

**STUDIES OF INSULIN SENSITIVITY USING THE EUGLYCAEMIC
HYPERINSULINAEMIC CLAMP**

**A THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF MEDICINE
IN THE FACULTY OF MEDICINE, UNIVERSITY OF GLASGOW**

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PREFACE

The work presented in this thesis was carried out during my appointments as a Clinical Research Fellow and Lecturer in the University Department of Medicine and Therapeutics, Western Infirmary, Glasgow.

I was primarily responsible for conducting and analysing the studies described and the preparation of this thesis was entirely my own work. I am particularly grateful to many colleagues in the Department of Medicine and Therapeutics for their encouragement and for invaluable assistance in performing the laboratory assays. Their help is formally acknowledged.

This work was supported by a mini-project grant awarded to Dr. Richard Donnelly and myself by the Scottish Home and Health Department. Several of the studies have been presented at scientific meetings and published or accepted for publication in peer-reviewed scientific journals. These communications and publications are listed on pages 16 and 17.

Andrew D. Morris

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PUBLICATIONS ARISING FROM THE THESIS

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Metabolic effects of lacidipine: a placebo-controlled study using the euglycaemic hyperinsulinaemic clamp.
British Journal Clinical Pharmacology, 1993, **35**, 40-45.

A.D. Morris, R. Donnelly, J.M.C. Connell, J.L. Reid. Acute and chronic effects of lacidipine on insulin sensitivity in essential hypertension.
Hormone and Metabolic Research; 1994, **26**, 257-259.

A.D. Morris, J. Petrie, J. Anderson, J.M.C. Connell, R. Donnelly.
The effects of angiotensin II on insulin sensitivity: a placebo-controlled study. *Clinical Science*; 1993, **85**, 431-436.

R. Donnelly, A.D. Morris. Drugs and insulin resistance: clinical methods of evaluation and new pharmacological approaches to metabolism.
British Journal of Clinical Pharmacology; 1994, **37**, 311-320.

A.D. Morris, J.M.C. Connell.
The clinical significance of insulin resistance. *American Journal of Medical Sciences*; 1994, **307**, S47-S52.

A.D. Morris, J. Petrie, J. Connell. Insulin and blood pressure. *Journal of Hypertension*; **12**, 633-642.

A.D. Morris, J. Petrie, S. Ueda, M. Small, H.L. Elliott, J.M.C. Connell, R. Donnelly.
Angiotensin II increases insulin sensitivity in non-insulin dependent diabetes: dissociation of metabolic and blood pressure effects.
Diabetes; in press.

A.D. Morris, J. Petrie, S. Ueda, J.M.C. Connell, H. Elliott, R. Donnelly.
The euglycaemic hyperinsulinaemic clamp: an evaluation of the methodology including arterialisation of venous blood. *European Journal of Clinical Investigation*; submitted for publication.

A.D. Morris, J. Petrie, S. Ueda, J.M.C. Connell, R. Donnelly, H. Elliott. The effects of trandolapril on insulin sensitivity in diabetic hypertensive patients: a placebo-controlled study. *British Journal of Clinical Pharmacology*; in preparation.

COMMUNICATIONS TO LEARNED SOCIETIES

A.D. Morris, R. Donnelly, J.M.C. Connell, J.L. Reid. The effects of lacidipine on insulin sensitivity: a placebo-controlled study using the euglycaemic hyperinsulinaemic clamp. British Pharmacological Society, London, December 1991. *British Journal of Clinical Pharmacology*, 1992; **33**: 537P.

A.D. Morris, J. Petrie, J.M.C. Connell, R. Donnelly. The effects of angiotensin infusion on insulin sensitivity in man. Medical Research Society, London, April 1993. *Clinical Science*, 1993, **85** (1), 12P.

A.D. Morris, J. Petrie, J.M.C. Connell, R. Donnelly. The effects of angiotensin infusion on insulin sensitivity in man: a placebo-controlled study. European Society of Hypertension, Milan, June 1993. *Journal of Hypertension*, 1993, **11**, S459.

A.D. Morris, S. Ueda, J. Petrie, H. Elliott, J.M.C. Connell, R. Donnelly. How reproducible is the euglycaemic hyperinsulinaemic clamp? Medical Research Society, London, November 1993. *Clinical Science*, **86**, 23P.

A.D. Morris, R. Donnelly, J. Petrie, S. Ueda, H. Elliott, M. Small, J.M.C. Connell. Angiotensin II increases insulin sensitivity in non-insulin dependent diabetes: dissociation of metabolic and blood pressure effects. British Diabetic Association, Bournemouth, March 1994, *Diabetic Medicine* **11**, S36.

SUMMARY

A series of studies which have utilised the euglycaemic hyperinsulinaemic clamp to measure insulin sensitivity is described. The methods for measuring insulin sensitivity have been evaluated and conclusions drawn about reproducibility and inherent limitations of the technique. The euglycaemic clamp has been used to examine the effects of angiotensin II and antihypertensive drugs on insulin sensitivity in normal subjects, patients with essential hypertension and patients with impaired glucose tolerance or non-insulin dependent diabetes mellitus (NIDDM). Each individual study was approved by the local Research and Ethical Committee and informed consent was obtained from each subject before participation in a study.

(i) Evaluation of the euglycaemic hyperinsulinaemic clamp technique.

It was recognised that despite widespread use of the euglycaemic clamp in clinical research, key aspects of the technique remained to be validated.

(a) The intra- and inter- subject variability in insulin sensitivity and the time for insulin mediated glucose disposal to reach steady state were evaluated in 18 healthy male subjects and 6 patients with essential hypertension who attended two study days when a euglycaemic hyperinsulinaemic clamp was performed.

Measurements of insulin sensitivity derived from the euglycaemic hyperinsulinaemic clamp were expressed as whole body glucose uptake (M), and the insulin sensitivity index (S_{IP}). M values at 120 mins were significantly less than at 180 mins in both the healthy subjects and in the hypertensive patients indicating a progressive increase in glucose utilisation over the 180 minutes. The intra subject coefficient of variation (c.v.) of M at 120 mins and 180 mins was 8% and 6% for healthy subjects and 14% and 5% for patients with essential hypertension respectively. The inter subject c.v. of M at 120 mins and 180 mins was 22% and 21% for healthy subjects and 43% and 38% for patients with essential hypertension. The intra subject

c.v. of the insulin stimulus was 10% in both groups. The intra subject c.v. of the S_{IP} was 21% for healthy subjects and 16% for patients with essential hypertension.

Thus, it was demonstrated that the clamp is highly reproducible within subjects but the inter subject variability is wide. Clamps of 120 min duration underestimate M by up to 10% and are not as reproducible in patients with essential hypertension. The S_{IP} is poorly reproducible; my data suggest that the M value is a better index for comparison of possible changes in insulin sensitivity.

(b) The need to arterialise venous blood with a heated hand box was evaluated in 6 healthy subjects. Each subject attended 2 study days when a euglycaemic hyperinsulinaemic clamp was performed and "arterialised" (AV; heated hand vein) and mixed venous blood (MV; antecubital fossa) were collected simultaneously for the measurement of plasma glucose and oxygen saturations

It was demonstrated that AV blood was effectively arterialised achieving oxygen saturations of $95\pm 2\%$, and that plasma glucose concentrations were less variable when compared with MV samples.

(c) The haemodynamic and metabolic effects of hand warming were evaluated in 6 healthy male subjects who attended 2 study days when a euglycaemic hyperinsulinaemic clamp was performed. On the first day AV blood was used to adjust the glucose infusion rate required to maintain euglycaemia; on the second study day MV blood was used. The results demonstrate that there was a significant trend for M values to be higher when arterialised blood was used when compared with mixed venous blood. My results suggest that hand warming may induce a blood pressure lowering effect that is associated with a confounding increase in the derived measurement of insulin sensitivity.

(ii) Evaluation of the effects of lacidipine on insulin sensitivity.

The acute and chronic (2 weeks) effects of the dihydropyridine calcium antagonist, lacidipine (4 mg daily), on insulin mediated metabolic responses was evaluated in a double-blind placebo-controlled crossover study in 12 healthy subjects and 6 patients with essential hypertension. Each subject attended 4 study days when insulin sensitivity was evaluated using the euglycaemic hyperinsulinaemic clamp.

In the healthy subjects, reflex tachycardia was observed after lacidipine administration but neither first dose nor 2 weeks of lacidipine treatment altered insulin sensitivity when compared with placebo. A significant decrease in fasting triglyceride concentrations was observed after 2 weeks lacidipine treatment. 95% confidence intervals excluded a 15% change in whole body glucose uptake.

In the hypertensive patients, lacidipine treatment was associated with reflex tachycardia and a trend towards decreased blood pressure but there was no significant effect on whole body insulin sensitivity.

(iii) Evaluation of manipulation of the renin-angiotensin system on insulin mediated metabolic responses

(a) The effects of 4 weeks treatment with the ACE inhibitor trandolapril on insulin sensitivity was evaluated in a double-blind, placebo-controlled, crossover study in 12 "multiple risk factor" patients with impaired glucose tolerance or NIDDM, and essential hypertension. Each subject attended 3 study days (once after a single blind placebo run-in, and after each crossover) when insulin sensitivity was evaluated using the euglycaemic hyperinsulinaemic clamp. The hypothesis under test was that ACE inhibition would increase insulin sensitivity via a vasodilatory mechanism and angiotensin II "sparing" effect.

Despite evidence of 70% ACE inhibition and significant blood pressure lowering effects of trandolapril, there was no evidence that trandolapril altered insulin

sensitivity when compared with placebo. Confidence intervals excluded a 12% increase in insulin sensitivity associated with ACE inhibition.

(b) To test the hypothesis that acute administration of the potent vasoconstrictor angiotensin II would decrease insulin sensitivity, twelve healthy male volunteers completed a double-blind, placebo-controlled crossover to evaluate the effects of a sub-pressor (1ng/kg/min) and a weakly-pressor (5 ng/kg/min) infusion of angiotensin II on insulin mediated metabolic responses. Each subject attended three study mornings when whole body insulin sensitivity measured using the euglycaemic hyperinsulinaemic clamp.

The higher dose of AII produced a significant rise in blood pressure and plasma angiotensin II concentrations were increased to within the pathophysiological range; 11 ± 5 , 27 ± 9 and 125 ± 28 pg/ml after placebo, low and high dose angiotensin II respectively. The corresponding values for insulin sensitivity were 10.5 ± 2.0 , 10.5 ± 2.2 and 10.9 ± 3.4 mg/glucose/kg/min (NS; 95% confidence interval -1.9, 1.1 for placebo vs. pressor angiotensin II). Thus, acute administration of angiotensin II with or without a pressor response and producing plasma angiotensin II concentrations within the pathophysiological range, has no effect on insulin stimulated glucose disposal in healthy subjects.

(c) As there is evidence that the renin-angiotensin system may be involved in the metabolic as well as the cardiovascular features of diabetes, the effects of angiotensin II on insulin-dependent pathways of metabolism were evaluated in patients with NIDDM. In a randomised, double-blind, placebo-controlled, crossover study 11 patients with (diet-controlled) NIDDM attended on 3 study days to evaluate the effects of a 3-hour infusion of subpressor (1ng/kg/min) and pressor (5 ng/kg/min) doses of angiotensin II on whole-body insulin sensitivity using the euglycaemic hyperinsulinaemic clamp.

Plasma angiotensin II levels were 8 ± 4 , 28 ± 9 and 162 ± 45 pg/ml after placebo, low dose and high dose infusions of angiotensin II. The higher dose of angiotensin II was associated with significant increases in blood pressure and plasma

aldosterone. Whole-body insulin sensitivity was 4.3 ± 2.3 mg glucose/kg/min after placebo, and 5.5 ± 2.3 and 4.9 ± 2.4 mg glucose/kg/min following low and high dose angiotensin II respectively ($p < 0.05$, ANOVA). Angiotensin II had no effect on hyperinsulinaemia-induced reductions in serum potassium and triglycerides.

In summary, contrary to general expectation, acute infusion of angiotensin II, with or without an increase in blood pressure, increases insulin sensitivity in normotensive patients with NIDDM.

The dissociation of metabolic and blood pressure effects of angiotensin II suggests that haemodynamic alterations and redistribution of cardiac output might not be the sole (or principal) underlying mechanism in patients with diabetes.

(iv) Determinants of insulin sensitivity

Having validated the euglycaemic hyperinsulinaemic clamp and characterised insulin sensitivity in 75 individual hypertensive, diabetic, diabetic hypertensive patients or healthy subjects, an attempt was made to identify which demographic, metabolic or biochemical factors account for the large inter subject variability in insulin sensitivity observed.

Demographic and morphometric data were collected for each subject and a standard 75 gram oral glucose tolerance test (OGTT) was performed at screening when blood samples were collected for serum insulin and plasma glucose profiles at baseline and at 30 minute intervals until 120 minutes. On at least one occasion, each individual underwent a euglycaemic hyperinsulinaemic clamp was performed using arterialised blood samples to derive the calculation of insulin sensitivity.

The relationship between all variables was investigated at two levels. Firstly, for individual variables by correlation analysis, and secondly for multiple variables by multiple stepwise linear regression analysis. Thus, insulin sensitivity was the dependent variable and the independent variables were modelled separately and in all combinations with each other to find the best fit.

For the group as a whole, age, body mass index, diastolic blood pressure, fasting insulin and fasting glucose concentrations were the best correlates of insulin sensitivity. In the 30 patients with impaired glucose tolerance or NIDDM, the waist hip ratio and body mass index were individually the best predictors of insulin sensitivity and insulin and glucose concentrations during an OGTT poor predictors.

CONCLUSIONS

This work has validated the euglycaemic hyperinsulinaemic clamp as a safe, reproducible method of evaluating insulin-stimulated metabolic responses, but has highlighted some inherent limitations of the technique; for example, the wide inter-subject variability in insulin sensitivity and the potential confounding metabolic and haemodynamic effects of hand-warming. The technique therefore lends itself to studies in which subjects act as their own control. Thus, in a series of double-blind, placebo-controlled crossover studies, treatment with the calcium antagonist lacidipine, and the ACE inhibitor, trandolapril, have been shown to have neutral effects on insulin sensitivity in man. Acute administration of sub-pressor and weakly pressor angiotensin II in healthy man had no effect on whole body glucose uptake; in contrast, in a group of patients with NIDDM, the same experimental protocol was associated with an increase in whole body insulin sensitivity suggesting a dissociation of haemodynamic and metabolic effects. In a regression analysis metabolic and haemodynamic data have been used to explore the most important determinants of insulin sensitivity in man.

CHAPTER 1

GENERAL INTRODUCTION

1.1 INSULIN RESISTANCE: METABOLIC ABNORMALITIES AND CARDIOVASCULAR DISEASE

Altered sensitivity to the biological actions of a hormone is a well recognised phenomenon in clinical medicine; for instance, resistance of peripheral tissues to thyroid hormone or refractoriness of end-organs to gonadotrophins. The pancreatic hormone insulin, through its integrated actions on carbohydrate, protein and lipid metabolism, plays a pivotal role in maintaining glucose homeostasis. Loss of sensitivity to the hormonal effects of insulin, i.e. insulin resistance, affects mainly liver, muscle and adipose tissues and stands out because of its uniquely high prevalence in a variety of physiological and pathological conditions [table 1.1]. In some cases, loss of tissue sensitivity can be viewed as a physiological adaptation to an environmental challenge; for example, the insulin resistance that develops after any stressful insult (trauma, surgery, infection) clearly serves the function of sparing glucose substrate from both oxidation and storage at the expense of fat sources of energy. When the stress subsides, the insulin sensitivity returns to normal. In other cases, insulin sensitivity subserves a general homeostatic regulation of tissue growth and substrate utilisation. For example, the insulin resistance associated with pregnancy or puberty may reflect the need for more insulin to sustain accelerated tissue growth without the associated danger of hypoglycaemia. These physiological examples of insulin resistance can often be explained by increased secretion of hormones with insulin antagonist properties (catecholamines, cortisol, growth hormone, sex steroids). In many cases, however, the biochemical mechanisms and pathophysiological implications of insulin resistance have not been clearly established (Moller and Flier, 1991), and the insulin resistance can not always be explained by an adaptive response. Thus, the insulin resistance found in diabetic or hypertensive individuals does not appear to serve any protective function. Indeed there is growing consensus that the insulin resistance associated with such conditions may be a marker of disease or even

<u>Physiological states</u>	<u>Intrinsic pathological defects in target tissues</u>	<u>Insulin resistance secondary to another endocrine/metabolic disorder</u>	<u>Insulin resistance associated with other diseases</u>
Puberty	NIDDM*	Ketoacidosis	Stress e.g. fever/sepsis
Old age*	Obesity*	Acromegaly*	Uraemia
Pregnancy*	Rare genetic disorders	Thyrototoxicosis	Cirrhosis
Fasting/starvation	e.g. Type A syndrome	Oestrogen/steroid therapy*	Hypertension*
Physical inactivity*	Leprechaunism		Cardiac failure
			Atherosclerosis

Table 1.1. Physiological and pathological conditions associated with insulin resistance and its metabolic sequelae.
* = conditions also associated with a rise in blood pressure.

play a pathophysiological role in the development of the disease.

A reduction in insulin-mediated glucose disposal leads to a compensatory hypersecretion of insulin; fasting and post-prandial plasma insulin and triglyceride concentrations increase, usually with a reduction in high-density-lipoprotein (HDL) cholesterol. If the pancreatic response is insufficient or defective, insulin resistance may precipitate glucose intolerance. Thus, in non-insulin dependent diabetes mellitus (NIDDM), the combination of reduced insulin action *and* impaired insulin secretion gives rise to hyperglycaemia (DeFronzo et al, 1992). By comparison, many obese, non-diabetic subjects are more insulin resistant but avoid overt diabetes - at least in the short term - by virtue of an adequate β -cell reserve.

Thus, considerable interest has recently focussed on putative pathophysiological roles of insulin resistance in the aetiology of various endocrine and cardiovascular disorders, including NIDDM (Reaven, 1988a), ovarian hyperandrogenism (Barbieri et al, 1988), hypertension (Daly and Landsberg, 1991) and in the genesis of atheroma (Stout, 1989). While it is fairly clear that insulin resistance is of primary importance in the development of NIDDM (DeFronzo et al, 1992), and some rare hereditary syndromes (Moller & Flier, 1991), the pathogenetic significance of insulin resistance (short of diabetes) and individual disorders [table 1.1] remains in doubt. What seems likely, however, is that insulin resistance contributes to some of the clinical, biochemical and metabolic features that often complicate these conditions; for example, glucose intolerance in pregnancy and hypertriglyceridaemia in obesity and diabetes (Reaven, 1988a).

This chapter explores three areas pertinent to the association of insulin resistance and essential hypertension. Firstly, the epidemiological and experimental data suggesting a putative role of insulin resistance and consequent hyperinsulinaemia in the genesis of hypertensive and atherosclerotic cardiovascular disease is reviewed.

Secondly, a description and critique of the presently available techniques for measuring insulin sensitivity *in vivo* will be discussed. To enable the insulin sensitivity of individuals or populations to be defined, and to allow the evaluation of any strategy which may ameliorate insulin resistance, it is essential that investigators have a very precise and reproducible technique to quantify the body's response to insulin.

Thirdly, the studies evaluating differential drug effects on insulin sensitivity and new pharmacological approaches to metabolism will be reviewed. One explanation for the association between insulin resistance and essential hypertension is that some of the commonly used drugs in hypertensive patients (thiazides and beta-blockers) may worsen insulin sensitivity.

1.1.1. A CLINICAL TRIAD: OBESITY, NON-INSULIN DEPENDENT DIABETES MELLITUS AND ESSENTIAL HYPERTENSION.

Simple clinical observation suggests that NIDDM, obesity and essential hypertension often occur together in the same patients. The extent to which they are associated has been clarified by several large epidemiological studies; for example, analysis of data from the 2930 individuals in the San Antonio Heart Study (Mitchell et al, 1990) suggests that, by the fifth decade, 85% of diabetics are hypertensive and obese and 80% of obese subjects have abnormal glucose tolerance and are hypertensive. Conversely, of the 287 patients (9.8%) who were hypertensive, three quarters were obese and half had either impaired glucose tolerance or NIDDM (Ferrannini et al, 1990) [figure 1.1]. The Framingham Heart Study confirmed that diabetic persons have an increased prevalence of hypertension, with as many as 50% having some degree of elevated blood pressure, and that the incidence of both diabetes and hypertension increases progressively with the degree of obesity (Kannel and McGhee, 1979). A high prevalence of hypertension was also noted in the United Kingdom Hypertension and Diabetes Study, with 46% of female patients and 35% of

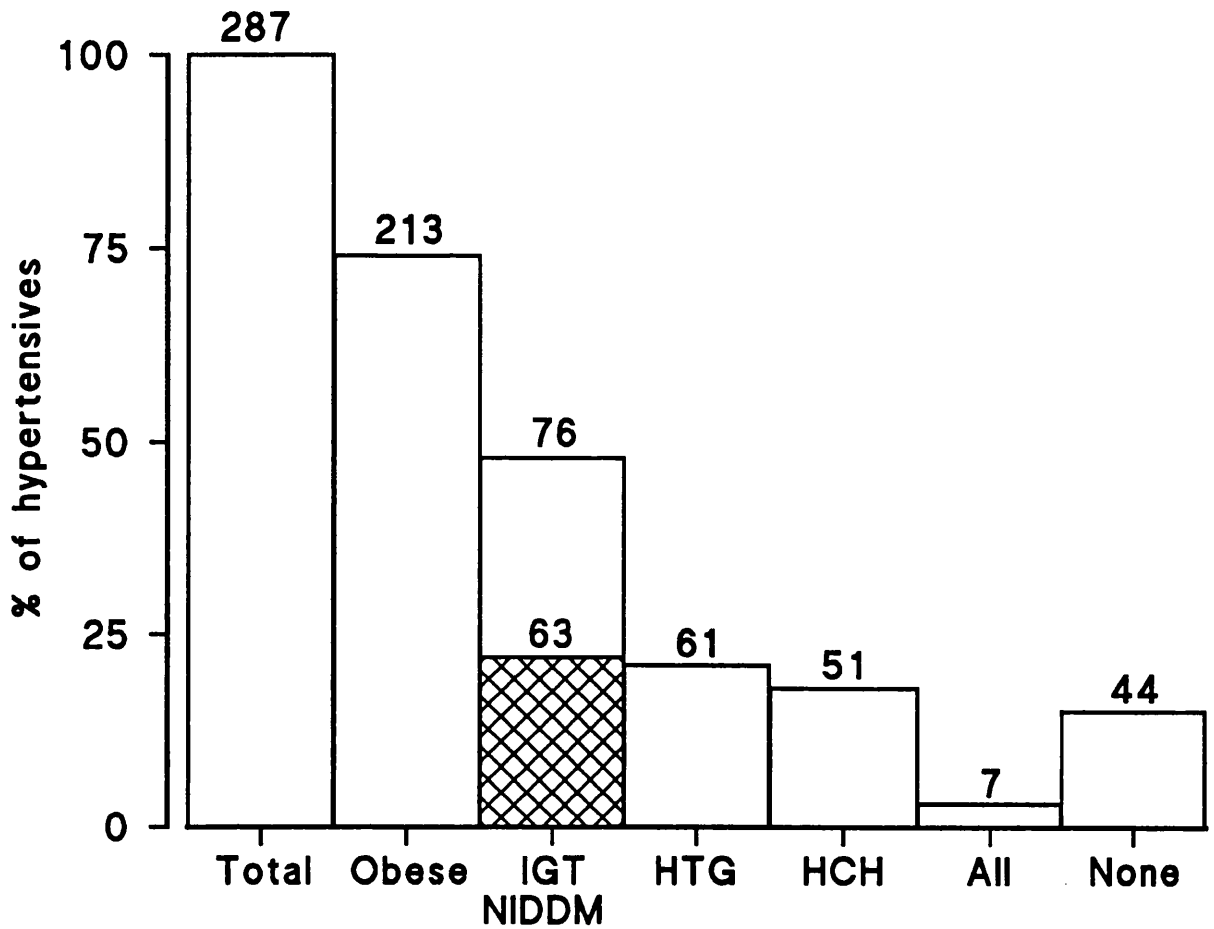


Figure 1.1. Patients in the San Antonio Heart Study with associated metabolic disorders. Of 2930 subjects in this study, 287 hypertensive patients were screened. Definitions are as follows: Obese = BMI > 27kg/m², IGT = impaired glucose tolerance, NIDDM = non insulin dependent diabetes mellitus (hatched area), HTG = hypertriglyceridaemia (>2.9 mmol/L), HCH = hypercholesterolaemia (> 6.48 mmol/L). Modified from Ferrannini et al, 1990.

males being affected (Hypertension in Diabetes Study Group, 1993). Finally, a study which screened over one million individuals (Stamler et al, 1978), estimated that hypertension was twice as prevalent in young overweight subjects and 50% more common in older obese individuals than in normal weight controls. Thus a wealth of epidemiological data confirm a striking degree of overlap between essential hypertension, obesity and NIDDM. Although the incidence of all three conditions increases with age, it has been established that the association between essential hypertension and NIDDM is independent of age, obesity and renal function (Teuscher et al, 1989; Kannel et al, 1991). When the two conditions coexist, hypertension predates the diagnosis of diabetes eight times more commonly than the reverse sequence (Lundgren et al, 1988).

Such clinical observations have prompted investigators to explore the pathophysiological mechanisms underlying these relationships. A primary abnormality in NIDDM is insulin resistance, and this is also a characteristic feature of obesity: over the last two decades substantial data has emerged indicating that resistance to the glucoregulatory effects of insulin with consequent hyperinsulinaemia are also features of essential hypertension. These metabolic abnormalities have therefore resulted in new perspectives in the investigation and treatment of hypertension.

1.1.2. INSULIN ACTION, HYPERINSULINAEMIA AND ESSENTIAL HYPERTENSION

The two major target tissues in insulin action are muscle and liver. Although the insulin-receptor interaction elicits a complex array of biochemical responses, by convention "insulin resistance" refers only to the effects of insulin on hepatic glucose production and peripheral glucose disposal (both oxidative and non-oxidative). Elevated basal free fatty acid levels and failure of insulin to suppress free fatty acid production reflect insulin resistance to lipid metabolism (Chen et al, 1987), which is

often secondary to impaired glucose oxidation. Thus, pathways of glucose, lipid and amino acid metabolism are closely inter-linked; resistance to glucose usually implies resistance to lipid and amino acid metabolism as well - but other effects of insulin, for instance on salt and water balance, may be spared in classic insulin resistant states. For example, it is still unclear whether insulin resistance extends to potassium disposal.

The concept of insulin resistance is not new; in 1936, Himsworth proposed that patients with diabetes mellitus were either "insulin-sensitive when the cause of the disease is deficiency of insulin", or "insulin insensitive when the cause of the disease is not lack of insulin, but the restriction, to a greater or lesser degree, of an unknown sensitising factor" (Himsworth, 1936). Thus, loss of tissue sensitivity to the actions of insulin leads to a compensatory hyperinsulinaemia which is a characteristic feature of both NIDDM and obesity.

