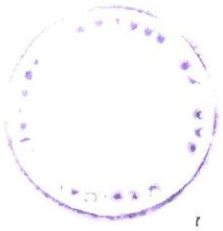
INSULIN RESISTANCE IN NON-OBESE AFRICAN HYPERTENSIVE SUBJECTS WITH NORMAL GLUCOSE TOLERANCE

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I HAVE READ THE DISSERTATION AND APPROVE IT FOR THE EXAMINATION.

SIGNED .....

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ABSTRACT

Insulin resistance and the concomitant compensatory hyperinsulinaemia, independent of obesity or abnormal glucose tolerance have been demonstrated to be associated with essential hypertension in Caucasians. This study was conducted to establish if insulin resistance and the accompanying hyperinsulinaemia is also associated with essential hypertension in Africans. A sample of 15 newly detected, untreated hypertensive non-obese subjects, together with 15 normotensive controls matched for age, sex and body mass index were studied. The modified Harano's insulin sensitivity test was employed. All subjects as well as the controls had normal oral glucose tolerance by WHO criteria. The mean systolic blood pressure in hypertensive subjects was 176±6 mmHg while the mean diastolic blood pressure was 111±2 mmHg. The controls had a mean systolic blood pressure of 131±3 mmHg and a mean diastolic blood pressure of 83±1 mmHg. The mean ages of the hypertensive subjects and controls were 38.5±1.7 years and 38.6±1.8 years respectively.

The mean (±sem) body weight in hypertensive subjects was 67.0±11.5 kg while that in controls was 64.6±11.3 kg. The mean height was 1.6±0.1 m in both groups, and the respective body mass indices were 24.6±0.9 kg/m^2 and 24.4±0.9 kg/m^2 which were comparable ($p = 0.92$).

Fasting blood glucose was significantly higher ($p = 0.04$) by about 7% in hypertensive subjects (mean = 4.4±0.1 mmol/l), than in controls (mean = 4.1±0.1 mmol/l). Steady state blood glucose concentration (SSBG) was significantly higher ($p = 0.009$) by about 26% in hypertensive subjects (mean = 5.0±0.3 mmol/l) than in controls (mean = 3.7±0.3 mmol/l). The mean metabolic clearance rate in hypertensive subjects (0.007± 0.001

ml/kg/min) was significantly lower ($p = 0.014$ by about 28.5% than that of controls (mean = 0.009±0.001 ml/kg/min.). There was no significant correlation between body mass index and systolic blood pressure in both groups ($r = 0.1$, $p > 0.7$ and $r = 0.3$, $p > 0.2$ respectively). A similar insignificant correlation was observed with diastolic blood pressure in the two groups.

There was a positive and significant correlation between waist: hip ratio and systolic blood pressure in hypertensive subjects ($r = 0.5$, $p < 0.05$) but not in the controls ($r = 0.2$ $p > 0.4$). No significant correlation was observed between waist: hip ratio and diastolic blood pressure in both groups ($r = 0.3$, $p > 0.2$, and $r = 0.2$ $p > 0.4$, respectively).

There was a negative correlation between metabolic clearance rate and systolic blood pressure in the control group which was statistically significant ($r = -0.5$, $p<0.05$), but not in the hypertensive subjects. No significant correlation was observed between steady state blood glucose concentration and both systolic and diastolic blood pressure in the hypertensives as well as controls. There was no significant difference in the mean fasting plasma insulin concentrations between the two groups. There was no correlation between fasting plasma insulin concentration and blood pressure in both groups.). These data indicate that there is an abnormality of insulin -mediated glucose metabolism in African hypertensives, suggesting an association between insulin resistance and essential hypertension.

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DECLARATION

I declare that the contents of this dissertation is my original work and it has not been submitted for a similar degree in any other University.

Date 2/10/92

Signature .....

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INTRODUCTION

A significant association has been demonstrated between insulin resistance and essential hypertension, independently of glucose intolerance or obesity [1,2,3,4,5,6,7,8,9]. This rather new observation has been preceded by a long period of appreciation of a close association between hypertension, obesity and glucose intolerance or non-insulin dependent (type II) diabetes mellitus [10,11,12,13,14], and confirmed by evidence gathered from epidemiological and clinical data [8,15,16].

The relationship between these three metabolic disorders exists in various combinations. For example, for a long time now clinicians have recognised the apparent increased prevalence of hypertension among diabetic patients [17,18,19,20,21]. Jarett et al [22] in 1978, studied the relationship between blood pressure and blood glucose concentration. A significant positive correlation was found between blood pressure and blood glucose which was independent of common associations with age, body mass index and heart rate. Many other workers investigating the prevalence of hypertension among diabetics have come up with more or less similar observations [23,24,25]. Hypertension worsens the complications already started by diabetes mellitus. The converse is also true. Hypertensive individuals who later become diabetic face a worse prognosis with considerable reduction in life expectancy than hypertensives without diabetes mellitus [21]. Both hypertension and diabetes mellitus have adverse effects on the coronary artery system, they are both atherogenic, increasing the frequency of ischemic heart diseases. Both conditions affect the kidney, eventually leading to renal insufficiency with albuminuria. The renal insufficiency in turn worsens the course of hypertension and diabetes mellitus. Treatment of hypertension in the presence of diabetes mellitus poses a great challenge. Many of the antihypertensive drugs interfere with the

metabolism of insulin and glucose [26,27,28,29].

Researchers therefore have many good reasons to justify the extensive studies trying to define the characteristics of the association of hypertension and diabetes mellitus.

Obesity is another metabolic disorder which has been found to correlate positively and significantly with elevations of blood pressure [5]. It has long been appreciated that obese individuals were more prone to develop high blood pressure [19] than non-obese subjects. Obesity is associated with decreased life expectancy and this becomes more marked when hypertension is superimposed. Obesity, like hypertension and diabetes mellitus is atherogenic, significantly predisposing one to coronary artery disease.

The prevalence of non-insulin-dependent diabetes mellitus in obese individuals has been extensively studied and shown to be much higher than in non obese persons (10,30).Obesity is present in 80-90% of adults with type II diabetes mellitus (31).

These observations point clearly to the overlapping relationship between obesity, diabetes mellitus and hypertension. It has now been realized that each of these conditions can and does influence the course of the other.

Studies have been conducted and others are still being conducted in search of a factor or factors which could account for the association among these three metabolic disorders: hypertension, type II diabetes mellitus and obesity. Some of these studies have pointed to a metabolic defect in the insulin stimulated glucose uptake as central to the apparent clustering of these disorders [19].

The role of insulin resistance in the pathogenesis and clinical course of non-insulin dependent diabetes mellitus was suggested almost 50

years ago by Himsworth and Kerr [32]. Since then, many studies have documented the existence of insulin resistance among individuals with impaired glucose tolerance or non insulin dependent diabetes mellitus [30,33,34]. Non insulin dependent diabetes mellitus is therefore considered to be a heterogeneous disorder characterized by impaired beta cell function and diminished tissue (liver and muscle) sensitivity to insulin [30,31]. Insulin resistance has been shown to be present in the vast majority of individuals with impaired glucose tolerance and in almost all patients with non insulin dependent (type II) diabetes mellitus who have fasting plasma glucose levels of 7.8 mmol/L or greater [31]. The insulin secretory response in these individuals is delayed and in many it is diminished. This defective insulin secretory response leads to inadequate suppression of hepatic glucose production and a decrease in glucose uptake by peripheral tissue during the period immediately following glucose ingestion [31]. The resultant post-prandial hyperglycemia provides a persistent stimulus to insulin secretion, leading to hyperinsulinemia which eventually returns the plasma glucose concentration to normal. However, fasting euglycemia will be maintained only at the expense of an increased plasma insulin concentration. With more time, the beta cell secretory response is exhausted, and the insulin response to elevated levels of plasma glucose becomes deficient. It is at this stage that type II diabetes mellitus sets in. Chronic hyperinsulinemia causes a downregulation in the number of insulin receptors, leading to more insulin resistance and more hyperglycemia. With severe hyperglycemia , blood glucose greater than 8.9-10 mmol/L, post binding abnormalities develop in addition to receptor downregulation, making the insulin resistance even more marked.

Insulin resistance has also been documented among individuals with

obesity [15,35]. It has been a long held view that obesity begets insulin resistance [21,36,37,38,39]. While this is true, the converse is also true, i.e, insulin resistance may cause obesity [39]. The hyperinsulinemia accompanying insulin resistance leads to increased concentration of insulin in the central nervous system and particularly in the appetite center in the hypothalamus to stimulate feeding. The insulin- stimulated overfeeding leads to obesity [10,40].

The apparent association of insulin resistance and obesity with hypertension has been defined by Modan et al [41]. In their study, investigating a link between hypertension, obesity and glucose intolerance which involved a random population sample of 2475, they found that insulin resistance was present in most patients with hypertension. It was suggested that these findings constituted a common pathophysiologic feature of obesity, glucose intolerance and hypertension.

Obesity was the first condition widely accepted to link insulin resistance to hypertension [41]. There is now sufficient evidence to suggest that, hypertension, obesity and type II diabetes mellitus can and do exist independently as overt clinical entities [4,10,19,42].

The first hints of a broader relation between insulin resistance and hypertension were provided from epidemiological analyses conducted by Dr Jeremiah Stamler and his colleagues [43] and published in 1975. Stamler and colleagues screened almost 40,000 young adult and middle-aged men and women, Caucasians and those of African heritage from the fall of 1967 through the Spring of 1972 for correlates of blood pressure. It was found among other things that weight, resting heart rate and plasma glucose concentration were independently related to both diastolic and systolic blood pressure. However, this study did not

provide evidence for a direct association of insulin resistance with hypertension. The evidence tendered was indirect with the assumption that the observed glucose intolerance in hypertensive patients was due to insulin resistance.

The first direct association of insulin resistance with essential hypertension was demonstrated by Ferrannini et al [1]. The subsequent findings by some workers of high insulin levels in hypertensive versus normotensive individuals, even when they are matched for body weight and glucose tolerance provide further support to the findings of Ferrannini and his colleagues. Studies have been showing a significant relationship between either systolic or diastolic blood pressure and both fasting and post glucose load plasma insulin levels [3,42] which has been used as evidence for the existence of insulin resistance. Other studies conducted in Israel, the USA, West German, Holland and Italy have demonstrated that as a group, patients with hypertension are insulin resistant and hyperinsulinemic [12]. The relationship between blood pressure and insulin resistance has also been demonstrated in children [23,44]. Most recently, the association of insulin resistance and hypertension, was broadened to include lipid abnormalities, namely increased concentrations of very low density lipoprotein (VLDL) triglycerides, and decreased concentrations of high density lipoprotein (HDL) cholesterol [19]. Review of the metabolic abnormalities resulting from insulin resistance has led to the demonstration of a cluster of factors all of which are considered to be important risk factors for the development of coronary artery disease. Put together, these factors constitute what is now referred to as Reaven's syndrome X (after Dr. Gerald Reaven of California [19]). The disorders in this syndrome include resistance to insulin stimulated glucose uptake, glucose

intolerance, hyperinsulinemia, increased VLDL- cholesterol, decreased HDL-cholesterol and hypertension [19,45,46,47,48,49]. The first variable in syndrome X: resistance to insulin stimulated glucose uptake is thought to be responsible for the pathogenesis and clinical course of each of the others [9,38,50,51,52,53,54]. Reaven and Hoffman have suggested that, abnormalities of insulin metabolism contributed to the development of hypertension. The pathological vascular changes seen in obesity, diabetes and hypertension could be due to insulin resistance and the resulting hyperinsulinemia [38,55].

The relation of insulin resistance to hypertension and the two conditions contributing to coronary artery disease has been further explained by each being an independent risk factor as defined in the Framingham study [21]. Insulin resistance is therefore considered to be an important link between the current life style in developed countries and the high prevalence of cardiovascular disease [19,52].

The purpose of this study was to determine if insulin resistance exists among African hypertensives independently of obesity and glucose intolerance as described in some studies among Caucasians. According to the available literature no studies of this nature have been performed in this continent.

The study also looked into the relationship between variables such as body mass index, plasma lipids, and waist-to-hip ratio with essential hypertension.

Although the demonstration of an association between insulin resistance and essential hypertension among Africans may not necessarily indicate a causal relationship, since it is possible that insulin resistance and elevation of blood pressure are all secondary to another genetic or metabolic defect that is expressed differently in

different individuals or racial groups, it will provoke more research in African subjects. It may also lead to a description of metabolic derangements whose interactions could lead to the establishment of essential hypertension. A clear description of any similarities or dissimilarities among hypertensives of different races is required to broaden our understanding on the etiopathogenesis of essential hypertension which is still surrounded by many gray zones today as it was more than 50 years ago.

BROAD OBJECTIVE

To establish whether insulin resistance is associated with essential hypertension, independently of glucose intolerance and obesity in Africans.

SPECIFIC OBJECTIVES

1. To determine the oral glucose tolerance test pattern and blood pressure in hypertensive subjects and controls.
2. To determine and compare the relationship between steady state plasma glucose concentration (SSPG) and glucose metabolic clearance rate (MCR), and blood pressure in cases and controls.
3. To determine and compare the relationship between body mass index and blood pressure in cases and controls.
4. To determine and compare the relationship between Waist-Hip ratio and blood pressure in cases and controls.
5. To determine and compare the relationship between plasma insulin and blood pressure.

REVIEW OF LITERATURE

EPIDEMIOLOGY OF HYPERTENSION

Hypertension is now a major public health in developed and developing countries [56]. Essential hypertension markedly increases the risk of death from coronary artery disease, cerebrovascular accident and renal failure [8,57,58].

Prospective epidemiological data suggest that in western societies most populations have blood pressure values above the optimum for risk of cardiovascular disease [58]. Some studies have indicated that about 17.7% of the entire US population of adults has definite hypertension [57]. Studies have demonstrated significant racial and geographic differences in both incidence and severity of essential hypertension [59]. For example in the US, the morbidity and mortality as well as the prevalence of essential hypertension are greater among African-Americans [56,57,59,60], particularly in early to middle adulthood, 34-54 years old [57]. About 25% of the African -Americans with hypertension are undiagnosed [66].

The higher incidence of hypertension among populations of African-Americans has been attributed to a higher prevalence of such cardiovascular risk factors as low socio-economic status, smoking, obesity, environmental factors and poor dietary practices [61].

Documentation of hypertension in Africa was made only about 50 years ago. Reports from this continent prior to 1940 indicated an insignificant prevalence of hypertension [62]. In 1929, Donnison [63] reported that the blood pressure of Kenyans remained normal and did not rise with age. He carried out investigations on natives living in one of the largest reserves in Kenya, namely South Kavirondo, situated on the shores of Lake Victoria, Nyanza. He noted that up to the fourth

decade, blood pressure levels among the Africans were similar to those of the Europeans. However, after the fourth decade the blood pressure, both systolic and diastolic tended to fall in the Africans, whereas in whites it continued to rise until the eighth decade.

Donnison's view prevailed up to the 1930's and early 1940's. During this period, hypertension was more the exception than the rule in Africa [33]. In 1934, 200 cases of hypertension were reviewed in Nairobi Kenya. It was found that all of them were Europeans and Asians, further supporting the earlier observation by Donnison that hypertension did not exist among Africans (of East Africa)[64].

A curious revolution in observations began in the late 1940's [65]. Williams and Sharper [65] noted that hypertension in East Africa, far from being rare was one of the common cardiovascular problems in urban populations. In 1941-42, Williams [65] conducted a blood pressure survey in Uganda. Out of 456 men and women aged 21-50 years surveyed, it was noted that their blood pressure levels remained normal throughout adult life, but did not fall with advancing age as Donnison had previously suggested. Two years later, that is in 1944, he (Williams) became the first researcher to record undoubted cases of essential hypertension in East Africa. The diastolic blood pressure levels in 23 patients he had examined during the previous four years rose above 95 mm Hg. However, there was still no reported case of malignant hypertension in East Africa. From the initial observations of Donnison and then Williams, it has now become common to document a rise in systolic and diastolic blood pressure with age in African men and women as has been shown in various population studies conducted in East and West Africa. African populations, especially in urban areas are increasingly showing a greater prevalence of obesity, hypertension

and hypertensive heart and kidney diseases [56,66], Hypertension is increasingly becoming a major public health problem in populations of African heritage world over in whom it tends to develop at an earlier age [56] being more aggressive with an accelerated course [67] and a much higher incidence of end-organ damage [67].

In 1978, Sedat et al [66] in a random house-to-house study of about 11000 urban Zulus of South Africa found an overall prevalence of essential hypertension of 25% (females 27% and males 23%). Women between the ages of 35 and 40 had a higher prevalence than men.

Philip [68] conducted a cross- sectional survey of arterial blood pressure in a Nigerian city in 1983. Approximately 15% of the studied population had borderline hypertension while 11.1% were definitely hypertensive. The highest age-specific prevalence as observed by Philip was in men aged 45-50 years.

The prevalence of hypertension in Swaziland, according to Mokhobo et al [69] has been estimated as 7%. Primary hypertension being the commonest, secondary hypertension accounting for only 8% of all cases of hypertension. He also found that 34.7% had one or more complications at initial presentation. Forty percent had cardiac involvement, 26.9% renal disease, 22% cerebrovascular disease and 11.1% retinopathy. Stroke emerged as the leading cause of disablement, accounting for 39% of all hypertension - related complications. Congestive heart failure was uncommon, present in only 0.93% of all hypertensives.

In 1986 June, Rene M' Buyamba - Kabangu et al [70] documented an overall prevalence of definite hypertension in adults of urban Kinshasa, Zaire of 9.9% (13.6% in males and 6.6% females). Of the hypertensives detected in that study, 69% were not aware of their elevated blood pressure, 31% were aware but only 13% were treated and 3% were

controlled. Another recent survey conducted in Zaire demonstrated a prevalence of hypertension in rural and urban Bantu of 14.2% and 9.9% respectively [71]. In this study, women were more affected in rural areas whereas men were more affected in urban communities. Vaughan et al [72] compared cardiovascular characteristics in two typically rural African communities in the Gambia and Tanzania as well as in a rural community in Jamaica of individuals of African descent. Both systolic and diastolic blood pressures were lowest in Gambians and highest in Jamaicans. No significant increase in either systolic or diastolic blood pressure with age was found in Tanzanian and Gambian men. All three female populations showed an increase in both pressures, systolic and diastolic with age, and these were statistically significant with the exception of that for diastolic blood pressure among Tanzanian women. The differences were not explicable in terms of body build.

In general, hypertension in Africa, contrary to the initial belief by Donnison and other earlier workers, is today an indisputable public health problem in populations of African descent the world over, whether they are living in Africa or outside Africa. More recent surveys continue to report a constantly increasing prevalence of hypertension throughout the African Continent [73]. A recent survey for example among Tanzanian executives revealed a prevalence of hypertension of about 30% (unpublished data). The rise in diastolic blood pressure being more striking than the rise in systolic blood pressure.

Studies show that Africans are more likely than Caucasians to develop hypertension at an early age [67]. Moreover, approximately 25% of the African hypertensives have uncontrolled hypertension, compared with only 16% of Caucasians [67]. It has been shown that blood pressure tends to rise more rapidly with advancing age in Africans than in

Caucasians, and that mortality from hypertension disease is approximately 6-13 times greater in Africans than in Caucasians [67]. End-organ damage secondary to hypertension likewise develops twice as fast and is more severe in the African hypertension [67].

Changing life style and eating habits, including increased dietary salt in take are thought to have contributed significantly to the changing epidemiology of hypertension in Africa. For example Trowell is said to have pointed to an increased salt in take in East Africa at the time high blood pressure began to be documented [74]. Sedentary life, with little physical exercise, increased smoking and drinking habits, and increasing mental stress due to the unstable socio-economic conditions are all other contributing factors.

