesium in a number of cellular ion channels and pumps that modulate peripheral vascular tone has been well documented. In all of these instances low intracellular magnesium levels or activity would potentiate calcium-dependent vasoconstriction and underlie a dual dysregulation of the sodium-magnesium exchanger that is present in patients with

essential hypertension (Fox, 2001; Resnick, 1984). Previous data in a similar group of South Africans found increased, albeit not significant platelet membrane ATPase activity, especially magnesium ATPase activity in hypertensive versus normotensive subjects (Touyz, 1993). Our own data seemingly fits the hypothesis that insulin resistance or hyperinsulinaemia is able to modulate trans membrane cation flux in susceptible patients and thereby influence vascular tone and also provide a unifying mechanism that explains the link between hypertension and glucose intolerance (Sowers, 1994).

CHAPTER 5

5.1 Conclusion

A standard 75 g OGTT with a single 2 hour capillary glucose measurement estimated on a reflectance meter is a simple, accurate and cost effective method for determining glucose tolerance in elderly patients with treated hypertension. Postprandial blood glucose is an independent risk factor for cardiovascular events in Type 2 diabetes suggesting that more attention should be paid to postprandial hyperglycaemia, particularly in women.

Our findings support the strong link between hypertension, insulin resistance, hyperinsulinaemia and regional adiposity. Dyslipidaemia was closely related to the use of thiazides, albeit in low dose. It is possible that the use of thiazide and ß-blocker therapy may have an added adverse impact on glucose tolerance/intolerance. The metabolic sequel to this relationship is manifest in the very high incidence of glucose intolerance and diabetes in elderly patients with essential hypertension. The calculated magnitude of the level of insulin resistance in this group of patients increased progressively as deterioration in glucose tolerance supervened. The indices of pancreatic beta cell dysfunction paralleled a reduction in insulin sensitivity and were characterized by increased and prolonged endogenous insulin secretion and perturbed beta cell processing manifest in the increase in stimulated serum insulin precursor peptide concentrations. The changes in intracellular cation activity reflected both the diabetic and hypertensive states respectively and conform to the unifying hypothesis that abnormal cation exchange, hypertension and insulin resistance co-exist.

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