Evidence that essential hypertension is associated with metabolic abnormalities first emerged in 1966 when Welborn and colleagues reported higher plasma insulin concentrations in hypertensive compared with normotensive subjects (Welborn et al, 1966). This initial evidence, based on only 19 patients, many of whom were on treatment, went largely unnoticed, but has more recently been substantiated by data from several large scale studies in which oral glucose tolerance tests have been performed in patients with essential hypertension. Thus, in a study of over 2000 individuals, Modan reported higher post-prandial insulin levels in both treated and untreated hypertensive patients than normotensive controls (Modan et al, 1985), a relationship which was independent of both glucose tolerance and obesity. A similar study by Asch in 1437 North Americans reported significantly higher post load insulin concentrations in hypertensive individuals, but the significance was lost when stratified for glucose intolerance and obesity (Asch et al, 1991). Berglund reported elevated fasting insulin concentrations and significantly higher blood glucose concentrations 60 minutes after a 100 g oral glucose tolerance test in 106 untreated

hypertensive patients as compared to 41 controls (Berglund et al, 1976). Parillo similarly reported higher serum glucose levels and serum insulin levels 60 to 180 minutes after a glucose tolerance test in hypertensives as compared to controls (Parillo et al, 1988). In a more physiological study, Singer reported that subjects with essential hypertension showed a higher insulin response after feeding despite similar changes in glucose levels when compared with normal subjects (Singer et al, 1985).

It is important to note, however, that not all hypertensive patients are hyperinsulinaemic and that many patients with hyperinsulinaemia and insulin resistance do not have hypertension. Ethnic differences may explain these discrepancies; for example, the Pima Indians in the United States, in whom insulin resistance and hyperinsulinaemia are common, do not have a high prevalence of hypertension (Saad et al, 1991). Mbanya reported similar insulin levels in hypertensive and normotensive subjects, and although obese subjects were hyperinsulinaemic, there was no significant correlation between hypertension and hyperinsulinaemia; in fact there was a tendency for a negative correlation between plasma insulin concentrations and blood pressure (Mbanya et al, 1988). Moreover, in a recently reported study from Mauritius, a positive relationship between insulin and blood pressure was wholly accounted for by obesity and age, and showed marked inter-ethnic variation (Dowse et al, 1993). Thus, there appear to be racial differences in the association of hyperinsulinaemia and blood pressure.

Another explanation for these discrepant results may be problems with conventional one-site radioimmunoassays for insulin used in these studies. Insulin is normally produced by processing of proinsulin in the β -cell yielding C-peptide and two, 2-amino acid fragments (figure 1.2). Conventional radioimmunoassays for insulin lack specificity for the hormone, and different kits show significant but variable cross-reactivity with proinsulin and its split products: insulin levels may therefore be variably overestimated (Heding, 1977). For example, Temple and colleagues, using two-site sensitive and specific immunoradiometric assays for insulin, intact proinsulin

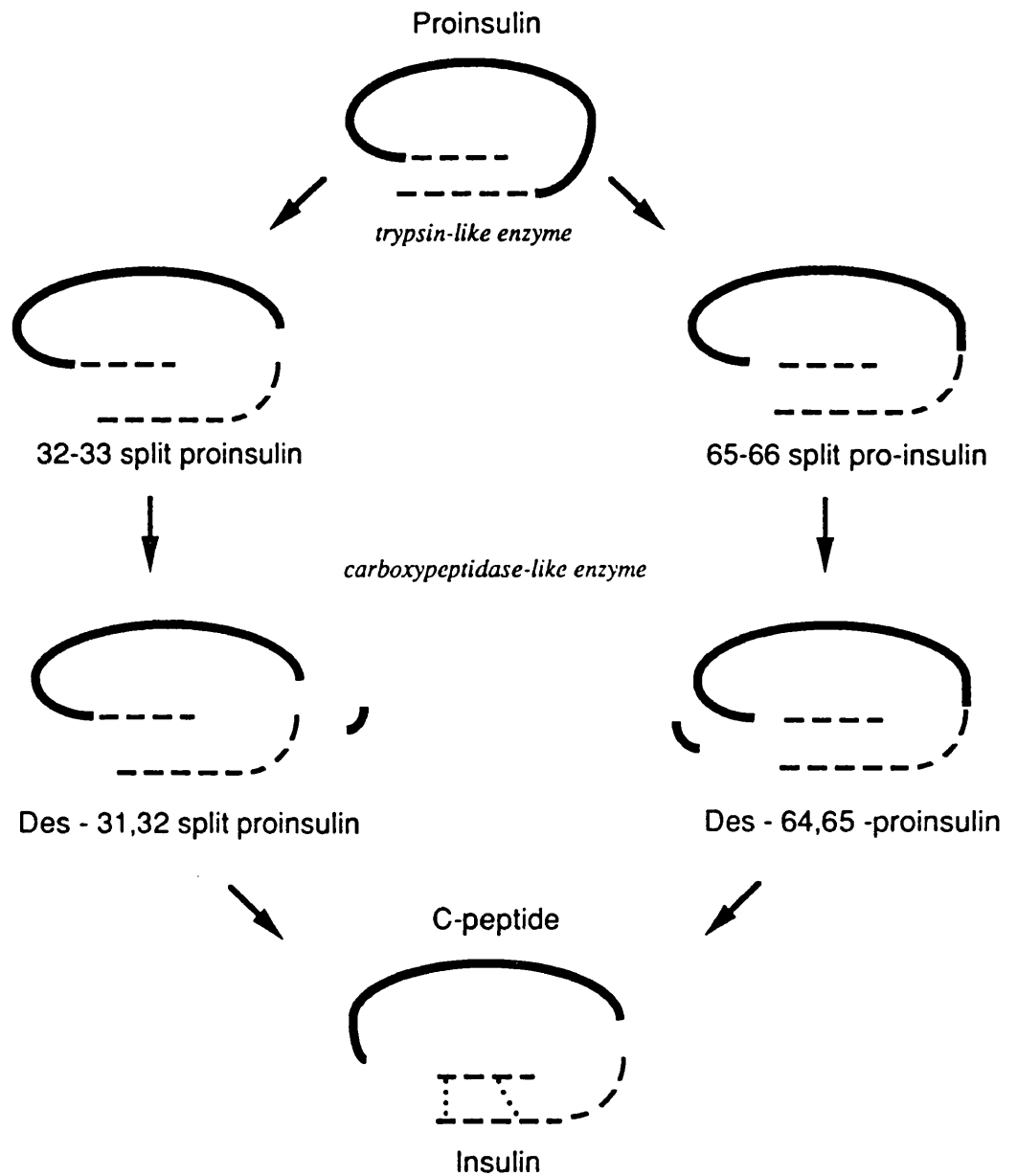


Figure 1.2: The insulin processing cascade. Pro-insulin is split in the beta-cell to yield insulin, C-peptide and two amino acid fragments.

and 32,33 split proinsulin, have shown that newly-diagnosed patients with NIDDM are hypoinsulinaemic and hyperproinsulinaemic (Temple et al, 1989; Temple et al, 1990). However, Reaven and Polonsky, using a specific proinsulin enzyme-linked immunosorbant assay and by subtracting from total immunoreactive insulin levels to give an estimate of "true" insulin levels, showed that the overestimation of insulin levels was modest in a similar group of patients (Reaven et al, 1993). Preliminary data (in abstract form) using a specific insulin assay to measure fasting insulin concentrations in a large Caucasian population suggest that, after adjustment for confounding factors (including weight and age), specific insulin levels but not those measured by a conventional radioimmunoassay are, indeed, related to blood pressure (Grootenhuys et al, 1993).

The variable association of insulin levels and blood pressure, the variable overestimation of insulin levels by conventional assays, and the lack of an obvious pressor effect of insulin, raise the possibility that proinsulin (and its split products) may have independent vascular and haemodynamic effects. Proinsulin has only 7% of the activity of insulin in stimulating glucose disposal in humans, but split-products have higher biological activities and are thought to bind to insulin receptors. Proinsulin has a longer elimination half-life than insulin and is converted to split-products, but not insulin, in the circulation (Galloway et al, 1992). Indirect evidence implicating proinsulin as an aetiological factor in human vascular disease has come from a clinical trial in which proinsulin was compared to insulin in the treatment of insulin-dependent diabetes mellitus (Spradlin et al, 1990). The trial was abandoned on safety grounds owing to an increase of myocardial infarctions in the proinsulin treated group, and as a result, proinsulin now has very limited availability for human study. Data on the relationships among proinsulin, insulin and insulin resistance in patients with essential hypertension and appropriately matched controls are still awaited.

Conflicting evidence from epidemiological studies may also have arisen

because they have used glucose tolerance test data which give only indirect information about insulin sensitivity, as the resultant insulin and glucose values are the product of secretion, distribution and elimination, and may be affected by various pathophysiological processes [see section 1.2.1]. The euglycaemic hyperinsulinaemic clamp technique, the minimal model technique of Bergman, and the insulin suppression test [see sections 1.2.2. to 1.2.5.] are more direct methods of assessing insulin dependent metabolic pathways. Using these techniques, steady state metabolic studies have been performed on hypertensive patients and it is now clear that the elevated insulin levels are a compensatory response to a peripheral defect in insulin-stimulated glucose uptake (Ferrannini et al, 1987; Pollare et al, 1990) [figure 1.3], that this defect persists even after blood pressure has been lowered with antihypertensive medication (Swislocki et al, 1989), and that the coexistence of either obesity or diabetes results in a further impairment in insulin-stimulated glucose disposal.

The metabolic abnormalities of essential hypertension are not restricted to carbohydrate metabolism. An abnormal lipoprotein profile associated with essential hypertension has been identified, namely an increase in triglycerides and a reduction in HDL-cholesterol (Shieh et al, 1987; MacMahon et al, 1985). Reaven has coined the term "syndrome X" to describe the combination of essential hypertension, dyslipidaemia, hyperinsulinaemia and insulin resistance (Reaven, 1988a). There are also data suggesting that elevated levels of fibrinogen (Kannel et al, 1987) and plasminogen activator inhibitor (PAI-1) (Landkin et al, 1990) are also features of the hypertensive state.

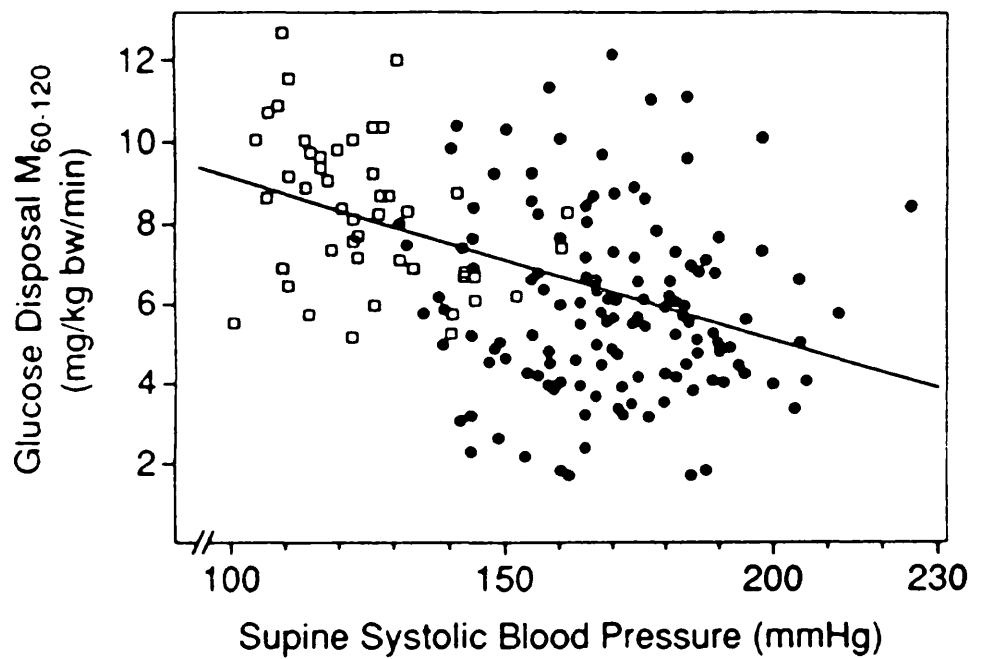


Figure 1.3.: Relationship between tissue sensitivity to insulin (glucose disposal rate as measured by glucose clamp technique) and systolic blood pressure in 143 normal-weight patients with essential hypertension (●) and 54 normotensive control patients (□). $p < 0.001$; $r = -0.42$. Modified from Pollare et al, 1989.

1.1.3. HYPERINSULINAEMIA IN ESSENTIAL HYPERTENSION: CAUSE OR CONSEQUENCE OF HIGH BLOOD PRESSURE, OR EPIPHENOMENON

It remains unclear whether the defect in glucose uptake and hyperinsulinaemia described in hypertensives is an epiphenomenon, a consequence of the hypertensive process itself, or whether it may precede and thus possibly promote the development of hypertension.

(i) Insulin sensitivity in primary and secondary hypertension.

Marigliano studied 36 patients with essential hypertension, 12 with renovascular hypertension, and 69 normotensive controls. He concluded that the essential hypertensives had significantly higher fasting insulin concentrations than those patients with renovascular disease or the controls (Marigliano et al, 1990). Shamiss evaluated insulin sensitivity in 5 patients with essential hypertension, 5 patients with renovascular hypertension, 5 patients with primary hyperaldosteronism and normotensive controls using the euglycaemic hyperinsulinaemic clamp (Shamiss et al, 1992). He concluded that the essential hypertensives had significantly lower insulin sensitivity than patients with secondary forms of hypertension. These data suggest, therefore, that insulin resistance is only found with essential hypertension and not in secondary forms of high blood pressure.

(ii) Longitudinal relationship between insulin resistance and essential hypertension.

The association between insulin resistance and essential hypertension appears to be long term, and to predate the development of clinically important high blood pressure. For example, further analysis of data from the San Antonio Heart Study suggests that changes in glucose tolerance and insulin concentrations coexist with

subclinical elevations in blood pressure in healthy subjects, eight years before the onset of essential hypertension (Haffner et al, 1992). Similarly, Zavaroni and colleagues subdivided workers in a factory into those who had relative hyperinsulinaemia and those with normal insulin concentrations (Zavaroni et al, 1992). The hyperinsulinaemic subjects had higher mean blood pressures without being hypertensive and also had increased levels of triglyceride and decreased HDL cholesterol concentrations than those with normal insulin levels. More recently, Ferrari and colleagues prospectively investigated insulin sensitivity, plasma insulin and glucose concentrations in 70 normotensive children of essential hypertensives as compared with 78 age and weight matched controls of normotensive families (Ferrari et al, 1991a). They concluded that the young normotensives with one hypertensive parent were apparently in excellent health, but were hyperinsulinaemic and had significantly reduced insulin mediated glucose disposal as compared with controls. In a similar study, Beatty and colleagues (1993) matched 15 normotensive subjects who had at least one hypertensive parent with 15 controls. Despite having similar blood pressures, the insulin mediated glucose disposal of the offspring with hypertensive parents as measured by the euglycaemic hyperinsulinaemic clamp was significantly lower than controls. The Bogalusa Heart Study has also shown that children demonstrate a relationship between blood pressure and metabolic changes at a relatively young age (Jiang et al, 1993). Finally in a Finnish study, Salomaa and colleagues reported that subjects with impaired glucose tolerance in 1968 had an increased risk of becoming hypertensive nearly 20 years later when compared with normoglycaemic subjects (Salomaa et al, 1991). Thus, the available clinical evidence suggests that a familial trait for essential hypertension would appear to co-exist with defects in carbohydrate metabolism, and that the metabolic abnormalities and hyperinsulinaemia may precede the development of hypertension. These metabolic abnormalities would not appear to be present in secondary forms of hypertension.

(iii) Animal Evidence.

Insulin resistance and hypertension are associated in some rodent models of high blood pressure. Reaven has documented the presence of *in-vivo* dyslipidaemia and hyperinsulinaemia, and reduced *in-vitro* insulin-mediated glucose uptake by adipocytes in two animal models of genetic hypertension; the spontaneously hypertensive rat (SHR) (Reaven et al, 1989a), and the Dahl salt-sensitive rat (Reaven et al, 1991a). In the SHR the development of insulin resistance precedes the development of the hypertension (Reaven & Chang, 1991b). In addition, the normal Sprague-Dawley rat, an animal with no genetic predisposition to hypertension, develops insulin resistance and hypertension when fed a fructose-enriched diet (Hwang et al, 1987). Reaven's group have also demonstrated in this model that the hypertension is attenuated by correcting the insulin resistance by physical training or by the administration of somatostatin (Reaven et al, 1989b). In contrast, rats made hypertensive by renal artery clipping do not become insulin resistant or hyperinsulinaemic (Buchanan et al, 1991). In an elegant series of experiments, Burstyn studied the *in-vivo* uptake of a glucose tracer in three animal models of hypertension; the SHR, the DOCA-salt animal and the 2-kidney, 1-clip model of renovascular hypertension and their controls (Burstyn et al, 1992). He concluded that the SHR's were hyperinsulinaemic and had reduced clearance of the glucose tracer compared with controls. In the two non-genetic models of hypertension, however, there were no differences between hypertensives and controls.

In another species, insulin resistance has been evaluated in a model of obesity hypertension developed by Rocchini. By feeding dogs cooked beef fat, he demonstrated that weight gain was directly associated with an increase in blood pressure and insulin levels (Rocchini et al, 1989).

In summary, it appears that insulin resistance and hyperinsulinaemia are

associated principally with essential hypertension in humans and equivalent animal models, and that the metabolic abnormalities predate the development of hypertension. This raises the possibility that hyperinsulinaemia contributes to the development of high blood pressure, and also suggests that an underlying genetic abnormality may predispose to both pathophysiological disorders. In this context it is of interest that increased red blood cell sodium/lithium counter transport is also associated with insulin resistance and other metabolic abnormalities in hypertension, such as triglyceride and uric acid levels (Hunt et al, 1991; Mattiasson et al, 1992; Canessa et al, 1980). This ion transport activity is a good marker of genetically determined essential hypertension, and reinforces the notion that inherited factors may determine insulin sensitivity in these subjects. However, other evidence suggests that environmental influences in very early development may also play a role. For example, Barker and colleagues have shown that low birth weight accurately predicts long term development of high blood pressure, coronary heart disease, NIDDM and a number of adverse metabolic factors including increased fibrinogen levels and insulin resistance in later life (Barker et al, 1993a). It has been proposed that foetal under-nutrition might be a common predisposing factor to these abnormalities, and the same group have proposed that abnormal pancreatic development might be a possible contributory cause to the abnormal insulin response (Barker et al, 1993b).

1.1.4. MECHANISMS AND CONSEQUENCES OF INSULIN RESISTANCE IN HUMAN DISEASE

Classically the term insulin resistance refers to a reduction in insulin stimulated glucose uptake [section 1.1.2]; the implication of this definition is that pathophysiological characteristics of insulin resistant states may differ if a selective resistance to insulin occurs. The high insulin levels needed to compensate for the defect in carbohydrate metabolism may cause increased effects in other pathways. Indeed, experimental evidence suggests that the insulin resistance of obesity, aging

and NIDDM show differential effects on other insulin mediated metabolic processes; for example, obesity is also associated with a reduction in glucose oxidation and potassium uptake (Bonadonna et al, 1990). In contrast, the insulin resistance of essential hypertension appears to be selective for glucose uptake. Using clamp studies, Ferrannini has suggested a "tissue specific" (predominantly located in skeletal muscle), and "pathway-specific" (impairment of insulin-stimulated glycogen synthesis, but unaffected lipid, protein and electrolyte metabolism) form of insulin resistance (Ferrannini et al, 1987), although these specific observations have not been independently confirmed. A number of possible mechanisms which may account for the insulin resistance observed in essential hypertension have been proposed.

(i) Altered regional blood flow.

Julius has argued that reduced delivery of glucose to skeletal muscle as a consequence of altered regional haemodynamics contributes to the insulin resistance of essential hypertension (Julius et al, 1991). Data which report an increase in insulin sensitivity after administration of antihypertensive drugs with a predominant vasodilatory action may support this hypothesis (Pollare et al, 1989a) [see sections 1.3.2 and 5.1.1) as may recent studies with angiotensin II (Buchanan et al, 1993; Townsend & DiPette 1993) [see chapter 6].

Altered vascular effects of insulin may also contribute to the suggested change in regional blood flow, and consequent alteration in delivery of glucose to target tissues in essential hypertension. Insulin has been shown to have vasodilator properties (at least in pharmacological doses) by some (Creager et al, 1985) but not all (Natali et al, 1991) workers. It opposes the direct vasoconstrictor actions of noradrenaline and angiotensin II *in vitro* (Yagi et al, 1988). Findings in humans are, however, conflicting: although Sakai and colleagues found that intra-arterial infusion of insulin attenuated the decrease in forearm blood flow caused by phenylephrine and

angiotensin II (Sakai et al, 1993), Naehring reported no effect in studies using a similar protocol with noradrenaline (Nearhing et al, 1993), while Lembo and colleagues have shown that insulin attenuates the vasoconstriction caused by reflex forearm sympathetic activation (Lembo et al, 1993). Insulin-mediated vasodilatation is decreased during euglycaemic hyperinsulinaemia in patients with either obesity or NIDDM, causing post-prandial blood flow to skeletal muscle to decrease and a corresponding reduction in net tissue glucose uptake (Baron et al, 1990; Baron et al, 1991a). In contrast, skeletal muscle blood flow during hyperinsulinaemia in hypertensive subjects has been reported to be no different from that in control subjects (Capaldo et al, 1991); however, data are conflicting as another study found that attenuation of insulin-stimulated blood flow was correlated with insulin resistance and blood pressure in healthy subjects (Baron et al, 1993). Most data which have addressed this point have been accrued from normal or obese subjects and further studies in patients with essential hypertension are required.

(ii) Altered tissue cellular characteristics.

Differences in glucose transport between skeletal muscle fibre types may be an important determinant of *in vivo* insulin action (James et al, 1986). For example, Lillioja and colleagues (1987) found that obesity was associated with capillary rarefaction and that capillary density was positively correlated with insulin mediated glucose disposal in a population of lean and obese non-diabetic men. In hypertensive patients it has also been suggested that there is a quantitative difference in the composition of muscle fibres as compared to controls; there are less type I (red) oxidative highly vascularised fibres which are more sensitive to the actions of insulin, than type IIb non-oxidative fibres which are less sensitive (Johlin-Dannfelt et al, 1979). Therefore both capillary density and insulin sensitivity may be determined by fibre composition. In addition, muscle fibre composition could determine insulin

sensitivity as a function of GLUT 4 transporter expression. This is because, in rats, muscles containing greater numbers of type I fibres express more GLUT 4 glucose transporters and display a greater capacity for insulin-mediated glucose uptake (Richardson et al, 1991; Hardin et al, 1993).

(iii) Cell signalling, glucose uptake and metabolic disposal of substrate.

The defect in glucose uptake in hypertensive animals appears to be unrelated to insulin receptor number, binding or tyrosine kinase activity (Reaven et al, 1989a). The major mechanism by which insulin activates glucose transport is via a rapid and dose-dependent recruitment of glucose transporter proteins from a large intracellular pool. In search of a post-receptor defect in insulin action, attention has focussed on this family of facilitative glucose transporters in the plasma membrane, particularly GLUT-4 which is primarily located in fat and skeletal muscle. For example, in a recent comprehensive study which examined the insulin sensitivity in athletes, Ebeling and colleagues (1993) concluded that the cellular mechanisms that explained the enhanced insulin sensitivity observed were an increase in GLUT-4 content and an increase in glycogen synthase activity. There is some evidence, using immuno-blotting and measurement of the corresponding mRNA, that reduced levels of GLUT 4 occur in skeletal and adipose tissue of patients with NIDDM and/or obesity (Dohm et al, 1991). However, in another study using human skeletal muscle biopsies, no difference could be found in the plasma membrane content of immunoreactive GLUT 4 between patients with NIDDM and normal subjects (Lund et al, 1993). Direct observations of glucose transporters or insulin receptors have not been reported in essential hypertension.

The studies of Ferrannini suggest that the abnormality in insulin-stimulated glucose uptake is restricted to non-oxidative pathways of glucose metabolism, suggest a defect in glycogen synthesis (Ferrannini et al, 1987). There is evidence in man that

alterations in glycogen synthase activity directly account for changes in insulin dependent glucose incorporation into glycogen (Bogardus et al, 1987). There are no studies which have measured glycogen synthase activity specifically in essential hypertension.

The role of glucocorticoids in the genesis of insulin resistance in essential hypertension may also be worth considering. Action of hormones at the type II corticosteroid receptor has a hyperglycaemic effect which may precipitate diabetes. A genetic variation linked with this (glucocorticoid) receptor locus has been reported to be associated with the insulin resistance of obesity (Weaver et al, 1992), and it is of interest that the same polymorphism, in a different study, was found to be increased in frequency in subjects with a high likelihood of developing hypertension (Watt et al, 1992). More recently, it has been shown that administration of pharmacological doses of glucocorticoid to pregnant rats results in development of hypertension in their offspring (Benediktsson et al, 1993): this has been ascribed to an imprinting effect of the steroid, and preliminary evidence suggests that this rise in blood pressure is associated with hyperinsulinaemia. Thus, there is some evidence that altered steroid hormone action, either *in utero* or in adult life, can lead to insulin resistance and hypertension.

(iv) Amylin.

Amylin is a recently described pancreatic peptide which is co-secreted with insulin (Cooper et al, 1988). Preliminary evidence suggests that amylin opposes a number of the cellular actions of insulin, and it has been suggested that increased amylin levels may contribute to the increased insulin resistance seen in subjects with NIDDM (Amiel, 1993). However, there is no information on the levels of amylin or the effects of this peptide in hypertensive patients or in animal models of hypertension and there remains doubt about the exact physiological role of the peptide.

In summary, the primary mechanisms underlying the reduced insulin sensitivity in essential hypertension remain unclear. Although there are a number of attractive hypotheses which may account for the abnormality, further careful physiological studies in hypertensive patients are required.

1.1.5. CLINICAL IMPLICATIONS OF HYPERINSULINAEMIA AND INSULIN RESISTANCE IN HYPERTENSION

No satisfactory single hypothesis has been proposed for the mechanism by which insulin resistance and the compensatory high plasma insulin levels (or other β -cell products) might produce a rise in blood pressure. Any plausible explanation needs to take into account the lack of hypertension in patients with insulin/proinsulin secreting tumours (Fujita et al, 1992) and also in patients with the polycystic ovarian syndrome (Zimmermann et al, 1992): this latter group of patients have profound insulin resistance with proportionate increases in insulin and proinsulin levels (Conway et al, 1993). A number of plausible biological explanations have been proposed by which insulin might produce a rise in blood pressure.

(i) Insulin induced antinatriuresis.

An increase in plasma insulin, within, or slightly above the physiological range markedly reduces fractional sodium excretion (DeFronzo 1981; Baum 1987), and during euglycaemic hyperinsulinaemia urinary sodium excretion may fall by as much as 50% (DeFronzo et al, 1975). The exact mechanisms for this antinatriuresis are unknown, but they appear to be related in part to a direct effect on renal tubular reabsorption (Gupta et al, 1992). In theory, if hyperinsulinaemia causes a sustained antinatriuretic effect, chronic hypertension could result from an expansion of the

extracellular fluid volume. Preliminary evidence suggests however that insulin's direct antinatriuretic actions are insufficient alone to elevate blood pressure (Hall et al, 1990). Brands has studied the effects of chronic hyperinsulinaemia on conscious dogs using a glucose clamp technique. He reported an antinatriuretic effect most pronounced in the acute stage which was due to increased renal tubular sodium reabsorption. Despite this transient sodium retention, mean arterial pressure actually decreased during the 7 days of insulin infusion (Brands et al, 1991). Furthermore, young patients with essential hypertension do not demonstrate an increase in body sodium content, nor do they show a reduction in plasma renin concentration which might be anticipated if there was expansion of extracellular fluid volume (Beretta-Piccoli et al, 1982).