EPIDEMIOLOGIC EVIDENCE FOR THE ASSOCIATION OF INSULIN RESISTANCE WITH ESSENTIAL HYPERTENSION

Several workers have conducted studies to find out whether insulin resistance exists in patients with essential hypertension. Preliminary data directly indicating that essential hypertension is an insulin resistant state comes from work conducted in 1987 by Ferraninni et al [1]. To test whether hypertension is associated with insulin resistance, independently of obesity and glucose intolerance, they measured insulin sensitivity using the euglycemic insulin-clamp technique in 13 young subjects with untreated essential hypertension, normal body weight and normal glucose tolerance. Total insulin-induced glucose uptake was markedly impaired suggesting the presence of insulin resistance. It was also found that total glucose uptake related inversely with systolic and mean blood pressure. Insulin resistance directly correlated with the severity of hypertension.

A closely related study was performed one year later, by Shen et al [75] who measured plasma glucose and insulin responses to a glucose challenge and insulin stimulated glucose uptake in age, weight and sex matched Chinese men . Hypertensive men, treated or untreated had significantly elevated plasma glucose and insulin responses to the oral glucose dose compared to normal men. Their results showed that patients with hypertension, treated or untreated were insulin resistant, hyperglycemic and hyperinsulinemic compared to a control group.

Bonita et al [4] in an attempt to determine if insulin resistance could be an antecedent to hypertension in a high risk population, studied normotensive and borderline hypertensive lean young individuals of African heritage, matched for body mass index and percent adipose mass. A significantly higher fasting plasma insulin concentration in borderline hypertensives independently of obesity or glucose intolerance was found. It was also found that insulin mediated glucose uptake correlated negatively with blood pressure that is, the lower the insulin mediated glucose uptake, the higher the blood pressure. From this study it was suggested that insulin resistance could be an antecedent of essential hypertension.

The prevalence of hyperinsulinemia in patients with high blood pressure has also been studied by Zavaroni et al [76]. 41% of the hypertensive patients were hyperinsulinemic suggesting the presence of insulin resistance. These subjects also had higher plasma glucose, triglyceride and lower HDL - cholesterol concentrations. Similar findings have been reported by Cacciatone et al [46].

Leonardo et al [3] conducted a study to determine whether a decreased sensitivity to insulin was involved in the pathogenesis of essential hypertension by measuring fasting blood glucose, serum

insulin, serum C-peptide and the glucose -insulin ratio in lean normotensives, overweight normotensives, lean hypertensives and overweight hypertensives. Compared with the lean normotensives, the patients who were overweight, those with hypertension alone and those who were both overweight and hypertensive showed increased fasting serum insulin and C-peptide levels and a lower glucose-to-insulin ratio, all pointing to the existence of insulin resistance in those subjects.

The relation between hypertension and hyperinsulinemia in the absence of insulin resistance has also been studied. O' Brien, et al [77], studying a large cohort of patients with insulinoma found an incidence of hypertension of 12%, which was not different from that expected from the general population. It was consequently concluded that hyperinsulinemia from insulinoma (not accompanied by insulin resistance)was not associated with hypertension. Sawick [78] in an independent study reported similar findings. Chronic hyperinsulinemia in patients with insulinoma was not associated with elevation of blood pressure. More evidence for the association of insulin resistance with hypertension, came from work by Lucas et al [15] who assessed factors in overweight persons that accounted for a tendency toward hypertension in very obese women, 26-77 years of age. Both systolic and diastolic blood pressure were significantly related to fasting serum insulin levels even when age, weight and blood glucose level were controlled. The relation between serum insulin and blood pressure was more pronounced in women with a positive family history of hypertension.

Eriksson et al [24] estimated insulin resistance by determining the 12 hour fasting insulin-to-glucose ratio during an oral glucose tolerance test controlled for body mass index in 4677 unselected subjects without

clinical coronary heart disease or previous diabetes. Insulin resistance correlated well with both systolic and diastolic blood pressure.

Caderhom et al [54] evaluated insulin resistance in 807 middle-aged subjects at a health survey, using an index measured in a 75g oral glucose tolerance tests. The mean value of insulin resistance was higher in a hypertensive group than among normotensives, independently of body mass index, physical activity, smoking, sex, age and thiazide treatment. One third of the hypertensives had a high resistance value. Another third of the hypertensives and also about one third of normotensives had slightly increased insulin resistance. The remaining third of the hypertensives had a normal-low resistance. The study showed that hypertensive had higher insulin resistance than normotensives with use of a simplified oral glucose tolerance test measure.

In obesity, the occurrence of hypertension is said to mark the presence of additional hyperinsulinemia and insulin resistance independent of any impairment of glucose tolerance [79].

To investigate if hypertension is associated with defects in carbohydrate metabolism, plasma glucose and insulin responses to oral glucose and resistance to insulin stimulated glucose uptake were compared by Reaven et al [80] in normal individuals, untreated hypertensives and treated hypertensives after matching for sex, age and degree of obesity. Plasma glucose and insulin responses were higher in patients with hypertension, treated or untreated, who were also insulin resistant as compared to normal subjects. Patients with hypertension were hyperglycemic and hyperinsulinemic as compared to normal subjects.

In another study Swilocki et al [81] reported findings similar to those of Reaven. Thomas et al [2] concluded in his study that insulin resistance was a characteristic feature of primary hypertension, independent of obesity, and that after correcting for a series of probable confounding factors, hypertension still emerged as part of a syndrome characterized by major abnormalities of carbohydrate, insulin and lipid metabolism, which independently or in concert may act as important risk factors for cardiovascular disease.

In 1985, Singer et al [82] demonstrated a significant post prandial hyperinsulinemia in patients with mild essential hypertension as compared with normal controls. Post-prandial hyperinsulinemia in patients with mild hypertension may possibly provoke lipid accumulation in the arterial wall and therefore may be a relevant risk factor for development of atherosclerosis.

Although many workers have come up with convincing evidence for the association of insulin resistance with hypertension independent of the common confounding factors e.g. age, glucose intolerance and obesity, some have found contrasting results. Jean-Claude et al [83] investigated the hypothesis that insulin resistance was concerned in the pathogenesis of essential hypertension. Patients with essential hypertension had normal fasting serum insulin values and normal fasting glucose /insulin ratios while the hypertensive non insulin dependent diabetic subjects had higher fasting serum insulin and lower glucose/insulin ratios than either normotensive diabetic or non diabetic patients. It was suggested that hyperinsulinemia was not causally related to essential hypertension but that it might contribute to the hypertension of non insulin dependent diabetes mellitus in association with low hepatic insulin clearance. Saad et al [10] also found no

association between insulin resistance and essential hypertension in untreated hypertensive Pima Indians. There was however a significant relation between hyperinsulinemia and hypertension in treated hypertensive subjects. This observation suggested that hyperinsulinemia resulted from the treatment rather than contributing to the pathogenesis of hypertension. Although hypertension and blood pressure were closely related to obesity and glucose intolerance in Pima Indians as in other populations, insulin did not appear to be a major determinant of hypertension or blood pressure regulation in this population. Although Pima Indians are commonly insulin resistant and hyperinsulinemic, they have a lower prevalence of hypertension than other ethnic groups.

Secondary hypertension on the other hand has been shown not to be an insulin resistant state [84].

INSULIN - GENERAL CONSIDERATIONS

Insulin is a polypeptide molecule containing in most species 51 amino acids arranged in 2 chains, one containing 21 amino acids (A or α-chain) and another containing 30 amino acids (B or ß- chain). The two chains are united by 2 disulphide bonds [85,86,87] Insulin was discovered in 1921 by Banting and Best [87] and its protein nature was described by Wintersteiner and his colleagues as early as 1928 [86]. However, it was not until 1955 after the extensive work of Sanger that the primary structure of insulin was elucidated [86]. Insulin occurs throughout the vertebrate kingdom, and immunological and histological evidence indicates its presence in the digestive systems of several invertebrate species including molluscs and echinoderms. Its molecular weight shows slight variation among animals, i.e. it is about 6000 for human insulin

[88], 5734 for bovine insulin [86,88] and 5778 for porcine insulin[89].

Certain structural features of the insulin molecule are conserved throughout nature. These include the positions of the three disulfide bonds, the N-terminal and C-terminal regions of the A-chain and the hydrophobic residues in the C-terminal region of the B-chain as well as others [86]. When the amino acid structures of beef, pork and human insulin are compared, differences are noted at positions 8, and 10 of the A-chain and position 30 of the B-chain. Human insulin differs from pork insulin by only one amino acid, that is threonine at position 30 in human insulin instead of alanine at this position in pork (porcine) insulin. Human insulin differs from beef (bovine) insulin by three amino acids: threonine, isoleucine and threonine at positions 8,10 and 30 respectively in human insulin instead of alanine, valine and alanine at these respective positions in bovine insulin [86]. Fish insulin maintains more than 50% homology with human insulin [89].

The biological activity of most mammalian insulin preparations ranges from 25-30 IU/mg dry weight. The bovine insulin standard of the International Union of Pure and Applied Chemistry is stated to have activity of 25 IU/mg dry weight

Insulin degrades in alkaline medium (pH >10) primarily due to its cysteine residues.

INSULIN BIOSYNTHESIS

Insulin is synthesized in the B-cells of the islets of Langerhans of the pancreas. The Islets of Langerhans form about 1-4% of the pancreatic mass with the majority being found in the tail [86,89]. The B-cells account for about 60- 80% of the total islet cell mass [89,90]. There are other cells as well in the Langerhans islets e.g. A -cells, accounting for

15-20%,and secreting glucagon, D-cells, forming 5-10% and secreting somatostatin and the pancreatic polypeptide cells forming 15-20% of the islet cell mass and secreting pancreatic polypeptide [86,87,89,90].

The insulin gene in man is located on the short arm of chromosome 11 [87,90] and contains 1789 base pairs [90]. It is present in all eukaryotic cells, although it is only transcribed in the pancreatic B-cell [90].

Synthesis of insulin is preceded by synthesis of proinsulin by the rough endoplasmic reticulum of the B- cells [81,82,85]. Synthesis of proinsulin is in turn preceded by synthesis of preproinsulin, a much longer polypeptide with more amino acid residues than proinsulin [86,87,90]. Preproinsulin once synthesized is rapidly converted to proinsulin by removal of the additional amino acid residues (24 residues in humans). Proinsulin, once formed is transported to the Golgi apparatus by a process requiring about 10-30 minutes [86] for packaging into clathrin -coated granules. At this stage, cleavage of proinsulin into insulin and connecting peptide (C-peptide) commences and continues throughout the life time of the granule.

Conversion of proinsulin to insulin by proteolysis is a strictly intracellular process. At least 95% of proinsulin is converted into insulin and C-peptide which are stored in secretion granules until both are released in blood. The C-peptide is that portion of the proinsulin molecule which links the carboxyl terminus of the B-chain to the amino terminus of the A-chain of the insulin molecule. Variations in length in the mammalian proinsulin occur only in the connecting peptide.

Intact proinsulin exhibits about 3-5% of the biological activity of insulin. Insulin within the clathrin-coated granules, spontaneously

forms hexamers with two zinc ions and one calcium ion. The granules containing insulin, proinsulin and C-peptide lose their clathrin coat as they move towards the cell surface along the microtubular system and ultimately expel their contents into the extracellular media by exocytosis.

MECHANISMS OF INSULIN SECRETION

There are still major gaps in the understanding of the precise mechanisms involved in the release of insulin from the B-cells. Insulin release is a result of an interaction between a variety of effector systems, whereby glucose serves as a major agonist [85].

BASAL INSULIN RELEASE

Studies show that basal insulin secretion rate is primarily determined by glucose, and is directly related to glucose level when all other factors are held constant [34]. However basal insulin is also regulated by many other factors, including parasympathetic and sympathetic neural tone, other substrates such as amino acids and hormones such as cortisol and growth hormone. Thus the resultant basal insulin and glucose levels represent an integrated response to all of these influences. It is glucose that provides for a feedback loop that maintains insulin and glucose levels constant in basal states.

GLUCOSE INDUCED INSULIN RELEASE

In addition to its role in the regulation of basal insulin secretion, glucose is an important physiologic stimulant of insulin secretion. However, there is still no agreement on exactly how glucose stimulates insulin secretion. Glucose may act by binding to a glucose receptor on the surface of the B-cell [85], although it seems more likely that it must

first enter the cell and be metabolized before insulin synthesis and secretion can be stimulated. The glucose metabolite responsible for induction of insulin release has not been precisely identified although it is probably a triose and phosphoenol pyruvate has been implicated [82].

When the extracellular glucose concentration is elevated, there is an immediate increase in glucose metabolism, with a subsequent increase in the ATP/ADP ratio which leads to closure of specific ATP-regulated K^+-channels in the B-cell membrane. These channels are also closed by sulphonylureas. Under basal conditions, the relatively high B-cell permeability to K+ions determines its membrane potential, which is typically maintained at -80 mV. Closure of the ATP-regulated K^+-channels depolarizes the cell. At a threshold of -40 mv, L-type Ca^{2+} channels open. Ca^{2+} enters the cell along its electrochemical gradient, and intracellular Ca^{2+} concentration increases. This leads to changes in the function of the Ca^{2+}-calmodulin regulated proteins and the eventual release of insulin secretory granules [104].

The pancreatic islet responds rapidly to hyperglycemia. An abrupt rise in plasma glucose level above 100 mg/dl ($\tilde{}$5-6 mmol/L) causes a near maximal rate of insulin secretion, which is attained within seconds to a few minutes [34]

Glucose stimulated insulin secretion shows a typical biphasic pattern [34]. The initial phase consists of a rapid rise in insulin level that begins 1-3 minutes after glucose level is raised, and is followed by a subsequent return toward baseline which is completed 6-10 minutes after the stimulus. The acute insulin response to glucose is directly related to the rate and amount of glucose administration, with a 20 g intravenous bolus being a maximum stimulus in man [34]. The initial

spike is later followed by a gradual increase in the release of insulin accounting for the second phase which is directly related to the degree and duration of glucose level elevation [34,82]. This phase persists for the duration of hyperglycemia.

Various explanations have been proposed for the biphasic pattern of insulin release and disagreement on this issue still prevails. The much favored explanation considers insulin as being segregated into two compartments within the B-cell. The compartments behave either as two distinct functional units or as anatomical reality (e.g. insulin granules located near the cell membrane and those present in the more central parts of the cell). The first compartment, presumed to be of limited size reacts rapidly on stimulation and discharges its contents into the extracellular space within a duration corresponding to the initial peak of insulin release [81,82]. The second compartment has slower kinetics but a larger capacity, and constitutes the late insulin response. Ongoing proinsulin biosynthesis as well as release of stored granules also contributes to the normal second phase response.

In short it can be stated that, the first phase represents the release of preformed insulin granules near the cell surface whilst the second phase is approximately 50% preformed granules and 50% newly synthesized insulin.

Nutrients other than glucose, particularly amino acids are also capable of stimulating insulin release. Arginine and leucine appear to be the most potent stimuli of insulin secretion [91].

Insulin secretion is also induced by vagal (parasympathetic) stimulation and inhibited by alpha-adrenergic stimulation . Beta-adrenergic stimulation induces insulin release.

Stressful states are associated with inhibition of insulin secretion, thus allowing more glucose to be available for non insulin mediated glucose uptake.

CELLULAR MECHANISMS FOR INSULIN ACTION

Insulin exerts many metabolic effects on different tissues. It regulates cellular metabolic reactions by its action on the plasma membrane, intracellular enzymes and nucleus [92].

One of the most widely accepted and fundamental action of insulin is its ability to remove a barrier for glucose entry into certain tissues, primarily muscle and adipose tissue [93]. Insulin also effects transmembrane transport of amino acids and ions. It enhances catecholamine release independent of any changes in plasma glucose levels, and acutely regulates sodium and water reabsorption mainly from the proximal renal tubule [16,87,94].

Insulin activates or deactivates a range of intracellular enzymes such as hormone sensitive lipase, or enzymes involved in the glycolytic pathways. Moreover, it regulates protein synthesis by directly influencing gene expression in the nucleus [95] and in this way promoting cell growth [96].

The exact mechanism whereby the transmembrane signalling mechanism(s) results in different insulin-mediated cellular effects is not known. However, the insulin receptor tyrosine kinase, the expression of second messengers and the action of protein kinase-C may either individually or in combination, mediate some of the insulin effects such as translocation and activation of glucose transporter proteins [92].

Although the cellular responses are diverse, and in many instances tissue specific, all are mediated by insulin receptor [96].

INSULIN RECEPTOR

The first stage in the propagation of the insulin signal is the coupling of insulin to its specific receptors situated in the plasma membrane of the target cells [92,96]. The existence of insulin receptors has been known for nearly 20 years now [87,95]. The insulin receptor gene is represented on the short arm of chromosome 19 [87,96]. It is a well characterized heterotetramer containing two 120 - 135kDa alpha-subunits with 719-731 amino acids and two beta-subunits with a mass of about 90-95 kDa and 620 amino acid residues [85,87,92,95,96]. The subunits are assembled in the cell membrane with the alpha-subunits extracellular and linked to the intracellular ß-subunits by disulfide bonds [87,95,96]. The B-subunit has tyrosine activity [85,87] and is capable of undergoing autophosphorylation. The extracellular alpha-subunits provide binding sites for insulin molecules. After binding insulin, the receptors cluster into coated pits on the cell surface [87,92]. Gordon et al using labelled insulin, demonstrated that these clustered coated receptors then undergo rapid internalization [97] leaving the intracellular domain of the ß-subunit in contact with cytosol [87].

The surface concentration of receptors varies widely, from as few as 40 on circulating erythrocytes to more than 200,000 on adipocytes and hepatocytes. However, insulin receptors have practically been demonstrated in all cells [96]. Down regulation of receptors occurs after continuous exposure to raised concentration of insulin. This may be implicated in the impairment of insulin action seen in certain hyperinsulinemic states [87].

Hypothetically, receptor internalization and conformational changes could represent the initial step in the insulin signalling system. However, it has been shown experimentally that receptor internalization

is not a prerequisite for insulin effect [87]. Receptor aggregation itself may initiate an insulin signal [98].

THE INSULIN SIGNAL

Recently, several signalling systems have tentatively been described, which alone or in combination could exert certain insulin effects. Two such systems are: Beta-subunit autophosphorylation and the second messenger system.

I. Tyrosine kinase and autophosphorylation of the beta subunit

Kasuga et al have demonstrated that insulin binding to the receptor-subunit induces autophosphorylation of the ß-subunit [85,99]. This further increases the tyrosine activity. Autophosphorylation occurs at several tyrosines on the ß-subunit. The subsequent intracellular targets of the receptor kinase differ depending on which tyrosine has been phosphorylated. This is thought to account for the great specificity of insulin activity [87]. The tyrosine kinase activity of the receptor beta-subunit is capable of phosphorylating both exogenous and endogenous substrates, and may therefore represent the initial step of a tentative phosphorylation cascade. The demonstration of regulation of several insulin-sensitive intracellular enzymes by phosphorylation/dephosphorylation events is consistent with this view [92].

Many studies have shown that the receptor tyrosine kinase has an important role in the insulin signal [100]. Mutant cells lacking receptor tyrosine kinase activity are also insulin resistant [101]. Cells obtained from insulin resistant diabetic patients may exhibit defective activation of the insulin receptor tyrosine kinase [102,103].

However, the exact role of the receptor tyrosine kinase and the

phosphorylation cascade is hotly debated. Experiments have shown that glucose transport activity may be initiated without activation of the receptor tyrosine kinase. That is, some, but not all of the metabolic effects of insulin may be regulated by the tyrosine Kinase.

II: Second Messenger

Experiments have described compounds which can initiate insulin-like responses in several intracellular enzymatic systems [92]. Insulin hydrolyses small water-soluble glycolipids which can be further hydrolysed to an inositol phosphate glycan and diacylglycerol [104,105]. Thus two different signals for insulin may be generated, since inositol-phosphate glycan may regulate the cyclic adenosine monophosphate phosphodiesterase, and diacylglycerol activates protein kinase C [87,104,120]. The exact mechanism whereby insulin initiates the hydrolytic process is not clear, but this could occur via stimulation of phospholipase-C.

Although it has been hypothesized that insulin mediates part of its intracellular actions by decreasing cyclic adenosine monophosphate concentration through increased cyclic adenosine monophosphate phosphodiesterase activity, there are several instances in which the actions of insulin can be dissociated from its ability to decrease cyclic adenosine monophosphate concentration. Larner et al [85,99] demonstrated that in muscle, glycogen synthetase was activated by insulin while cyclic adenosine monophosphate concentration was either unaffected or actually increased. In adipocytes, the antilipolytic action of insulin was observed under conditions in which cyclic adenosine monophosphate concentration was unaffected [85,99,106].

Thus, a decrease in the intracellular concentration of cyclic adenosine monophosphate by insulin under certain conditions may

explain some, but not all of the effects of insulin [85]. On the other hand, there is some evidence to suggest that inositol phosphate and diacylglycerol may have an important role in the insulin signal system. Both substances are produced in the cells following insulin exposure [107]. However, insulin does not stimulate protein kinase-C in all cell type. Thus the hydrolysis of glycolipids and stimulation of protein kinase-C may not explain all the different effects of insulin.

In summary, the current hypothesis on the mechanisms of insulin action is that insulin binds to one of the two binding sites on the extracellular portion of the heterotetrameric insulin receptor. This is followed by conformational changes transmitted through the transmembrane B-subunit, increasing the tyrosine activity even more. Communication with the guanine nucleotide regulatory protein (G-protein system) may occur via tyrosine kinase activity or via conformational changes within the cell membrane. The stimulated G-protein system in turn activates phospholipase-C which then acts on phosphotidyl inositol to release inositol triphosphate. The released inositol triphosphate then activates Ca^{2+}-dependent protein kinase and diacylglycerol which in turn activates the protein kinase-C. The end result of all these events is activation of key enzymes of glucose metabolism, regulation of gene expression and enhancement of rate of the translocation of glucose transporters from intracellular stores to the plasma membrane.

INSULIN RESISTANCE

DEFINITION OF INSULIN RESISTANCE

Insulin resistance may be defined as a state in which a given concentration of insulin produces less than the expected biologic effect [88,108]. Clinically, insulin resistance is said to be present when more

than 200 units of insulin per day for more than two days are necessary to maintain adequate control of blood glucose in the absence of acute ketoacidosis or coma [109]. Patients requiring more than 60 but less than 200 units per day are designated as insulin insensitive [109]. These definitions are based on clinical grounds rather than on laboratory data of quantitative measurements of insulin sensitivity. Secondary insulin resistance occurs in such disease states as infections; endocrinopathies such as acromegaly adrenal cortical hyperfunction and thyrotoxicosis; impaired liver function especially cirrhosis and hemochromatosis, chronic lymphocytic leukemia and lipo-atrophic diabetes mellitus [88,108,109]. Insulin resistance of short duration (usually less than 48 hours) is associated with diabetic ketoacidosis or coma [109]. When insulin resistance develops in the absence of any other disease, it is referred to as primary or idiopathic.

MECHANISMS OF INSULIN RESISTANCE

Insulin has diverse cellular actions that may result from more than one biochemical mediator and that may even involve more than one receptor type. As a result, resistance to one action of insulin, e.g. its glucose lowering effect need not necessarily be associated with resistance to other important actions such as antilipolysis, amino acid uptake or growth stimulation [88].

Insulin resistance can be classified according to pathophysiological abnormalities. One such approach considers three general causes of insulin resistance: Abnormalities of the insulin molecule; circulating antagonists of insulin action and target cell (receptor) defects in the pathways for insulin action. However, it should be noted that there exists substantial overlap between these categories.

I: Abnormal Insulin molecule

An apparent insulin resistant hyperinsulinemic state can exist because of secretion of an abnormal beta cell product (insulin) with defective biologic activity [87,88,92,108,119]. Bruce et al [119] have reported on the impaired biologic activity of a 51 year-old non-obese diabetic patient's circulating insulin due to a structural abnormality. Defective insulin's biologic activity could result from mutation in the structural gene for insulin. This has also recently been described in an adult type II diabetic with fasting hyperglycemia and hyperinsulinemia but with surprisingly normal sensitivity to exogenous insulin. This patient's insulin had normal immunologic, chromatographic and electrophoretic properties, but it displayed a markedly reduced ability (60% reduction) to bind to insulin receptors and its bioactivity potency in isolated adipocytes was reduced by about 85% [88,120]. A leucine for phenylalanine substitution was demonstrated at position 24 of the beta insulin chain, which is the heart the of active site of the insulin molecule [120] and which accounted for the 60% reduction in its receptor binding affinity [102].

Further more, two families with familial hyperinsulinemia have been described. In this syndrome, the proteases within the beta-cell secretory granule cannot effectively convert proinsulin into insulin plus C-peptide because of a mutation in the proinsulin molecule. Consequently, an increased amount of proinsulin is secreted which has only about 5% of the biologic activity of insulin. Because proinsulin is immunoreactive in the insulin radioimmunoassay, this syndrome leads to an apparent hyperinsulinemic state [120]. The prevalence of insulin resistance due to abnormalities in the insulin molecule is probably low.

II: Circulating Insulin antagonists

Insulin resistance may be caused by circulating insulin antagonists. These antagonists may be hormonal or non hormonal (antibodies) in nature [120]. Antibodies could be directed against insulin molecule or insulin receptor [88].

ANTIBODY -INSULIN ANTAGONISTS: Virtually all patients treated with exogenous insulin (bovine,porcine or even human) develop insulin binding IgG antibodies within a few months [88,120]. These antibodies have not proven significant in most patients. In a minority (0.1%) antibody titres rise, and clinically important insulin resistance ensues [88,102]. This resistance is often self limited and may be treated by substitution of less immunogenic forms of insulin (e.g. porcine vs bovine) or by therapy aimed at the immune response itself, e.g. prednisolone. These antibodies appear to limit the access of insulin to its receptors.

HORMONAL INSULIN ANTAGONISTS: The hormonal antagonists consist of all known counter regulatory hormones such as growth hormone, cortisol, glucagon and catecholamines. Insulin resistance resulting from circulating hormonal antagonists is however very rare. Examples are the insulin resistance seen in clinical syndromes due to hypersecretion of these hormones e.g. Cushing's syndrome and acromegaly and that seen in states of stress [88,102].

III. Insulin receptor defect

This can be classified into two categories: insulin resistance due to loss of insulin receptors and that due to post-receptor defects.

INSULIN RESISTANCE DUE TO LOSS OF INSULIN RECEPTORS: Insulin action at the level of the target tissue consists of a complex sequence

of events. Because the first step in insulin action involves binding to the receptor, it is apparent that a decrease in the number of cellular insulin receptors could lead to insulin resistance [30,87,88]. The reduction of insulin binding in obesity was shown to be due to a decrease in the number of available receptors, with all other binding parameters (affinity and temperature) being normal [88,102]. However, the potential relationship between receptor and insulin action is not a straightforward one because of the fact that cells possess spare receptors. The spare-receptor concept is based on the observation that a maximal insulin effect is achieved at an insulin concentration that occupies fewer than the total number of cellular receptors. For example it has been shown that in isolated adipocytes, maximal stimulation of glucose transport occurs when only 10% of the adipocyte insulin receptors are occupied. Therefore 90% of the normal complement of receptors are spare. These spare receptors are all potentially functional, with only statistically random events determining which receptors are occupied at any given time. Any group of occupied receptors amounting to 10% of the total will lead to the same metabolic response [88]. The only time a decrease in the number of insulin receptors can lead to a decrease in maximal insulin action is when the reduction in receptor number is sufficiently great that fewer than 10% of the normal complement of receptors are present [88]. Studies have demonstrated a reduction in insulin binding capacity in the basal state in obese individuals [88,102]. The reduction of insulin binding in obesity was shown to be due to a decrease in the number of available receptors [102].

POST RECEPTOR DEFECTS: Insulin resistance could also result from a post receptor defect in the intracellular pathway of glucose metabolism

[88,102]. The precise biochemical locus of the this intracellular abnormality is not known, but recent in vitro experiments suggest, at least for adipocytes,that prolonged exposure to high concentrations of insulin may induce resistance to insulin not only by causing loss of insulin receptor but by impairing post receptor steps as well [88].

A post receptor defect results in a proportionate decrease in insulin action at all insulin concentrations, including maximally effective hormone concentrations [110]. This abnormality is referred to as a decrease in responsiveness, in contradistinction from that resulting from loss of insulin receptors which has been referred to as insulin insensitivity [92,110].

In obese animals, although the number of cellular insulin receptors is decreased, the marked decrease in insulin responsiveness indicates that the major abnormality responsible for the cellular insulin resistance is a post receptor defect in insulin action. The greater the degree of hyperinsulinemia or insulin resistance, the greater the post receptor defect [120]. In summary, it can be stated that the specific mechanisms underlying insulin resistance are heterogenous A continuum of defects exist. In patients with mild insulin resistance,the impairment in insulin action is due to a decreased number of insulin receptors leading to decreased insulin sensitivity. In patients with severe insulin resistance, decreased numbers of insulin receptors and a post receptor defect in insulin action co-exist, but the post receptor defect is the predominant abnormality. Causes of insulin resistance could be summarized as follows [121]:

Abnormal B-cell secretory product (insulin)

- Abnormal insulin molecule
- Incomplete conversion of proinsulin to insulin

Circulating insulin antagonists

- Elevated levels of counterregulatory hormones (growth hormone, cortisol etc)
- Anti-insulin antibodies

Target tissue defects:

- Insulin receptor defects (decreased receptor number, anti-receptor antibodies)
- Post receptor defects.

THE ROLE OF INSULIN RESISTANCE IN THE PATHOGENESIS OF ESSENTIAL HYPERTENSION

Normal blood pressure is arbitrarily defined as a systolic blood pressure equal to or below 140 mm Hg with a diastolic (the 5th phase) blood pressure equal to or below 90mmHg [122]. The average normal blood pressure in the brachial artery in young adults in the sitting or supine position at rest is approximately 120/70 mmHg [123].

Hypertension in adults is arbitrarily defined, according to the formal definition of hypertension produced by the WHO Expert Committee in 1978, as a systolic blood pressure equal to or greater than 160 mmHg and/or diastolic (5th phase) blood pressure equal to or greater than 95 mmHg, on at least three consecutive and separate occasions [122,124]. The term borderline hypertension is used to denoted blood pressure values between the normal and hypertensive ranges as described above

The most common form of hypertension is called essential hypertension or primary hypertension, indicating that its development cannot be explained by a specific abnormality. Over 90% of hypertensive individuals have essential hypertension and only less than

10% have an identifiable cause for the elevation of blood pressure [19] and hence it is termed secondary hypertension.

The majority of hypertensive patients, about 70%, have mild hypertension defined as diastolic blood pressure between 95 and 104 mmHg. Of the remaining, 20% have moderate hypertension: diastolic blood pressure between 105 and 114 mmHg, and 10% have severe hypertension, diastolic blood pressure equal to or greater than 115 mmHg [53].

The dividing line between normotension and hypertension is artefactual. According to Pickering [125], no clearly distinct dividing line can be drawn between normotensive and hypertensive levels. For example, the risk of stroke and ischemic heart disease, the two main complications of hypertension has been demonstrated in some societies to rise steadily with every increment of diastolic blood pressure above 70 mmHg [53].

The basic problem in hypertension is therefore the tendency for blood pressure to rise with age. The rise of blood pressure with age has been shown by epidemiological data to occur exclusively and invariably in salt-eating societies [126]. It occurs in about 93% of men and 85% of women who survive to age of retirement in urban Australia [126].

When blood pressure rises with age in salt eating cultures, it also displays a continuous distribution and the whole range of elevated blood pressure is pathological, with what is formally defined as hypertension occurring only at the upper end, i.e. occupying the apex of the pyramid [126].

BLOOD PRESSURE REGULATION - PHYSIOLOGICAL CONSIDERATIONS

Arterial blood pressure is a product of cardiac output and peripheral vascular resistance. Cardiac output is influenced by heart rate, central venous pressure, autonomic nervous activity and the intrinsic state of the heart itself [87,123]. Peripheral vascular resistance is influenced by autonomic nervous activity, renin-angiotensin-aldosterone activity, endogenous vasodilators (e.g. bradykinin and prostaglandins) salt intake and extracellular volume [87]. Any condition affecting cardiac out, peripheral vascular resistance or both will affect blood pressure regulation as well. Insulin is one such factor which can affect these regulatory mechanisms to bring about elevation of blood pressure.

A relation between insulin and blood pressure has been noted as early as 1966, when it was observed that plasma insulin levels were elevated in patients with essential hypertension compared to a control group [127]. In 1976, Gans et al [128] observed a higher body mass index, a greater degree of glucose intolerance and a higher fasting serum levels in untreated hypertensive men than normotensive controls.

Resistance to insulin stimulated glucose uptake is now believed to play an important role in the pathogenesis and clinical course of several cardiovascular diseases in addition to glucose intolerance and diabetes mellitus. Most prominent of these diseases are hypertension and coronary artery disease [42]. There are several mechanisms by which insulin may interfere with the regulation of blood pressure to induce hypertension. These include: renal sodium handling, vasopressive reactivity, the sympathetic nervous system; the renin-angiotensin-aldosterone system; atrial natriuretic peptide and the kallikrein-kinin system.

I: Effects of insulin on renal sodium handling

Sodium is the most abundant extracellular cation and is essential for maintaining adequate blood volume and organ perfusion [53]. Abnormal sodium metabolism has been shown to be crucial in the pathogenesis of certain forms of hypertension, particularly salt-sensitive hypertension [53,73,124]. Individuals at high risk for development of salt sensitive hypertension include normotensive patients with strong family histories of hypertension, elderly patients, patients of African descent and those with low-renin hypertension [53,73].

Animal studies show that elevated sodium and reduced potassium concentrations within the arterial wall elevate the systemic vascular resistance, leading to established chronic hypertension [5]. The central role of sodium in the pathogenesis of hypertension is further underlined by studies which have shown that persons who feed on low sodium and high potassium diets, who tend to be lean and active, have little tendency to develop hypertension with increasing age. It has been shown that if the dietary intake of sodium is less than 30 mmol/day, hypertension is almost not found [53]. If dietary sodium is between 30 and 60 mmol/day, about 3%develop hypertension [53]. Severe restriction of sodium to less than 20 mmol/day has been shown to normalize blood pressure in 30-50% or more of patients with salt sensitive essential hypertension [53]. More evidence now suggests that even modest sodium excess, whether due to increased dietary in take or abnormal renal sodium retention/handling, induces hypertension in genetically susceptible individuals [53]. Increases in insulin concentration have been shown to induce renal sodium retention [128,129]. There is still a conflict of opinions regarding the actual site at which insulin acts to enhance renal sodium reabsorption. Most authors seem to agree that insulin

increases sodium reabsorption from the distal tubule (118,119).
The distal tubule of man has been shown to posses the highest density of insulin receptors [128]. Moreover, insulin stimulates Sodium Potassium ATPase, a cation transport system that is abundantly present in the distal tubule, and the activity of which correlates very closely with the sodium reabsorptive capacity of this tubular segment [130].

Studies done in animals, however, contrast observations made in humans. Studies in rabbits [131] and dogs [132] have shown that the active site for insulin induced renal sodium reabsorption is the proximal tubule. On the other hand, some workers have also recently suggested that insulin in man exerts its antinatriuretic effect at the proximal and not the distal tubule [130] .

Insulin also enhances sodium reabsorption indirectly though its effects on the activity of the sympathetic nervous system and the renin-angiotensin-aldosterone system.

Insulin may therefore work together with other factors such as genetically determined renal sodium excretion abnormalities and environmental factors like salt-eating habits to cause increased extracellular sodium concentration and blood volume expansion which may eventually lead to elevation of blood pressure. Accumulation of sodium in the extracellular compartment may also lead to accumulation of sodium in the vascular smooth muscle cells. This inhibits a postulated sodium -calcium exchange mechanism resulting in a rise in free intracellular calcium ion concentration and enhanced vascular contractility and responsiveness to vasoconstrictor factors such as norepinephrine, angiotensin II and arginine vasopressin. This eventually leads to vascular structural changes with vessel wall thickening and increased peripheral vascular resistance. The long term effect of which

would be a sustained elevation of blood pressure [118].

Sodium restriction is therefore an important primary non-pharmacologic treatment in the majority of patients with essential hypertension. It has been suggested that sodium restriction reduces the systemic vascular resistance by altering peripheral vasoconstrictive responses in the short and long term which in turn reduces blood pressure.

II: Effect of insulin on sympathetic nervous system

Insulin administration provokes an adrenergic response if hypoglycemia is not prevented [87,123, 128]. However, when euglycemia is maintained, acute administration of insulin is still capable of activating the sympathetic nervous system [87,128]. Oral glucose administration increases sympathetic outflow in close parallel with an increase in serum insulin levels [133]. The mechanism whereby insulin stimulates the sympathetic nervous system is unknown, but it may be related to insulin-mediated glucose metabolism within insulin-sensitive areas of the hypothalamus [128]. On the other hand, the increase in sympathetic activity could represent a counterregulatory mechanism to prevent or offset a fall in blood pressure as a result of nonspecific vasodilatory action of insulin [123,128].

Sympathetic nervous over-activity seems to play a role in some patients with essential hypertension, particularly during the early rise in blood pressure.

Stimulation of the sympathetic nervous system increases heart rate, central venous tone and myocardial contractility. These factors lead to an increase in cardiac output and peripheral vascular resistance and thence to elevation of blood pressure. Norepinephrine, released from

stimulated sympathetic neurones acts on alpha 1 and alpha 2 receptors located on the surfaces of a number of cells including vascular smooth muscle cells. Interaction of norepinephrine with its receptors leads to hydrolysis of phosphatidyl inositol to inositol triphosphate and diacylglycerol. Inositol triphosphate and diacylglycerol cause release of calcium from sarcoplasmic reticulum. The free calcium increases vascular contractility and peripheral vascular resistance leading to elevation of blood pressure [87].

However, even if chronic hyperinsulinemia is able to maintain increased sympathetic activity in man, the overall effect on blood pressure appears to be too small to be of major significance [128].

Effects of insulin on the renin-angiotensin-aldosterone system

Renin is produced principally in the kidney. It cleaves a renin substrate present in plasma to produce the decapeptide angiotensin I. Angiotensin-I-converting enzyme is widely distributed in the body but is present in particularly high concentration in the lungs. Angiotensin-I-converting enzyme removes a further two amino acids from angiotensin-I to form the octapeptide angiotensin I. At physiological concentrations, angiotensin II causes potent vasoconstriction.

The action of angiotensin II on vascular smooth muscle cells is like that of norepinephrine, receptor mediated. Its intracellular effects are also transmitted by the inositol triphosphate messenger system [87]. Angiotensin II also causes a direct stimulation of aldosterone secretion which cause salt and water retention leading to plasma volume expansion and elevation of blood pressure.

There is a communication between the renin-angiotensin- aldosterone system and the sympathetic nervous system. The normal kidney is

supplied with sympathetic afferents via the renal nerves. Stimulation of these nerves, via beta-adrenergic receptors causes renin secretion [87,123,128]. On the other hand, angiotensin receptors are found in the brain stem [87]. Just as the autonomic nervous system can modulate the secretion of renin by the kidney, so may angiotensin, acting centrally influence the activity of the autonomic nervous system.