(ii) Activation of the sympathetic nervous system.

The second mechanism by which hyperinsulinaemia may elevate blood pressure is via activation of the sympathetic nervous system. Acute insulin infusion elevates plasma noradrenaline levels (Rowe et al, 1981), but the interpretation of these studies is difficult because pharmacological doses of insulin have been used. Anderson recently used physiological insulin concentrations in normal volunteers and reported increased sympathetic activity with decreased forearm vascular resistance but no increase in blood pressure (Anderson et al, 1991). Furthermore, in normal subjects insulin infusion into the forearm opposes the vasoconstriction caused by sympathetic activation (Sakai et al, 1993).

(iii) Altered vascular smooth muscle structure and function.

Insulin can be shown *in vitro* to increase vascular smooth muscle cell growth, acting via IGF-1 receptors (Banskota et al, 1989). If this occurs *in vivo*, long term

hyperinsulinaemia might promote vascular hypertrophy, which will act as an amplification mechanism for the maintenance and development of high blood pressure. However, the concentrations of insulin which are required to show this effect on vascular cells *in vitro* are much higher than those found under physiological or pathophysiological conditions.

The relationship between insulin action and intracellular cation concentrations is also complex. Insulin causes relaxation of pre-constricted cultured vascular smooth muscle cells, and this appears to relate to an enhancement of vascular cell recovery from intracellular calcium loads (Zemel et al, 1992). However, Resnick has proposed that insulin resistance is a primary consequence of altered intracellular calcium/plasma membrane calcium handling (Resnick, 1993), and a recent study by Ohno showed that *in vivo* insulin sensitivity was negatively associated with intracellular platelet calcium concentrations in subjects with essential hypertension (Ohno et al, 1993).

The vasodilator action of insulin has been discussed above [section 1.1.4], and may relate to the effects of insulin on intracellular calcium handling. Limitation of this action of insulin (i.e. to reduce intracellular calcium concentrations) in subjects with hyperinsulinaemia or insulin resistance, might account for the reported loss of insulin-related vasodilatation in this circumstance, and provide an explanation for the apparently contradictory finding of Ohno of increased levels of intracellular calcium in patients with hyperinsulinaemia.

(iv) Interactions with endothelial dependent relaxation and contraction.

The effects of insulin and proinsulin on endothelial function remain unclear. There is evidence that subjects with essential hypertension have reduced vascular endothelial dependent relaxation (Panza et al, 1990). While there are reports of impaired endothelial function in insulin resistant states including NIDDM (McVeigh et al, 1992) and hypercholesterolaemia (Creager et al, 1990), no direct relationship

with either insulin levels or insulin sensitivity has been shown. Indeed, insulin has vasodilator properties in some vascular beds [section 1.1.4] and there is evidence from the San Antonio cohort that individuals who went on to become diabetic (and hence insulin resistant) had a hyperdynamic circulation (Stern et al, 1992). In patients with insulin dependent diabetes mellitus (IDDM) who are without vascular complications, endothelial dependent vasorelaxation appears normal (Calver et al, 1992). However, in patients with microalbuminuria a defect in endothelial dependent relaxing factor (EDRF) activity, which would be consistent with abnormal generation of nitric oxide, is reported (Elliott et al, 1993). As other studies show that microalbuminuria is closely associated with insulin resistance (Yip et al, 1993), the role of hyperinsulinaemia and insulin resistance in this abnormality merit further study.

Insulin is also reported to increase the expression of receptors for endothelin *in vitro* (Oliver et al, 1991), and levels of this vasoconstrictor peptide rise during acute hyperinsulinaemia in man (Wolpert et al, 1993). Again, there are no data on the effects of hyperinsulinaemia on endothelin-mediated vasoconstriction in human vascular tissue.

In summary, there are several plausible mechanisms by which hyperinsulinaemia and insulin resistance may influence blood pressure control; however, much of the evidence is circumstantial and direct studies in patients with essential hypertension are required.

1.1.6. LONG TERM CONSEQUENCES OF INSULIN RESISTANCE AND HYPERINSULINAEMIA

The chronic vascular complications of hypertension result from a complex interaction among the haemodynamic changes of high blood pressure with a variety of other metabolic abnormalities. It has been proposed that hyperinsulinaemia is one of

the factors associated with hypertension which promotes development of atherosclerosis; there are both experimental and clinical data that support the notion that insulin *per se* is atherogenic, independent of glucose (Stout 1989).

(i) Experimental studies.

The atherosclerotic plaque is characterised by excessive amounts of lipid, collagen, foam cell macrophages and proliferated smooth muscle cells. The effects of insulin on the constituents and formation of the atheromatous plaque has been examined in cell and tissue culture and in animal studies. For example, long term treatment with insulin produces lipid-containing lesions and thickening of the arterial wall in experimental animals (Stout 1970); insulin inhibits the regression of diet-induced experimental atherosclerosis (Marquie 1978); insulin in physiological concentrations stimulates proliferation and migration of cultured arterial smooth muscle cells (Stout et al, 1975) and also stimulates cholesterol synthesis and LDL binding in both arterial smooth muscle cells and monocyte macrophages (Krone et al, 1988). Thus, under experimental conditions, insulin has been reported to have a number of effects on the constituents of atherosclerotic plaques that are consistent with our understanding of the progression of atherosclerosis.

(ii) Clinical studies.

As the formal techniques for measuring insulin sensitivity such as the euglycaemic hyperinsulinaemic clamp and insulin suppression test are labour intensive [section 1.2.1], simple tests, such as fasting serum insulin and C-peptide concentrations or measurements after an oral glucose tolerance test, have been used in epidemiological studies to indirectly infer the presence of insulin resistance.

Two large cross-sectional studies have examined the relationships between

insulin concentrations and cardiovascular disease. Lichtenstein and colleagues (1987) in a study of 2512 men aged 45-59 years from Caerphilly reported an association between fasting insulin and the prevalence of coronary artery disease independent of age, body mass index, blood pressure and serum triglyceride levels. Similarly, in a Finnish population, Ronnema and colleagues (1991) in a study of 1373 non-diabetic men aged 45-64 years reported increased fasting insulin concentrations in those subjects with clinical manifestations of coronary artery disease, but this association was lost when corrected for body mass index, blood pressure, triglyceride levels and HDL cholesterol levels.

There are three large and two small prospective studies which have examined the relationship between plasma insulin levels and subsequent coronary artery disease. In the Helsinki Policeman study, 982 men aged between 35 and 64 years and free of coronary artery disease at baseline received an oral glucose load and were followed up for ten years. Both fatal and non-fatal myocardial infarctions were more common in those with the highest quintile of fasting, 1 hour, 2 hour and total (AUC) insulin responses to glucose. On multivariate analysis this association was independent of blood glucose, plasma lipids and body mass index (Pyolara 1979). Similarly, the Paris Prospective study of 7038 male civil servants aged 43-54 years examined the incidence of fatal and non-fatal myocardial infarctions and has reported at 5 years (Ducimetiere et al, 1980), 10 years (Eschwege et al, 1985), and 15 years (Fontbonne et al, 1991). At baseline, plasma insulin and glucose concentrations were measured fasting and 2 hours after an oral glucose tolerance test. At 5, 10 and 15 years there was no relationship between the plasma glucose concentrations and incidence of coronary artery disease, but the fasting and 2 hour insulin levels were related to the incidence of coronary artery disease independent of glucose tolerance and blood pressure, and were greater in obese compared with than non-obese subjects. In the Busselton study blood samples were collected for insulin levels 60 minutes after a 50 gramme oral glucose tolerance test in 3390 Australian male and female adults aged 21 to 70 years.

The individuals were not instructed to fast. In a univariate analysis, insulin was shown to be associated with the 6 year incidence and the 12 year mortality rate from coronary vascular disease in men aged 60 to 69 years, but not in women of any age; using multiple linear regression analysis, 13 year all-cause mortality rate (but not coronary vascular mortality) was significantly and positively related to insulin concentrations in those men aged 40 to 70 years at entry (Cullen et al, 1983). In a smaller study of 895 Swedish men born in 1913, Welin and colleagues (1992) performed oral glucose tolerance tests and collected samples for insulin at baseline, 1 hour and 2 hours. They demonstrated that the incidence of coronary artery disease was related to triglyceride, cholesterol and fasting, 60 and 120 minute plasma glucose concentrations, but that the fasting serum insulin concentration was of borderline significance: in fact when known diabetic patients were excluded from the study only serum cholesterol and triglyceride levels emerged as statistically significant risk factors. Lastly, the Edinburgh study of 107 men aged 40 years at baseline examined the 12 year incidence of coronary artery disease. In those who developed coronary heart disease, the fasting insulin concentration was higher but not significantly so. Body mass index and abdominal skin folds were stronger indicators of subsequent disease. This is the only large study where HDL cholesterol was measured at baseline: regression analysis demonstrated a significant inverse relationship with subsequent coronary artery disease (Hargreaves et al, 1992).

These studies have correlated absolute insulin levels to the incidence of atherosclerotic complications; there are no data available on the relationship of insulin resistance *per se*, using more direct measures of insulin sensitivity. All of these studies (with one exception) only included white middle aged males and the effects of all but the highest concentrations of insulin on cardiovascular events were relatively minor. It must be borne in mind that hyperinsulinaemia and insulin resistance are associated with other potential metabolic risk factors for coronary artery disease including fibrinogen, lipoprotein (a) and plasminogen activator inhibitor levels. Many of these

variables were not measured in the above major epidemiological studies and it is possible that insulin is acting as a surrogate marker for other, more potent coronary risk factors.

1.2. **CLINICAL ASSESSMENTS OF INSULIN SENSITIVITY.**

There are various reasons for measuring insulin sensitivity *in vivo*. Firstly we may wish to define the importance of diminished insulin sensitivity as opposed to other associated factors such as exhausted beta-cell reserve. Secondly, the demonstration of impaired insulin sensitivity *in vivo* allows the identification of conditions in which a specific defect in insulin action should be sought. Lastly, the ability to measure insulin sensitivity accurately and reproducibly facilitates the evaluation of the efficacy of therapeutic regimens, for example, either drug treatments or a change in lifestyle.

The expression of insulin action in any isolated tissue is measured by establishing a dose response relationship between the concentration of insulin and some index of insulin action. Such data may then be amenable to standard analysis such as the application of Michaelis-Menten kinetics to express insulin action in terms of V_{max} or K_m . Thus the satisfaction of these needs ideally requires a stable, quantitative relationship between plasma insulin concentration and some measurable insulin-dependent metabolic response. Three techniques, each with a different set of limitations, fulfil this requirement, and have found widespread use in clinical research: the euglycaemic hyperinsulinaemic clamp, the insulin suppression test, and the frequently sampled intravenous glucose tolerance test with model analysis. Unfortunately, each of these techniques is relatively labour intensive and not suitable for large scale measurement of insulin resistance in epidemiological studies. Thus, simple tests such as the measurement of serum insulin or C-peptide levels in the

fasting state or after an oral glucose load have been used to infer indirectly the presence of insulin resistance.

1.2.1. ORAL GLUCOSE TOLERANCE TEST

In the 1930's, Himsworth introduced the first standard approach to measuring insulin sensitivity *in vivo* (Himsworth, 1936 & 1939). Two oral glucose tolerance tests (OGTT) were performed on individual subjects: one with and one without an intravenous insulin injection. Insulin sensitivity was expressed as the ratio of the areas under the respective glucose tolerance curves, but without direct measurements of plasma insulin concentrations Himsworth could only infer differences in tissue insulin sensitivity, e.g. between type I (insulin dependent) and type II (non-insulin dependent) diabetic patients. Thus, he was unable to exclude differential effects of exogenous insulin on glucose absorption and suppression of endogenous insulin release, or differences due to variation in insulin clearance.

Since its initial use by Himsworth, the OGTT has been used by many investigators to examine metabolic responses in a variety of disorders. It can provide a simple, albeit indirect, estimation of whole-body insulin sensitivity. Inappropriately elevated plasma insulin concentrations, e.g. 2 hours after an oral or intravenous glucose load, in the face of normal or above-normal plasma glucose concentrations, is generally accepted as evidence for decreased overall tissue sensitivity to insulin. However, the heterogeneity of OGTT profiles in both normal and diabetic subjects has been widely emphasised (Reaven & Miller, 1968), and it is essentially impossible to draw conclusions about insulin sensitivity when an intervention (e.g. drug) or disease (e.g. NIDDM) under investigation is potentially associated with changes in insulin secretion as well as insulin action. For example, changes in peripheral insulin sensitivity affect plasma glucose concentrations, which in turn affect glucose-induced insulin release, making it impossible to differentiate between pancreatic and

extra-pancreatic effects. Thus, an OGTT characterises the net effect of several glucoregulatory mechanisms but provides only limited information about individual pathways and insulin sensitivity; it is nevertheless useful for larger studies, when absolute measurements of insulin-mediated glucose uptake are not required, and when the study design uses individuals as self-controls.

1.2.2. INTRAVENOUS GLUCOSE TOLERANCE TEST.

A conventional intravenous glucose tolerance test (IVGTT) characterises the early and late phases of insulin secretion following bolus injection of a glucose load. The net fractional glucose disappearance rate (K value) is easily calculated from the profile of plasma glucose concentrations, but differences in K cannot distinguish between effects on insulin secretion and insulin action.

Bergman and colleagues have described a modification of the IVGTT - the so-called "minimal model" or frequently sampled IVGTT - that provides a quantitative index of insulin sensitivity (Yang et al, 1987). An intravenous bolus of glucose is followed 20 minutes later by an injection of insulin or tolbutamide 500mg; some 30 blood samples are then collected over 180 minutes for glucose and insulin measurements which are analysed using computer-based model predictions of insulin's effect on glucose kinetics. The crucial piece of software, marketed and apparently understood only by the authors, calculates an index of insulin sensitivity with few details of how the measurement was derived. Despite the complexity of this method, parameters derived from the minimal model correlate well with other techniques (Bergman et al, 1987).

1.2.3. EUGLYCAEMIC HYPERINSULINAEMIC CLAMP AND HYPERGLYCAEMIC CLAMP TECHNIQUES.

Andres (1966) was the first to propose that a dose-response measure of insulin action in the whole body could be derived by maintaining a stable plasma insulin concentration, thus disrupting the glucose-insulin feedback mechanism which regulates insulin action and secretion.

Thus, the widely popular, though labour intensive, euglycaemic hyperinsulinaemic clamp was initially described by Andres and he evaluated it further in collaboration with DeFronzo (DeFronzo et al, 1979). The technique uses a constant intravenous infusion of soluble insulin (e.g. 1.5 mU/kg/min) to increase circulating insulin concentrations by about 100uU/ml. Many rates of insulin infusion have been used, and the resultant hyperinsulinaemia also depends on the metabolic clearance rate of insulin itself. However, as insulin clearance is rapid, "steady state" insulin concentrations are usually attained within 30 minutes in healthy subjects.

Normally the glucose level would decline under these conditions, reflecting the action of insulin to increase glucose utilisation by some tissues and to reduce hepatic glucose output. However, a substantial decline is prevented by making rapid and frequent measurements of the plasma glucose during the procedure and by the administration of a variable-rate infusion of 20% glucose to maintain plasma glucose at a target value, usually 5-6 mmol/L. Although the increase in plasma insulin concentration is within 30 minutes, there is a time lag of 1-2 hours before the maximal hypoglycaemic stimulus is observed. Thus, during a typical clamp, the glucose infusion rate is increased progressively until a steady state equilibrium is achieved, usually at around 120 minutes [figure 1.4]. Calculations of insulin sensitivity are based on a 30-40 minute period of steady state infusion when the rate of glucose input approximates to whole body insulin sensitivity (M value expressed in mg glucose/kg/min). Thus it is necessary to maintain the infusions for at least 3 hours.

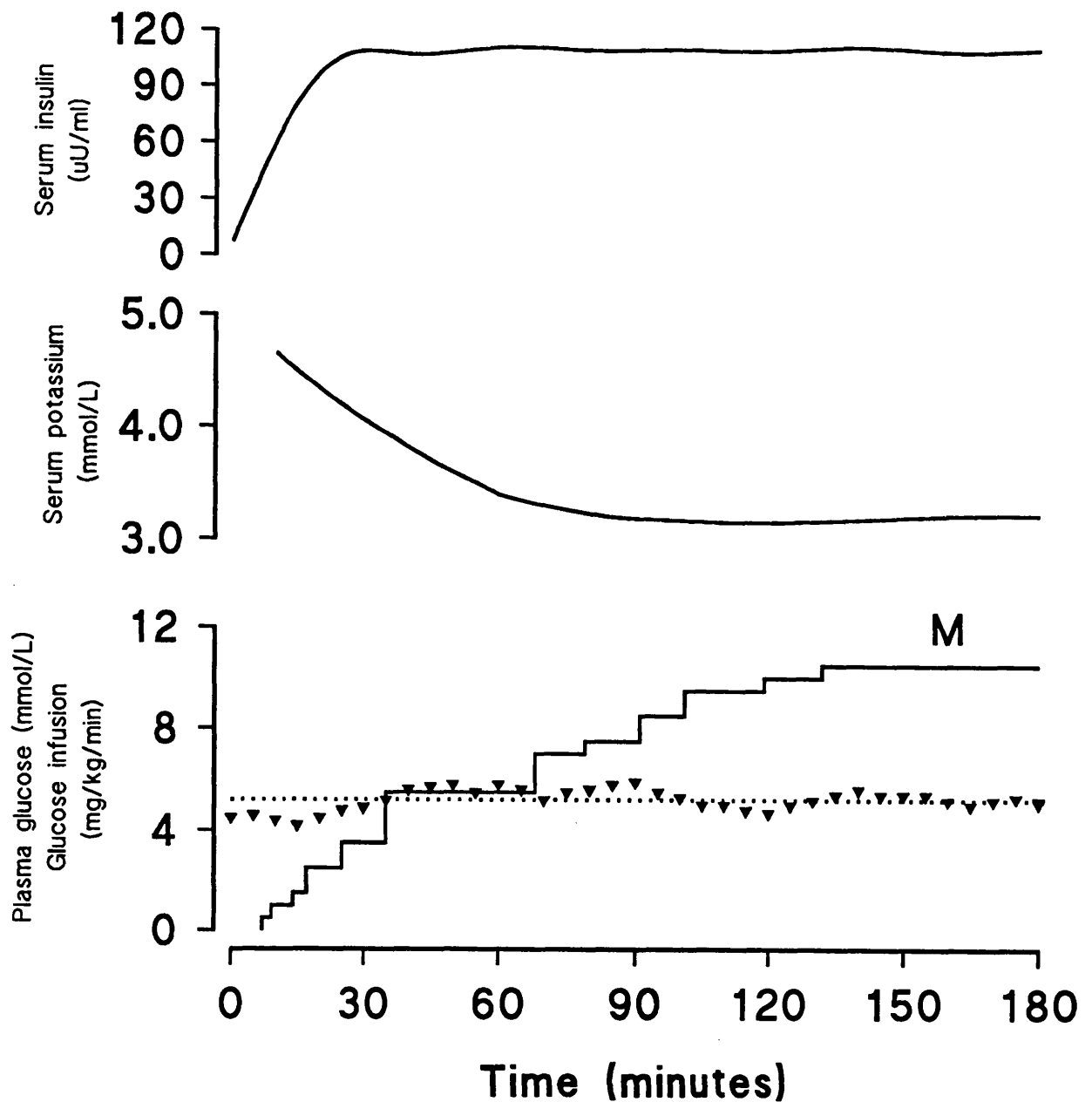


Figure 1.4.: Representative euglycaemic hyperinsulinaemic clamp in a healthy volunteer. The exogenous insulin infusion (1.5 mU/kg/min) produces a "steady-state" serum insulin concentration approx. 100uU/ml above the basal fasting value (top panel). A dose dependent fall in serum potassium is observed (middle panel). Whole body glucose disposal, M, in mg glucose/kg min is calculated during "steady state" at the end of the 3 hour clamp. ▼ = plasma glucose concentration (lower panel).

The glucose infusion rate can be adjusted manually in response to rapid measurements of plasma glucose at the bedside, or automatically with a computerised glucose-controlled infusion system derived from mathematical formulae [Biostar] (Ponchner et al, 1984). Manual corrections are perfectly adequate, but as they can be influenced by subjective bias, estimated to be up to 15% (Greenfield et al, 1981), in clinical pharmacological studies it is still important that the investigator remains blind to drug administration. Rebound hypoglycaemia is a potential complication if glucose and insulin infusions are stopped simultaneously. This is easily avoided by continuing intravenous glucose for an extra 20 minutes and promptly feeding the subject at the end of the infusions. *In vivo* insulin infusion with maintenance of euglycaemia causes a dose-dependent fall in plasma potassium concentrations by promoting potassium uptake into the liver and peripheral tissues. However, below a threshold potassium level the splanchnic potassium exchange switches from a net uptake to a net release, so preventing further hypokalaemia (DeFronzo et al, 1980). Thus, potassium replacement in the infusate is usually unnecessary.

The clamp provides an overall measurement of whole-body insulin sensitivity, which represents the net balance of peripheral glucose utilisation and endogenous hepatic glucose production, but there has to be some concern that measurements derived under such artificial conditions might not be a true reflection of physiological responses to insulin. In insulin resistant states, especially NIDDM, it is unlikely that the insulin stimulus will completely suppress glucose secretion from the liver. However, by combining the clamp with a constant infusion of radiolabelled tracer ($[^3\text{H}]$ - or $[^2\text{H}]$ - glucose), it is possible to quantify rates of glucose appearance (R_a) and disappearance (R_d) which correspond to hepatic glucose production and peripheral glucose utilisation respectively (Finegood et al, 1987). These methods depend upon steady-state conditions at the time of blood sampling but some investigators doubt whether a true equilibrium is achieved after 3 hours, raising questions about the accuracy and reproducibility of these measurements.

The glucose taken-up by skeletal muscle is processed through oxidative (i.e. glycolytic) or non-oxidative (e.g. glycogen synthetic) pathways of metabolism, and by combining the euglycaemic clamp with a [³H]-glucose infusion and indirect calorimetry (Ferrannini et al, 1989), it is possible to quantify peripheral glucose utilisation in terms of oxidative and non-oxidative rates of disposal.

The glucose clamp technique is a measure of insulin sensitivity but provides no information on insulin secretion. The hyperglycaemic clamp is a method based on the same principle but devised to quantify beta-cell sensitivity; the plasma glucose concentration is acutely raised to a plateau (e.g. 14 mmol/L) by a priming infusion of glucose, and the resultant biphasic insulin response measured (DeFronzo et al, 1979).

The euglycaemic hyperinsulinaemic clamp is discussed in more detail in chapter 2.3 and chapter 3.

1.2.4. INSULIN SUPPRESSION TEST.

The principle of the insulin (or pancreatic) suppression test involves pharmacological inhibition of endogenous insulin secretion, and other counter-regulatory hormones (e.g. glucagon and growth hormone), during constant infusions of glucose and insulin. Endocrine suppression was originally achieved using intravenous infusions of epinephrine and propranolol (Porte, 1967), but lately this has been replaced by somatostatin (Harano et al, 1987), or the somatostatin-analogue octreotide, which is more widely available and appears to be better tolerated. As with the clamp, plasma glucose and insulin concentrations gradually reach a plateau [figure 1.5]; the steady state plasma glucose concentration (SSPG) is a reflection of whole body insulin sensitivity.

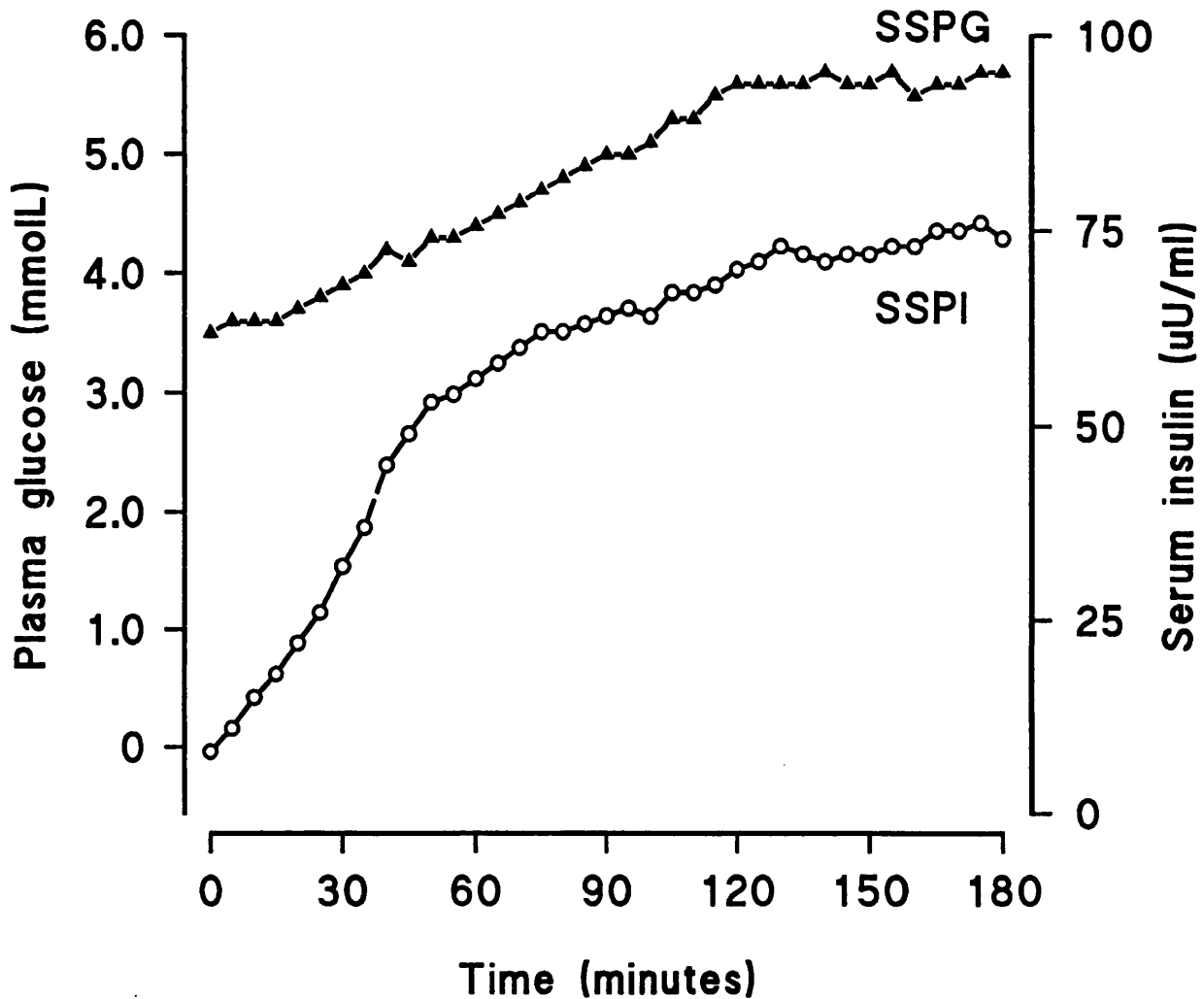


Figure 1.5: Representative insulin suppression test in a healthy volunteer. After around 120 minutes infusion of insulin, glucose and somatostatin the steady state plasma insulin **SSPI**, and steady state plasma glucose **SSPG** are calculated. **o** = plasma glucose (mmol/L), **▲** = serum insulin (uU/ml).