The effects of insulin on the renin-angiotensin-aldosterone system have received remarkably little attention [128]. In the two studies that have addressed this issue, conflicting effects on the renin-angiotensin-aldosterone system have been observed: Plasma renin activity and angiotensin II levels increased in a dose dependent manner, but despite these rises, no concomitant increase in plasma aldosterone levels was noted [134]. All these effects appear to be related to the insulin- induced decrease in plasma potassium levels, since no changes occurred when the fall in plasma potassium was prevented.

Since angiotensin II, not only exerts a pressure effect. but also effects renal sodium retention, involvement of the renin-angiotensin-aldosterone system in the pathogenesis of essential hypertension has been suggested [38]. Studies on type II diabetes and hypertension suggest that the renin-angiotensin-aldosterone system is unlikely to be involved in the pathogenesis of hypertension occurring in insulin resistant states [128] although it has been shown that renin-angiotensin-aldosterone system activity in patients with essential hypertension exhibits a continuum, with low renin hypertension at one extreme, and high-renin hypertension at the other.

IV: Effects of insulin on cardiovascular reactivity

An exaggerated cardiovascular reactivity to norepinephrine or angiotensin has been demonstrated in most patients with borderline hypertension [87]. In diabetes mellitus, the increased cardiovascular reactivity is even more pronounced and is already present at an early stage of the disease before blood pressure has been elevated [135]. The increase in cardiovascular reactivity may well contribute to the development and maintenance of an elevated blood pressure in both non-diabetic and diabetic hypertensive patients [128]. An enhanced cardiovascular reactivity to norepinephrine, but not to angiotensin II has been demonstrated even in the normotensive offspring of hypertensive families [128]. Some experimental data also indicate that the increase in vascular smooth muscle sensitivity to vasoactive hormones may actually precede or parallel the development of hypertension [128].

Insulin is known to affect several transmenbranous cation exchange systems, thereby influencing intracellular cation homeostasis,including the regulation of intracellular calcium content. Since intracellular calcium is considered to be the final common mediator of vascular tone, it is tempting to relate the increase in cardiovascular reactivity in essential hypertension and diabetes to insulin induced changes in intracellular cation content especially calcium. Insulin induces the growth of human vascular smooth cells. The resulting hypertrophy of vessel walls causes structural encroachment on the lumen, there by increasing vascular resistance. Vascular hypertrophy also amplifies the response of the vessel wall to vasoactive stimulation. The long-term effect of this is elevation of blood pressure. Insulin has chronotropic and inotropic properties, independently of changes in plasma

catecholamine levels [87,123]. On the other hand, insulin appears to have vasodilatory properties [128]. Hypotension has been observed after intravenous insulin administration, particularly in the upright position in diabetics with autonomic neuropathy, in patients with progressive autonomic failure and after ganglion blockade. All these features share the common feature of impaired baroreflex, low plasma noradrenaline levels and an inability to increase plasma noradrenaline levels so as to counteract a fall in blood pressure.

Non-specific vasodilation and or capillary recruitment as a result of the insulin-stimulated tissue metabolism of glucose, i.e. glucose oxidation is the most likely mechanism for the vasodilatory action of insulin [128]. However, physiological hyperinsulinemia does not appear to cause any acute hemodynamic effects in healthy subjects.

The increased vascular responsiveness to noradrenaline in essential hypertension can be normalized by potassium supplementation or calcium reentry blockade [128].

V. Effects of insulin on atrial natriuretic peptide (ANP)

Atrial natriuretic peptide is considered to be the physiological counterpart of the sympathetic nervous system and the renin-angiotensin-aldosterone system. However, it has not yet been established to what extent ANP participates in the day to day regulation of sodium excretion and blood pressure regulation under both normal and abnormal conditions [128].

Endogenous ANP has been shown to be elevated in essential hypertension, and a compensatory role for the hormone in sodium excretion in this condition has been suggested. Elevated levels of ANP have been demonstrated in patients with insulin dependent diabetes

mellitus and hypertension [136] and preliminary results suggest that ANP may also be elevated in non insulin dependent diabetes mellitus with hypertension [128].

In obese subjects, weight loss, known to lower insulin levels is accompanied by changes in ANP that correlate directly with changes in blood pressure. Limited information is available concerning the effects of insulin on ANP levels. Trevisan et al have shown that insulin administration raises ANP levels [136]. ANP has been shown to have no effect on insulin secretion but it delays insulin degradation with a consequent increase in insulin levels. The relevance of these observations with regard to blood pressure regulation is not yet known.

VI. Effects of insulin on the kallikrein - kinin system

The kallikrein-kinin system forms the endogenous vasodilator system of the body. The role of vasodilatory substances in the control of blood pressure is less well understood and is still a matter of debate [87,128]. However the ability of bradykinin and prostaglandins to dilate the vasculature is well recognized [87]. There is some evidence to suggest that this system is involved in both the regulation of blood pressure and in the insulin sensitivity of peripheral tissues [128]. Release of bradykinin appears to be implicated in the increase in local blood flow and the stimulation of insulin induced glucose oxidation and protein synthesis after muscle work. The subsequent vasodilatory response accounts for the blood pressure lowering effect.

Both in patients with essential hypertension and in patients with non insulin diabetes mellitus, such a vasodilatory response after muscle work cannot be demonstrated, suggesting that activation of the kallikrein-kinin system is impaired [128]. This could contribute in part

to the elevation of blood pressure.

An improvement in insulin sensitivity and a consequent reduction in insulin levels, has been observed in hypertensives and in normotensives after treatment with the angiotensin-I- converting enzyme inhibitor class of antihypertensives [128].

In summary, a causal role for insulin resistance in the pathogenesis of hypertension has been proposed and apparently supported by a number of experiments. However, it should be noted that the finding of a defect in one insulin action in a certain disease state, does not necessarily imply a similar defect in another apparently related condition For example, although essential hypertension, obesity and type II diabetes mellitus are all characterized by a whole-body, decrease in glucose uptake, patients with diabetes are still sensitive to the antilipolytic and potassium uptake-promoting actions of insulin. Obese individuals are resistant to all effects of insulin with the possible exception of lipid oxidation and potassium uptake into cells.

In patients with essential hypertension the effects of insulin on hepatic glucose output, glucose oxidation, lipid oxidation, suppression of lipolysis and potassium uptake are all preserved. Therefore, if one assumes a causal role for insulin in the pathogenesis of hypertension associated with the various insulin-resistant states, sensitivity to at least some of the possible blood-pressure elevating actions of insulin should be maintained, despite the presence of resistance to the glucose-lowering action of the hormone [128]. Since hypertension does not develop in all obese or insulin-resistant subjects, the relationship must be modulated by other genetic or environmental factors.

METHODS OF ASSESSING INSULIN SENSITIVITY

The first standard approach to measuring insulin sensitivity in vivo was introduced by Himsworth and Kerr [110] in the 1930s. Two oral glucose tolerance tests were performed in individual subjects: One with and a second without a concomitant intravenous insulin injection. Insulin sensitivity was expressed as the ratio of the areas under the respective glucose tolerance curves. On the basis of the plasma glucose response, Himsworth divided patients with diabetes into two groups insulin-sensitive and insulin-insensitive. However, this approach of demonstrating insulin sensitivity was viewed as inferential because of the absence of specific sensitive measurement of plasma insulin. The development of the radioimmunoassay (RIA) by Yalow and Berson[111] in the 1960s satisfied the need for an accurate specific measure of plasma insulin and as a consequence, several techniques have been described for assessing insulin sensitivity. Some of these techniques include:

I. Glucose tolerance test (oral or intravenous).

This is the simplest method for assessing insulin sensitivity in an individual. Glucose tolerance test oral or intravenous, is performed in the usual way and glucose and insulin levels are simultaneously determined. The demonstration of inappropriately elevated plasma insulin in the face of normal or supranormal plasma glucose indicates diminished overall tissue sensitivity to insulin [110].

II. Insulin tolerance test

This is another simple method which can be employed to determine tissue sensitivity to the action of insulin. In this approach, as employed by Bonora et al [127], a bolus of intravenous injection of

regular insulin (0.1U/Kg body weight) is given to a subject after at least 12-hour overnight fast. Blood samples are collected at 15 min before and 3,6,9,12,15,20 and 30 min after insulin injection. Glucose is injected at 30 min to stop the fall in plasma glucose. The rate constant for plasma glucose disappearance (Kitt) during the 3-15min period following insulin injection is a measure of insulin action and is calculated from the formula 0.693/t1/2. The plasma glucose t1/2 is calculated from the slope of the least square analysis of the plasma glucose concentrations from 3-15min after insulin injection, when the plasma glucose concentration declines linearly. Plasma insulin is also measured throughout the test.

Insulin tolerance test has been widely used for several years and has yielded useful information about insulin sensitivity in several clinical states [127]. The measure of in vivo insulin sensitivity achieved by insulin tolerance test has proved to correlate with insulin sensitivity in vitro, insulin binding to its receptors and intravenous glucose tolerance [127].

However, these two simple methods for assessing insulin sensitive (glucose tolerance test and insulin tolerance test) cannot quantify insulin sensitivity adequately because of variations in insulin levels and hormonal counterregulation [32]. As regards to the insulin tolerance test, it has been claimed that the counterregulatory hormone responses to insulin-induced hypoglycemia blunt the fall in plasma glucose concentrations and that the rate at which the plasma glucose concentration decreases after insulin administration is not a simple function of insulin action [127]. On the other hand, other investigators argue that the glucose fall occurring in the first 15 minutes after insulin injection reflects only the action of insulin, since during this

period counterregulatory hormone concentrations remain at the basal level [115]. Another draw back of this test, particularly in the normoglycemic subjects is the marked hypoglycemia that sometimes follows insulin injection. However hypoglycemia and its unpleasant symptoms rarely occur earlier than 20-30min after insulin administration and can be prevented by the intravenous injection of glucose 15-20 min after insulin administration [127].

In order to avoid problems encountered with the two methods above, more sophisticated techniques have been designed. These include:

III. Insulin suppression test:

In this method as employed by Heine et al [32] and also documented by Bergman et al [110], two cannulae are positioned intravenously, one in an antecubital fossa for all infusions and a second, inserted retrogradely and distally in the other hand for sampling arterialized venous blood. The hand for sampling arterialized venous blood is maintained at 55 oC in a thermoregulated plexiglas box. Throughout the 150 minute study period, 20% glucose (200 g/L) is infused at 6mg/Kg/min together with insulin at 50mU/Kg/hr diluted to 30ml with hemaccel. Inhibition of endogenous secretion of insulin, and hepatic glucose out put the two variables which may confound the results during exogenous insulin and glucose infusion is achieved pharmacologically by concomitant infusions of epinephrine and propranolol respectively. Epinephrine is infused at 6ug/min while propranolol is given initially as a bolus of 5mg intravenously followed by an infusion at 0.08mg/min, all for 150 min. All the infusion are delivered from different syringes.

Once the experiment system has been fit, blood samples for glucose and serum insulin are taken twice basally, every 15minutes for two hours

and then every 5 minutes from 120 - 150 minutes.

The epinephrine/propranolol infusion may be replaced by cyclic somatostatin infused at a rate between 125 and 250mg/hr, diluted with normal saline to 30ml [116]. This approach of substituting cyclic samatostatin for epinephrine/propranolol infusion has been referred to as Haranos' technique of insulin suppression test [116].

During the insulin suppression test, glucose achieves a new steady state value within 90 minutes of the beginning of the combined infusion [110,116]. The steady state plasma glucose concentration is defined as the average glucose concentration during a steady state observation period [110].In this technique, it is assumed that the concomitant infusion of glucose, insulin, epinephrine and propranolol (or somatostatin in Haranos technique), act in concert to totally suppress hepatic glucose output as well as pancreatic insulin secretion. Under these conditions the exogenous glucose infusion represents the total entry of glucose into the body glucose pool. Exogenous insulin acts to increase glucose mobility into insulin dependent tissues. At steady state period, glucose appearance (entry) into the glucose pool must equal total glucose utilization, and the resultant glucose level which is attained, that is, the steady state plasma glucose concentration (SSPG) value will be the plasma glucose concentration which allows these two fluxes to be equal [116].

The SSPG value for a given rate of glucose infusion (6 mg/Kg /min in most published studies [110], will depend on first, suppression of hepatic glucose output, second, insulin independent glucose utilization (central nervous system, red blood cells, renal medulla), and third, the net effect of the infused insulin to regulate glucose uptake by insulin dependent tissues [110,117]. Because the steady state plasma insulin

levels (SSPI) are the same in all subjects, the height of the steady state plasma glucose (SSPG) levels provides a quantitative estimate of the ability of insulin to stimulate disposal of a glucose load [110,117]. Thus, as resistance to insulin action increases, so too will the SSPG value [110]. There are factors which may confound the interpretation of the SSPG value. These include, the insulin independent glucose disappearance, and the extent of hepatic glucose output suppression. Limited and conflicting evidence exists as to whether hepatic glucose uptake is suppressed during the insulin suppression test. Some have found that hepatic glucose output was 80% suppressed in normal individuals and 69% suppressed in non diabetic obese subjects [110]. The failure to totally suppress hepatic glucose output will likely cause an increase in SSPG proportional to the remaining hepatic glucose output [117,127]. Insulin resistance is likely to be overestimated in subjects with incomplete suppression of the hepatic glucose output. The interpretation of SSPG will therefore be limited under conditions of extreme insulin resistance [110]. When SSPG is very high, the renal glucose threshold will be exceeded glucose will start appearing in urine. The urinary loss of glucose will also distort the true value of SSPG. In insulin sensitive individuals, glucose levels may be sufficiently depressed to excite counterregulatory responses. The resulting secretion of counterregulatory hormones (catecholamines, glucagon, cortisol, growth hormone) support the SSPG level and would result in underestimation of insulin sensitivity [114]. It can be clearly seen that SSPG may be heavily influenced by factors other than insulin sensitivity. These confounding factors may be particularly important in groups with highly elevated or very low SSPG.

One approach to cope with the widely varying SSPG value is to use the glucose metabolic clearance rate (MCR) [110]. Metabolic clearance rate for glucose is calculated by dividing the glucose infusion rate by the steady state plasma glucose concentration i.e

$$MCR = \frac{\text{Glucose infusion rate}}{\text{steady state plasma glucose concentration.}}$$

Unlike SSPG, glucose clearance is a linear function of steady state plasma insulin concentration up to a saturating insulin value [110] and does not depend on the ambient plasma glucose [32]. Also while SSPG is very dependent on the insulin independent glucose clearance, MCR is not entirely free from some draw backs. For example, MCR fails to correct for such factors as variable effects of the alpha-adrenergic stimulation, nonlinearities in the effects of insulin and glucose on glucose utilization, variable and incomplete suppression of hepatic glucose output, urinary glucose loss and counterregulatory hormones.

IV. Modified Harano's technique:

Studies have shown that the inhibition of endogenous insulin release as well as hepatic glucose output by pharmacologic agents: epinephrine; propranolol or somatostatin is not necessary and may carry an added risk due to side effects of these drugs [32]. It has been found that adequate inhibition of endogenous insulin release and hepatic glucose output during the insulin suppression test can be derived by the use of exogenous infusions of glucose and insulin alone [23,110,112,116] and therefore excluding cyclic somatostatin from the original Harano's protocol. This technique has been referred to as modified Harano's technique for assessing the insulin sensitivity in vivo. This technique has the advantage of simplicity to perform, not requiring very sophisticated equipments, or highly trained man power and yet yielding

results which are adequately accurate [32]. This is the technique which has been used in this study, the details of which have being described under the section of methodology.

V. Glucose Clamp

The glucose damp technique is an application of the negative feedback principle to the system regulating the blood glucose [110].

The glucose clamp technique is now regarded as the most valid technique for measurement of in vivo insulin action, mainly because it provides information about the amount of glucose metabolized by peripheral tissues during insulin stimulation [127]. With the use of the glucose clamp, the two primary variables governing glucose homeostasis in man beta-cells response to glucose and sensitivity of body tissue to insulin can be accurately assessed [117]. The beta-cells sensitivity to glucose can be assessed by the hyperglycemic clamp technique while the tissue sensitivity to the action of glucose can be assessed by euglycemic clamp technique [117].

(a). Hyperglycemic clamp technique:

This technique has been described by De Fronzo et al [117]. The goal of the hyperglycemic clamp is to raise the plasma glucose concentration acutely to a fixed hyperglycemic plateau and to maintain it at that level for 2 hours. This is accomplished by an intravenous glucose infusion consisting of two phases: (1) a 15- min priming dose needed to raise the glucose level in plasma and in extravascular glucose compartments to the desired plateau and (2) a maintenance dose that is computed at 5-min intervals throughout the study. The pattern of the priming dose is determined through trial and error and is empirically set as a constant for all subjects. The dose is computed per square meter body surface

area, eg a dose of 9,622mg/m^2 or 240mg/kg body weight will raise the plasma glucose concentration by 125mg/dl during the first 14 minutes. The maintenance dose is determined by computation of the periodic adjustments in the glucose infusion made every 5 minutes required to maintain the glucose level at the hyperglycemic state, and is based on the negative feedback principle of the actual glucose concentration. When the plasma glucose concentration is higher than the goal, the infusion is decreased and vice versa. The computations are based on the use of a 20% glucose in water infusate via a calibrated infusion pump. Because the plasma glucose concentration is held constant, the glucose infusion rate is an index of glucose metabolism [117,127]. Under the conditions of constant hyperglycemia, the plasma insulin response is biphasic with an early burst of insulin release during the first 6 min [117] followed by a gradually progressive increased in plasma insulin concentration. In this way, the sensitivity (response) of the pancreatic beta cells to plasma glucose is assessed.

On the other hand, the amount of glucose infused to maintain hyperglycemia at the desired goal, corrected for urinary glucose loss and glucose added to or removed from the glucose space is considered as the amount of glucose metabolized [127]. The mean plasma insulin concentrations during steady state hyperglycemia (20-120min) can be calculated and the ratio between the metabolized glucose and the steady state plasma insulin concentration; taken as a measure of tissue sensitivity to insulin [127]. The hyperglycemic glucose clamp can therefore be used to assess the sensitivity of the pancreatic beta cells to plasma glucose as well as the sensitivity of insulin dependent tissues to the action of insulin.

(b). Eugycemic clamp:

The goal of the euglycemic (hyperinsulinemic) clamp, a technique which was developed by Andres and colleagues[116], is to raise the plasma insulin concentration acutely to a new plateau and to maintain it at that level through out the study period, as described by De Fronzo et al [117]. The technique consists of an infusion of predetermined fixed dose of insulin and a variable rate of glucose infusion. The euglycemic clamp technique, as applied by Greenfield et al [116] can be described as follows: After an overnight fast, two cannulae are inserted one antegradely in the antecubital fossa vein used for all infusions and the second, retrogradely in the other hand vein, kept warm by use of a thermoregulated plexiglas box for sampling arterialized venous blood. A priming intravenous infusion of insulin (800 mU over 10 minutes) is followed by a constant infusion at 40mU/m^2/min. According to De Fronzo et al [117], this maintains the plasma insulin level at 100uU/ml above basal level for 120 minutes. The glucose infusion is initiated 4 minutes after the insulin had been started (t+4) at an empirically determined rate of 1-2mg/kg/min.This is increased to 2.5 - 3.5mg.kg/min at t+10 minute. Plasma glucose is determined every 5 minutes, and subsequent adjustments in the rate of glucose infusion needed to maintain the basal plasma glucose level (at euglycemic level) are based on a negative feedback algorithm. The glucose infusion is continued for 120 minutes and the amount of glucose metabolized (M) during the period 20 - 120 min is calculated from the amount of glucose infused (I), with corrections made for changes in glucose pool size. 'M' defines the amount of glucose that must be metabolized in order to prevent an insulin induced fall from basal plasma glucose concentration. As such 'M' provides a quantitative estimate of insulin resistance. To determine the

efficiency at which this glucose is metabolized, the metabolic clearance rate (MCR) of glucose is calculated by dividing the amount of glucose metabolized (M) by the plasma glucose concentration. Metabolic clearance rate is a better index of insulin sensitivity than the amount of metabolized glucose (M) [110,116,177,127].