1.2.5. MODEL DERIVED PARAMETERS OF INSULIN SENSITIVITY

Mathematical models have been derived to describe the complex kinetics of glucose and insulin under physiological conditions, to derive parameters for peripheral insulin sensitivity and to evaluate β -cell dysfunction. The technique of HOMA (homeostatic model assessment), for example, uses measurements of fasting plasma glucose and insulin to derive an index of insulin sensitivity (S) which shows good correlation with parameters derived from infusion techniques (Turner et al, 1979; Matthews et al, 1985). Since basal (fasting) insulin concentrations are particularly subject to assay variability, Hosker and colleagues (1985) attempted to improve on the assessment of insulin sensitivity from HOMA by stimulating endogenous insulin secretion by a constant infusion of glucose. Thus, CIGMA (continuous infusion of glucose with model assessment) defines insulin sensitivity from the steady state levels of glucose and (endogenous) insulin after 2 hours. These "structural" model predictions have many advantages - not least that measurements reflect physiological responses to endogenous insulin, rather than "pharmacological" infusions - but as with all models there are some inherent assumptions that may be over simplistic; for example, that various tissues in different subjects behave similarly with respect to glucose metabolism, and many of the variables used to calculate insulin sensitivity are not individualised for each subject.

1.2.6. ARTERIALISED BLOOD SAMPLING.

For both of the principal infusion techniques for assessing insulin sensitivity, the euglycaemic hyperinsulinaemic clamp and minimal model, it has been emphasised that blood used to measure plasma glucose concentrations should preferably be sampled from the arterial side of the circulation. Thus, during hyperinsulinaemic stimulation glucose extraction across the capillary bed is high, resulting in a

significant arterio-venous difference in glucose concentration. As arterial cannulation is associated with increased morbidity, cannulation of a dorsal hand vein surrounded by a heated box is often preferred. The hand is warmed either by hot air (McGuire et al, 1976) or a heated pad (Forster et al, 1972). The theory of this manoeuvre is that the hand has relatively little muscle bulk and the local application of heat will create a physiological arteriovenous shunt (Roddie et al, 1956), thus minimising glucose extraction.

The methods for arterialisation of venous blood are discussed in more detail in chapter 2 [section 2.3] and chapter 3.

1.2.7. GLUCOSE CLAMP VERSUS IVGTT VERSUS MINIMAL MODEL: WHICH IS BETTER?

None of the three methods for evaluating insulin sensitivity can categorically be said to be "better" as they were developed for different purposes. The clamp is frequently the method of choice when resources are available which allow a controlled laboratory environment. The procedure is compromised however by being relatively time-consuming and labour intensive and therefore studies using the clamp are usually restricted to a relatively small number of subjects. Nevertheless, the performance of a series of clamp experiments within an individual gives an excellent index of insulin sensitivity and allows accurate assessment of both peripheral glucose utilisation and hepatic glucose output. The inventors of the minimal model claim it has attributes not possessed by the clamp. It allows simultaneous assessment of β -cell secretory function and requires less blood sampling and less intervention than the clamp. It therefore would be the method of choice in studies requiring assessment of insulin sensitivity in a large number of subjects. Likewise, the insulin suppression test is less time-consuming than the clamp technique and may be preferable in larger scale studies.

1.3 EFFECTS OF PHARMACOLOGICAL AND NON PHARMACOLOGICAL INTERVENTIONS ON INSULIN SENSITIVITY.

The notion that an improvement in insulin sensitivity may reduce cardiovascular risk and perhaps lower blood pressure has focussed attention on the effects of both pharmacological and non-pharmacological interventions on insulin resistance.

1.3.1. NON-PHARMACOLOGICAL METHODS OF MODIFYING INSULIN SENSITIVITY

(i) Physical exercise.

It is well established that physical exercise can improve glucose tolerance in obese individuals, patients with diabetes and in normal subjects. A relationship between physical fitness and insulin-stimulated glucose disposal has been established in both cross-sectional and exercise training studies (Rosenthal et al, 1983; Rodnick et al, 1987); regular aerobic exercise is antihypertensive and is associated with an improvement of insulin sensitivity and progressive lowering of plasma insulin responses to a glucose challenge (Bogardus et al, 1984; DeFronzo et al, 1987). The relationship of physical training to enhanced insulin sensitivity is independent of obesity, as brief training bouts can improve insulin sensitivity in the absence of any changes in body weight in patients with NIDDM (Rogers et al, 1988) and essential hypertension (Saito et al, 1992). The improvement in insulin sensitivity observed appears to be shortlived; for example, trained athletes who do not exercise for several days show a deterioration in insulin sensitivity in the absence of changes in body weight (Heath et al, 1983).

Thus, there is ample evidence that regular exercise can improve insulin

sensitivity in humans. The associated benefits of regular exercise of reduction in blood pressure and plasma triglyceride concentrations make it an important non-pharmacological measure in the reduction of cardiovascular risk.

(ii) Obesity and weight loss.

There is much evidence suggesting a relationship between insulin resistance and obesity (Després et al, 1990; Cambien et al, 1987). Several studies in obese patients both with and without diabetes have shown that weight reduction, whether induced by low-calorie diets (Olefsy et al, 1974; Calle Pascual et al, 1991; Fukagawa et al, 1990) or gastric bypass surgery (Hughes et al, 1984; Hale et al, 1988) results in improvement in glycaemic control and insulin sensitivity. The mechanisms underlying these changes remain poorly defined. What is evident is that the improvement in insulin sensitivity observed with weight loss is unrelated to the baseline body weight or to the absolute or relative weight loss achieved. For example, Olefsky and colleagues (1974) demonstrated that a weight loss of 10kg in moderately obese individuals resulted in approximately a 37% increase in insulin sensitivity whether the subjects weight decreased from 108kg to 97kg or 86kg to 75kg.

A recent randomised study in 64 obese hypertensive men which compared the effects of dietary weight reduction versus antihypertensive drug therapy upon insulin, glucose, lipid metabolism and blood pressure reported that dietary therapy was inferior in controlling hypertension but superior in lowering plasma insulin concentration and improving the serum lipid profile (Faberberg et al, 1992).

Much recent work has focussed on the relationships between regional fat distribution, most commonly waist-hip ratio, and insulin resistance. It has been shown that a subjects with upper body fat distribution are more insulin resistant than subjects with the same body mass index, but with lower body fat distribution (Evans et al, 1984; Peiris et al, 1988).

(iii) Smoking.

Cigarette smoking is associated with the lipid abnormalities of increased plasma triglyceride concentrations and decreased HDL cholesterol concentrations. A recent cross-sectional study which evaluated the insulin sensitivity of 20 normotensive, glucose tolerant male smokers as compared to 20 age and weight matched non-smoking controls suggested that the smokers were both hyperinsulinaemic and insulin resistant (Facchini et al, 1992). Further studies are required to define the relationship between both acute and chronic effects of cigarette smoking on *in vivo* insulin action

1.3.2 PHARMACOLOGICAL METHODS OF MODIFYING INSULIN SENSITIVITY

Numerous drugs affect glucose tolerance (Pandit et al, 1993), usually via changes in insulin secretion, while some exert peripheral effects on insulin target tissues. In general, the nature and magnitude of drug effects on insulin-dependent metabolic pathways have been poorly characterised and sometimes misrepresented. Non-pharmacological measures that improve insulin sensitivity, e.g. weight loss and physical exercise [chapter 1.3.1] are effective in lowering glucose and triglyceride levels but few drugs, other than the biguanides, enhance insulin signalling. Much recent research has focussed on the metabolic effects of drugs used to treat hypertension. This section will review the studies evaluating the effects of antihypertensive drugs on glycaemic control. The effects of the currently available oral hypoglycaemic agents and novel insulin sensitising drugs which are undergoing clinical development will also be discussed.

The notion that insulin resistance and hyperinsulinaemia might increase the risk of atherosclerosis has focussed attention on the differential effects that

antihypertensive drugs may exert on glucose homeostasis and insulin resistance.

These studies have largely been prompted by the hypothesis that the use of the more traditional antihypertensive agents may explain the "paradox" that treatment of mild hypertension fails to reverse the excess risk of coronary heart disease (Pollare et al, 1989b; Poulter et al, 1990). For example, a recent meta-analysis suggests that the mean reduction in diastolic blood pressure of 5-6mm Hg in the large hypertension trials results in virtually all the epidemiologically predicted stroke reduction (42%), but that coronary heart disease was reduced by only half (14%) of that expected (Collins et al, 1990). Data from these trials, however, suggest that clinically important glucose intolerance occurred in only 2.5% of patients taking thiazides and 1% of those taking propranolol or placebo over the 5 year period (MRC Trial, 1985). Other authors have therefore seriously questioned the clinical importance of "iatrogenic" worsening of insulin resistance (Yudkin 1991).

Many of the studies in the literature are unfortunately compromised by poor trial design, lack of full placebo data, various indirect measurements of insulin sensitivity and heterogeneous patient groups in whom the biochemical mechanisms of insulin resistance may not be the same.

(i) Thiazide diuretics.

Diuretic therapy is of proven efficacy in the control of hypertension and has been part of the therapeutic regimen used in most of the major blood pressure intervention trials. It has long been recognised that diuretic therapy for the treatment of hypertension may be associated with impaired glucose tolerance. This complication was highlighted in a study published in 1982 which performed serial oral glucose tolerance tests over a 14 year period in diuretic treated hypertensive patients (Murphy et al, 1982). They demonstrated substantial deterioration in glucose tolerance but the results are difficult to interpret in the absence of a control group. Diuretic therapy has recently undergone considerable scrutiny and data from numerous controlled trials are

now available which have established a relationship between diuretic treatment and impaired glucose tolerance. For example, in the single blind MRC trial in mild hypertension (1985), bendrofluazide (10mg daily) was compared with placebo and oral glucose tolerance tests were performed if the random plasma glucose was greater than 8.3 mmol/L or the fasting value was greater than 6.0 mmol/L. Over the 3 years, the incidence of impaired glucose tolerance (per 1000 treated patient years) was 9.4 for bendrofluazide vs. 2.5 for placebo in men, and 6.0 vs. 0.8 in women. Similar results have been reported in large scale double blind studies. For example, in the Systolic Hypertension in the Elderly Programme (SHEP) trial (1988) blood sugar was significantly higher after 12 months of treatment with chlorthalidone. In the European Working Party on High Blood Pressure in the Elderly (EWPHE) trial (Amery et al, 1989), fasting blood sugar was increased by diuretic therapy after 12 months and remained significantly elevated (by approximately 6%) after 3 years. Therefore, there is good evidence that long term diuretic treatment is associated with increased incidence of impaired glucose tolerance in hypertensive patients.

Peripheral insulin resistance has been implicated as one possible mechanism of thiazide induced glucose intolerance but in contrast to the wealth of evidence that impaired glucose tolerance occurs with diuretic treatment, there is a surprising lack of clinical trials examining the effects of thiazides on insulin sensitivity. In Pollare's study comparing captopril to hydrochlorthiazide at an average dose of 40mg/day for 14 weeks, it was reported that the diuretic significantly impaired insulin sensitivity, but this study was not placebo-controlled (Pollare et al, 1989a). Swislocki also reported impaired peripheral insulin sensitivity with thiazides in an uncontrolled study (Swislocki et al, 1989). However, a recent crossover study using the euglycaemic hyperinsulinaemic clamp showed no effect of bendrofluazide 1.25mg or 5mg on insulin sensitivity after 12 weeks (Harper et al, 1993).

(ii) Angiotensin converting enzyme inhibitors.

Incidental observations appeared in the mid-1980's suggesting an improvement in glucose tolerance after treatment with an ACE inhibitor. Anecdotal reports from diabetic clinics also described a reduction in insulin requirements or discontinuation of oral hypoglycaemic therapy after the introduction of either captopril or enalapril (Ferriere et al, 1985; McMurray & Fraser, 1986); even hypoglycaemic episodes were attributed to ACE inhibition (Rett et al, 1988). When given acutely to hypertensive patients with NIDDM (Turlone et al, 1991), or normotensive individuals (Jauch et al, 1987), captopril has been reported to increase insulin-mediated glucose disposal. Jauch proposed that the increase in insulin sensitivity is secondary to increased glucose uptake into skeletal muscle; the amount of glucose taken up 30 minutes after a single oral dose of captopril was 2.22uM/100g/min compared with 0.83 uM/100g/min after placebo. In a similar study but in patients with insulin dependent diabetes mellitus, Helve and colleagues (1993) reported no acute effect of captopril on insulin sensitivity using the euglycaemic hyperinsulinaemic clamp.

The most widely quoted study of ACE inhibitors and insulin sensitivity is by Pollare and colleagues (Pollare et al, 1989a). This study was performed in 50 non-diabetic hypertensive subjects to compare captopril and hydrochlorothiazide, each administered for 16 weeks, in a randomised, cross-over design. Despite a 4-week washout phase, carry-over effects hampered the intended analysis, so results were presented for two parallel groups during the first treatment phase only. There was no difference in fasting plasma glucose and insulin levels, or glycosylated haemoglobin, but insulin stimulated glucose disposal increased marginally from 5.7 to 6.3 mg glucose kg/min after captopril, compared with a change from 6.4 to 5.7 mg glucose/kg/min with the diuretic. In a similar study with enalapril (Prince et al, 1988), haemoglobin A_{1c} decreased after 4 weeks of the ACE inhibitor but there was no change in the glucose disposal rate. Likewise, a placebo-controlled open trial which

evaluated the effects of 3 months therapy of the ACE inhibitor cilazapril on glucose tolerance and insulin sensitivity in 20 patients with essential hypertension reported no effect of chronic ACE inhibition on insulin sensitivity, substrate oxidation or thermogenesis (Santoro et al, 1992).

Thus, there are conflicting data which demonstrate striking inconsistencies concerning the effects of ACE inhibitors on insulin sensitivity. For example, of 34 studies in which some parameter of glycaemic control was assessed in patients with NIDDM and hypertension receiving an ACE inhibitor, improvements were noted in only 13: 3 out of 9 studies with enalapril and 8 out of 24 with captopril (Stein & Black, 1991). However, few, if any, studies of the metabolic effects of ACE inhibitors have been properly controlled, and there are no direct comparisons with placebo.

(iii) Calcium channel blockers

Many investigators have reported the effects of calcium antagonists on glucose homeostasis, but often these studies are not comparable because of different design and heterogeneous patient groups. Furthermore, the calcium antagonists are a heterogeneous group of compounds (i.e. dihydropyridines, benzothiazepines, phenylalkalamines) which may exert differential effects on the peripheral vasculature and cardiac conduction system. Acute treatment may influence glucoregulatory control differently from chronic treatment; this is especially important for the dihydropyridines which are characterised by sympathetic augmentation on acute dosing.

In two placebo-controlled acute experiments, neither single therapeutic doses of oral nifedipine (Joffe et al, 1983) nor a bolus of intravenous verapamil (Rodjmark et al, 1980) affected the fasting glucose concentrations or plasma insulin levels in healthy normotensive subjects. These findings have been confirmed in other studies. In contrast, several studies examining the "short-term" effects of different calcium

antagonists on glucose tolerance, as assessed by either an oral or intravenous glucose challenge, have yielded conflicting results. For example, a detrimental effect of verapamil was reported in a small placebo-controlled study of 7 healthy subjects when 5g of intravenous glucose was administered with an infusion of verapamil [9.6mg/hr] (Giugliano et al, 1981). The disappearance rate of glucose and the insulin output were both diminished. However, when 20g of glucose was administered under similar experimental conditions by the same group, glucose and insulin levels remained unchanged. Likewise, the areas under the curve of glucose and insulin were similar after a 100g OGTT with or without concomitant infusion of verapamil [5mg/hr over 2 hours] (Andersson & Rodjmark, 1981). In contrast, an almost identical protocol led to a significant increase in blood glucose and a decrease in plasma insulin between 30 and 120 minutes after the verapamil infusion was started (De Marinis & Barbarino, 1980).

Such conflicting results are not unique to verapamil; studies with dihydropyridines have caused similar confusion. Two placebo-controlled studies reported no change in the profiles of blood glucose and insulin profiles when 10mg (Donnelly & Harrower, 1980), and 20mg (Pasanisi et al, 1986) of nifedipine were given after a 50g OGTT and IVGTT in healthy volunteers and patients with essential hypertension, respectively. However, in another study, when repeated doses of nifedipine were given after a 100g OGTT (20mg of nifedipine in total) the AUC glucose profile was significantly increased but the AUC insulin profile remained unchanged (Ferlito et al, 1980).

Several studies have examined the effects of long term administration of calcium antagonists on glucose homeostasis. Patients with angina pectoris or essential hypertension undergoing long term treatment with calcium antagonists (6 weeks to 62 weeks) have been studied. Trials of 5 different calcium antagonists, nifedipine (Landmark, 1985), verapamil (Lang et al, 1981), diltiazem (Pool et al, 1985) and nitrendipine (Ferrara et al, 1985) have concluded that there is no adverse effect on

blood glucose, plasma insulin and C-peptide concentrations. Again, there are conflicting data; Oehman and colleagues (1985) studied 25 hypertensive patients in a placebo-controlled crossover study of nifedipine over a 6 week period. They concluded that blood glucose and insulin levels were both significantly elevated, but conversely, the glycosylated haemoglobin concentration was significantly lower after 6 weeks of treatment. Another study with diltiazem and metoprolol in 52 non-diabetic hypertensive patients (Hedner et al, 1990), concluded that diltiazem had a significant detrimental effect, but the endpoint was fasting blood sugar concentration and the resultant values were still within the normal range.

Few studies of calcium antagonists have used direct measurements of insulin sensitivity. In two studies using the euglycaemic hyperinsulinaemic clamp, neither diltiazem (Pollare et al, 1989b) nor verapamil (Berne et al, 1991) had any effect on insulin sensitivity.

In single dose or short term experiments of patients with NIDDM, conflicting results have also been reported. Most studies, with or without a glucose challenge, report a neutral effect; for example, when 10mg of nifedipine was administered to 8 elderly diabetics, no effect on fasting blood sugar and plasma insulin levels was found (Donnelly & Harrower, 1980). A similar neutral effect has been reported by other groups for nifedipine (Collins et al, 1986), verapamil (Ferlito et al, 1982) and diltiazem (Wada et al, 1982). In contrast, a beneficial effect of an intravenous infusion of verapamil on the AUC of glucose following an OGTT has been reported in two placebo-controlled studies (Andersson & Rodjmark, 1981; Rodjmark et al, 1981). The plasma insulin concentrations remained unchanged however. Other studies have reported significantly increased blood glucose concentrations and reduced plasma insulin concentrations for nifedipine, (Deedwania et al, 1984; Sando et al, 1983), but the doses studied were high. A poorly controlled single blind study of felodipine (Kjellstrom et al, 1991) reported a significant increase in the glucose AUC following an OGTT.

Long term studies have generally only studied small numbers of patients. Despite anecdotal reports that a deterioration in glucose tolerance may occur in individual cases when a calcium antagonist is prescribed (Bhatnagar et al, 1984; Iversen et al, 1990), the majority of controlled studies, for example, with nifedipine (Winocour et al, 1986), verapamil (Whitcroft et al, 1986) and nitrendipine (Trost et al, 1987) have detected no deterioration in fasting blood sugar levels, fasting insulin levels or glycosylated haemoglobin concentration. Similar results have been reported following an oral glucose challenge with diltiazem (Wada et al, 1982) and nitrendipine (Odigwe et al, 1986). A recent double-blind placebo-controlled crossover study, using the euglycaemic clamp, evaluating the effects of long term isradipine administration on peripheral insulin sensitivity also reported a neutral effect (Klauser et al, 1991).

In summary, currently available data suggest that calcium antagonists do not alter glucose homeostasis when given acutely in therapeutic doses, although some short term studies in non-diabetic hypertensive patients have reported increased fasting blood sugar levels and reduced insulin sensitivity with the dihydropyridines. Interpretation of many of these studies is difficult because of uncontrolled study designs.

(iv) Beta-adrenoceptor blockers.

The suggestion that β -adrenergic blocking agents may increase the risk of developing diabetes mellitus in patients with essential hypertension first emerged from longitudinal studies which compared treated hypertensive patients with a healthy normotensive control group and attributed the higher incidence of diabetes in the hypertensive patients to their therapy (Bengston et al, 1984; Skarfors et al, 1989). Such longitudinal, poorly controlled reports should be viewed with caution; for example, in a large cross-sectional study Halkin and colleagues (1989) reported no

difference in the prevalence of glucose tolerance and diabetes mellitus between an untreated hypertensive group and patients treated with β -adrenergic blocking agents.

Nevertheless, β -adrenergic blocking agents have been shown in smaller studies to increase fasting blood sugar and to decrease glucose tolerance in diabetic and non diabetic hypertensive subjects (Helgeland et al, 1984; Struthers et al, 1985). The release of insulin from the pancreas is modulated by β -adrenergic receptors and β -adrenergic blocking agents are reported to inhibit insulin secretion by as much as 50% (Kendall et al, 1988).

Whether β -adrenergic blocking agents influence insulin sensitivity remains uncertain. In a study using the euglycaemic hyperinsulinaemic clamp, Pollare and colleagues (1989c) reported a deterioration in insulin sensitivity in hypertensive patients after treatment with the β 1-adrenergic blocking agents metoprolol and atenolol, but the results may have been confounded by weight gain in the β -blocker treated group. In contrast, in subjects with impaired glucose tolerance, metoprolol and atenolol have been reported to improve insulin sensitivity as compared with placebo (Faberberg et al, 1990).

(v) Alpha receptor blockers

Alpha₁ -adrenergic receptor antagonists such as prazosin, terazosin and doxazosin effectively reduce blood pressure without affecting lipid or glucose control. A study evaluating the metabolic effects of prazosin reported an improvement in insulin sensitivity but this was not placebo controlled (Pollare et al, 1988).

In summary, a disappointing feature of many of the published trials which have evaluated the effects of antihypertensive therapy on glucoregulatory control mechanisms is that many are uncontrolled or have used "indirect" endpoints such as fasting glucose and insulin concentrations to infer information about changes in

insulin resistance. There are few, if any, well controlled studies using direct, reproducible measurements of insulin sensitivity.

1.3.3. ORAL HYPOGLYCAEMIC AGENTS.

(i) Metformin.

Metformin is widely used in the treatment of NIDDM, both as monotherapy and in combination with sulphonylureas. The glucose-lowering effect occurs without stimulation of insulin secretion and several mechanisms have been suggested, including delayed gastrointestinal absorption of glucose (Czyzyk et al, 1968), enhanced insulin sensitivity (Gin et al, 1983) and suppression of hepatic glucose production through inhibition of gluconeogenesis (Wollen & Bailey, 1988). Although the principal underlying mechanism remains unclear, several studies have shown increases in insulin-stimulated glucose uptake and oxidative metabolism in muscle (Fantus & Bousseau, 1986; De Fronzo et al, 1991; Widen et al, 1992). However, this is not a universal finding (Jackson et al, 1987), and many believe that the hypoglycaemic effect is largely due to a reduction in hepatic glucose output (Wollen & Bailey, 1988). Consistent with its putative insulin-mimetic actions, metformin has other beneficial effects on lowering triglyceride levels, body weight and even blood pressure.

(ii) Sulphonylureas.

These drugs form the mainstay of oral therapy for NIDDM but there is still some debate about whether sulphonylureas lower glucose levels by mechanisms in addition to stimulation of insulin secretion (Feldman & Lebovitz, 1969). Part of the controversy about extrapancreatic effects stems from studies evaluating insulin responses during chronic therapy; hypoglycaemic effects were observed with little or no change in plasma insulin concentrations (Barnes et al, 1974). In addition, several

studies have demonstrated improved insulin sensitivity (Kolterman et al, 1984; Simonson et al, 1984) and increased receptor binding (Beck-Nielsen et al, 1979). However, at present, the balance of evidence is probably against significant extrapancreatic effects; for example, sulphonylureas do not lower plasma glucose levels in patients with IDDM or patients with NIDDM rendered insulinopaenic with somatostatin (Widen et al, 1991), and it is quite possible that the apparent increase in insulin sensitivity is a non-specific response secondary to improved glycaemic control, rather than any direct peripheral effect of the drug itself.

1.3.4. NEW PHARMACOLOGICAL APPROACHES TO NON-INSULIN DEPENDENT DIABETES MELLITUS.

NIDDM is characterised by a triad of metabolic abnormalities: peripheral and hepatic insulin resistance, impaired insulin secretion and hepatic glucose overproduction. There is some debate about which disorder arises first but insulin resistance, affecting multiple pathways of glucose and lipid metabolism, is generally regarded as the principal defect (DeFronzo et al, 1992). It therefore seems somewhat anomalous that sulphonylurea drugs, which are primarily insulin secretagogues, should form the mainstay of oral therapy; the ideal treatment should logically focus on reversing the peripheral defects in glucose and lipid oxidation. This is supported by the relatively high rates of secondary failure of sulphonylurea therapy (Groop & Pelkonen, 1984), which presumably reflect islet cell exhaustion in the face of sustained tissue insulin resistance. The last decade has seen enormous progress in defining the biochemistry of NIDDM, and has led to a variety of new pharmacological innovations, some of which are now entering early clinical development.

The thiazolidinedione derivatives represent a new structural class of antidiabetic compounds. Included in this group are ciglitazone, pioglitazone, englitazone and troglitazone. At least three are at present undergoing phase I and II

studies, and a recent report demonstrated hypoglycaemic efficacy in patients with NIDDM (Suter et al, 1992; Iwamoto et al, 1991; Kuzuya et al, 1991). Analogues of this class lower plasma glucose, insulin and triglyceride levels in a variety of rodent models in which insulin resistance is a conspicuous feature, and biochemical studies have shown insulin-mimetic effects in each of the key target tissues (Hofmann & Colca, 1992). Thus, thiazolidinediones increase glucose oxidation in adipose tissue and muscle, increase glycogen and lipid synthesis from glucose and decrease glycogenolysis (Steiner & Lien, 1987; Colca & Morton, 1990). *In vitro* studies have shown that this group of drugs act as insulin-sensitisers; for example, insulin-stimulated uptake of deoxyglucose in isolated adipocytes from KKA^y mice was markedly more sensitive to insulin after pretreatment of these insulin sensitive animals with pioglitazone (Hofmann & Colca, 1992), and thiazolidinedione effects (like those of metformin) are dependent upon the presence of insulin (Hofmann et al, 1991). In addition to their hypoglycaemic effects, these drugs have other properties that may be particularly useful in patients with NIDDM, including effects on HDL-cholesterol and possibly blood pressure (Yoshioka et al, 1993).

Dichloroacetate (DCA) represents a potentially novel class of oral hypoglycaemic agents. DCA has no effect on insulin secretion but reduces plasma glucose levels via inhibition of hepatic glucose production and stimulation of peripheral utilisation (Stacpoole & Greene, 1992). The peripheral effect is due mainly to the stimulation of pyruvate dehydrogenase, which is the rate-limiting enzyme of glucose oxidation. In patients with NIDDM, short-term treatment with DCA was associated with a significant fall in triglycerides (Stacpoole et al, 1978). Clinical trials with DCA were discontinued due to one case of reversible neuropathy, but new DCA derivatives are now available (Stacpoole et al, 1987).

Elevation of free fatty acid levels is a well recognised feature of NIDDM, and there is evidence that this may be causally related to hyperglycaemia. Fatty acid release from adipocytes is dependent on the net balance between lipolysis and

re-esterification. In NIDDM there is resistance to insulin-mediated suppression of lipolysis, which is the rate limiting step in free fatty acid re-esterification. The principal mechanism by which free fatty acids may be causal in hyperglycaemia is in stimulating gluconeogenesis in the liver. In addition, Randle and colleagues (1963) showed that free fatty acids exacerbate insulin resistance in skeletal muscle.

Insulin therapy and other inhibitors of lipolysis, e.g. nicotinic acid and its analogue acipimox, reduce levels of free fatty acids and glucose (Dulbecco et al, 1989). Clinical trials with acipimox have shown useful effects on triglyceride levels and glycaemia (Fuccella et al, 1980), but rebound effects with these anti-lipolytic agents has focussed attention on different approaches to lowering elevated free fatty acid levels. One approach is with novel adenosine A-1 receptor antagonists, for example, GR79236 (Strong et al, 1993), which inhibit catecholamine induced lipolysis. In addition, a series of inhibitors of free fatty acid oxidation are presently in various stages of development. In particular, a group of drugs that irreversibly inhibit a key mitochondrial enzyme involved in long-chain fatty acid oxidation, carnitine palmitoyltransferase-1 (CPT 1) have entered clinical development (Foley 1992). For example, preliminary studies with etomoxir in patients with NIDDM using the euglycaemic hyperinsulinaemic clamp showed a 33% increase in insulin-stimulated glucose disposal (Ratheiser et al, 1991).

1.4 SCOPE OF THE THESIS.

In recent years it has become recognised that clustering of metabolic abnormalities is an inherent feature of the hypertensive individual; patients with untreated essential hypertension have higher than normal plasma insulin concentrations, are resistant to insulin-stimulated glucose uptake, and often have accompanying lipid abnormalities. The extent to which insulin resistance is involved in the initiation and maintenance of high blood pressure remains unknown, but these

observations have potentially important implications for long term hypertensive complications, as insulin has mitogenic properties and can potentiate vascular smooth muscle growth thus promoting structural changes and atherosclerosis.

Much recent interest has focussed on the potential confounding or beneficial effects of drugs on metabolic function. Antihypertensive and oral hypoglycaemic agents may have differential effects on carbohydrate metabolism and insulin resistance but their effects have often been poorly characterised because of concerns about the methodology used to assess insulin sensitivity and limitations of study design.

This thesis incorporates a series of studies of insulin sensitivity using the euglycaemic hyperinsulinaemic clamp in healthy male volunteers, patients with essential hypertension and patients with NIDDM.

The studies consist of 211 individual clamp procedures performed in 75 individuals. Chapter 3 is a series of 3 experiments performed in 24 healthy male volunteers and 6 patients with essential hypertension designed to evaluate critically the euglycaemic hyperinsulinaemic clamp technique. This research technique has been used for over 20 years for the *in vivo* determination of insulin mediated metabolic responses. Despite its widespread use, several aspects of the method have been poorly documented. This chapter firstly describes the validation of the technique in my hands, secondly evaluates the use of a heated hand box to achieve "arterialisation" of venous blood, and lastly analyses the metabolic and haemodynamic effects associated with euglycaemic hyperinsulinaemia and arterialisation of venous blood with a heated hand box.

Few studies in the literature which have evaluated the effects of antihypertensive drugs on insulin sensitivity have used a placebo-controlled, crossover study design. Chapters 4 and 5 evaluate the effects of two new antihypertensive agents on insulin induced metabolic responses. Chapter 4 evaluates the effects of the new dihydropyridine calcium antagonist, lacidipine, on whole body insulin sensitivity in 12 healthy volunteers and 6 patients with essential hypertension. As the dihydropyridine

calcium antagonists are associated with reflex sympathetic augmentation on acute dosing, both the acute and chronic effects of drug administration on insulin sensitivity are evaluated. Chapter 5 evaluates the effects of the ACE inhibitor trandolapril on whole body insulin sensitivity and metabolic parameters in 12 patients with both essential hypertension and impaired glucose intolerance or NIDDM.

At both *in vitro* and *in vivo* levels, numerous studies have suggested important pathophysiological links between glucose and insulin metabolism and the neuroendocrine systems involved in cardiovascular regulation, particularly the renin angiotensin system and the sympathetic nervous system; for example, the insulin antagonist effects of the catecholamines are well established. Insulin augments the aldosterone response to angiotensin II, there is *in vitro* evidence that angiotensin II may possess some glycogenolytic and gluconeogenic properties, and there are limited data that suggest that ACE inhibitor drugs may enhance insulin sensitivity, but the *in vivo* effects of angiotensin II on insulin mediated metabolic responses have not been previously reported. Chapter 6 examines the effects of angiotensin II on insulin stimulated metabolic responses using the euglycaemic clamp technique in 12 healthy male volunteers and 11 patients with NIDDM.

It is well recognised that even within a group of age matched healthy volunteers, insulin sensitivity may vary by more than threefold. Using data collected from all 75 subjects in whom insulin sensitivity was determined by the euglycaemic clamp technique, chapter 7 uses a multiple linear regression analysis to investigate the inter subject variability in insulin sensitivity and identifies factors which may be of clinical importance in predicting the insulin sensitivity of an individual.

CHAPTER 2

GENERAL METHODS

2.1. GENERAL CLINICAL PROTOCOL

Healthy male volunteers were recruited by advertising. Patients with essential hypertension were recruited from the cardiovascular risk factor clinic at the Western Infirmary and patients with non-insulin dependent diabetes mellitus (NIDDM) were recruited from Dr. M. Small's diabetic clinic at Gartnavel General Hospital or from the joint dietetic/medical obesity clinic at the Western Infirmary. In total, seventy-five subjects gave informed consent to participate in the projects, all of which were individually approved by the Research and Ethical Committee of Greater Glasgow Health Board (Western District).

Hypertensive patients were either newly diagnosed and previously untreated, or patients in whom current antihypertensive therapy was ineffective or poorly tolerated. Each patient discontinued any previous medication and, after a treatment-free period of at least 4 weeks, was entered into a study if blood pressure on three consecutive occasions was within the range 160/90 to 210/115 mmHg. Patients with secondary forms of hypertension, co-existent disease requiring regular prescription medication, or with a history of myocardial infarction in the preceding 12 months were excluded from participation.

All patients with NIDDM were diagnosed either by a documented random blood sugar of greater than 14 mmol/L, or by a 75 gram oral glucose tolerance test (OGTT) (WHO Study Group, 1987) [section 2.1.4]. No patients were receiving any oral hypoglycaemic agent or insulin therapy.

Before entering a study, all patients and volunteers underwent full clinical screening, performed by myself, including physical examination, routine biochemistry, haematology, urinalysis and an electrocardiogram to exclude other significant cardiovascular disease, or significant end-organ damage. All subjects had a 75 gram OGTT at screening.

After informed consent was obtained, each individual was asked to refrain from any strenuous exercise for the duration of the study and asked to maintain his or

her usual diet. The diabetic patients were advised to adhere to an isocaloric diabetic diet throughout the study consisting of approximately 55% carbohydrate, 25% fat and 20% protein.

The general clinical protocol for each study was similar. On each study day, following an overnight fast, each subject attended the CIRU at 8 a.m. After 20 minutes supine rest, baseline blood pressure and heart rate were recorded before the insertion of indwelling cannulae into a left antecubital vein and a right dorsal hand vein. The 3 hour euglycaemic hyperinsulinaemic clamp was then commenced, during which blood pressure and heart rate were measured at regular intervals. A standard light lunch was provided at the end of the procedure.

2.2. CLINICAL AND MORPHOMETRIC MEASUREMENTS.

2.2.1. Body mass index.

Body weight and height were measured with subjects in undershorts and without shoes to the nearest 0.5kg of weight and to the nearest 0.5cm of height. Body mass index (BMI) was calculated as:

$$\frac{\text{Body weight (kg)}}{\text{Body height (m)}^2}$$

2.2.2. Waist hip ratio.

Hip circumference, at the greater trochanter, and waist circumference, at the umbilicus, were measured to the nearest 0.5cm while the subject was standing.

2.2.3. Blood pressure and heart rate.

During all the clinical studies the technique of blood pressure and heart rate measurement was uniform. Systolic and diastolic blood pressure and heart rate were measured using a previously validated (Johnson & Kerr, 1985) Datascope Accutorr Semiautomatic Sphygmomanometer, maintained and calibrated at regular intervals by the Clinical Physics Department at the Western Infirmary. This machine uses an oscillometric technique for indirect blood pressure and direct heart rate measurement. All subjects had supine and erect measurements taken at screening. On each study day, duplicate recordings were made 30 minutes before and at 15 minute intervals during the euglycaemic hyperinsulinaemic clamp.

2.2.4. Oral glucose tolerance test.

An OGTT was performed in all subjects in the morning after ten hours of overnight fasting. The subjects were resting in the semi-recumbent position and took 75 grams of glucose orally with 100 mls of water after 20 minutes of rest. Blood was withdrawn from a peripheral venous cannula at time zero and at 30 minute intervals throughout the test for 2 hours for measurement of plasma glucose and serum insulin concentrations. The diagnosis of diabetes was made if the fasting plasma glucose was greater or equal to 7.8 mmol/L and/or the 2-hour post-load plasma glucose was greater or equal to 11.1 mmol/l. The diagnosis of impaired glucose tolerance was made if the fasting plasma glucose was less than 7.8 mmol/L and the 2-hour post load plasma glucose was between 7.8 and 11.1 mmol/L (WHO Study Group, 1987).

2.3. THE EUGLYCAEMIC HYPERINSULINAEMIC CLAMP.

In each individual study, the whole-body insulin sensitivity was assessed using a modification of the euglycaemic hyperinsulinaemic clamp described by DeFronzo et al (1979).

2.3.1. Infusions of insulin and glucose.

Two 18 gauge intravenous cannulae (*Venflon*, Helsinborg, Sweden) were inserted: the first retrogradely into a dorsal hand vein for blood sampling and the second antegradely into the contralateral antecubital fossa for administration of insulin and 20% Dextrose.

The infusion of soluble insulin (Actrapid, Novo Nordisc, Denmark) was prepared in 45 mls of 0.9% NaCl plus 5 mls of the patients' own blood to prevent adsorption of insulin to plastic surfaces, and was administered using a Braun Perfusor pump.

The insulin was administered as a primed, constant rate infusion at a rate of 1.5 mU/kg/min for 180 minutes, with the aim of achieving a steady state serum insulin concentration approximately 100 uU/ml above the basal fasting level. The primed infusion regimen used was as follows:

0-4 minutes	4.5 mU/kg/min
4-7 minutes	3.0 mU/kg/min
7-180 minutes	1.5 mU/kg/min

A variable rate infusion of 20% dextrose was administered to maintain euglycaemia (5.2 mmol/l). The 20% dextrose was infused from 4-180 minutes using an IMED IV infusion system (IMED, Abingdon, UK). The infusion rate was adjusted for body weight for each individual and expressed as mg glucose/kg/min. For

example, the infusion rate of 20% Dextrose equal to 1mg glucose per kilogramme per minute for a 70kg individual would be calculated as:

$$60 \text{ mins} + 200\text{g glucose} \times 70 \text{ kg} = 21 \text{ mls } 20\% \text{ Dextrose/hour.}$$

A 3 way tap enabled simultaneous infusion of the insulin and 20% Dextrose solutions.

At 5 minute intervals, 2 ml blood samples were collected from the cannulated dorsal hand vein surrounded by a heated box (55°C) with the aim of arterialisation of venous blood for plasma glucose measurements [section 2.3.3].

2.3.2. Calculation of whole body insulin sensitivity from the euglycaemic hyperinsulinaemic clamp.

Under steady state plasma glucose conditions, usually the last 40-60 mins of the clamp, the glucose infused must equal the glucose being removed out of the glucose space (i.e. glucose metabolised, M) provided that endogenous glucose production is completely suppressed. If hepatic glucose synthesis was quantified using labelled glucose, it would be possible to calculate peripheral glucose disposal; as this was not done, the measurements equate with whole body glucose disposal.

In practice, the glucose infusion (I) must be modified by two factors before it can be equated with M:

$$M = I - UC - SC$$

where I is the glucose infusion rate, UC is the correction for urinary glucose loss (only relevant in a hyperglycaemic clamp), SC is the space correction for inevitable variations from absolute euglycaemia, and all values are calculated in units of mg glucose/kg/min. This "space correction" (in mg glucose/kg/min) is calculated as

follows (De Fronzo et al, 1979):

$$SPACE\ CORRECTION = (5.2 - G) \times 17.86 \times 0.095$$

G = Ambient glucose concentration over last 40 minutes of the clamp in mmol/L.

17.86 = Conversion factor to mg/dl.

0.095 = Space correction.

Obviously if SC is negative (i.e. the glucose level has fallen), the correction is added to I and M is greater than I. If too much glucose has been infused, SC is subtracted from I. In clamps of good quality the SC is usually small; approximately 0.1 mg/kg/min. To calculate I the infusion rate over the last 40 minutes of the clamp is averaged. For example, if I was calculated between 120-140 minutes:

<u>Clamp Time</u>	<u>Infusion Rate</u>		
118mins	5.0		
120-----			
131	5.5	11x5	= 55
136	5.5	5x5.5	= 27.5
139	6.0	3x5	= 15
		1x6	= 6
140-----			
Total			103.5

$$I = \frac{103.5}{20\text{ mins}} = 5.17\text{ mg/kg/min}$$

2.3.3. Arterialisation of venous blood

A dorsal hand vein was cannulated in a retrograde fashion to enable placement of the hand into a cylindrical heated box (Department of Physiology,

Nottingham University, UK) which under thermostatic control maintains the ambient temperature at 55°C. The patency of the cannula was maintained with an infusion of 0.9% NaCl which was attached to a 3 way tap to facilitate venous sampling. During the euglycaemic hyperinsulinaemic clamp, approximately 100mls of 0.9% NaCl was administered via the dorsal hand vein cannula. The rationale behind the use of the heated hand box is discussed in chapter 1.3.3. The metabolic and haemodynamic effects of the heated hand box are discussed in chapter 3.

2.4. LABORATORY METHODS.

Venous blood samples for laboratory assay were withdrawn from the indwelling right dorsal hand cannula and collected into normal clotted, and chilled lithium heparin and EDTA tubes. Plasma was separated by centrifugation at 4°C for 15 minutes at 3000 rpm and stored at -70°C until assay. Whenever possible, within each study, the samples for each individual were performed in batches.

2.4.1. Serum insulin and C-peptide concentrations.

Human insulin and proinsulin originate as a single polypeptide chain known as proinsulin, which is formed on the surface of the rough endoplasmic reticulum in the beta cells of the islet of Langerhans. Proinsulin is then transported to the Golgi apparatus where it is packed into granules. It is in these granules that proinsulin is cleaved proteolytically into insulin and C-peptide. Insulin and C-peptide are stored in these beta granules until their secretion is stimulated, at which time approximately equimolar amounts of each are released into the portal vein. While insulin has a pervasive influence on the body, affecting virtually every organ and biochemical component, C-peptide has no known physiological function.

Serum insulin and C-peptide concentrations were measured using radioimmunoassay (INCSTAR) kits. To perform these equilibrium assays, samples are

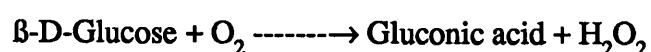
combined with an antibody and a tracer and incubated for 16 hours at 5°C. A pre-precipitated second antibody complex is then added as a single reagent. The assay is then centrifuged and decanted after 20 minutes incubation at 20°C.

The normal range for fasting serum C-peptide in our laboratory is 0.5-3.0 ng/ml. The normal range for fasting serum insulin in our laboratory is less than 25 uU/ml. The within-assay and between-assay coefficients of variation for C-peptide are 6.7% and 14.3% respectively. The within-assay and between-assay coefficients of variation for serum insulin are 6.4% and 8.5% respectively. For each study, all samples for each individual were performed in batch analysis.

2.4.2. Plasma glucose concentrations.

At baseline and at 30 minute intervals during the OGTT, and at 5 minute intervals throughout the euglycaemic hyperinsulinaemic clamp, blood samples were collected for plasma glucose measurements. Samples were collected into 1.5ml tubes and loaded into a Beckman microfuge (Beckman Instruments, Fullerton, California, USA) situated at the bedside. Samples were spun for 60 seconds. An Accustroke Pipet was then used to aspirate 10 microlitres of plasma. The pipetting technique included a post aspiration rinse of the disposable pipet in sterile water to avoid carryover effects from material retained on the outside of the tip. Samples were analysed using a Beckman Glucose Analyser II (Beckman Instruments, Fullerton, California, USA) which measures the rate of change in oxygen consumption when a sample is injected into an enzyme reagent sample.

When a sample is injected into the enzyme reagent solution, β -D-glucose from the sample combines with dissolved oxygen from the solution according to the reaction:



In the reaction, oxygen is consumed at the same rate as glucose reacts to form gluconic acid. At all times during the reaction, the rate of oxygen consumption is directly proportional to the concentration of glucose present in the reaction cup. The maximum observed rate has been shown to be a direct measure of the concentration of glucose originally in the sample at the time of injection (Kadish et al, 1968). The analyser measures and holds this maximum rate and permits the digital readout to be scaled, providing a number corresponding to glucose concentration in mmol/L.

On each study day, the Beckman glucose analyser was calibrated to 8.3mmol/L with an aqueous standard, and typically, checked once every ten samples during the euglycaemic hyperinsulinaemic clamp. The intra-assay coefficient of variation in plasma glucose concentrations was 3.6%.

2.4.3. Serum aldosterone concentrations.

Serum aldosterone concentration was measured by radioimmunoassay (Biodata, Milan) according to the technique described by MacKenzie and Clements (1974). Samples were incubated for 3 hours at 20°C with a tracer (¹²⁵I - Aldosterone); these two compete for binding sites on the antibody. After incubation the amount of tracer bound to the antibody is inversely proportional to the amount of aldosterone present in the plasma sample. Separation of the bound fraction is performed by addition of a second antibody coupled to magnetic particles and the immunocomplex is collected by the application of a magnetic field: the quantity of antibody-ligand complex is measured by radioactive counting using a gamma camera.

The normal range for serum aldosterone in the supine position in our laboratory is 12-150 pg/ml. The within-assay and between-assay coefficients of variation are 5.0% and 5.4% respectively.

2.4.4. Plasma renin activity.

Plasma renin activity was determined by radioimmunoassay of angiotensin I (Biodata, Milan). Before radioimmunoassay, plasma was incubated so that renin generated angiotensin I under standardised conditions. This then competes with a radioactive tracer (^{125}I - Angiotensin) for the binding sites of the antibody. After incubation the amount of tracer bound to the antibody is inversely proportional to the amount of renin present in the plasma sample. The separation of the bound from the free fraction is performed by addition of a double antibody coupled to magnetic particles. The application of a magnetic field allows the sedimentation of immunocomplexes, avoiding the centrifugation step. The normal range for plasma renin activity in the supine position in our laboratory is 0.12 - 1.59 ng/ml/AI/hour. The within-assay and between-assay coefficients of variation are 4.9% and 7.8% respectively.

2.4.5. Plasma catecholamine concentrations.

Plasma noradrenaline was assayed by high performance liquid chromatography with electrochemical detection (HPLC-ED) as previously described by Howes et al (1985). Catecholamines were extracted from plasma by acid-washed alumina (50mg) and were then eluted by vigorously mixing the alumina with 250ul of 0.2 M perchloric acid. A 200 ul aliquot of the eluate was injected into the liquid chromatograph onto a home packed 5 micron spherisorb octadecylsilane (ODS) reversed phase column. The normal range for resting supine plasma noradrenaline and plasma adrenaline is 0.3 - 7.5 nmol/l and 0 - 1.0 respectively. The within-assay and between-assay coefficients of variation are 15% and 13% respectively.

2.4.6. Plasma angiotensin II concentrations.

Plasma angiotensin II concentration was determined by radioimmunoassay according to the technique as described by Dusterdieck and McElwee (1971). Venous blood was collected into EDTA/phenanthroline to inhibit converting enzyme and angiotensinase enzymes. Angiotensin II was extracted from plasma using cartridges containing a reverse phase non-polar medium. The cartridges were pretreated with methanol and then water. Angiotensin II was eluted from the column with aqueous 20% methanol. The extracts were dried and redissolved in Tris buffer for assay. The normal range for plasma angiotensin II in the supine position in our laboratory is 3.0 - 12.0 pg/ml. The within-assay and between-assay coefficients of variation are 6.4% and 8.8% respectively (Morton and Webb, 1984).

2.4.7. Serum potassium concentrations.

Serum potassium ion activity was measured by a direct ion selective electrode on a Corning Model Na/K analyser (Ciba Corning Diagnostics, Halstead, Essex, UK).

2.4.8. Blood gas analysis.

Samples for blood gas analysis were collected into heparinised syringes and immediately placed on ice. Oxygen saturation and oxygen concentration were measured on a Corning 288 analyser (Ciba Corning Diagnostics, Halsted, U.K.).

2.4.9. Glycosylated haemoglobin concentrations.

Glycosylated haemoglobin (HbA_{1c}) was determined by electroendosmosis using a Corning Glytrac Haemoglobin Set (Corning 470064, U.S.A.). The reference range of the Biochemistry Laboratory at Gartnavel General Hospital Glasgow, derived from the endogenous population, is 4.8% to 7.8%.

2.4.10. Plasma angiotensin converting enzyme activity.

Angiotensin converting enzyme (ACE) converts the decapeptide angiotensin I to the octapeptide angiotensin II through cleavage of the carboxy-terminal dipeptide histidyl-L-leucine. The assay that was used to determine plasma ACE activity is based on an HPLC technique for measuring the rate of release of hippuric acid from an artificial substrate of angiotensin I (Chiknas,1979). One unit of enzyme generates one nanomole of hippuric acid per minute and the normal range in our laboratory for plasma ACE activity is 5-32 EU/ml. The inter- and intra-assay coefficients of variation are 6% and 2% respectively, with a limit of detection of 0.1 EU/ml.

2.4.11. Plasma lipid and lipoprotein concentrations

Plasma and lipoprotein cholesterol concentrations were measured using a CHOD-PAP kinetic method (Boehringer Mannheim, East Sussex, U.K.), standardised with primary cholesterol standards (Reference Laboratory for Cholesterol Assays, Bilthoven, the Netherlands). Triglycerides were measured using an enzymatic method (Merck Diagnostics, Darmstadt, Germany). Measurements were performed using a Cobas Bio Analyser (Roche Diagnostics, Welwyn Garden City, U.K.).

2.5. STATISTICAL ANALYSIS.

All data were formally checked to ensure a Gaussian distribution by plotting normal plots using the statistical package Minitab. Coefficients of variation (c.v.) were calculated according to the method of Bland (1987).

Whole-body insulin sensitivity in mg/kg/min was calculated under steady

state conditions during the last 40 minutes of each 'clamp' (DeFronzo et al, 1979). Insulin sensitivity values for individual subjects on each study morning were compared between treatments by analysis of variance (ANOVA) using the statistics package, Rummage, and the Bonferroni method for calculating 95% confidence intervals. To ensure that the euglycaemic clamps were of good quality, the c.v. of glucose measurements from time 0-180 minutes of all the clamps, and the mean plasma glucose levels over the last 40 minutes of the clamps for each treatment period were calculated.

Measurements of serum insulin, C-peptide, triglyceride, potassium, plasma renin activity, plasma aldosterone and plasma noradrenaline at individual time points were compared between study mornings by repeated measures ANOVA.

Blood pressure and heart rate measurements were evaluated by repeated measures ANOVA.

Linear regression analysis was used to investigate the relationship between the derived value for insulin sensitivity, and factors such as patients age, body mass index, fasting serum insulin concentration and baseline blood pressure [see chapter 7.2].

Results throughout are expressed as mean \pm standard deviation.

CHAPTER 3

THE EUGLYCAEMIC HYPERINSULINAEMIC CLAMP:
AN EVALUATION OF THE METHODOLOGY INCLUDING THE
ARTERIALISATION OF VENOUS BLOOD

3.1. INTRODUCTION:

The euglycaemic clamp [chapter 1.2.3] has been used extensively over the last 15 years to quantify *in vivo* insulin sensitivity. Despite some recognised limitations of this technique (Bergman et al, 1989), the clamp has found widespread application in both cardiovascular and endocrine research but a number of important technical aspects of the method have not been clearly documented. For example:

(1) since the original report by DeFronzo and colleagues (1979), in which reproducibility was assessed in only 4 volunteers, few investigators have evaluated the intra-subject variation in metabolic responses across a range of glucose disposal rates (and without the confounding influence of dietary or drug interventions).

(2) whether insulin sensitivity should be expressed in terms of glucose uptake (M) or the insulin sensitivity index (S_{IP}) is controversial, and the merits of these two parameters have not been directly compared in the same individuals.

(3) the optimal duration of the clamp is unclear: although glucose requirements may increase steadily for up to 5 hours (Doberne et al, 1981), infusions are usually maintained for either 2 or 3 hours, but differences in insulin sensitivity measurements from 120 versus 180 minute clamps have not been clearly evaluated.

(4) the importance of sampling "arterialised" blood from a heated dorsal hand vein - a cumbersome and sometimes painful procedure - is disputed (Andrews et al, 1984), and few studies have assessed the overall impact of hand-warming on both the haemodynamic and metabolic response to a euglycaemic clamp.

Before embarking on the main series of studies that comprise this thesis, the aim of this study was to address some of these methodological issues pertinent to the

euglycaemic hyperinsulinaemic clamp itself. In particular, (1) to evaluate the reproducibility of insulin sensitivity (expressed as M and S_{IP}) in subjects with a range of glucose disposal rates - i.e. both insulin sensitive and insulin resistant; (2) to compare the quality of information obtained from clamps of 120 and 180 minutes duration; and (3) to assess the haemodynamic and metabolic effects of hand-warming using a purpose-built warm air device (manufactured commercially by the Department of Physiology and Pharmacology, University of Nottingham).

3.2. SUBJECTS AND METHODS.

3.2.1. General methods.

A total of twenty-four healthy male volunteers (age range 20-34 years, BMI 22.1 ± 2.2 kg/m²), and six patients (4M, 2F), with mild to moderate essential hypertension (age range 49-59 years, BMI 26.2 ± 4.6 kg/m²), gave written consent to participate in these studies. The 6 patients and 12 volunteers in chapter 4 [table 4.1] who underwent two euglycaemic hyperinsulinaemic clamps while taking placebo therapy are included in the reproducibility analysis (protocol A).