The euglycemic clamp is considered the most accurate technique for assessing insulin sensitivity in vivo [32,116,118] Unfortunately this technique is considered primarily a laboratory procedure because it requires sophisticated equipment, highly trained personnel and it is costly and labor intensive.

VI. Minimal Model Method for Assessing Insulin Sensitivity Index:

In this technique, a computer is used to calculate insulin dynamics observed during the frequently sampled intravenous glucose tolerance [118]. The frequently sampled intravenous glucose tolerance test is performed as followed [118]: After an overnight fast, two intracatheters are placed one in an antecubital vein and another in a hand vein cannulated retrogradely and kept in a heating pad to provide arterialized venous blood. Four basal samples are collected over 20 minutes after which glucose (0.3g/kg) is injected over 1 min. An additional injection of tolbutamide is given over 20 seconds, 20min after glucose. The blood samples are then collected at the following times after glucose injection at t = 0: 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 24, 25, 27, 30, 40, 50, 60, 70, 90, 100, 120, 140, 160, and 180 min. The insulin sensitivity index is then calculated from there results using a computer program.

This method is quite accurate and has been found to correlate well with the Andres's euglycemic clamp technique [116,117,118].

It can be realized from the discussion above that a variety of

approaches have been put forth to yield indices of in vivo insulin sensitivity. Some workers have determined the correlations between some of these method. Strong correlations have been found between the eughycemic clamp technique and the insulin suppression test, with and without somatostatin [32,117]. Bergman et al [118] have found that the minimal model method and eughycemic glucose clamp are equivalent for the assessment of overall insulin sensitivity in normal and insulin - resistant non diabetic subjects. Bonora and colleagues [112] compared the insulin tolerance test with eugycemic and hyperglycemic glucose clamp studies. A strong correlation between this methods could be documented. The choice of an appropriate method for assessing insulin sensitivity in man is therefore mainly dependent upon the local circumstances surrounding the investigator including especially equipment and personnel. At Muhimbili for example, we do not have sophisticated equipment required in the glucose clamp technique. The author had therefore to use the modified Haranos technique which needs fairly simple equipment and averagely trained personnel while producing results which are significant comparable to those obtained from other methods.

RESEARCH DESIGN METHODS AND MATERIALS

SELECTION OF SUBJECTS

Between 1st April and 31st August 1991, a total of 280 employees of Kilimanjaro Hotels Ltd, the biggest tourist hotel in the city of Dar es Salaam with a work force of approximately 800 workers, were screened for hypertension. Out of these, 30 African subjects, 15 cases and 15 controls were enrolled for the study after obtaining informed consent from each individual.

INCLUSION CRITERIA

1. Newly diagnosed hypertension defined as blood pressure equal to or greater than 160/95 mmHg on at least two occasions.
2. Body mass index less than 30 kg/m^2.
3. Normal glucose tolerance test according to 1985, WHO criteria.

EXCLUSION CRITERIA

1. Individuals with: known hypertension on treatment, diabetes mellitus; heart disease on cardiovascular medicines; renal diseases; liver disease and endocrine disorders.
2. History of excessive alcohol consumption (15 pints of beer per week).
3. Current use of contraceptive pills.
4. Abnormal electrolyte (Na^+,K^+) and serum creatinine levels suggesting secondary hypertension.

1.3 CONTROL GROUP

PHYSICAL EXAMINATION

The recruited subjects underwent a complete physical examination to exclude possible secondary causes of hypertension. Blood pressure,

weight, height, body mass Index, waist circumference, hip circumference and waist-hip ratio were recorded as follows:

BLOOD PRESSURE Blood pressure was measured on three different occasions. After measuring blood pressure on day 1, a second measurement was obtained about a week later and a third measurement, 1-3 days later. On each occasion, at least two readings were taken and the mean value recorded. An appropriate adult cuff mercury sphygmomanometer was applied 2-3 cm above the antecubital fossa of the right arm. Blood pressure was measured between 2 pm and 4 pm, and recorded to the nearest 2 mmHg, reading the calibration below the meniscus with the subject in the sitting position. Systolic and diastolic blood pressure were read at the 1st and 5th Korotkoff's phases respectively. The mean of the three blood pressure values obtained from the three visits was taken as the subject's true blood pressure. Hypertension was defined as systolic or diastolic blood pressure of equal to or greater than 160 mmHg and 95 mmHg respectively, according to the WHO expert Committee (1978) criteria.

WEIGHT Weight was measured using a seca bathroom weighing scale, after the subject had removed his shoes, coat and jacket and was left with light clothing. It was recorded to the nearest 0.5 kg.

HEIGHT Height was measured after the individual had removed his shoes and /or cap. It was recorded in meters to the nearest decimal place.

BODY MASS INDEX (BMI) After obtaining the weight in kilograms and height in meters, BMI for each individual was calculated by dividing the weight by the square of the height, and expressed in Kilograms per meter squared (kg/m^2)

WAIST CIRCUMFERENCE Waist circumference was taken as the maximum abdominal girth and recorded to the nearest centimeter.

HIP CIRCUMFERENCE Hip circumference was taken as the maximum circumference at the level of the greater trochanter and recorded to the nearest centimeter.

WAIST/HIP RATIO This was calculated by dividing the waist circumference in centimeters by the hip circumference (in centimeters).

3.1 LABORATORY TESTS

The following laboratory tests were performed in all subjects:

ORAL GLUCOSE TOLERANCE TEST/INSULIN SECRETION TEST

All subjects, cases and controls underwent a 75 g oral glucose tolerance test, performed according to the WHO 1985 recommendations. After an overnight fast (10-14 hours), preceded by an unrestricted last evening carbohydrate meal, subjects reported at the study center by 8 am. After a short rest, during which time the subjects were not

allowed to smoke, blood was collected from an antecubital vein (or other convenient vein) and immediately placed in a fluoride oxalate containing bottle. Whole blood glucose (fasting-0 min) was determined on the spot by a glucose oxidase method using Yellow Springs instruments (Yellow Springs, Ohio, USA). Then 75 g of glucose dissolved in 300 ml of water was given to the subject who was instructed to drink it in about 5 minutes. Blood samples for determination of blood glucose were then collected at 15, 30, 60 and 120 minutes and analysed by the same Yellow Springs instrument. Results were interpreted according to 1985, WHO criteria. Blood samples for plasma insulin analysis were kept in ice-block containers and within 4 hours of collection; they were centrifuged and kept frozen at -40°C until they were finally transported to Newcastle upon Tyne, U.K. for analysis by radioimmunoassay.

For the purpose of quality control, a further aliquot was stored and frozen, for analysis by hexokinase fluorimetric method using a Cobas Bio Centrifugal analyser in Newcastle upon Tyne, U.K.

INSULIN SENSITIVITY TEST

Insulin sensitivity was assessed in both cases and controls. The modified Harano's technique (glucose-insulin infusion technique) was employed. Subjects reported at the study center on the morning of the procedure at around 8 am. after an overnight fast (10-14 hours). A teflon cannula was inserted retrogradely in a dorsal hand vein for sampling venous blood which was arterialized by warming the hand at an approximate temperature of 55°C using a thermoregulated plexiglass box. Use of the Plexiglass box ensures arterialization of venous blood within 20 to 40 minutes. A butterfly needle was inserted on the other hand vein and used for administration of all infusions (glucose solution

and insulin). Twenty percent glucose solution (200 g/l) was used. This was infused at a rate of 6 mg/kg/min together with human soluble insulin (Actrapid) at 50 mU/kg/hr, diluted to 30 ml with hemaccel throughout the 180 minutes of the study period.

After 30 minutes of cannulation, a basal blood sample was drawn (-30 min sample). A further basal sample was drawn 15 minutes later (-15 min. sample). After a further 15 minutes, another blood sample was taken (0-min sample) and the glucose and insulin infusions started. Thereafter blood samples were collected at 30, 60, 90, 120, and 150 min., and then every 5 min. from 150 to 180 min. Blood glucose was analysed on the spot by a glucose oxidase method (Glucostat, Yellow Springs instrument Co., Yellow Springs, Ohio, USA). Blood samples for plasma insulin analysis were kept in ice-block containers and within 4 hours of collection, they were centrifuged and kept frozen at -40°C until they were finally transported to Newcastle upon Tyne, U.K. for analysis by radioimmunoassay.

DATA ANALYSIS

Previous studies have indicated that after 150 minutes of intravenous glucose, 6 mg/kg/min and insulin, 50 mU/kg/hr infusion, a steady state plasma concentrations for glucose (SSPG) and insulin (SSPI) are reached. Steady state plasma glucose concentration reflects total body insulin sensitivity. Because the SSPI are the same in all subjects, the height of the SSPG level provides a quantitative estimate of the ability of insulin to stimulate disposal of a glucose load. It is a measure of insulin stimulated glucose uptake. The SSPG was determined from the mean of 3 blood glucose values only, ie. from min 150 to min 165. (steady state period) for each subject since the plasma glucose

concentration started varying widely from min 170 to min 180. The metabolic clearance rate for glucose at steady state, a much better index for measuring insulin resistance, was calculated by dividing glucose infusion rate (GIR) by steady state plasma glucose concentration (SSPG). Microcomputer using the Statistical Package for the Social Sciences (SPSS/PC) was employed for data enalysis. Comparison of the means was performed using the unpaired t-test.

RESULTS

Table 1 below shows the age and sex distribution of hypertensive and control subjects. The mean age in hypertensive subjects and controls was similar, 38.5 ± 1.7 years, and 38.6 ± 1.8 years respectively. Among the hypertensives and controls the range was from 27 to 51 years. There were four females in each group.

Hypertensive subjects (table 2) had a systolic blood pressure range of 143 to 232 mmHg and a diastolic blood pressure range of 100 to 133 mmHg, and the respective mean values (±sem) were 178 ±6 mmHg and 111 ± 2mmHg. Controls had systolic and diastolic blood pressures ranging from 110 to 150 mmHg and 71 to 88 mmHg respectively. Mean systolic blood pressure (±sem) was 131 ±3 mmHg and diastolic blood pressure was 83 ±1 mmHg The difference in mean systolic and diastolic blood pressure between the two groups was statistically significant ($p < 0.001$).

Table 1: Age and sex distribution of hypertensive subjects and controls

	Hypertensive subjects		Controls	
s.no	age (yrs)	sex	age (yrs)	sex
1	29	f	28	f
2	50	f	51	f
3	41	m	42	m
4	35	m	34	m
5	39	m	39	m
6	27	f	27	f
7	30	m	31	m
8	40	m	40	m
9	39	m	40	m
10	40	m	39	m
11	38	m	38	m
12	38	m	41	m
13	38	m	38	m
14	51	m	51	m
15	40	f	40	f
mean	38.5		38.6	
±sem	1.7		1.8	

Table 2: Distribution of blood pressure in cases and controls

	hypertensives		controls	
s.no.	SBP	DBP	SBP	DBP
1	143	110	131	86
2	161	102	125	81
3	192	111	150	88
4	173	111	126	86
5	169	102	113	82
6	143	101	144	88
7	149	98	110	83
8	232	133	142	87
9	205	118	135	77
10	204	121	113	71
11	202	119	149	86
12	179	119	124	73
13	177	114	137	84
14	205	122	129	88
15	176	100	147	82
mean	178±6	111±2	132±3	83±1

Key: SBP = Systolic blood pressure (mm Hg)
DBP = Diastolic blood pressure (mm Hg)

Table 3 shows the distribution of weight, height and body mass indices in hypertansive subjects and controls. Weight in hypertensives ranged from 42.5kg to 85 with a mean value (±sem) of 67 ± 11.5kg while in the control group the range was 49 kg to 81.5kg with a mean value (±sem) of 64.6±11.3kg. Height in hypertensive subjects ranged from 1.5m to 1.8 with a mean value (±sem) of 1.6±0.1m while the range in controls was 1.4 to 1.8m with a mean (±sem) of 1.6±0.1m. Hypertensive had body mass ranging from 18.1kg/m^2 to 29.7 kg/m^2 with a mean (±sem) of 24.6±0.9kg/m^2. Body mass indices in controls ranges from 19.1kg/m^2 to 29.7kg/m^2 with a mean (±sem) of 24.4±0.9kg/m^2 which was not significantly different from that of hypertensive subjects ($p = 0.92$).

The waist circumference (table 4) in hypertensive subjects ranged from 64.5 to 101.0 cm with a mean (±sem) of 85.2±10.9cm, while the range in controls was 65.0 to 86.0cm with a mean (±sem) of 80.6±10.0cm. The hip circumference ranged from 81.5 to 108.0cm in hypertensives and 77.0 to 108.0cm in controls with means (±sem) of 95.8±7.3 and 92.4±9.2cm respectively. The waist/hip ratio ranged from 0.76 to 0.95 in hypertensive and 0.80 to 0.94 in controls with respective means (±sem) of 0.89±0.07 and 0.87±0.04 which were comparable ($p > 0.5$).

Table 3: Distribution of weight, height and body mass indices (BMI) in hypertensive subjects and controls

	Hypertensives			Controls		
s.no.	wt (kg)	ht (m)	BMI kg/m^2	wt (kg)	ht (m)	BMI Kg/m^2
1	68.5	1.6	29.9	61.5	1.5	29.3
2	70.0	1.5	29.7	87.0	1.7	29.7
3	85.0	1.7	27.9	81.5	1.7	28.2
4	76.5	1.8	24.1	62.0	1.6	23.2
5	53.5	1.6	21.7	49.0	1.5	20.7
6	54.0	1.6	21.5	72.5	1.8	22.6
7	65.5	1.7	22.5	57.0	1.6	21.6
8	70.0	1.6	27.5	72.5	1.6	28.1
9	69.0	1.6	25.0	65.5	1.6	25.2
10	76.5	1.8	24.8	67.5	1.6	25.6
11	79.0	1.7	27.2	69.0	1.6	27.3
12	57.0	1.6	22.1	68.0	1.8	22.1
13	80.5	1.7	28.0	54.0	1.4	27.1
14	58.0	1.7	20.8	54.0	1.6	19.9
15	42.5	1.5	18.1	49.0	1.6	19.1
mean ±sem	67.0± 11.5	1.6± 0.1	24.6± 0.9	64.6± 11.3	1.6± 0.1	24.4± 0.9

Table 4: Distribution of waist and hip circumferences and waist/hip ratio among cases and controls

s.no.	Hypertensives			Controls		
	waist (cm)	hip (cm)	waist/ hip	waist (cm)	hip (cm)	waist/ hip
1	80.0	103.0	0.78	75.0	88.0	0.85
2	101.0	108.0	0.94	95.0	108.0	0.87
3	96.0	102.0	0.94	96.0	104.0	0.92
4	89.0	98.0	0.91	84.0	89.0	0.94
5	77.0	88.0	0.88	70.0	81.0	0.86
6	71.0	93.0	0.76	76.0	95.0	0.80
7	86.0	93.0	0.92	71.0	87.0	0.81
8	96.0	101.0	0.95	90.0	102.0	0.88
9	91.0	94.0	0.97	79.0	92.0	0.85
10	92.0	99.0	0.93	87.0	101.0	0.86
11	92.0	102.0	0.90	95.0	100.0	0.95
12	71.0	87.0	0.82	82.0	94.0	0.87
13	93.0	99.0	0.93	65.0	77.0	0.86
14	78.0	88.0	0.87	78.0	86.0	0.90
15	64.5	81.5	0.79	70.0	82.0	0.85
mean	85.2	95.8	0.89	80.6	92.4	0.87
±sem	10.9	7.3	0.07	10.0	9.2	0.04

Table 5 below shows the results of glucose tolerance test. Fasting blood glucose in hypertensive subjects was significantly higher (mean ±sem = 4.4±0.1mmol/l) than in controls (mean ±sem = 4.1± 0.1 mmol/l) with a `p' value of 0.04. The remaining blood glucose levels (at 15min, 30min, 60min and 120 min) did not show any significant statistical difference between the two groups (p=0.09 - 0.97). On average, the hypertensive subjects had a fasting blood glucose concentration about 7% higher than the controls.

Table 5: Oral glucose tolerance test results in hypertensives and controls.

	hypertensives					controls				
	time in minutes									
s.no	0	15	30	60	120	0	15	30	60	120
1	4.7	5.6	6.6	6.5	4.4	3.4	4.6	4.6	4.8	5.6
2	3.9	4.7	5.5	4.2	4.8	4.0	4.8	6.0	7.3	4.4
3	4.4	5.4	6.6	5.0	5.0	4.6	5.3	9.5	9.0	9.4
4	4.4	6.4	8.5	10.6	6.0	4.7	4.3	6.5	7.5	5.7
5	3.9	5.1	5.8	5.8	4.5	3.7	6.2	9.1	8.3	5.9
6	4.0	4.1	5.4	7.1	5.5	4.1	4.5	4.7	6.4	6.2
7	4.4	5.7	5.2	6.0	5.4	3.4	5.1	8.0	7.3	3.1
8	4.3	4.6	5.2	6.7	4.8	4.0	7.3	8.6	6.8	5.9
9	4.6	6.1	7.2	6.6	5.5	4.3	4.9	6.4	6.4	3.6
10	4.8	6.8	8.3	8.5	5.6	4.0	5.4	7.1	8.1	6.2
11	5.0	7.4	8.8	9.7	6.1	4.8	4.6	6.2	8.4	6.2
12	4.2	4.9	7.5	6.7	5.9	3.7	3.5	5.6	9.0	4.8
13	5.3	-	7.3	9.0	5.0	4.1	4.1	6.2	6.6	6.5
14	4.0	6.4	6.7	4.3	4.1	4.6	4.2	5.1	7.5	5.4
15	4.3	4.3	6.5	5.3	4.1	3.7	5.0	7.2	6.5	4.5
mean	4.4	5.5	6.7	6.8	5.1	4.1	4.9	6.7	7.3	5.4
±sem	0.4	1.0	1.2	1.9	0.7	0.5	0.9	1.5	1.1	1.5

Table 6 shows a rise in blood glucose concentration from the basal level (mean±sem of 4.1±0.5mmol/l) to a peak (mean ±sem of 7.0 ± 1.0 mmol/l) after 30 min, which was maintained for 30 minutes before falling towards a basal level which was not yet achieved by the end of 120 minutes.

Table 6: Blood glucose concentration during the first 2 hours of the 3 hour insulin sensitivity test in hypertensive subjects.