In each study, the general clinical protocol was similar. All subjects attended the Clinical Research and Investigation Unit (CIRU) at 8 am following an overnight fast. In each study, whole-body insulin sensitivity was assessed with the euglycaemic hyperinsulinaemic clamp as described in chapter 2.3. At baseline, and at 60-minute intervals during the 3-hour clamp, blood samples were collected for measurements of serum insulin, C-peptide and potassium concentrations. In addition, in 4 healthy subjects, insulin concentrations were measured at 5 minute intervals for the first 60 minutes of the clamp to evaluate the serum insulin profile induced by the priming phase of the insulin infusion (chapter 2.3). Measurements of supine blood pressure and heart rate were recorded at 15 minute intervals during the clamp.

3.2.2. Study designs

Three separate studies were performed:

Protocol A: Specific aim: to evaluate the reproducibility of M values and S_{IP} values derived from the euglycaemic clamp.

Eighteen healthy volunteers and six patients with essential hypertension attended for 2 study mornings at least 1 week apart. On each occasion, a 3 hour clamp was performed with blood sampled from a heated dorsal hand vein for the determination of insulin sensitivity. In both the volunteer group and the patient group, the inter-subject and intra-subject variability of whole body insulin sensitivity was assessed by calculating the coefficient of variation (c.v.) [chapter 2.5]. In addition, insulin sensitivity was calculated using data from two time periods of the 3 hour clamp; 80 to 120 minutes, and 140 to 180 minutes. The reproducibility of S_{IP} and the insulin stimulus were also calculated for the same time periods.

Protocol B: Specific aim: to evaluate the effects of hand warming on plasma glucose concentrations and oxygen saturation of venous blood.

Six healthy volunteers attended for 2 study mornings at least 1 week apart. On each occasion, a 3 hour clamp was performed with the following modifications: (1) a cannula was inserted into the antecubital fossa of *both* arms for infusions of glucose and insulin [right arm], and for sampling of mixed venous blood [left arm]. In addition, a cannula was inserted into a dorsal hand vein of the right arm which was heated with the warm air box (55°); and (2) at 10 minute intervals antecubital and "arterialised" blood samples were collected simultaneously for analyses of plasma glucose and (every 60 minutes) oxygen saturation. The glucose infusion rate was adjusted according to the arterialised plasma glucose concentrations.

Protocol C: Specific aim: to evaluate the effects of hand warming on haemodynamic and metabolic responses.

Six healthy volunteers attended for 2 study mornings 1 week apart. On each occasion, a 3 hour clamp was performed. On the first study day arterialised blood (i.e. with the warm air box) from a heated dorsal hand vein was used to adjust the glucose infusion rate required to maintain euglycaemia. On the second study day, mixed venous blood (i.e. without the warm air box) from the antecubital fossa was used to guide the glucose infusion and calculate insulin sensitivity. The order of the 2 study days was randomised.

For each study, whole-body insulin sensitivity (M) in mg glucose/kg/min was calculated under steady state conditions during the last 40 minutes of each clamp, as described in chapter 2.3. The S_{IP} was calculated as the steady state ratio of the increment in glucose uptake (ΔR_d) to the increment in plasma insulin concentration (ΔI), normalised to the ambient plasma glucose concentration (G) at which the clamp was performed (Bergman et al, 1989): $S_{IP} = 100 \times \Delta R_d / (\Delta I \times G)$. Values for M and S_{IP} for individual subjects on each study morning were compared between treatments by analysis of variance [chapter 2.5].

3.3. RESULTS.

3.3.1. Evaluation of priming phase of the insulin infusion.

Individual profiles of serum insulin in 4 healthy subjects during the first 60 minutes of the clamp are shown in figure 3.1. The priming protocol tended to cause an "overshoot" in serum insulin concentration within the first 10 minutes, but a steady-state plateau was achieved thereafter, with insulin concentrations approximately 80uU/ml above baseline (fasting) levels (figure 3.1).

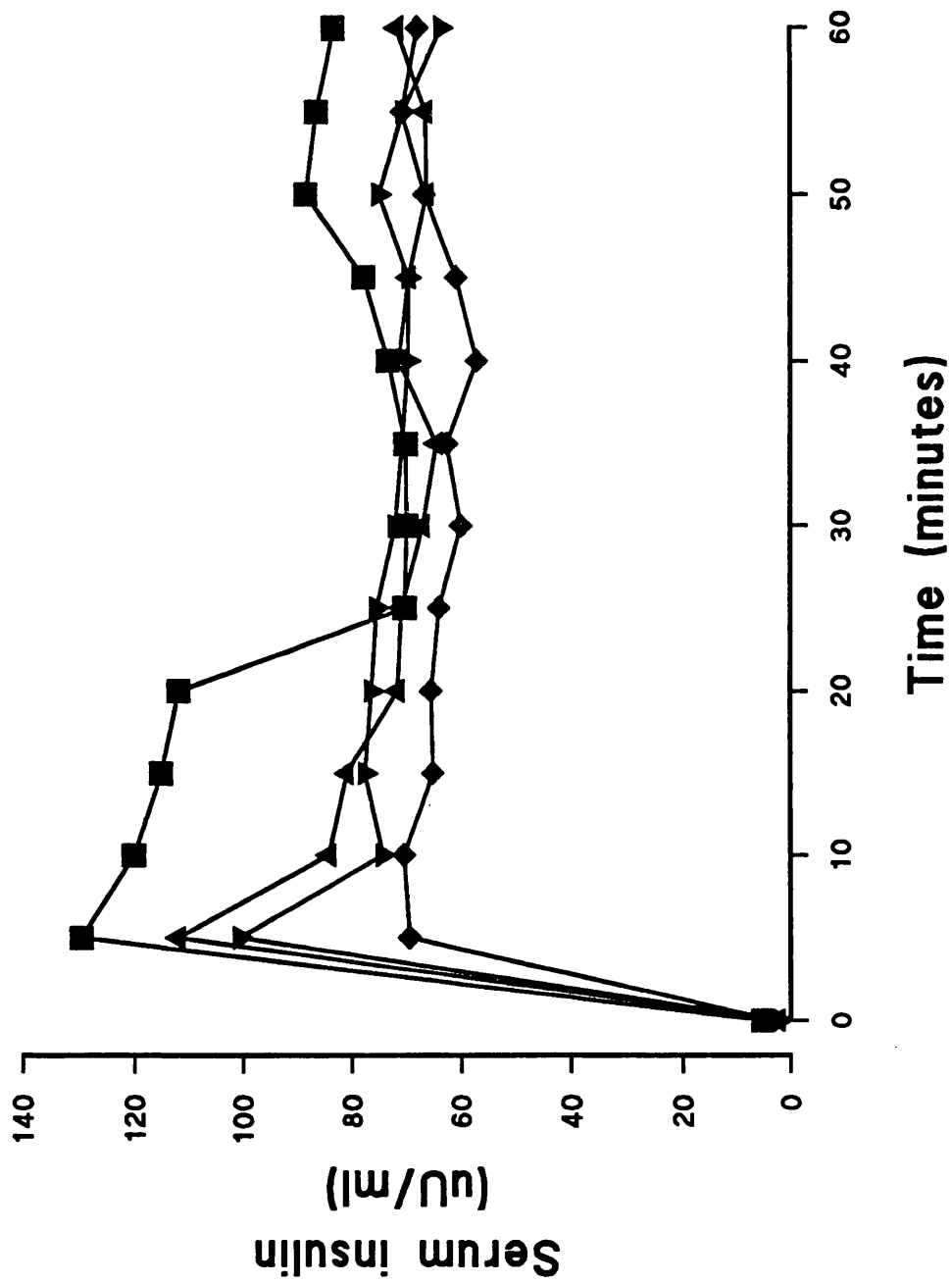


Figure 3.1: Individual serum insulin profiles of 4 healthy subjects during the first 60 minutes of a euglycaemic clamp

3.3.2. Protocol A: Evaluation of the reproducibility of the technique.

There was no significant difference between the steady state plasma glucose concentration at 80-120 minutes compared with 140-180 minutes; mean values were 5.1 ± 0.2 and 5.2 ± 0.4 mmol/L, respectively, for the healthy volunteers and correspondingly 5.3 ± 0.4 and 5.2 ± 0.2 mmol/L for the hypertensive patients. Satisfactory euglycaemia was maintained throughout the clamps as reflected by the mean c.v. of plasma glucose concentrations of 7% for healthy subjects and 8% for hypertensive patients. In both healthy male volunteers and patients with essential hypertension mean values for M and S_{IP} calculated for the period of 140-180 minutes were significantly higher than values obtained at 80-120 minutes; for example, in healthy volunteers M was 9.8 ± 2.0 mg/kg/min at 180 minutes compared with 9.0 ± 1.9 mg/kg/min at 120 mins ($p < 0.001$). S_{IP} values for the corresponding time points were 8.1 ± 2.6 and 8.8 ± 2.7 mg/kg/min per uU/ml (x 100) respectively ($p < 0.001$) (table 3.1). Intra-subject reproducibility was the best for M values derived from clamps of 180 minutes duration; for example, the c.v. was 6% in the healthy subjects and 5% in patients with essential hypertension. S_{IP} values, however, demonstrated greater variability; the corresponding c.v.'s for S_{IP} values at 180 minutes were 21% and 13% respectively (table 3.1).

There was wide inter-subject variability in insulin sensitivity in both groups of subjects. In healthy subjects and essential hypertensives the inter-subject c.v. in M at 180 minutes was 30% and 47% respectively. The corresponding c.v for the inter-subject variability in the insulin stimulus was 20% and 36% respectively (table 3.1). There were no significant changes in blood pressure or heart rate during the hyperinsulinaemic stimulus in the normotensive or hypertensive subjects.

3.3.3. Protocol B: Evaluation of arterialisation of venous blood.

"Arterialised" samples had higher and less variable oxygen saturations than

		HEALTHY		SUBJECTS	
		MEAN	RANGE	INTERSUBJECT C.V. (%)	INTRASUBJECT C.V. (%)
M VALUES mg kg/min	120 mins	9.0 +/- 1.9	5.3 - 13.0	22	8
	180 mins	9.8 +/- 2.0	5.6 - 13.8	21	6
INSULIN SENSITIVITY INDEX	120 mins	8.1 +/- 2.6	3.0 - 16.3	32	21
	180 mins	8.8 +/- 2.7	3.7 - 18.1	30	21
INSULIN STIMULUS uU/ml	120 mins	118 +/- 26	93 - 220	22	12
	180 mins	117 +/- 24	88 - 208	20	10
		HYPERTENSIVE		PATIENTS	
		MEAN	RANGE	INTERSUBJECT C.V. (%)	INTRASUBJECT C.V. (%)
M VALUES mg kg/min	120 mins	5.9 +/- 2.5	1.5 - 10.0	43	14
	180 mins	6.6 +/- 2.5	2.0 - 10.3	38	5
INSULIN SENSITIVITY INDEX	120 mins	5.4 +/- 2.6	0.7 - 8.7	48	17
	180 mins	6.4 +/- 3.0	1.0 - 9.9	47	13
INSULIN STIMULUS uU/ml	120 mins	121 +/- 40	90 - 221	33	15
	180 mins	115 +/- 41	82 - 197	36	10

Table 3.1: Table summarising the reproducibility of M values and insulin sensitivity index values derived from time periods 80-120 minutes and 140-180 minutes during the euglycaemic hyperinsulinaemic clamp in 18 healthy subjects and 6 patients with essential hypertension.

Insulin sensitivity index is expressed as the increase in glucose uptake divided by the increment in plasma insulin concentration (mg/glucose/kg/min per uU insulin x 100).

mixed venous blood withdrawn simultaneously (figure 3.2). For example, at 180 minutes O₂ saturation was 95±2% in arterialised venous blood compared with 79±18% in mixed venous blood. In keeping with this, there was a difference between arterialised and mixed venous plasma glucose concentrations; 5.2±0.2 vs. 4.5±0.4 mmol/L at 150 minutes (figure 3.3). This difference in plasma glucose was smallest at the beginning of the procedure and increased progressively until 60 minutes. The difference between arterialised and mixed venous blood glucose concentrations correlated significantly with the difference in arterialised and mixed venous oxygen saturations ($r=0.609, p<0.01$).

3.3.4. Protocol C: Evaluation of metabolic and haemodynamic effects of hand warming.

Mean steady state plasma glucose concentrations during the two clamps were 5.2±0.4 and 5.1±0.4 mmol/L for the arterialised and non-arterialised day respectively. Mean steady state increments in serum insulin concentration were similar in both studies; the mean c.v. of the insulin stimulus was 7% and 8% for the arterialised and non-arterialised days respectively. Individual values obtained for M during the clamp studies are seen in table 3.2. It is apparent that individual values for M were quite comparable, but there was a small but significant trend for the derived measurement of insulin sensitivity to be higher using arterialised as compared with mixed venous blood; 11.7±3.2 mg/kg/min compared with 10.5±3.0 mg/kg/min ($p<0.05$). Confidence intervals for this observation were 0.4,1.9. Blood pressure profiles of the two days are shown in figure 3.4. There was a significant reduction in blood pressure on the days when arterialised venous blood was used; e.g. 6 mmHg reduction in diastolic blood pressure at 120 minutes ($p<0.05$), and a corresponding increase in heart rate (figure 3.4).

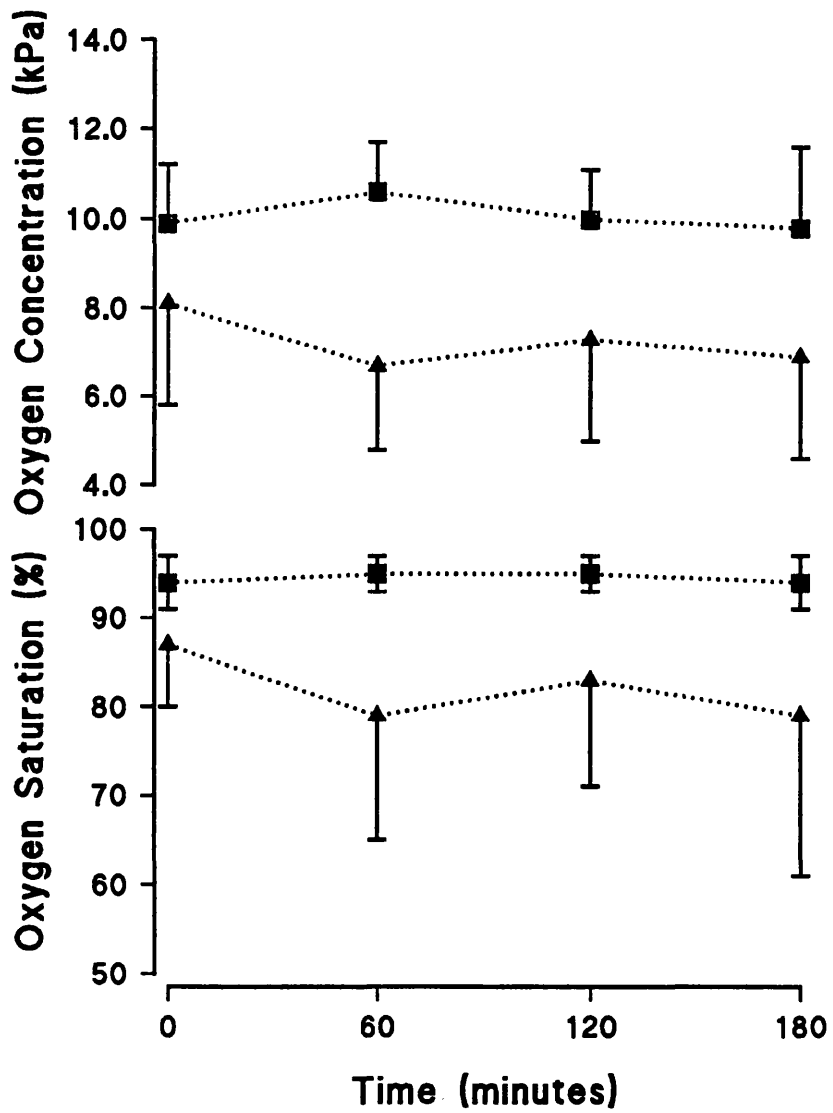


Figure 3.2: Oxygen saturations during euglycaemic hyperinsulinaemic clamp
 ■ = arterialised venous blood, ▲ = mixed venous blood.

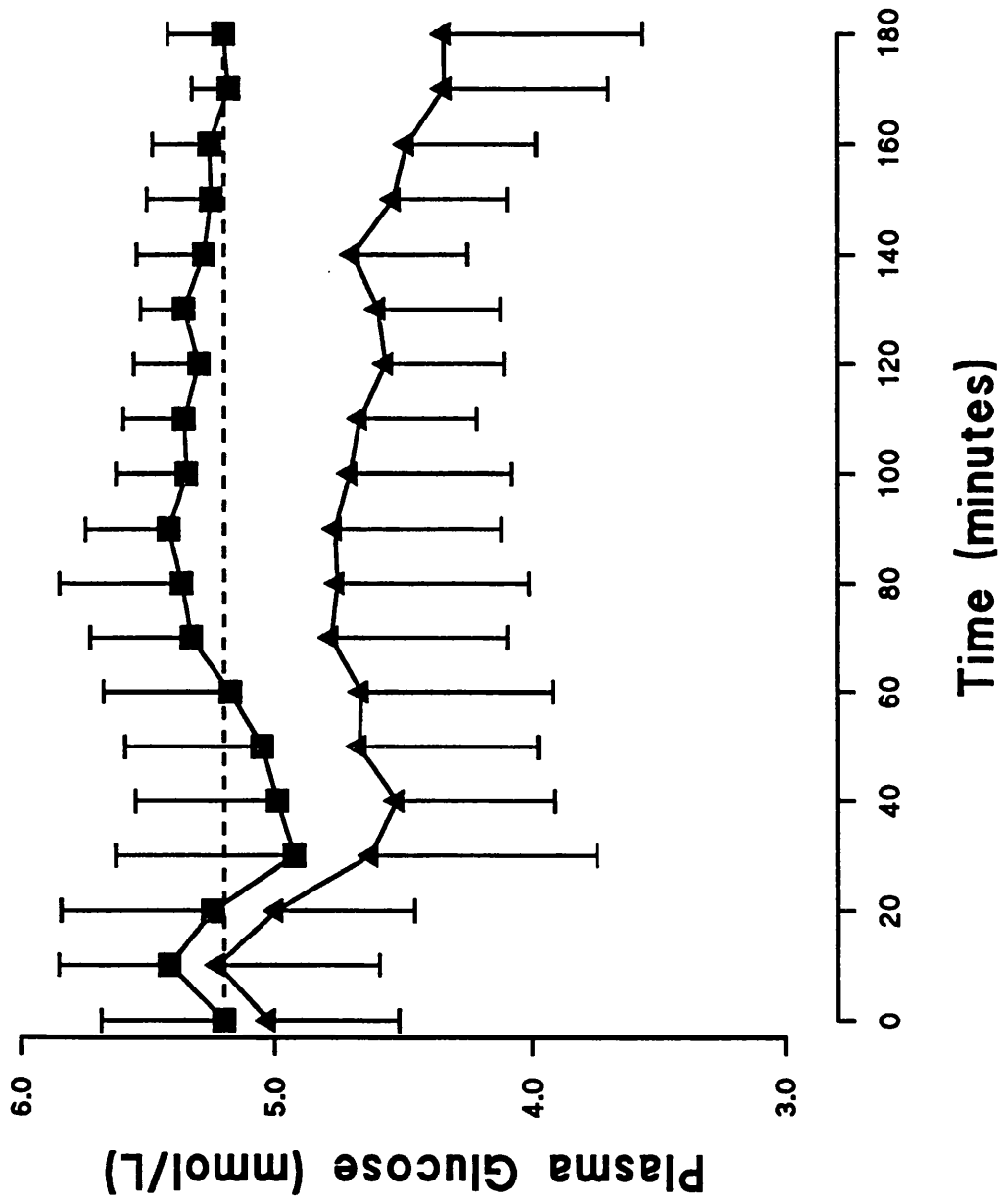


Figure 3.3: Plasma glucose concentrations during euglycaemic clamp.
 ■ = arterialised venous blood, ▲ = mixed venous blood.

Subject No.	M value Arterialised Blood	M value Mixed venous Blood	Difference
1	11.6	11.4	0.2
2	12.8	11.5	1.3
3	13.1	11.4	1.7
4	10.0	9.6	0.4
5	6.6	5.2	1.4
6	16.2	14.1	2.2
Mean S.D	11.7 3.2	10.5 3.0	1.2 0.7

Table 3.2: Individual M values (mg glucose/kg/min) of 6 healthy subjects who underwent 2 euglycaemic hyperinsulinaemic clamps one week apart. Insulin sensitivity values derived from the clamp were significantly greater on the day when arterialised blood was used to adjust the glucose infusion rate.

($p < 0.05$) 95% confidence intervals; 0.4, 1.9.

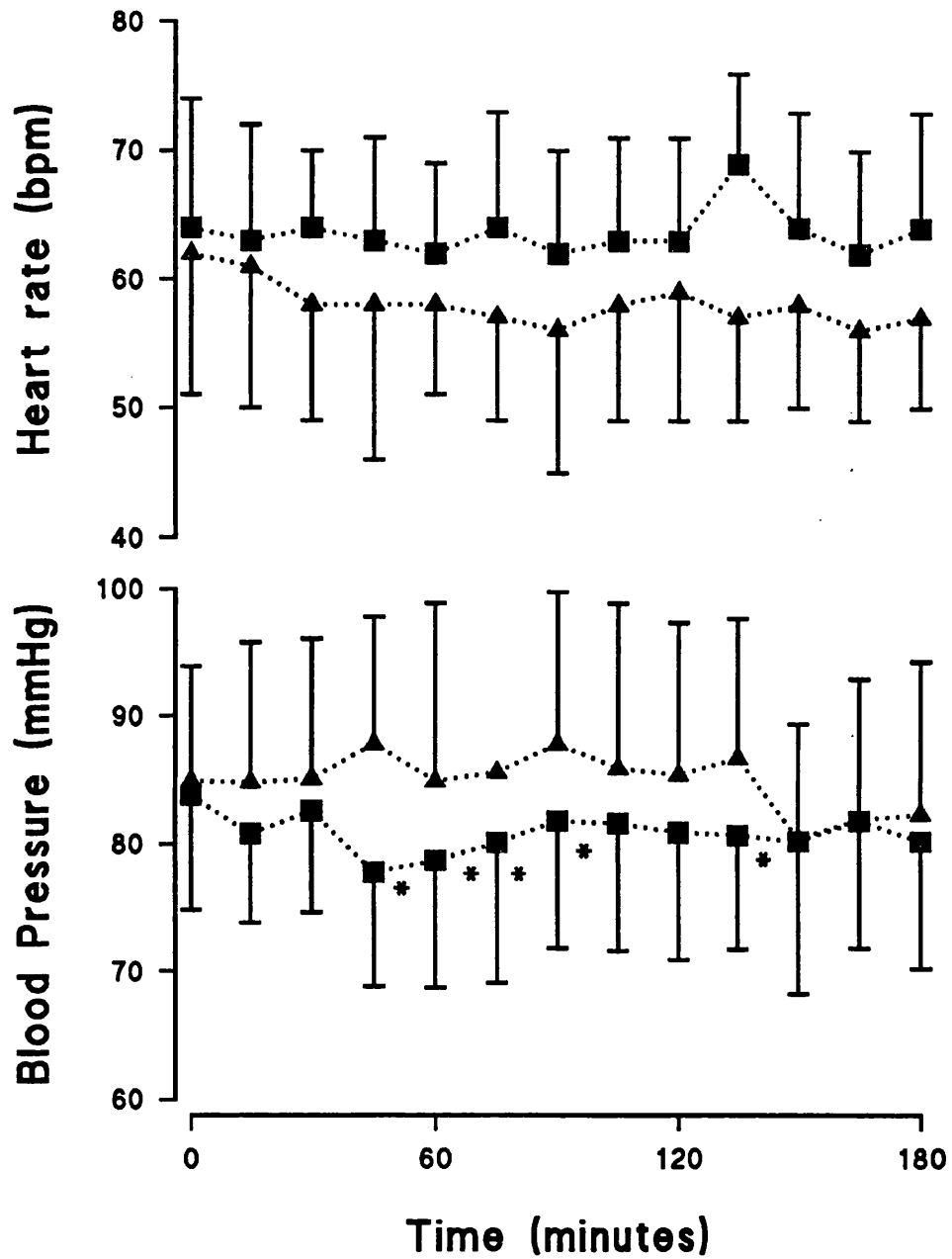


Figure 3.4: Changes in mean blood pressure and heart rate in 6 healthy subjects during the euglycaemic clamp performed with and without arterialisation of venous blood

■ = arterialised venous blood, ▲ = mixed venous blood.

* = $p < 0.05$ vs. corresponding mixed venous blood timepoint

3.4. DISCUSSION.

Although the euglycaemic hyperinsulinaemic clamp has been widely practised for over 15 years, important aspects of the methodology have been poorly validated. With more widespread application of the technique it is important that the methodology is subjected to critical evaluation. To the best of my knowledge, this is the largest study to report the intra-subject reproducibility of M and S_{IP} values derived from the clamp. The only available data comes from DeFronzo's original description of the technique. He studied 4 healthy subjects, 3 to 4 weeks apart, and reported mean M values of 8.5 ± 0.8 mg/kg/min on the first study day, compared with 8.4 ± 0.8 mg/kg/min on the second day. My studies have therefore demonstrated good reproducibility of M values derived from the clamp in both healthy subjects and hypertensive patients, but have confirmed large inter-individual variations; insulin mediated glucose disposal varied almost threefold in healthy subjects and more than fivefold in patients with hypertension. This variation in insulin sensitivity in healthy subjects is consistent with that reported by others (Reaven, 1988a) and cannot entirely be explained by differences in BMI or age. These results serve to highlight the importance, whenever possible, of using a crossover rather than parallel group design when evaluating insulin mediated metabolic responses with the clamp. This large variation in glucose disposal rates may in part be related to the variation in the insulin stimulus observed; even in this group of apparently homogeneous male subjects, the weight adjusted insulin infusion produced steady-state insulin concentrations which varied almost threefold [table 3.1]. Insulin clearance rates are known to vary with obesity (Meistas et al, 1983) and age (Minaker et al, 1982) and these variables may account for the differences observed in the hypertensive patients but not in the group of healthy subjects.

A notable feature of this study is the poor reproducibility of S_{IP} values despite the clamps achieving stable euglycaemia and glucose disposal rates as demonstrated by the acceptable coefficients of variation of the glucose measurements from 0-180

minutes. Bergman (1989) advocates the use of S_{IP} when reporting values of insulin sensitivity derived from clamp experiments. He argues that a number of difficulties are associated with the use of M values derived from clamp experiments as the value of M obtained is highly dependent on the ambient glucose and insulin concentrations obtained which are difficult to match among groups. Bergman warrants that the S_{IP} is not confounded by non-insulin mediated glucose uptake and does not change over the physiological range of insulin or glucose (Bergman et al, 1989). Thus, the S_{IP} is widely used but there are no published data on its reproducibility. My results demonstrate marked variability in the S_{IP} within an individual from day to day. My data therefore suggest that despite the theoretical advantages of using S_{IP} values from clamp experiments, M values are less variable and are probably a better index for comparison of possible changes in insulin sensitivity derived from clamp experiments.

The utility of the euglycaemic hyperinsulinaemic clamp technique is limited by its labour intensiveness; furthermore, it is time consuming for patients and volunteers. Many investigators have attempted to minimise discomfort to subjects by performing 2 hour clamps. My data suggest that 2 hour clamps have acceptable reproducibility but may underestimate insulin sensitivity by up to 10% when compared with the 3 hour clamp. In contrast, Reaven's group reported an underestimation of up to 20% in obese subjects and patients with NIDDM (Doberne et al, 1981). It is notable however that the reproducibility of the 2 hour clamp in the hypertensive subjects was the poorest at 14% - this may reflect a longer time to reach steady state in these insulin resistant subjects; a phenomenon observed in insulin resistant obese individuals (Reaven et al, 1988b). Therefore, a three hour clamp is optimal when evaluating insulin sensitivity, especially in groups of patients with insulin resistance.

In analysing whole body glucose metabolism, measurement of arterial rather than venous glucose concentrations has been preferred because arterial kinetic data represent an average of the processes occurring in the tissues comprising the total body, whereas venous data, although more easily obtainable in man, will be affected by the particular tissue which is being drained. During the hyperinsulinaemic stimulus

of the clamp, there is increased extraction of glucose into skeletal muscle, which gives rise to a large and variable arterio-venous difference in plasma glucose concentration. Arterial cannulation would therefore be desirable during the clamp but tends to be avoided in clinical research protocols because of increased risks compared with venous cannulation. Investigators have compromised by sampling from a heated hand vein (McGuire et al, 1976). As the hand has relatively little skeletal muscle bulk, heating effectively shunts blood from the arterial to the venous side of the circulation. The extent to which this is effective, or necessary is controversial; additionally, it remains unknown whether this local application of heat may have systemic effects on cardiovascular or metabolic variables. For example, Liu and colleagues (1992) evaluated the effects of the warm air box in 6 healthy subjects during hyperinsulinaemic euglycaemia and hypoglycaemia. They concluded that the heated hand technique sufficiently arterialises venous blood so that the glucose measurement in the arterialised blood gives a reasonable estimate of the true arterial value. They noted however that venous blood from the opposite forearm was also markedly arterialised, probably reflecting a generalised vasodilator effect of heating. There were no haemodynamic measurements in this study. Gallen and Macdonald (1990) evaluated the effects of two methods of hand heating for 60 minutes on body temperature, forearm blood flow and oxygen saturation. Again, adequate arterialisation was achieved by the use of the warm air box, but a consistent (non-significant) increase in forearm blood flow was recorded above baseline values.

My study demonstrates that the arterialisation technique used can sufficiently arterialise venous blood and achieve a blood oxygen saturation close to the true arterial value as reported by others (Liu et al, 1992). The variability in saturation in the non-arterialised blood was much higher when compared with arterialised blood; 54%-97% and 91%-97% respectively. The high non-arterialised blood oxygen saturation in our study is likely to be, in some individuals at least, a result of heating the contralateral hand for 180 minutes resulting causing a vasodilating effect on the forearm superficial vasculature with increased cutaneous blood flow (Gallen &

MacDonald, 1990).

As the heated hand box possibly exerts a confounding influence on the results derived from euglycaemic hyperinsulinaemic clamps, my last study addressed; (1) whether results obtained from the use of mixed venous blood in clamp studies differ from results using arterialised venous blood; and, (2) the overall impact of hand warming on haemodynamic variables. Previous studies have reported that results are not appreciably diminished if mixed venous blood is substituted for arterialised blood (Andrews et al, 1984; Wahab et al, 1993). However, in the study reported by Wahab and colleagues (1993), "mixed venous" blood was sampled in the antecubital fossa of the arm ipsilateral to the heated hand; therefore it may have been partially arterialised, obscuring any possible difference. My results suggest, however, that in healthy subjects, values of insulin sensitivity derived from mixed venous blood are significantly lower than clamps using arterialised blood. This is somewhat surprising in view of the results of my second study which demonstrate effective arterialisation of venous blood by heating the dorsal hand vein: in this study the plasma glucose concentration of the mixed venous blood was consistently lower than that of arterialised blood. It would therefore be expected that if mixed venous blood glucose concentrations are used to alter the glucose infusion rate, the calculated value for insulin sensitivity would be consistently higher than the value derived from arterialised blood samples. The haemodynamic data (i.e. an increase in heart rate and decrease in blood pressure) may provide an explanation for this apparent paradox: there is evidence that skeletal blood flow is an important determinant of glucose disposal in man (Julius et al, 1991) [chapter 1.1.4]. It is possible that the heating of the hand for 3 hours may cause a significant increase in perfusion of skeletal muscle blood flow by reducing systemic blood pressure via sympathetic deactivation. There are reports of increased forearm blood flow when the contralateral hand is heated with a hot blanket (Abramson et al, 1965) and in the study of Gallen and MacDonald (1990). Interestingly, there is evidence that lower body negative pressure may induce acute insulin resistance in the human forearm by a decrease in blood flow and reflex

sympathetic activation (Jamerson et al, 1993). Without direct measures of forearm blood flow or sympathetic tone it is impossible to assess however whether dorsal hand heating affects insulin sensitivity by altering muscle perfusion.

In summary, this study has evaluated the methodology of the euglycaemic clamp technique. In particular:

(1) My studies have confirmed the reproducibility of the clamp in a group of subjects with a range of glucose disposal rates but have questioned the value of the S_{IP} as a measure of insulin sensitivity derived from the clamp. The use of a 2 hour clamp is reproducible but underestimates insulin sensitivity by 10%. It is important that study designs take account of the large inter-individual variations in insulin sensitivity and steady-state insulin concentrations observed even in a group of homogeneous healthy male subjects. Whenever possible, a crossover study design should be employed and the investigator should remain blind to the treatment under investigation.

(2) The heated hand box effectively provides arterialised blood samples with oxygen saturations similar to those of arterial blood. My data suggest, however, that the heated hand box may exert independent confounding effects on whole body insulin sensitivity via vasodilatory and haemodynamic actions. Further studies incorporating measurements of skeletal muscle blood flow will be required to evaluate the systemic vasodilator effects of the heated hand box.

CHAPTER 4

THE EFFECTS OF THE DIHYDROPYRIDINE CALCIUM ANTAGONIST
LACIDIPINE ON INSULIN SENSITIVITY
IN NORMOTENSIVE AND HYPERTENSIVE SUBJECTS

4.1. GENERAL INTRODUCTION

Although the metabolic effects of antihypertensive drugs have been widely reported, few, if any, previous studies with calcium antagonists have used a placebo-controlled, cross-over design and many have obtained only indirect measurements of insulin sensitivity from oral glucose tolerance tests [chapter 1.3.2]. Thus, inadequate study designs may partly explain why there are so many conflicting reports about the effects of dihydropyridine calcium antagonists on insulin sensitivity and glycaemic control; for example, nifedipine has been shown to impair glucose tolerance in both diabetic (Bhathagar et al, 1984) and non-diabetic subjects (Oehman et al, 1985), whereas others have reported neutral metabolic effects with nifedipine (Landmark 1985), nisoldipine (Odigwe et al, 1986) and amlodipine (Ferrari et al, 1991b). There is also evidence that dihydropyridines may enhance insulin sensitivity following a glucose load (Beer et al, 1993; Giugliano et al, 1980).

This placebo-controlled, crossover study evaluates the effects of a new long-acting dihydropyridine, lacidipine, on insulin sensitivity in normotensive and hypertensive subjects.

4.1.1. Clinical pharmacology of lacidipine.

Lacidipine (Glaxo, Italy) is a new calcium antagonist which has been granted a licence in the United Kingdom as once daily therapy for the treatment of hypertension. It has a pharmacological profile typical of the dihydropyridine group of agents; vascular selectivity and extensive first pass metabolism. It causes a dose-related fall in vascular resistance accompanied by reflex tachycardia at single doses of 3-5mg in normal subjects (Hall et al, 1991). Lacidipine does not affect sino-atrial or atrioventricular dysfunction in healthy subjects or patients with atrial fibrillation (Frank et al, 1990). Lacidipine is rapidly absorbed and undergoes extensive first pass

metabolism to inactive metabolites. Absolute bioavailability is 2-9% and reported half-life 1-3 hours; protein binding is greater than 90% (Hall et al, 1991). In acute dose studies of lacidipine in essential hypertension, single doses of 4-6 mg were required to produce a definite pharmacodynamic effect (Perelman 1991). Despite its remarkably short half-life there are data suggesting lacidipine has a sustained duration of action with 24 hour blood pressure control following single daily dosing (Lyons et al, 1994; Heber et al, 1990). For example, after one weeks treatment, lacidipine 4-6 mg resulted in a significant reduction in blood pressure 24 hours after the last dose when compared to placebo. After single doses, lacidipine 4 mg has similar efficacy to that of nifedipine 10 mg but a longer duration of action (Perelman 1991). In chronic therapy, lacidipine 4-6 mg has been compared with hydrochlorothiazide (Chiariello, 1991; Leonetti et al, 1991a), atenolol (United Kingdom Lacidipine Study Group, 1991) and nifedipine (Leonetti et al, 1991b) in a series of double-blind, parallel group dose titration studies. Efficacy at the end of the dosing interval was similar to that of hydrochlorothiazide 25 - 50 mg daily, atenolol 50 - 100 mg daily or slow release nifedipine 20 - 40 mg daily. However, in the comparator groups, response rate was generally higher and fewer patients required dose titration.

Thus, the available data suggest that lacidipine may have comparable antihypertensive efficacy to a range of conventional alternative agents. Despite its short half-life there are data suggesting that a sustained antihypertensive response may be achieved across 24 hours following once-daily dosing. This long duration of action has been attributed to accumulation in a cellular lipid compartment, allowing continuous release into the binding site of the calcium channel (Herbette et al, 1993).

There are no studies evaluating the effects of lacidipine on glucoregulatory mechanisms. This placebo-controlled study evaluates the effects of single and multiple doses of lacidipine on whole-body insulin sensitivity in healthy male subjects and in patients with essential hypertension using the euglycaemic hyperinsulinaemic clamp.

4.2. SUBJECTS AND METHODS.

Twelve healthy male volunteers and six patients with a diagnosis of essential hypertension gave written informed consent to participate in the study (table 4.1.).

Following routine screening as outlined in chapter 2.1.1, each subject received lacidipine 4 mg once daily (Glaxo, Italy) and matching placebo, each for 2 weeks, in a randomised, double-blind crossover design with a 2-week washout period between each treatment phase (figure 4.1).

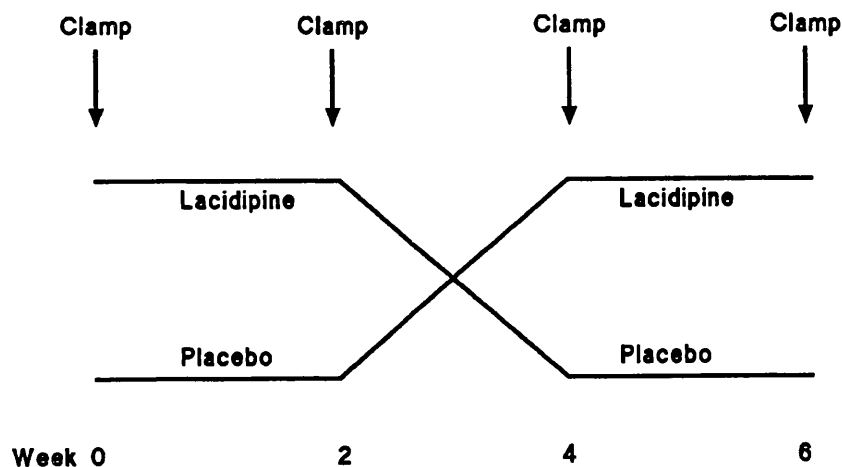


Figure 4.1: Schematic of study design.

Each subject attended four 5-hour study mornings to evaluate the metabolic effects of acute and chronic lacidipine and the corresponding placebo administrations. On each occasion, following an overnight fast, subjects attended the CIRU at 0745 am. After 20 minutes supine rest, baseline blood pressure and heart rate measurements were recorded (i.e. 24 hours after the last dose). Venous cannulae were inserted and subsequently lacidipine or placebo was administered orally with 100 mls water.

<u>VOLUNTEER</u> <u>No.</u>	<u>AGE</u>	<u>BMI</u> <u>(kg/m²)</u>	<u>SEX</u>
1	25	22.8	M
2	20	24.2	M
3	23	19.6	M
4	20	20.1	M
5	23	25.9	M
6	20	21.0	M
7	24	26.5	M
8	20	28.7	M
9	29	21.6	M
10	23	21.6	M
11	18	22.0	M
12	20	22.22	M

<u>PATIENT NO.</u> <u>No.</u>	<u>AGE</u>	<u>BMI</u> <u>(kg/m²)</u>	<u>SEX</u>	<u>Screen BP</u>	<u>Duration of</u> <u>Hypertension</u>	<u>Previous</u> <u>Treatment</u>
1	59	26.7	F	182/88	5 years	nil
2	66	29.3	F	184/82	8 years	nil
3	60	34.3	M	162/93	5 years	amlodipine 10mg o.d.
4	46	21.0	F	148/91	16 years	labetolol 100mg b.d.
5	46	25.2	F	156/98	18 years	nil
6	49	27.2	M	159/94	5 years	nil

TABLE 4.1: Details of hypertensive patients and healthy subjects in lacidipine study

On each study day, whole-body insulin sensitivity was calculated using the euglycaemic hyperinsulinaemic clamp as described in chapter 2.3. The clamp was commenced 60 minutes after drug administration. Systolic and diastolic blood pressure and heart rate (HR) were recorded in duplicate 60 minutes before and at 15 minute intervals during the clamp. At baseline, and at 60-minute intervals during the 3-hour clamp, additional blood samples were collected for measurements of serum insulin, C-peptide, serum potassium, triglyceride, plasma noradrenaline, plasma renin activity and serum aldosterone concentrations.

It was calculated from data in chapter 3 that the volunteer arm of this study would have greater than 80% power to detect a 15% change in insulin sensitivity.

4.3. RESULTS: (I) NORMAL VOLUNTEERS.

4.3.1. Tolerance

Lacidipine was generally well tolerated; apart from transient headache in one subject, no adverse events were reported, there were no significant adverse biochemical events, e.g. hypoglycaemia or hypokalaemia, and there were no withdrawals from the study. One subject had an episode of micturition syncope at time 138 minutes following acute lacidipine administration. The plasma glucose at the time of symptoms was 5.1 mmol/L; his supine blood pressure was 74/50, pulse rate 47 bpm. The subject made a prompt recovery.

4.3.2. Blood pressure and heart rate.

There were no statistically significant differences in supine blood pressure between the 4 study days in this group of normotensive volunteers. For example, average blood pressure between 0-180 mins was 124/63 mmHg after the first dose of

lacidipine compared with 125/62 mmHg following placebo (figure 4.2). There was a trend towards higher heart rates following active treatment: on average (0-180 mins) 68 and 69 bpm after the first dose and two weeks of lacidipine compared with 62 and 61 bpm following placebo (figure 4.3). The increase in heart rate was maximal and significantly greater than the corresponding placebo 30 - 75 minutes after the start of the clamp, i.e. 90 - 135 minutes after drug administration, and was observed following acute dosing and after 2 weeks of drug administration.

4.3.3. Serum insulin and C-peptide concentrations.

Serum insulin levels increased to a plateau within the first 60 minutes of the infusion and there were no significant differences in insulin concentration between the 4 study days (figure 4.4); for example, at 120 minutes, serum insulin concentrations were 125.3 ± 55.4 and 116.9 ± 37.2 uU/ml for acute and chronic lacidipine respectively, compared with 122.6 ± 35.7 and 116.3 ± 29.8 uU/ml on the corresponding placebo study days. The acute rise in serum insulin concentration was associated with a significant decrease in endogenous insulin release. C-peptide concentrations at 120 -180 minutes were 25-30% lower than baseline fasting levels (figure 4.4.) ($p < 0.001$).

4.3.4. Plasma glucose and whole-body insulin sensitivity.

Lacidipine had no significant effect on insulin-stimulated glucose uptake. Mean values for insulin sensitivity were 8.9 and 9.6 mg/kg/min after first dose and 2 weeks lacidipine respectively, compared with 9.1 and 9.7 mg/kg/min following placebo. Confidence intervals for this observation were -0.9,1.3 for 2 weeks placebo versus 2 weeks lacidipine. Individual values for whole body insulin sensitivity on the four study days are shown in table 4.2.

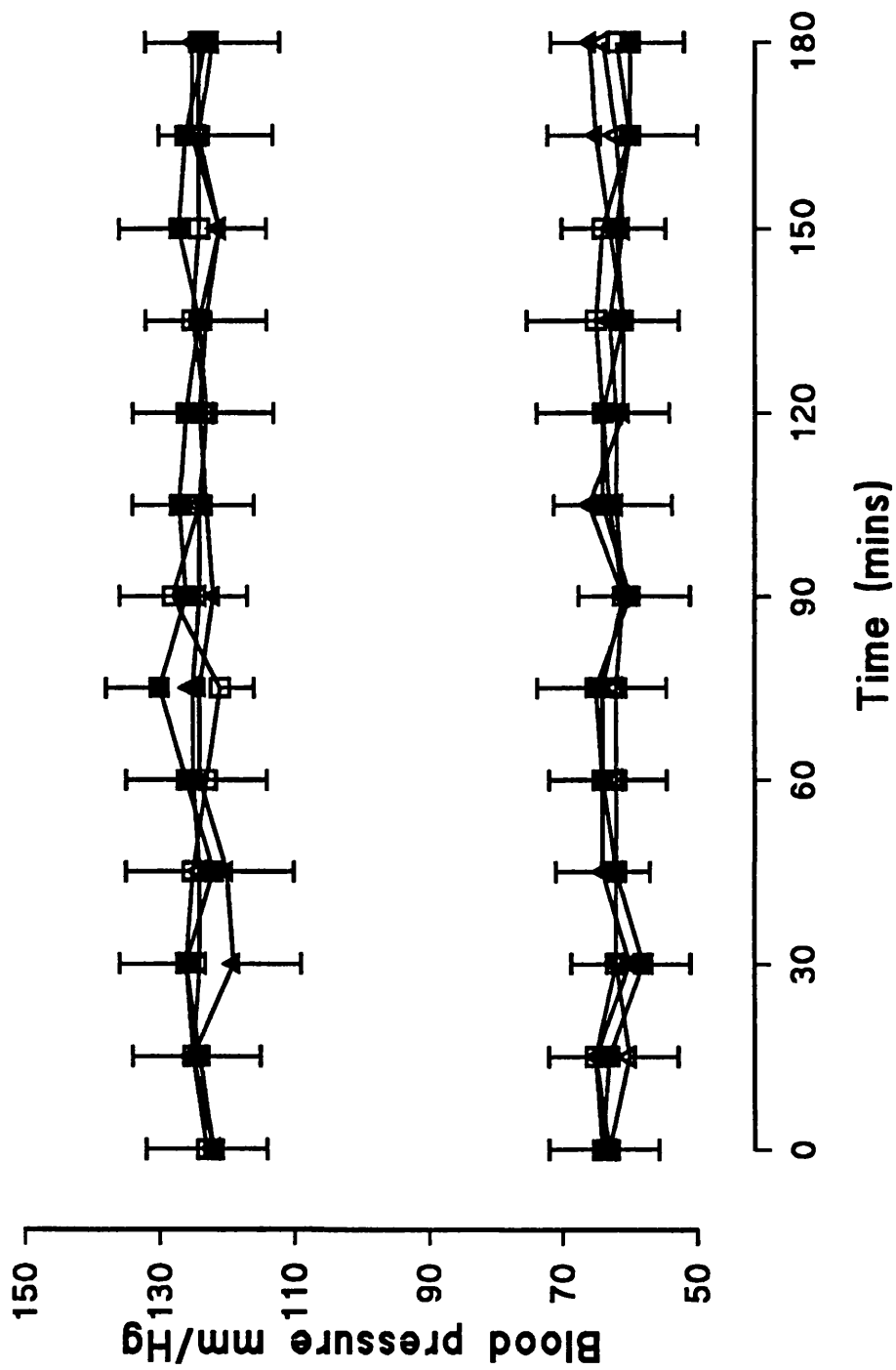


Figure 4.2: Mean profiles of supine systolic and diastolic blood pressure of healthy subjects during euglycaemic clamp in lacidipine study.

■ = acute placebo ▲ = 2 weeks placebo

□ = acute lacidipine Δ = 2 weeks lacidipine

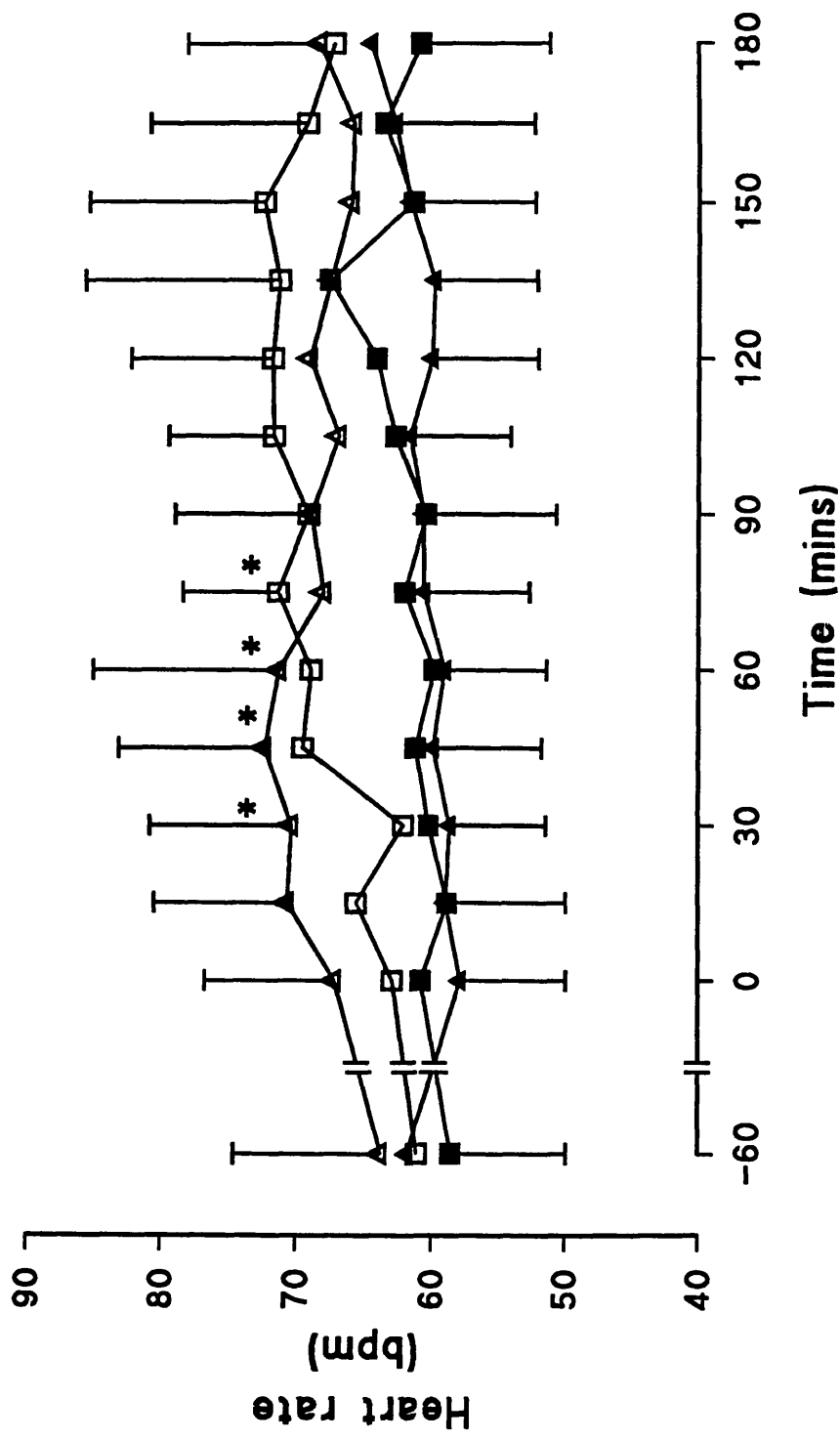


Figure 4.3: Mean profiles of supine heart rate of volunteers during euglycaemic clamp in lacidipine study
 ■ = acute placebo ▲ = 2 weeks placebo □ = 2 weeks lacidipine Δ = 2 weeks lacidipine
 * = p<0.001 lacidipine versus corresponding placebo administration

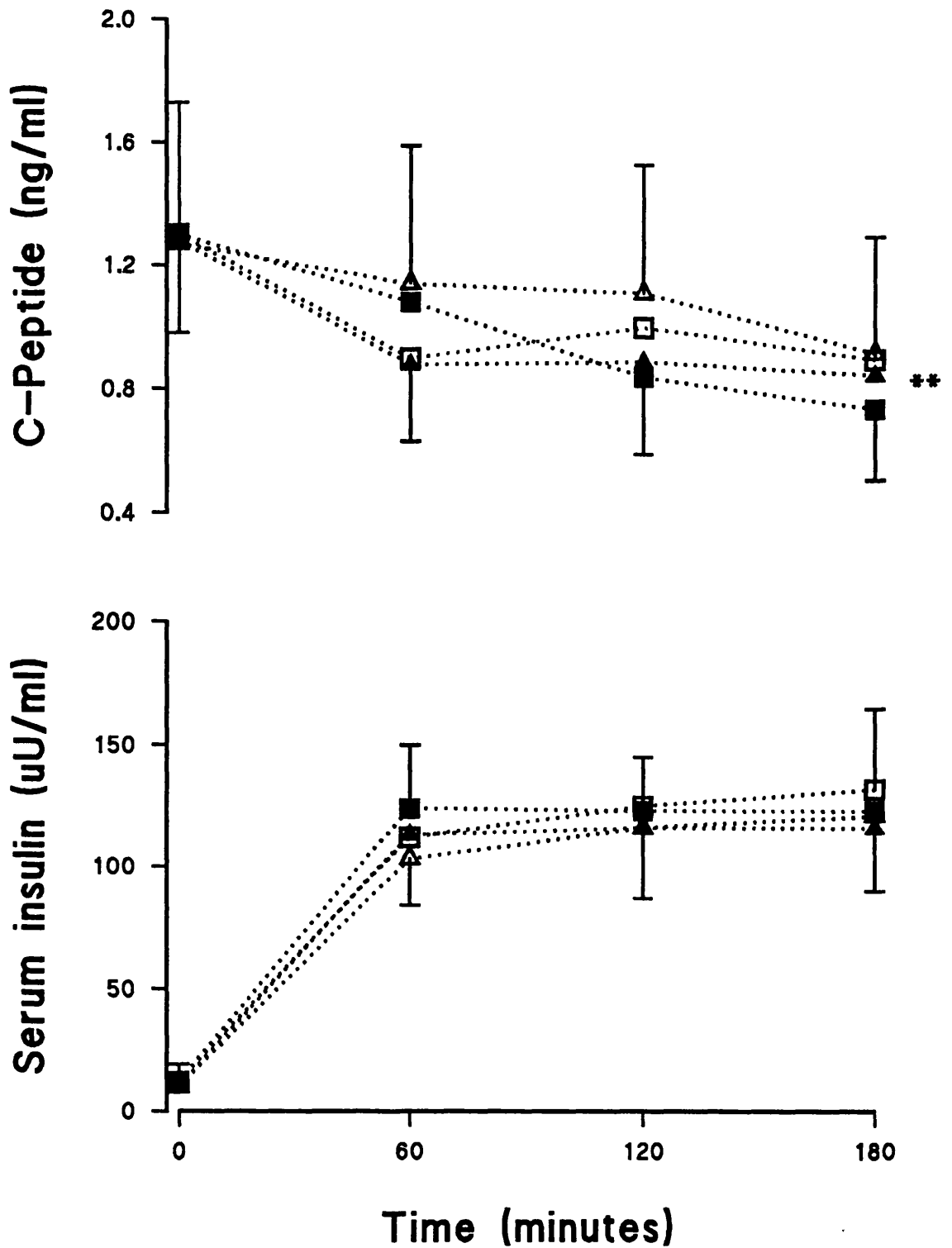


Figure 4.4: Mean profiles of insulin and C-Peptide of healthy subjects during euglycaemic clamp in lacidipine study

■ = Acute placebo ▲ = 2 weeks placebo

□ = Acute lacidipine. △ = 2 weeks lacidipine

** = $p < 0.001$ 180 minutes versus baseline fasting values

SUBJECT	FIRST DOSE PLACEBO	2 WEEKS PLACEBO	FIRST DOSE LACIDIPINE	2 WEEKS LACIDIPINE
1	5.6	6.8	6.3	7.0
2	8.7	7.4	9.4	8.3
3	9.6	10.0	10.0	12.0
4	11.0	11.7	11.4	12.0
5	8.8	8.9	9.4	8.5
6	8.5	10.2	5.9	7.1
7	10.6	10.4	9.0	11.0
8	7.5	9.8	8.4	6.7
9	9.9	10.4	9.2	8.5
10	11.7	11.8	13.0	11.8
11	7.9	10.3	6.5	10.5
12	9.6	9.2	8.9	11.5
MEAN	9.1 +/- 2.0	9.7 +/- 1.5	8.9 +/- 1.6	9.6 +/- 2.1

Table 4.2: Individual values for whole body insulin sensitivity of healthy subjects in lacidipine study.
Values expressed as mg glucose/kg/min.