	Blood glucose concentration (mmol/l)						
s.no	-30	-15	0	30	60	90	120
1	4.5	4.7	4.7	8.4	8.6	8.3	7.4
2	4.0	4.0	3.9	5.7	6.1	5.2	4.5
3	4.5	3.6	3.9	7.1	7.5	7.9	6.9
4	4.8	4.8	4.8	8.2	8.6	7.5	6.5
5	3.3	3.5	3.4	6.4	7.2	6.5	6.1
6	4.2	4.2	4.1	6.8	6.9	6.7	6.6
7	4.0	3.7	3.7	7.1	6.1	5.4	4.8
8	3.5	3.3	3.3	5.0	8.4	9.0	9.1
9	4.5	3.9	4.2	7.2	7.7	7.2	6.7
10	5.1	5.0	5.1	8.8	9.8	9.5	8.0
11	4.4	4.1	4.2	4.7	6.3	5.8	4.9
12	3.7	3.8	3.4	5.3	4.7	3.7	3.5
13	4.1	4.1	4.3	7.6	7.0	6.8	6.2
14	4.8	4.7	4.7	7.3	5.7	4.7	4.4
15	4.1	4.0	4.5	5.6	5.1	4.3	3.6
mean	4.2	4.1	4.1	7.0	7.0	6.6	6.0
±sem	0.4	0.5	0.5	1.0	1.4	1.7	1.6

Table 7 shows a rise in blood glucose concentration to a peak value (mean ±sem = 6.4 ± 1.1 mmmol/l) after 30 min which was mantained for another 30 min. The glucose concentration started falling after 90 min.

Table 7: Blood glucose concentration during the first 2 hours of the 3 hour insulin sensitivity test in normotensive controls.

	blood glucose concentration (mmol/l)						
s.no	-30 min	-15 min	0 min	30 min	60 min	90 min	120 min
1	4.3	3.9	3.8	5.3	4.6	3.6	3.1
2	3.7	3.6	3.8	6.3	4.3	3.9	3.5
3	4.6	4.7	4.8	7.9	8.7	7.1	5.4
4	3.8	3.9	4.1	8.0	7.8	6.8	5.3
5	3.9	3.9	3.9	5.5	4.5	4.2	2.5
6	3.7	3.7	3.5	7.0	7.8	7.9	7.7
7	4.1	3.6	3.6	5.8	5.7	4.4	3.2
8	4.0	4.5	4.0	4.6	7.1	6.2	5.1
9	4.6	5.5	4.9	7.2	4.9	4.2	3.0
10	3.4	3.7	3.4	7.6	8.7	8.9	4.2
11	3.8	3.8	4.0	6.2	5.2	-	4.7
12	4.0	4.0	3.6	6.1	8.8	3.8	3.4
13	4.0	3.7	3.6	5.1	4.3	4.2	3.3
14	3.8	3.7	3.7	5.9	4.9	4.6	3.3
15	3.7	4.7	4.6	7.3	6.7	4.1	-
mean	4.0	4.1	4.0	6.4	6.3	5.3	4.7
±sem	0.3	0.5	0.5	1.1	1.7	1.7	1.6

Table 8 shows a steady state for blood glucose concentration in hypertensive subjects, during the last 30 min. of the 3 hour insulin senstivity test, which was well achieved only from min 150 to min 165. The mean (±sem) blood glucose concentration at steady state phase (min 150 to 165) was 5.0 ± 0.3 mmol/l.

Table 8: Blood glucose concentration during the steady state period of the insulin sensitivity test in hypertensive subjects.

	blood glucose concentration (mmol/l)							
s.no	150	155	160	165	170	175	180	SSPG
1	6.4	6.1	6.0	5.9	5.7	5.6	5.4	6.1
2	3.5	3.5	3.6	3.4	3.2	3.2	3.1	3.5
3	5.9	5.8	5.5	5.5	5.1	4.9	5.0	5.7
4	5.5	5.2	4.9	4.9	4.5	4.3	4.2	5.1
5	5.7	5.5	5.2	5.1	5.0	4.9	4.4	5.4
6	5.5	5.5	5.2	5.2	5.0	5.0	4.8	5.3
7	3.8	4.5	4.4	4.3	4.3	3.9	4.1	4.3
8	8.2	7.9	7.8	7.6	7.5	7.0	7.0	7.9
9	6.8	6.8	6.4	6.5	6.5	6.6	6.5	6.6
10	5.7	5.2	5.6	5.4	4.7	5.0	5.1	5.5
11	4.7	4.4	4.3	4.3	4.2	4.1	3.9	4.4
12	2.8	2.6	2.6	2.5	2.6	2.5	2.5	2.6
13	5.2	5.1	4.9	-	-	-	-	5.1
14	4.2	4.4	4.4	4.2	3.6	3.4	3.2	4.3
15	3.2	3.2	3.3	3.2	3.4	3.2	3.1	3.2
mean	5.1	5.0	5.0	4.9	4.7	4.5	4.5	5.0
sem±	1.5	1.4	1.2	1.3	1.3	1.3	1.3	0.3

Table 9 shows the steady state for blood glucose concentration in controls during the last 30 min. of the 3 hour insulin sensitivity test which was well achieved only from min 150 to 165. The steady state blood glucone concentration during this phase (min 150 to 165) had a mean value (±sem) of 3.7±0.3 mmol/l.

Table 9: Blood glucose concentration during the steady state period of the insulin sensitivity test in normotensive controls.

	blood glucose concentration (mmol/l)							
s.no	150	155	160	165	170	175	180	SSBG
1	2.6	2.5	2.4	2.3	2.1	2.1	2.0	2.4
2	3.5	3.4	3.7	3.5	3.4	3.4	3.4	3.5
3	5.0	4.6	4.4	4.1	4.1	3.9	3.7	4.5
4	5.3	5.2	4.8	4.7	4.4	4.3	4.0	5.0
5	2.7	2.4	2.6	2.5	2.4	2.7	2.9	2.5
6	6.3	6.4	6.0	5.9	5.7	5.6	5.2	6.1
7	3.2	3.0	3.0	3.1	2.8	3.1	2.9	3.1
8	4.1	4.0	3.6	3.6	3.3	3.3	3.1	3.8
9	3.7	4.2	3.7	4.3	3.5	4.4	5.0	4.0
10	-	-	-	-	-	-	-	-
11.	4.1	4.2	3.9	3.8	3.8	3.8	3.5	4.0
12	3.5	3.2	3.1	3.1	3.0	2.9	3.2	3.1
13	2.9	2.6	2.3	-	-	-	-	2.6
14	2.8	2.9	3.0	3.0	-	-	-	2.9
15	3.4	4.8	4.3	4.9	5.5	5.6	-	4.7
mean	3.8	3.8	3.6	3.8	3.8	3.8	3.5	3.7
±sem	1.1	1.2	1.0	1.0	1.2	1.1	0.9	0.3

Table 10 shows the results of insulin sensitivity test. Hypertensive subjects had a steady state blood glucose concentration ranging from 2.6 to 7.9mmol/l while the range in the controls was 2.4 to 6.1 mmol/l. The steady state blood glucose concentration for hypertensives and controls were significantly different (mean ±sem = 5.0 ± 0.3mmol/l and 3.7 ± 0.3mmol/l respectively, p = 0.01), with hypertensives showing a higher mean steady state blood glucose concentration than controls by about 26%. The metabolic clearance rate in hypertensives ranges from 0.002 to 0.013 ml/kg/min with a mean (±sem) value of 0.007 ± 0.001 ml/kg/min. The metabolic clearance rate in controls ranged from 0.007 to 0.08ml/kg/min with a mean value (±sem) of 0.009ml/kg/min. Hypertensives had a lower metabolic clearance rate by 28.5% as compared to the controls and this was significant (p = 0.01).

Table 10: Steady state blood glucose concentration and metabolic clearance rate in hypertensive and control subjects.

s.no	hypertensives		controls	
	SSBG (mmol/l)	MCR (ml/ kg/min)	SSBG (mmol/l)	MCR (ml/ kg/min)
1	6.1	0.005	2.4	0.014
2	3.5	0.009	3.5	0.009
3	5.7	0.006	4.5	0.007
4	5.1	0.007	5.0	0.007
5	5.4	0.006	2.5	0.013
6	5.3	0.006	6.1	0.054
7	4.4	0.008	3.1	0.011
8	7.9	0.004	3.8	0.009
9	6.6	0.002	4.0	0.008
10	5.5	4.002	-	-
11	4.4	0.008	4.0	0.082
12	2.6	0.013	3.1	0.011
13	5.1	0.007	2.6	0.003
14	4.3	0.008	2.9	0.011
15	3.2	0.01	4.7	0.007
mean	5.0	0.007	3.7	0.009
±sem	0.3	0.001	0.3	0.001

SSBG = steady state blood glucose concentration (mmol/l)
MCR = metabolic clearance rate (mmol/kg/min)

Table 11 shows the results of insulin secretion test in hypertensive subjects. The mean (±sem) fasting plasma insulin concentration of 8.1 ± 4.4 was within the normal range (8.0 ± 2mU/l)[15]. Peak insulin secretion was achieved after 30 min.

Table 11: Results of insulin secretion test in the hypertensive subjects.

	plasma insulin concentration(mU/l)				
s.no.	0 min	15 min	30 min	60 min	120 min
1	5.3	29.0	42.6	11.4	-
2	4.7	18.5	25.8	17.8	6.9
3	-	13.0	-	29.5	21.1
4	6.9	7.6	28.7	58.2	32.2
5	2.5	26.8	45.8	57.1	53.2
6	13.5	6.5	4.0	53.5	20.5
7	6.3	29.3	56.3	48.8	36.7
8	13.8	20.0	64.2	89.2	54.4
9	-	-	-	-	116.9*
10	-	77.9*	-	50.6	-
11	15.1	59.7*	114.1*	-	-
12	42.0*	44.9*	68.8	62.8	33.1
13	-	22.7	89.9	-	76.4
14	5.1	27.6	53.3	-	8.9
15	7.5	10.0	47.0	33.9	9.2
mean	8.1	19.2	52.2	49.3	26.6
sem±	4.4	8.7	19.0	11.8	16

NB: * Plasma insulin concentration values considered to be 'outliers'

Table 12 shows the results of insulin secretion test in the normotensive controls. The mean (±sem) fasting plasma insulin concetration showed a wide variation (5.1 ± 3.2). Peak insulin secretation was achieve after 60 min.

Table 12: Results of insulin secretion test in normotensive controls

	plasma insulin concentration (mU/l)				
s.no.	0 min	15 min	30 min	60 min	120 min
1	3.4	13.0	27.0	71.1	10.8
2	4.7	5.8	32.3	24.9	35.4
3	10.7	18.9	65.2	118.5	108.9*
4	4.5	19.8	22.0	63.7	51.9
5	3.8	17.5	-	43.2	39.3
6	-	-	-	-	-
7	2.5	24.1	84.0	111.8	16.4
8	10.4	35.1	49.9	73.1	60.9
9	<1*	6.2	14.3	33.7	22.5
10	8.0	54.3	59.5	109.2	78.1
11	7.6	-	31.2	14.5*	17.6
12	1.9	5.9	38.8	-	63.8
13	-	-	-	-	-
14	1.7	6.5	6.3	16.1*	24.6
15	2.4	26.0	58.4	-	85.0
mean	5.1	19.4	40.7	72.1	42.2
sem ±	3.2	14.4	23.0	35.0	25.2

Table 13 shows a steady increase in the plasma insulin concentration during the first 2 hours of the insulin sensitivity test. There was a wide variation in plasma insulin concentration between individuals.

Table 13: Plasma insulin concentrations during the first 2 hours of the 3 hour insulin sensitivity test in hypertensive subjects

	plasma insulin concentration (mU/l)						
s.no	-30 min	-15 min	0 min	30 min	60 min	90 min	120 min
1	-	5.8	4.5	-	68.5	-	96.2
2	-	<1.0*	1.8	42.6	-	47.7	64.3
3	-	2.7	7.1	44.4	90.1	128.	164.8
4	-	-	<1.0*	59.6	47.8	-	94.2
5	-	2.3	<1*	74.4	82.0	-	83.8
6	-	8.0	4.5	-	-	-	-
7	-	-	3.8	-	32.7	-	36.7
8	-	<1.0*	1.0	-	73.0	-	76.0
9	-	8.9	9.1	47.7	70.1	-	99.6
10	-	6.9	10.0	-	123.8	-	160.0
11	-	6.9	8.1	-	65.7	-	143.4
12	-	2.5	2.7	-	47.9	-	43.8
13	2.1	1.4	2.2	-	74.9	-	71.5
14	6.6	6.3	2.3	-	89.2	-	152.7
15	-	<1.0*	2.3	3.4	53.4	-	4.9
mean		5.0		54.0	71.0	-	88.0
sem±		0.6		13.3	23.3		40.0

Table 14 shows a progressive increase in the plasma insulin concentration during the 120 minutes of the test. The plasma insulin concentrations observed at 60 and 120 minute intervals were not significantly different from those observed in the hypertensive subjects.

Table 14: Plasma insulin concentrations during the first 2 hours of the 3 hour insulin sensitivity test in normotensive controls.

	plasma insulin concentration (mU/l)						
s.no	-30 min	-15 min	0 min	30 min	60 min	90 min	120 min
1	2.0	-	3.5	-	58.5	-	51.8
2	-	8.0	5.9	-	95.5	-	93.0
3	-	-	8.3	-	98.3	-	-
4	-	5.0	2.8	-	75.0	-	87.3
5	3.8	1.3	3.8	-	55.8	-	55.5
6	-	-	-	-	-	-	-
7	-	3.0	3.1	-	39.3	-	32.8
8	-	4.8	2.3	-	39.4	-	82.5
9	-	1.3	<1*	20.3	22.4	-	30.7
10	-	-	7.5	-	119.0	-	112.5
11	6.5	8.3	5.9	-	78.5	-	87.2
12	-	2.5	2.7	-	47.9	-	43.8
13	2.1	1.4	2.2	-	74.9	-	71.5
14	6.6	6.3	2.3	-	89.2	-	152.7
15	-	<1*	2.3	3.4	53.4	-	44.9
mean		4.5	-		67.7	-	72.8
sem±		0.8			27.0		35.0

Table 15 shows moderate achievement of steady state plasma insulin concentration during the period 150 to 180 minutes.

Table 15: Plasma insulin concentration during the steady state period ofthe insulin sensitivity test in hypertensive subjects.

	plasma insulin concentration (mU/l)							
s.no	150 min	155 min	160 min	165 min	170 min	175 min	180 min	SSPI
1	109.3*	63.1*	-	87.0	86.0	88.9	89.1	87.8
2	94.2*	79.4*	-	52.4	50.9	55.4	25.2*	53.0
3	91.0	84.2	75.7*	94.7	59.8*	-	110.3*	90.0
4	80.2	76.0	-	70.6	76.9	54.0*	61.1*	76.0
5	79.2*	96.3*	60.4	71.4	60.0	63.3	56.5	64.3
6	-	-	-	-	-	70.2	51.0	-
7	29.9*	55.8	34.7*	-	58.1	65.4	44.7	56.0
8	71.7	70.8	78.0	87.5	84.5	73.3	94.0*	77.3
9	85.3	88.3	80.0	94.9	76.9	116.8*	102.6*	83.6
10	97.0	102.4	106.9	87.7	93.8	94.2	99.2	97.3
11	121.0*	116.8*	108.9*	81.7	83.2	79.3	77.4	80.4
12	55.8	170.8*	44.5	33.7*	44.7	55.2	60.1	52.1
13	67.4*	40.6	38.9	-	-	-	-	-
14	171.6*	94.9*	108.9	134.3	130.6	152.0	-	124.6
15	-	17.0*	-	28.1	4.9	29.5	29.8	29.1
mean	80.0	74.0	74.0	81.0	77.5	68.3	65.3	72.4
sem±	16.0	20.0	27.0	27.0	24.0	20.0	24.7	25.1

* Outliers, not considered for calculation of steady state plasma insulin concentration.
SSPI=steady state plasma insulin concentration.

Table 16 shows good achievement of steady state plasma insulin concentration in controls unlike that achieved in hypertensives. Although plasm insulin concentration were higher in hypertensives than in controls at all intervals ofthe 30 minute period, the difference between these two groups was not statistically significant ($p>0.5$).

Table 16: Plasma insulin concentration during the steady state period of the insulin sensitivity test in normotensive controls.

	plasma insulin concentration (mU/l)							
s.no	150 min	155 min	160 min	165 min	170 min	175 min	180 min	SSPI
1	61.5	51.0	59.8	71.5	53.0	31.1*	52.5	55.6
2	93.0	95.3	96.8	75.5*	84.3	85.7	90.5	91.0
3	-	93.2	77.0	78.3	83.4	75.0	60.2	81.4
4	81.0	81.0	78.8	73.5	81.3	73.5	73.5	78.0
5	68.2	66.0	53.5	72.5*	52.8	54.5	58.0	59.0
6	-	-	-	-	-	-	-	-
7	41.2	46.9	51.4	54.3	62.0	-	51.3	51.2
8	74.0	69.5	72.0	59.5	69.0	80.5	65.3	69.4
9	31.8	33.1	34.3	32.8	34.3	33.2	34.3	33.4
10	-	-	-	-	-	-	-	-
11	87.3	89.3	98.5	92.8	86.5	80.5	103.0*	59.1
12	73.8	69.8	72.5	79.3	85.8	76.8	74.8	76.1
13	-	-	-	-	-	-	-	-
14	61.0	58.5	50.0	36.5*	-	-	-	56.5
15	53.7	40.5	51.0	53.0	13.0*	51.0	-	50.0
mean ±sem	66.0 18.7	66.2 20.8	66.3 18.0	66.1 18.0	68.8 17.3	67.8 17.6	62.5 17.3	63.4 16.1

* Refers to outliers, not considered for calculation of steady state plasma insulin concentration.

Figure 1 shows the relation between systolic blood pressure and body mass indices in the hypertensive subjects, while figure 2 shows a similar relationship in the controls. There was no significant correlation between systolic blood pressure and body mass indices in both groups (r=0.1 and r=0.3 p>0.7, and 0.2 respectively).

Figure 1: The relationship between systolic blood pressure (mmHg) and body mass indices (kg/m^2) in the hypertensive subjects.

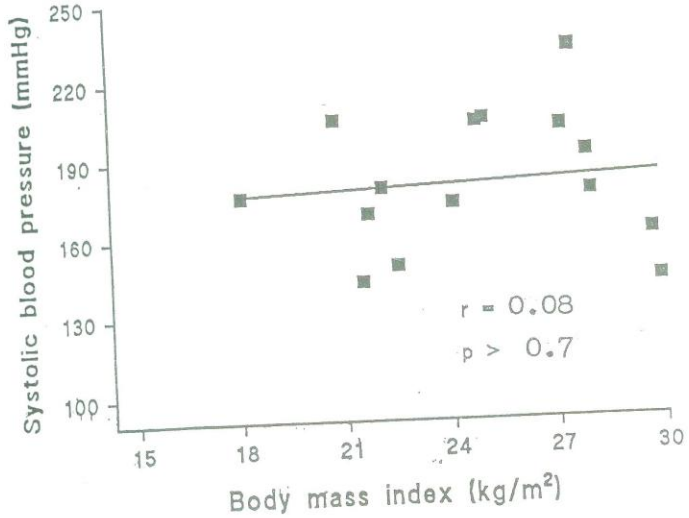
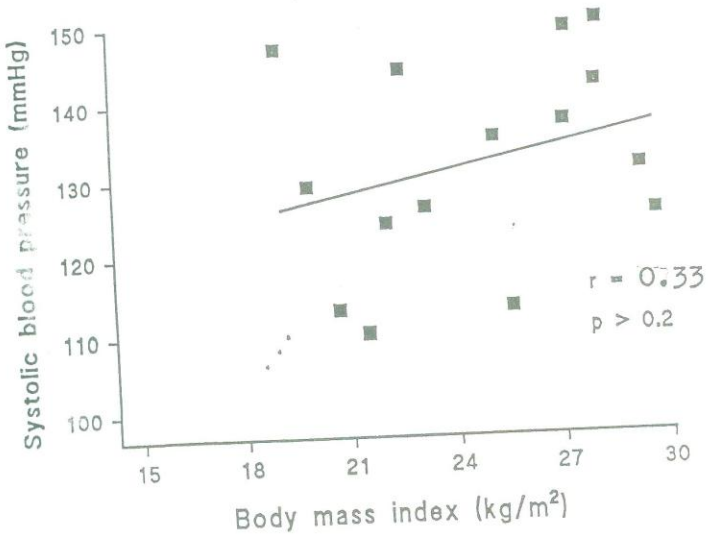


Figure 2: The relationship between systolic blood pressure (mmHg) and body mass indices (kg/m^2) in the controls.



Figures 3 and 4 show the relationship between diastolic blood pressure and body mass indices in the hypertensive subjects and the control group respectively. There was no significant correlation between diastolic blood pressure and body mass indices in the two groups (r=0.1in both groups, p>0.6).

Figure 3: Relationship between diastolic blood pressure (mmHg) and body mass indices (kg/m^2) in hypertensive subjects.

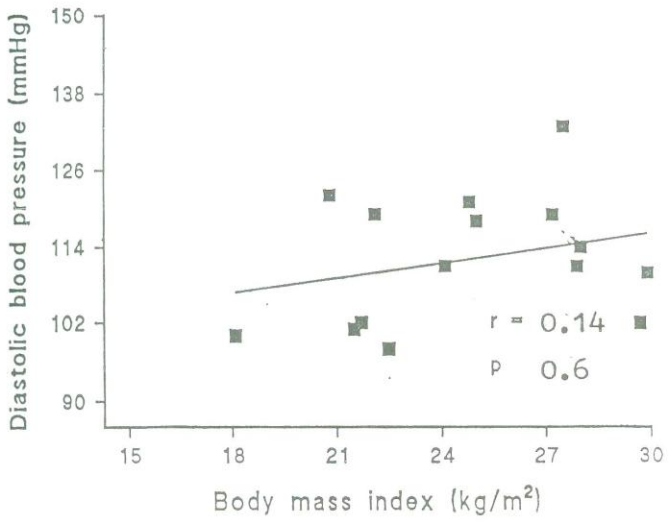
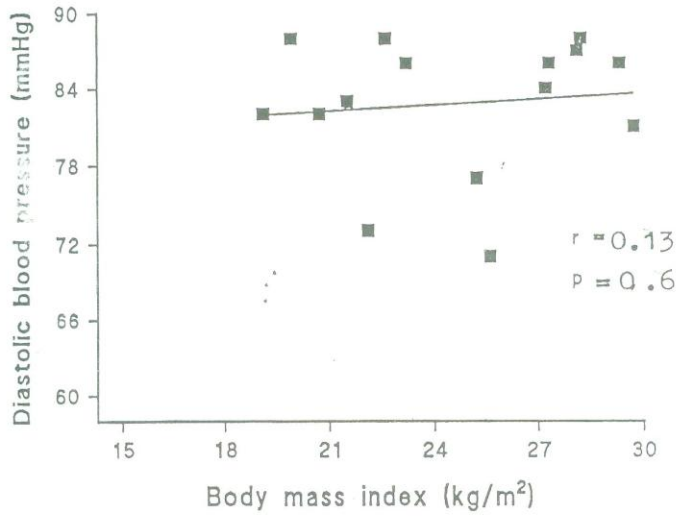


Figure 4: Relationship between diastolic blood pressure (mmHg) and body mass indices (kg/m^2) in the control group.



Figures 5 and 6 show the relationship between systolic blood pressure and waist-hip ratios in the hypertensive subjects and the control group respectively. There was a significant positive correlation between systolic blood pressure and waist-hip ratios in the hypertensive subjects ($r=0.5$ $p<0.05$) but not in the control group ($r=0.2$, $p>0.5$).

Figure 5: Relationship between systolic blood pressure (mmHg) and waist-hip ratios in the hypertensive subjects.

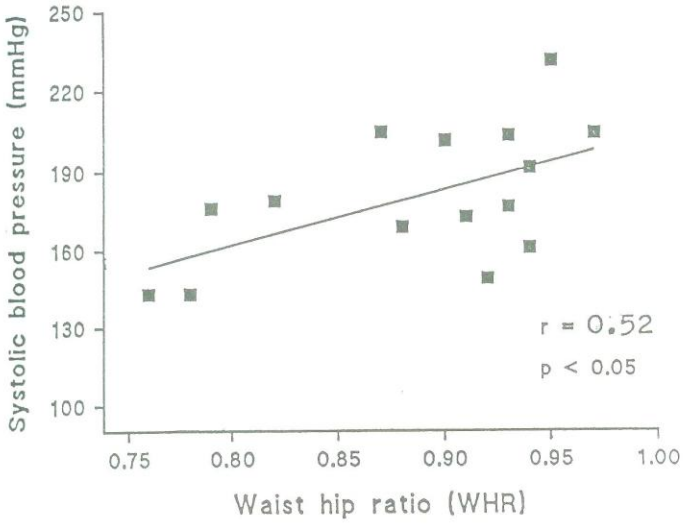
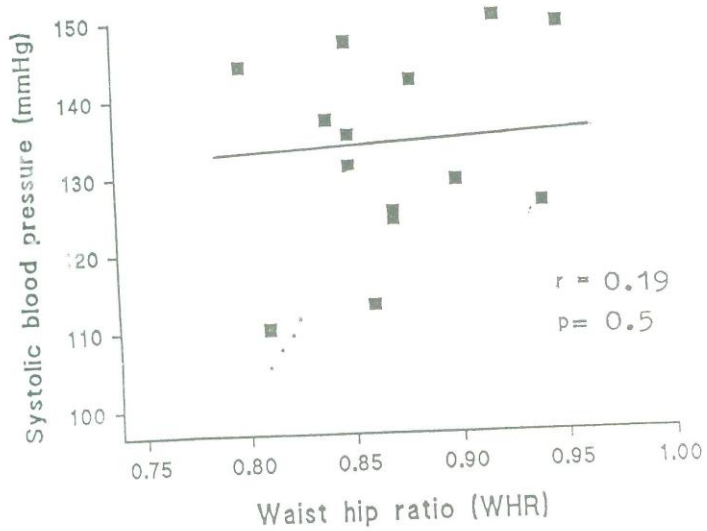


Figure 6: Relationship between systolic blood pressure (mmHg) and waist-hip ratios in the control group.



Figures 7 and 8 show the relationship between diastolic blood pressure and waist hip ratios in the hypertensive subjects and the control group respectively. There was no significant correlation in the two groups (r=0.3 and r=0.2, p>0.2 and 0.4 respectively).

Figure 7: Relationship between diastolic blood pressure and waist-hip ratios in the hypertensive subjects.

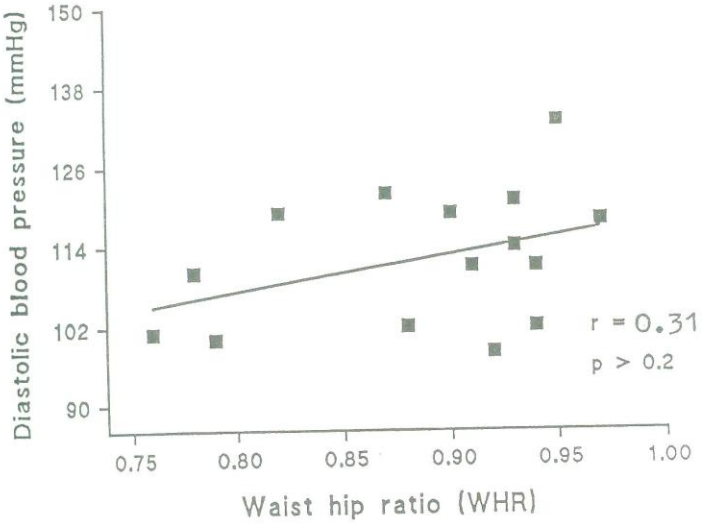
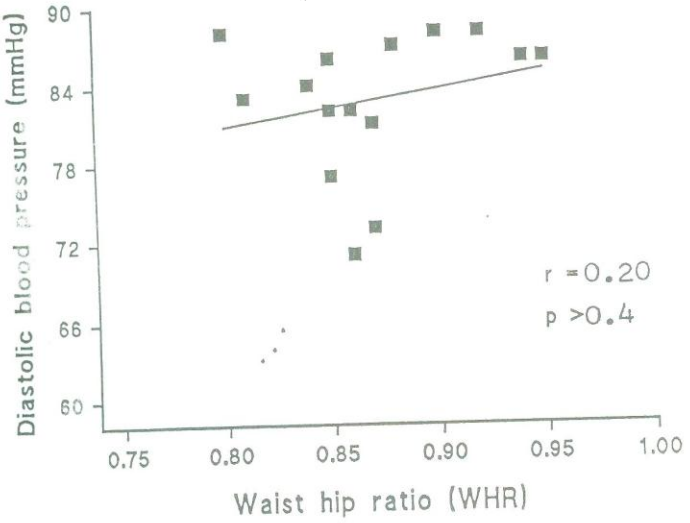


Figure 8: Relationship between diastolic blood pressure and waist-hip rations in the controls.



Figures 9 and 10 show the relationship between systolic blood pressure and steady state plasma glucose concentrations (SSBG) in the hypertensive subjects and the controls respectively. There was no significant correlation in the hypertensive group ($r=0.1$, $p>0.6$) while the correlation was significant in the control group, ($r=0.5$, $p<0.05$)

Figure 9: Relationship between systolic blood pressure (mmHg) and steady state blood glucose concentration (mmol/l) in hypertensive subjects.

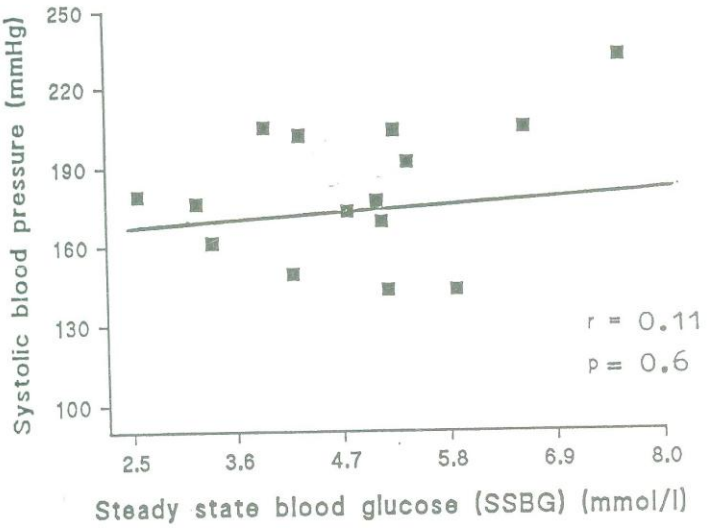
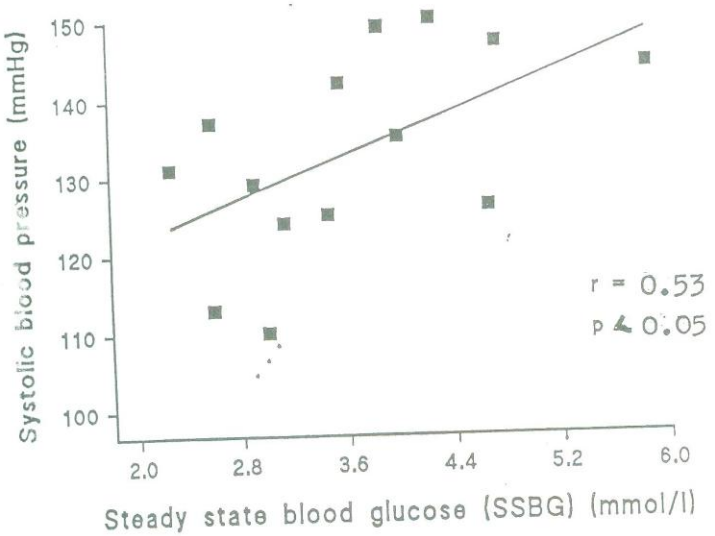


Figure 10: Relationship between systolic blood pressure (mmHg) and steady state blood glucose concentration (mmol/l) in controls.



Figures 11 and 12 show the relationship between diastolic blood pressure and steady state blood glucose concentration (SSBG) in the hypertensive subjects and their controls respectively. There was no significant correlation in the two groups (r=0.1 and r=0.4 respectively, p=0.7 and 0.4 respectively).

Figure 11: Relationship between diastolic blood pressure (mmHg) and steady state blood glucose concentration (mmol/l) in hypertensives.

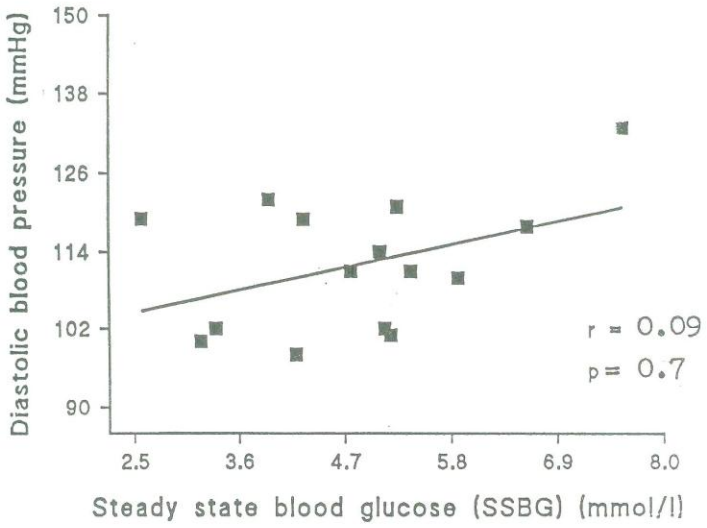
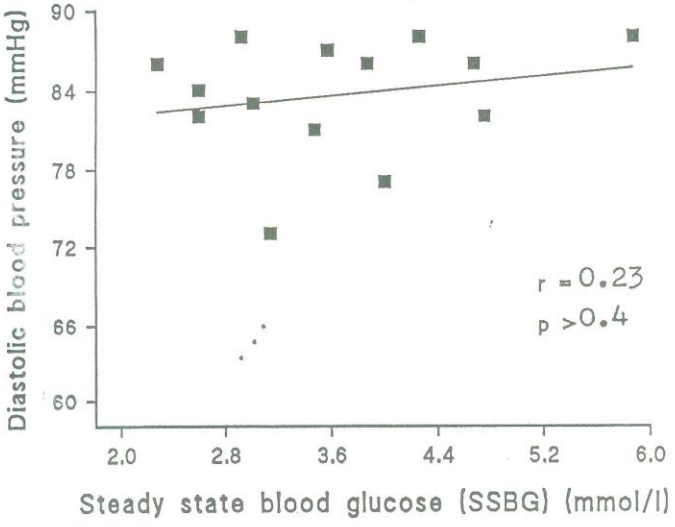


Figure 12: Relationship between diastolic blood pressure (mmHg) and steady state blood glucose concentration (mmol/l) in controls.



Figures 13 and 14 show the relationship between systolic blood pressure and metabolic clearance rates in the hypertensive subjects and the controls. There was no significant correlation in the hypertensive subjects (r=-0.1, p = 0.7), while there was a significant negative correlation in the control group (r=-0.5, p<0.05).

Figure 13: Relationship between systolic blood pressure (mmHg) and metabolic clearance rates (mmol/kg/min) in hypertensive subjects.

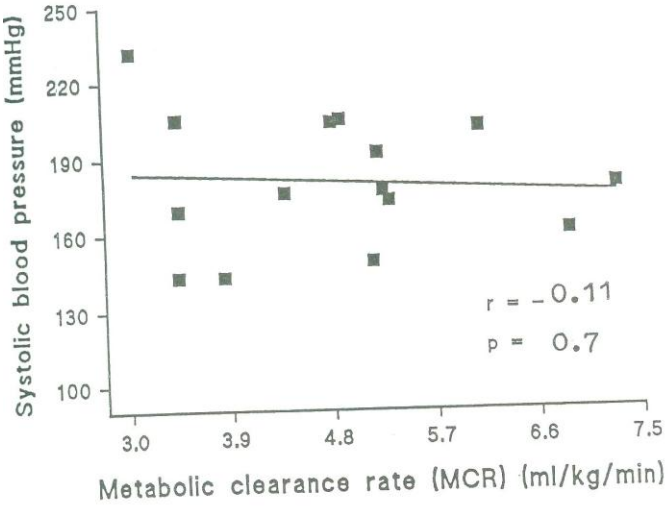
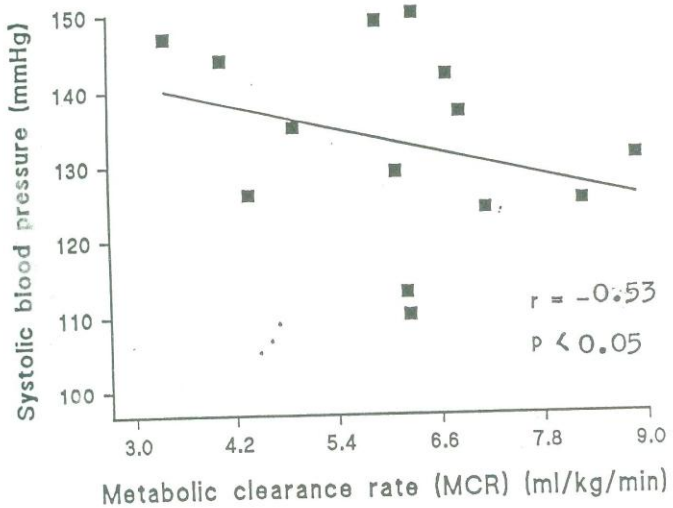


Figure 14: Relationship between systolic blood pressure (mmHg) and metabolic clearance rates (mmol/kg/min) in controls.



Figures 15 and 16 show the relationship between diastolic blood pressure and metabolic clearance rates in the hypertensive subjects and the control group respectively. Diastolic blood pressure did not correlate significantly with metabolic clearance rates in both groups (r=-0.1 and r=-0.2 respectively p>0.6).

Figure 15: Relationship between diastolic blood pressure (mmHg) and metabolic clearance rates (mmol/kg/min) in hypertensive subjects.

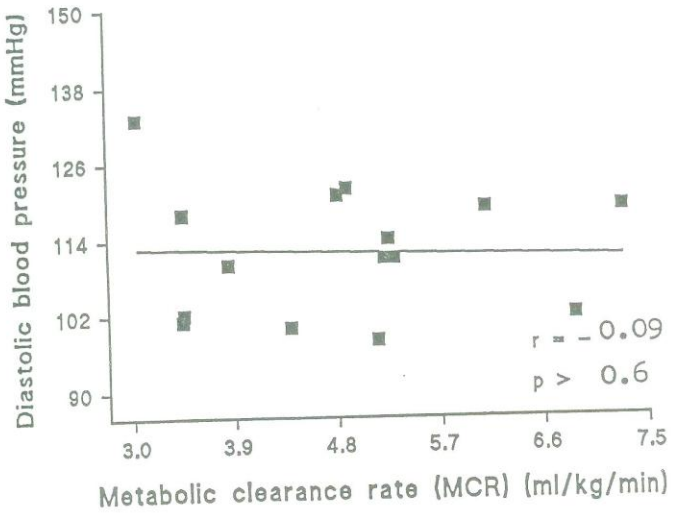
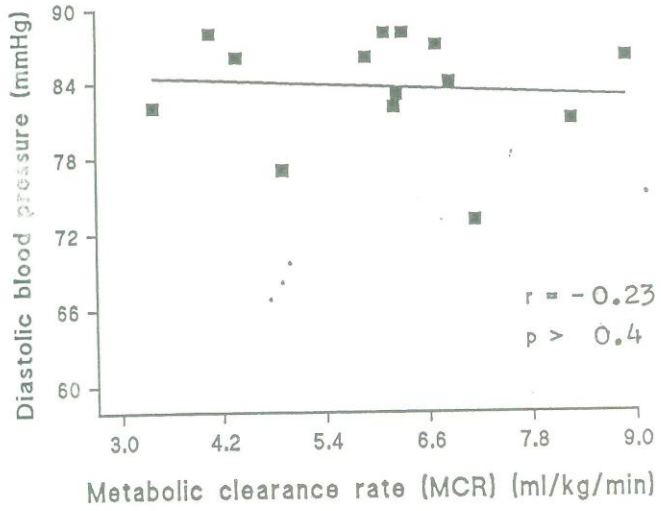


Figure 16: Relationship between diastolic blood pressure (mmHg) and metabolic clearance rates (mmol/kg/min) in controls.



Figures 17 and 18 show the relationship between fasting plasma insulin concentration and systolic blood pressure in the hypertensive subjects and the control group respectively. There was no significant correlation between fasting plasma insulin concentration and systolic blood pressure in the hypertensive subjects as well as in the control group (r=0.3 in both groups, p>0.3).

Figure 17: Relationship between fasting plasma insulin concentration (mU/l) and systolic blood pressure (mmHg) in hypertensive subjects.

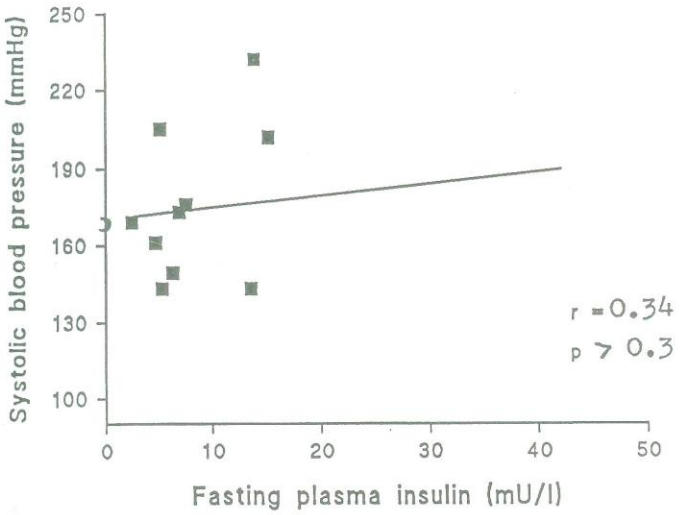
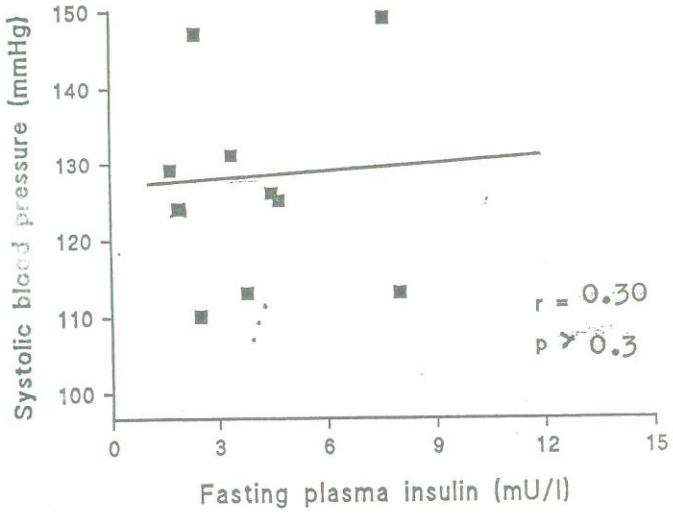


Figure 18: Relationship between fasting plasma insulin concentration (mU/l) and systolic blood pressure (mmHg) in controls.



Figures 19 and 20 show the relationship between fasting plasma insulin concentration and diastolic blood pressure in the hypertensive subjects and the control group respectively. There was no significant correlation between fasting plasma insulin concentration and diastolic blood pressure in both groups (r=0.3, p>0.3 in both groups).

Figure 19: Relationship between fasting plasma insulin (mU/l) concentration and diastolic blood pressure (mmHg) in hypertensive subjects.

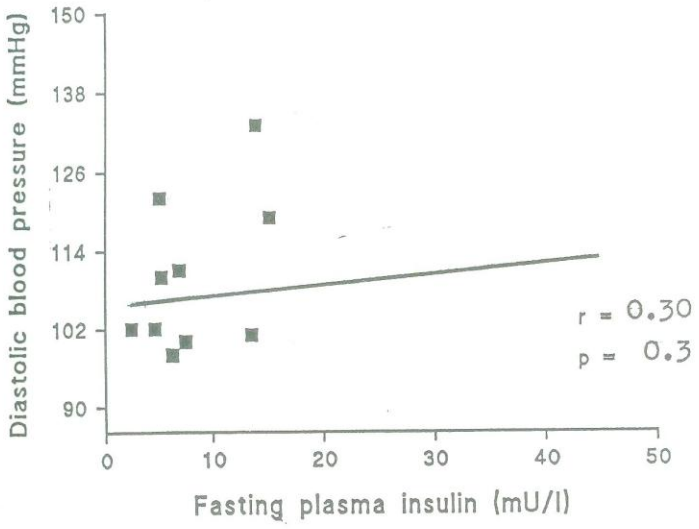
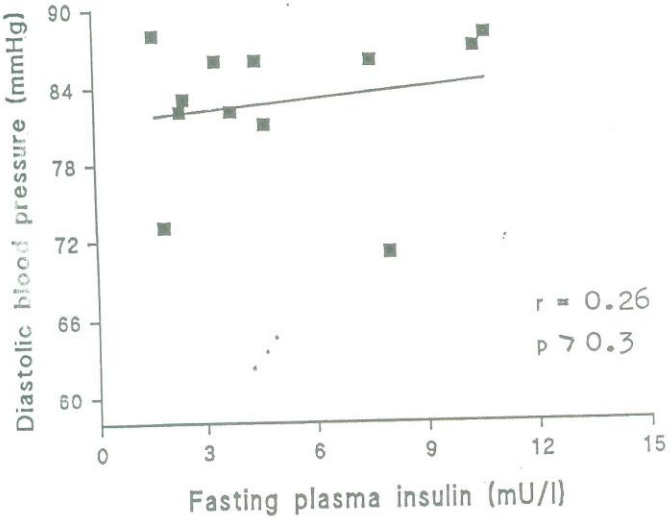


Figure 20: Relationship between fasting plasma insulin (mU/l) concentration and diastolic blood pressure (mmHg) in controls.



DISCUSSION

This study analyzed the insulin sensitivity pattern in 15 newly detected, untreated hypertensives, as well as in 15 age, sex and body mass index matched normotensive controls, using the modified Harano's (glucose/insulin infusion) technique. The results, showed that the hypertensives (cases) had abnormalities in glucose metabolism pointing to the existence of insulin resistance compared to the normotensive controls. This was indicated by the finding of higher levels of steady state plasma glucose concentration (SSPG) and Lower metabolic clearance rate (MCR) among hypertensives compared to normotensives.

In the modified Harano's technique for assessing insulin sensitivity, glucose and insulin are infused continuously at 6 mg/kg/min and 50 IU/KG/hr respectively for a period of 180 minutes. Previous studies have indicated that, after 150 minutes of infusing glucose and insulin at this rate steady state phase for glucose and insulin is achieved [137]. During the steady state phase, the net glucose and insulin disposal is zero. The height of the plasma glucose concentration at steady state phase therefore reflects the sensitivity of the tissue to the insulin stimulated glucose uptake. A much better index for measuring this tissue insulin sensitivity is (glucose) metabolic clearance rate, which will be low in the presence of insulin resistance. Since cases and controls had comparable mean body weights, their glucose infusion rates were also comparable. That is, glucose and insulin were on average infused at similar rates among cases and controls. The differing SSPG and MCR between cases and controls therefore reflects differing states of tissue sensitivity to insulin stimulated glucose disposal.

The study shows that on average, hypertensives had an elevation of SSPG of about 26% compared to the normotensives, implying a significant

decrease in insulin sensitivity. It was not possible in this study to compute directly the average glucose uptake among cases and controls. Thomas et al [2], found a 14% decrease in glucose uptake among cases compared to controls, using an euglycemic insulin clamp technique. It seems safe to assume that the decrease in glucose uptake among cases in this study was comparable to that found by Thomas and his colleagues.

The rate at which glucose was metabolized (MCR) was lower among hypertensives by about 28.5% as compared to normotensives. Findings by other workers agree with this observation [2,4].

The hypertensives in this study had a fasting blood glucose levels which were significantly higher than that of normotensives by about 6%. There were no differences in the plasma glucose levels between cases and controls, during the rest of the two- hour period of the oral glucose tolerance test. These observations contrast with those of Shen et al [85], who found higher plasma glucose concentrations throughout the entire 2- hour period of the oral glucose tolerance test in patients with hypertension, treated or untreated and significantly higher plasma glucose response among hypertensives compared to that in the normal group. In yet another study, it was found that, in the fasting post absorptive state, venous plasma glucose levels were similar in the hypertensive and control groups, but after 75 g of glucose, plasma glucose concentrations at 2-hours were significantly higher in the hypertensive than in the normotensive subjects [87].

A relationship between plasma glucose and blood pressure existed positively but was not statistically significant. Victoria et al [137], found a stronger relationship of plasma glucose to blood pressure in individuals with higher heart rates.

Body mass index is another significant correlate of blood pressure and insulin resistance as demonstrated by many studies [4]. A positive relationship between body mass index and blood pressure was observed but was not statistically significant. Failure to demonstrate a statistically significant correlation between body mass index and blood pressure even after considering the total study population could be explained by the small sample size. The metabolic clearance rate correlated negatively with blood pressure, but this was not statistically significant in the hypertensive group while a significant negative correlation between systolic blood pressure and MCR was observed in the control group. Thomas and colleagues [2], analysing a much bigger sample size (51 normotensives, 58 non obese hypertensives and 85 obese hypertensives) clearly demonstrated a significant negative correlation between insulin sensitivity and systolic blood pressure. On the other hand, Saad et al [50], also analysing a large sample found an insignificant negative correlation between glucose disposal and blood pressure among blacks and Pima Indians. The negative correlation between glucose disposal and blood pressure was however found to be significant in whites only.

Another significant correlate of blood pressure and insulin resistance which was considered in this study was waist to hip ratio (WHR). Waist to hip ratio is a measure of central localization of adipose tissue [129] and is associated with increased free fatty acid (FFA) concentrations and plasma triglyceride levels, elevated plasma glucose and insulin levels following an oral glucose load, and an increased disposition to diabetes mellitus [2]. The WHR varies with age. Black females for example aged 20-34 years have been found to have a ratio of 0.86 which was significantly different from the 0.95 ratio of black females over age

50 [138]. The mean WHR among hypertensives and controls in this study was not significantly different and there was a positive correlation only with systolic blood pressure in the hypertensives.

The relevance of WHR as an indicator of the increased response of abdominal adipocytes to stimuli promoting lipolysis and release of FFA directly into the portal vein to contribute to an increased synthesis of very low density lipoprotein in the liver is a clearly documented phenomenon [2].

In this study, plasma insulin concentrations were similar in the fasting state in the hypertensive subjects and the normotensive controls. A similar observation was made in caucasians by Manicardi et al [79]. This study has not demonstrated a significant correlation between fasting plasma insulin concentrations and systolic as well as diastolic blood pressure in both hypertensive subjects and controls. Lucas et al [15] found that both systolic and diastolic blood pressure were significantly correlated with fasting serum insulin. Pollare et al [2] in addition to demonstrating a positive correlation between blood pressure and fasting plasma insulin, also found that hypertensive groups had higher fasting plasma insulin concentration and insulin glucose ratio compared with normotensive controls. Falkner et al [4] in a study which involved young black men also made similar observations.

The sample size not with standing, this study has demonstrated the occurrence in newly diagnosed hypertensive of insulin resistance at body mass index levels below 30 kg/m2 and in the absence of impaired glucose tolerance. It has joined other studies in providing evidence that resistance to insulin stimulated glucose uptake exists in patients with hypertension independently of obesity and glucose intolerance (1,2,3,4,5,6,).

Investigators have provided evidence to suggest that peripheral tissue,and not the liver is the principal site of insulin resistance [1,2,83]. Skeletal muscle is thought to account significantly for the site of insulin insensitivity in hypertension [1].

The association between decreased insulin sensitivity and hypertension is not a simple one since pharmacologic reduction of blood pressure is sometimes followed by deterioration in insulin sensitivity, for example after administration of beta-adrenergic blockers or thiazide diuretics [2]. Hypertension is a well recognized risk factor for coronary artery disease, and the apparent inability to modify morbidity and mortality from coronary artery disease with treatment of hypertension remains enigmatic.

Obviously, there is much more to be learned about the relationship between the metabolic changes, antihypertensive medication and the increased risk of coronary artery disease in patients with hypertension. Of the two principal pathways of intracellular glucose metabolism; complete oxidation and non oxidative disposal, only the latter has been found to be reduced in a study conducted by Ferranini et al [1], accounting for virtually all the decrease in the total glucose utilization. Glucose oxidation in hypertensive subjects was unchanged in the fasting state and was normally stimulated by insulin. Energy output was essentially normal. In quantitative terms, Ferranini's study pointed out that the defect in non-oxidative glucose disposal (glycogen and lipid synthesis) was by far the dominant metabolic characteristic of the patients with essential hypertension. The table below summarizes the characteristics of insulin resistance in hypertension, obesity and non insulin dependent diabetes mellitus as found in Ferranini's study.

EFFECT OF INSULIN	HYPERTENSION	OBESITY	NIDDM
Whole body glucose uptake	+	−	−
Suppression of glucose output	±	−	±
Glucose oxidation	±	−	−
Lipid oxidation	±	+	±
Non oxidative glucose disposal	−	−	−
Suppression of lipolysis	±	±	−
Promotion of potassium uptake	±	−	−

Key: − = Decreased; + = Increased; ± = Unchanged

This study, like similar previous studies raises the question whether there is a causal relation between hypertension and insulin resistance.

Reduced sodium - potassium ATPase (Na+ - K+ ATPase) activity is suspected to be an inherent cellular malfunction in essential hypertension [1,139,130,141]. Unsaturated free fatty acids have been implicated as inhibitors of the sodium pump [1]. Insulin lowers plasma levels of free fatty acids and directly stimulates the Na+-K+ ATPase thought to represent a key insulin - regulated enzyme which plays a critical role in maintaining the normal intracellular electrolyte milieu [25], extruding sodium, in exchange for potassium in a ratio of 3:2. Obesity, diabetes and hypertension all represent insulin resistant states, with respect to glucose metabolism. If this insulin resistance were to extend to the enzyme sodium-potassium-adenosine triphosphatase(Na^+-K^+ ATPase), sodium would accumulate within the cell. In patients with essential hypertension, there is evidence that both intracellular Na+ content and transmembrane Na+ transport rate are diminished in leucocytes and erythrocytes [1,25,134,135]. The activity of the Na+ - k+ ATPase has been reported to be reduced in various cell systems in human essential hypertension [136,137]. Reduced activity of the sodium-potassium pump (Na^+ pump) also has been reported in other insulin resistant states including obesity and diabetes mellitus. It has been hypothesized that in insulin resistant states, the Na+ -K+ pump may not be normally responsive to insulin. However several recent observations of intact transmembrane insulin stimulated K^+ transmembrane handling in patients with essential hypertension, suggest that an abnormality in the Na+ -K+ ATPase pump is unlikely to explain the elevation in blood pressure in patients with essential hypertension [25]. Another cell membrane pump that has received considerable attention in the

pathogenesis of essential hypertension is the sodium-hydrogen exchanger (Na^+-H^+ exchanger) [25] which is considered to be equivalent to the sodium-lithium (Na^+-L^+) co-transport system. Insulin stimulates the activity of the Na^+- H^+ exchanger in skeletal muscle and adipocytes. This Na^+- H^+ exchanger has also been shown to be linked to calcium exchange and to play a critical role in the maintenance of intracellular pH [25].

The Na+-H+ exchanger could be responsible for the elevation of blood pressure observed in insulin resistant states such as hypertension, diabetes mellitus and obesity [144,145]. It has been suggested that the accompanying hyperinsulinemia of insulin resistant states augments Na+-H+ exchange, assuming that this pump maintains normal sensitivity to insulin [146]. Intracellular accumulation of sodium and calcium would be expected to enhance the sensitivity of the vascular smooth musculature to the pressor effects of norepinephrine, angiotensin and sodium chloride loading. Enhanced Na+- H+ exchange also will lead to an increase in cell pH. Intracellular alkalosis is a known stimulator of protein synthesis and cell proliferation and eventually could lead to the characteristic hypertrophy of resistance vessels that is observed in established hypertension. Intracellular alkalosis also is known to directly increase smooth muscle contractility [145,146].

Several clinical observations are consistent with the above hypothesis. First, the Na^+-H^+ exchanger is the only known genetic marker for essential hypertension, with many investigators demonstrating increased erythrocyte Na^+-H^+ countertransport in hypertensive versus non-hypertensive individuals [25,144,145].

It has been postulated that excessive Na^++-H^+ activity is an inherited trait but in itself is not sufficient to cause hypertension. Only in

individuals with insulin resistance and secondary hyperinsulinemia will the phenotypic expression of the Na+- H+ exchanger become manifest. Insulin acting directly or indirectly through the stimulation of growth factors , such as insulin - like growth factor - 1 (IGF-1),also may contribute to the development of hypertension by causing hypertrophy of the vascular wall and narrowing of the lumen of the resistance vessels involved in the regulation of systemic blood pressure. The components of vascular hypertrophy include increases in the size and number of myocytes and in the amount of contractile protein, DNA and collagen, all of which can be increased by the actions of insulin and IGF-1. Receptors for IGF-1 and insulin have been identified on blood vessels [25].

Insulin can also elevate pressure via renal sodium retention, sympathetic nervous system activation and renin- angiotensin - aldosterone system activation as discussed elsewhere in the literature review.

Victoria et al [137], have shown that high carbohydrate high sodium diets have synergistic effects on the induction of blood pressure elevation. Sodium excess has been shown to enhance renal vascular reactivity to angiotensin II.

Some workers have suggested that the clinical entity of essential hypertension might evolve by a step ladder sequence in which small rises in blood pressure, perhaps due to increased sympathetic activity produce changes in the kidney and possibly else where, which maintain the raised pressure and become the basis for further elevation [146].

In conclusion, it can be stated that although investigations have been successful in demonstrating the independent associating among insulin resistance and essential hypertension,no clear documentation of a cause and effect relationship has been made so far. Many theories

have been advanced but they are all matters for debate and none is free from criticism.

This study is the first to document the existence of insulin resistance in African hypertensives. More studies are being conducted trying to define the possible role for insulin resistance in the etiopathogenesis of essential hypertension. If a cause and effect relationship between insulin resistance and hypertension becomes clearly documented, then at least in this aspect, a similarity in the characteristics of hypertension between populations of African heritage and Caucasians will have been demonstrated. This will raise a hope for an approach of managing hypertension common to both populations. Management of hypertension from its root cause may have a better outcome than the current approach based on ill-defined pathogenetic mechanisms which often do not show a significant reduction in morbidity and mortality and in some cases have resulted in more deleterious metabolic changes worsening the prognosis.

More studies of this nature should be performed especially in Africans in whom essential hypertension has been shown to have significantly different clinical characteristics from those observed in Caucasians. For example, in Africans hypertension tends to be more severe and more refractory to a number of conventional as well as modern antihypertensive drugs. Hypertension in Africans tends to take a rapid course with early development of end-organ failure or damage despite the use of antihypertensives. This means that hypertension in Africans is not yet managed according to a clear understanding of its pathogenesis and the associated metabolic changes.

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