4.3.5. Serum triglyceride concentrations.

After 2 weeks treatment with lacidipine there was a significant reduction in fasting serum triglyceride concentrations at baseline: 0.7 ± 0.3 mmol/l vs 0.9 ± 0.6 ($p < 0.01$). In addition, there were significant reductions in serum triglyceride concentrations during each clamp; for example, after 2 weeks of lacidipine, triglyceride levels fell from 0.7 ± 0.3 to 0.5 ± 0.3 mmol/l at 180 mins, compared with a corresponding change of 0.9 ± 0.6 to 0.7 ± 0.5 mmol/l after placebo. There was no significant effect of lacidipine on insulin-stimulated reductions in triglyceride concentration during the clamp (figure 4.5).

4.3.6. Serum potassium concentrations.

There was a significant reduction in serum potassium concentration at the end of each clamp: on average 0.5mmol/l (figure 4.5). However, the insulin-stimulated reductions in serum potassium concentration were not significantly different between the four study days. The reduction in serum potassium was maximal within 60 minutes of commencing the infusion; there were no further significant reductions thereafter.

4.3.7. Plasma noradrenaline, plasma renin activity and serum aldosterone.

Baseline plasma renin activity was significantly higher after 2 weeks lacidipine compared with placebo; for example, 3.25 ± 0.60 ng A1/ml/hr after 2 weeks of lacidipine compared with 1.82 ± 0.79 ng A1/ml/hr following 2 weeks placebo (figure 4.6). There were no significant differences in plasma renin activity, serum aldosterone or catecholamines during the clamp (figure 4.7).

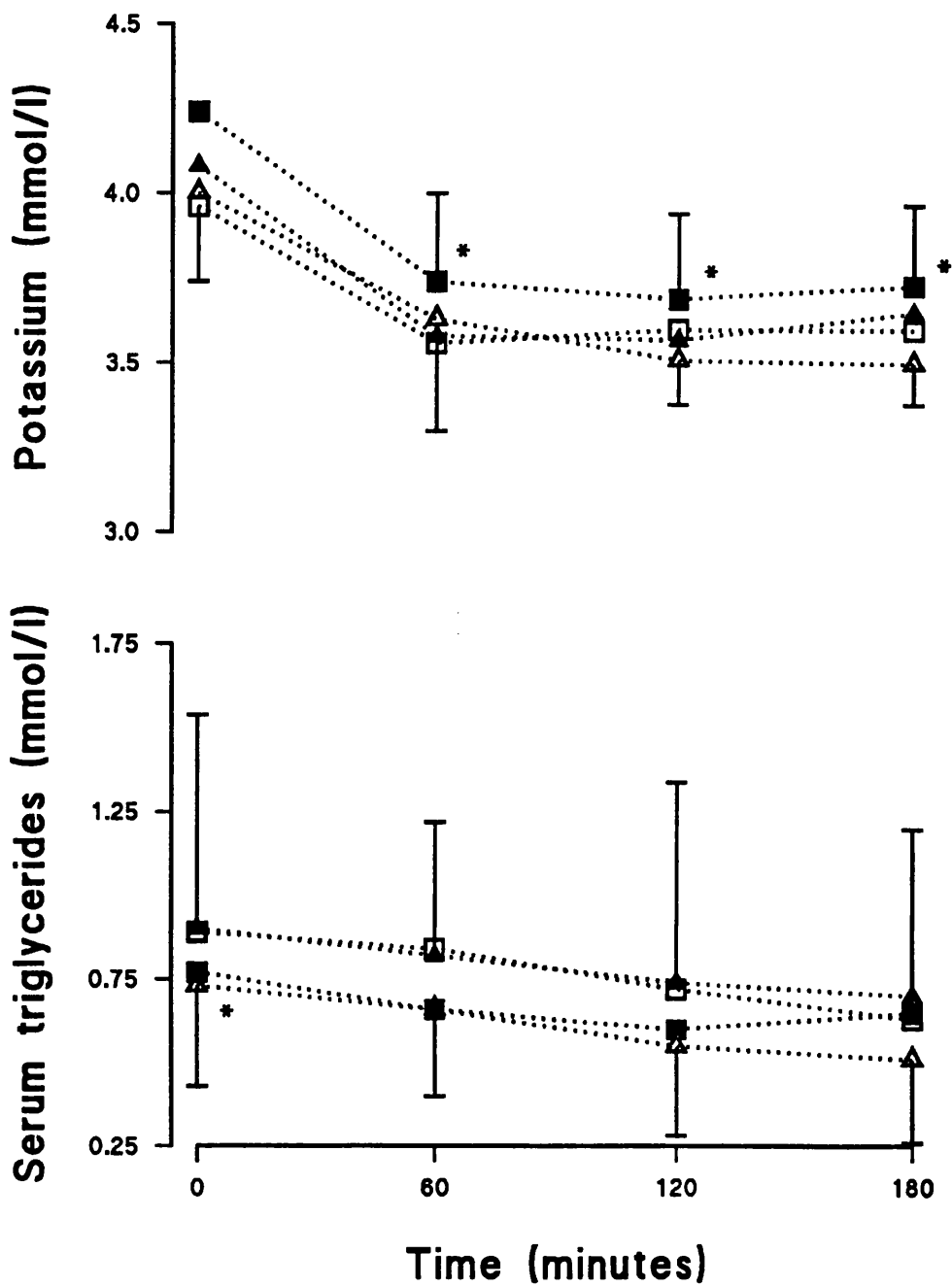


Figure 4.5: Mean profiles of serum potassium and serum triglyceride concentrations of healthy subjects during euglycaemic clamp in lacidipine study.

■ = acute placebo ▲ = 2 weeks placebo □ = acute lacidipine △ = 2 weeks lacidipine
 * = $p < 0.01$ versus corresponding baseline value

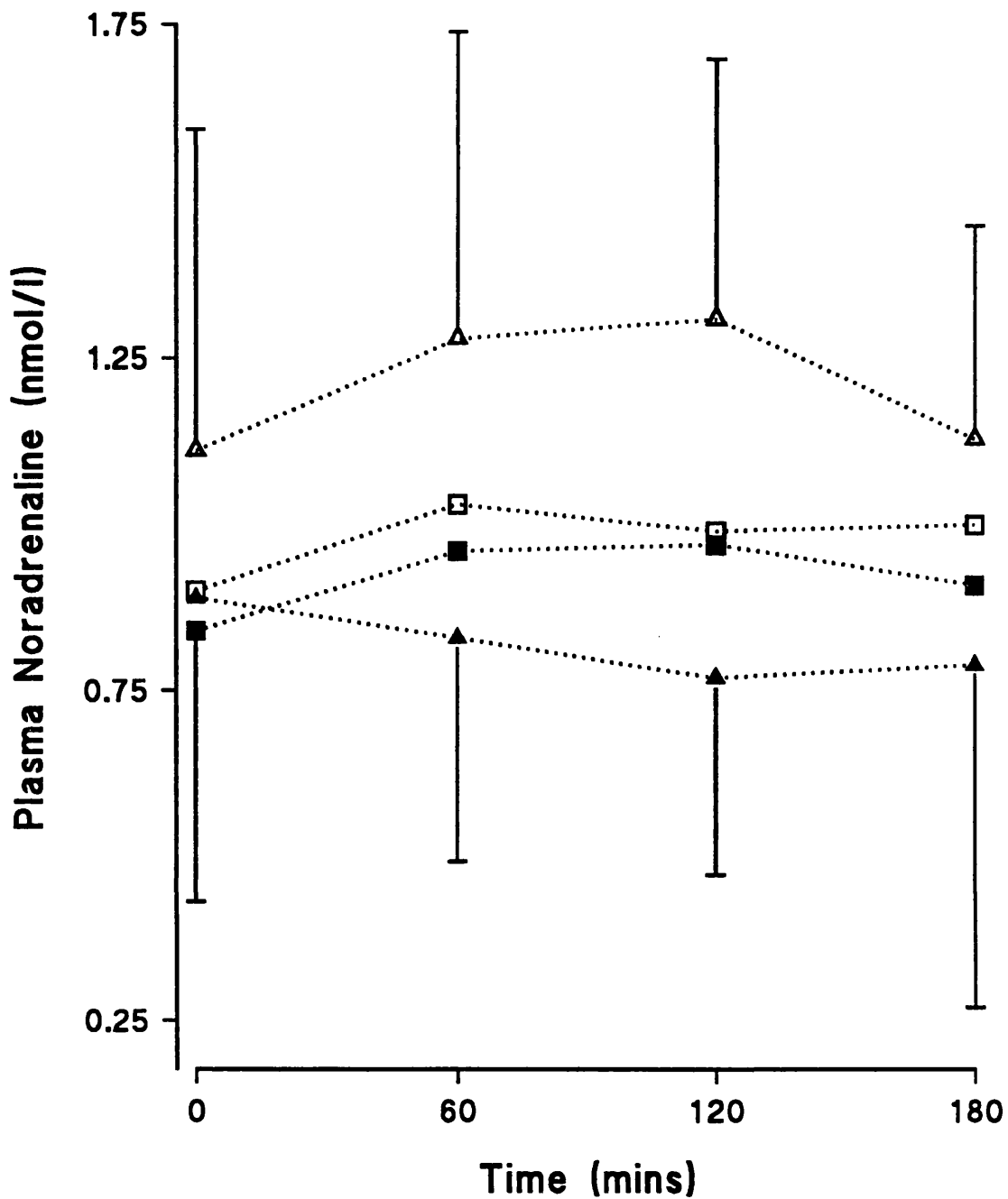


Figure 4.6: Mean profiles of noradrenaline concentrations of healthy subjects during euglycaemic clamp
 ■ = acute placebo ▲ = 2 weeks placebo
 □ = acute lacidipine △ = 2 weeks lacidipine

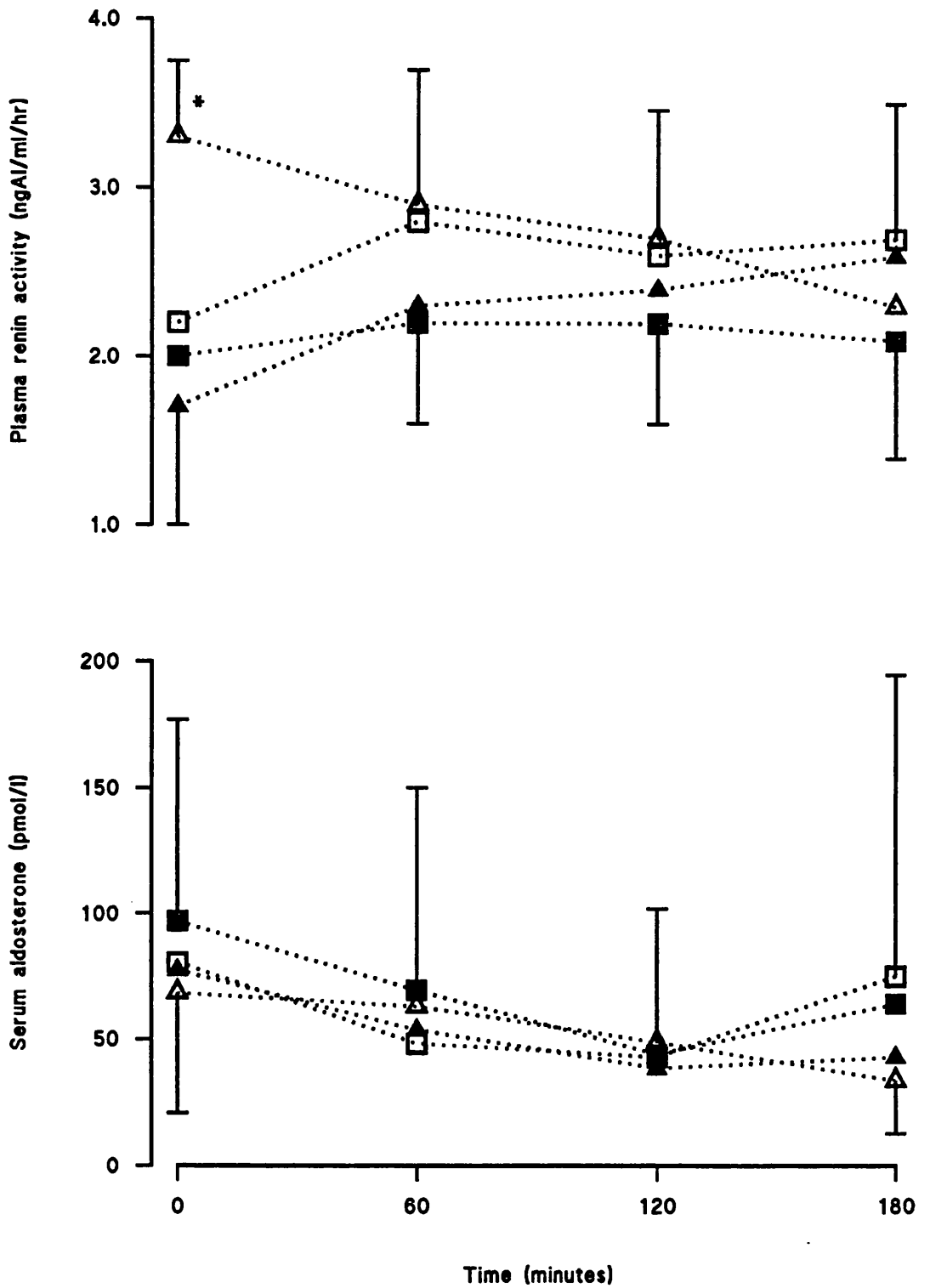


Figure 4.7: Mean profiles of aldosterone and plasma renin activity of healthy subjects during euglycaemic clamp.

■ = acute placebo ▲ = 2 weeks placebo
 □ = acute lacidipine △ = 2 weeks lacidipine
 * = p < 0.05 baseline lacidipine vs. baseline placebo

4.4. RESULTS (II): HYPERTENSIVE PATIENTS.

4.4.1. Tolerance.

Lacidipine was generally well tolerated and there were no withdrawals from the study. In one patient (patient 2, study day 2) the euglycaemic hyperinsulinaemic clamp was discontinued after 120 minutes for safety reasons because of failure of the Beckman glucose analyser. The plasma glucose at the time was 5.8 mmol/l. The hormonal data from all four study days are included for this patient but no value for whole body glucose disposal was obtained. Two patients complained of headache on acute dosing with lacidipine, but these symptoms had resolved after 2 weeks.

4.4.2. Blood pressure and heart rate.

After 2 weeks treatment with lacidipine there was a significant reduction in baseline systolic blood pressure, i.e. 24 hours after the last dose, compared with screening values ($p < 0.05$) but no significant difference compared with placebo. After 2 weeks lacidipine, supine values were $136/79 \pm 16/12$ following lacidipine compared with $143/78 \pm 22/12$ following 2 weeks placebo. Profiles of blood pressure during the 3 hour period of intervention and hyperinsulinaemia were not significantly different between study days (figure 4.8). Reflex tachycardia was observed after both first dose and after 2 weeks of lacidipine treatment; this increase in heart rate was maximal 60 minutes after drug administration (figure 4.9).

4.4.3. Serum insulin and C-peptide.

Lacidipine had no significant effect on fasting serum insulin concentrations; for example, 10.3 ± 6.6 uU/ml after 2 weeks lacidipine, compared with 6.5 ± 3.0 uU/ml

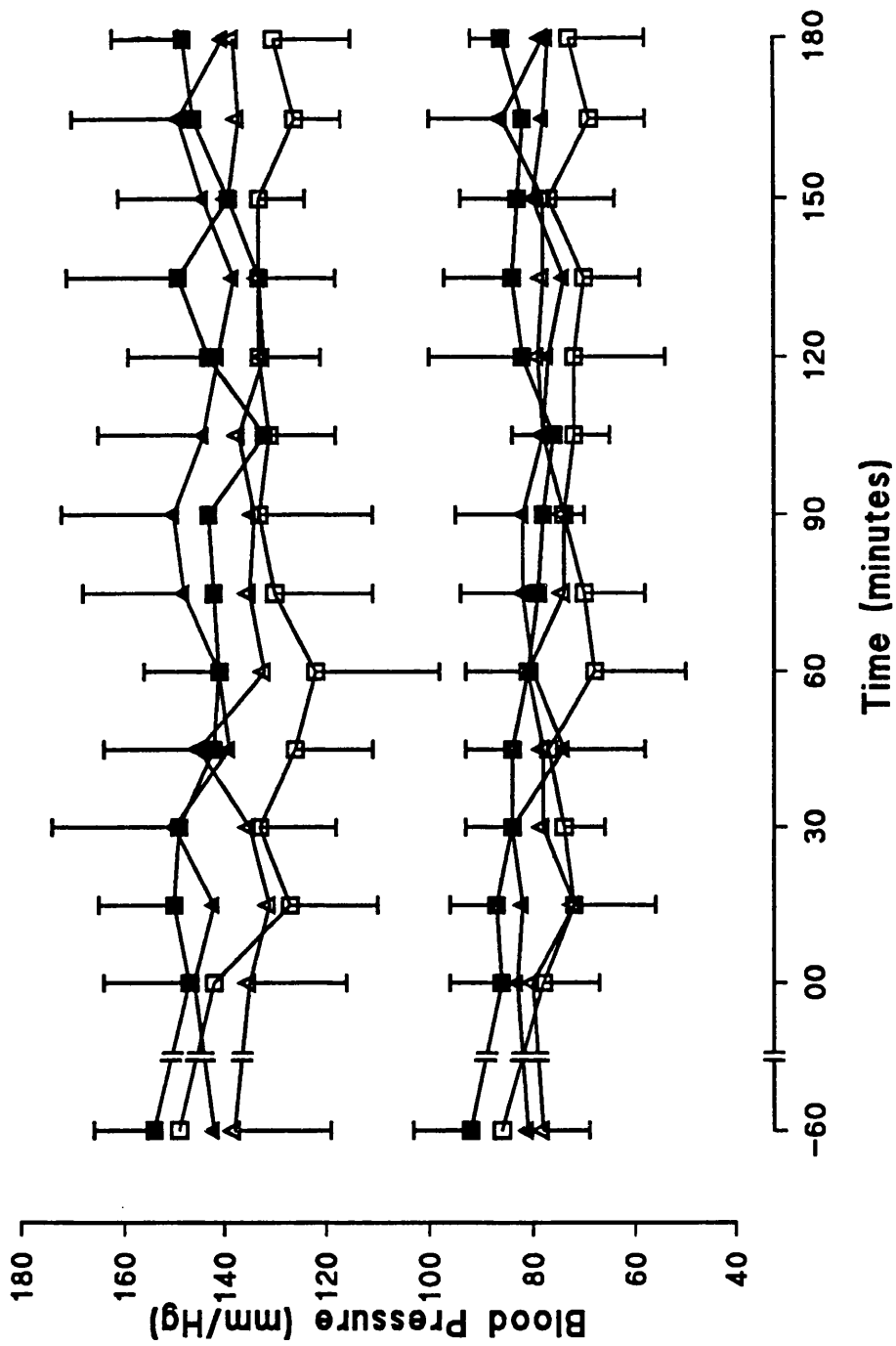


Figure 4.8: Mean profiles of supine systolic and diastolic blood pressure of hypertensive patients during euglycaemic clamp in lacidipine study

■ = first dose placebo. ▲ = 2 weeks placebo. □ = first dose lacidipine. △ = 2 weeks lacidipine.

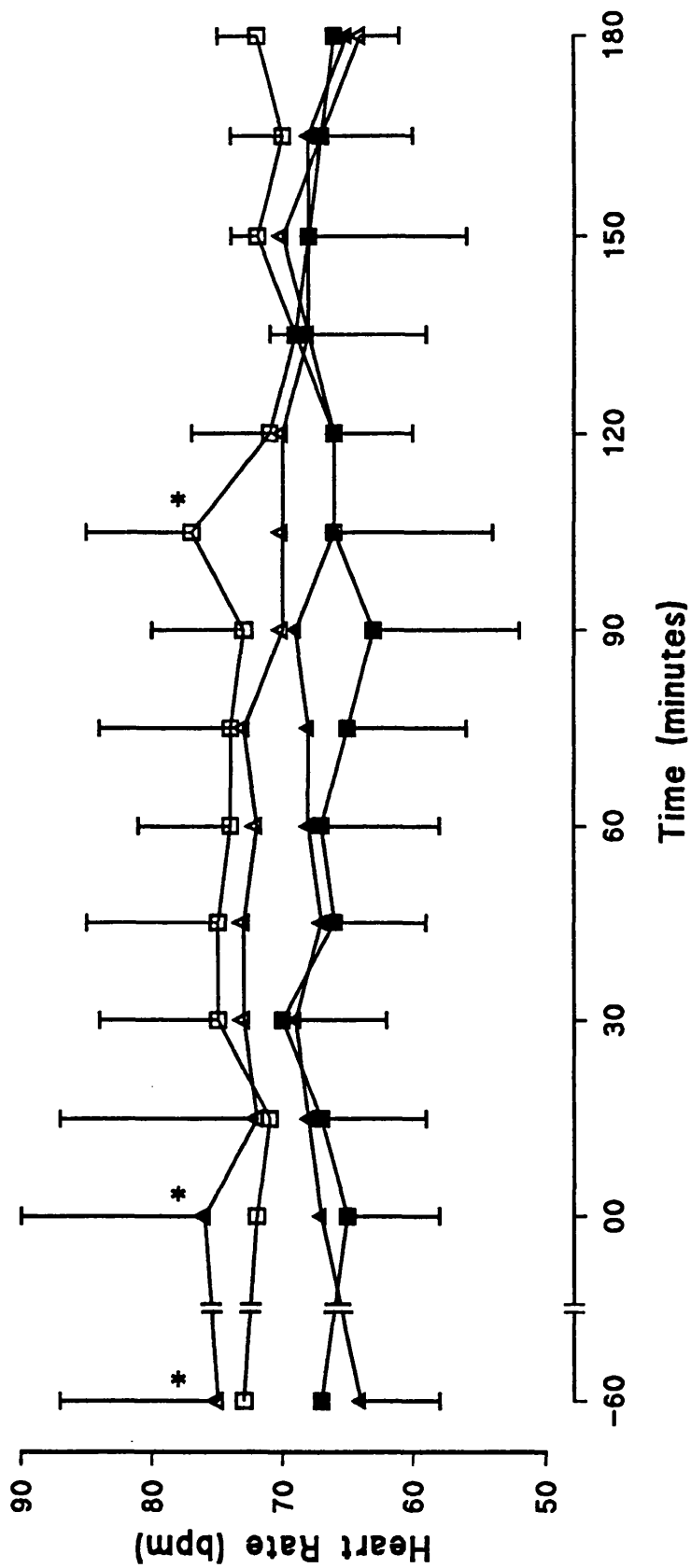


Figure 4.9: Mean profiles of supine heart rate of hypertensive patients during euglycaemic clamp in lacidipine study

■ = first dose placebo. ▲ = 2 weeks placebo □ = first dose lacidipine. △ = 2 weeks lacidipine

* = p < 0.01 lacidipine versus corresponding placebo administration

after 2 weeks placebo. Serum insulin levels increased to a plateau within the first 60 minutes of the insulin infusion and there were no significant differences in insulin concentration between the 4 study days (figure 4.10); for example, at 120 minutes, serum insulin concentrations were 106.8 ± 31.4 and 98.7 ± 29.8 uU/ml for first dose and 2 weeks lacidipine respectively, compared with 119.9 ± 28.2 and 100.2 ± 21.4 uU/ml on the corresponding placebo study days. As with the volunteers, the acute rise in serum insulin concentration was associated with a significant decrease in C-peptide concentrations; for example at 180 minutes C-peptide levels were about 15% lower than baseline fasting levels (figure 4.10) ($p < 0.05$).

4.4.4. Plasma glucose and whole body insulin sensitivity.

Lacidipine had no significant effect on fasting plasma glucose concentrations; for example, 5.6 ± 0.9 mmol/L after 2 weeks lacidipine, compared with 5.2 ± 0.4 mmol/L after 2 weeks placebo. The individual values of whole body insulin sensitivity on the four study days are shown in table 4.3. Lacidipine had no significant effect on insulin-stimulated glucose uptake. Mean values for insulin sensitivity were 6.6 ± 3.0 and 5.7 ± 2.2 mg/kg/min after first dose and 2 weeks respectively, compared with 6.6 ± 2.4 and 6.5 ± 2.0 mg/kg/min following placebo. Confidence intervals for this observation were (-2.0, 3.0) for 2 weeks lacidipine versus 2 weeks placebo.

4.4.5. Serum triglyceride and potassium concentrations.

Lacidipine had no effect on fasting serum triglyceride concentrations at baseline. There were, however, significant reductions in both triglyceride and potassium concentrations during each clamp; for example, after 2 weeks of lacidipine, triglyceride levels fell from 2.3 ± 2.0 at baseline to 1.6 ± 2.0 mmol/l at 180 mins, compared with a corresponding change of 2.2 ± 1.9 to 1.9 ± 1.6 mmol/l after placebo

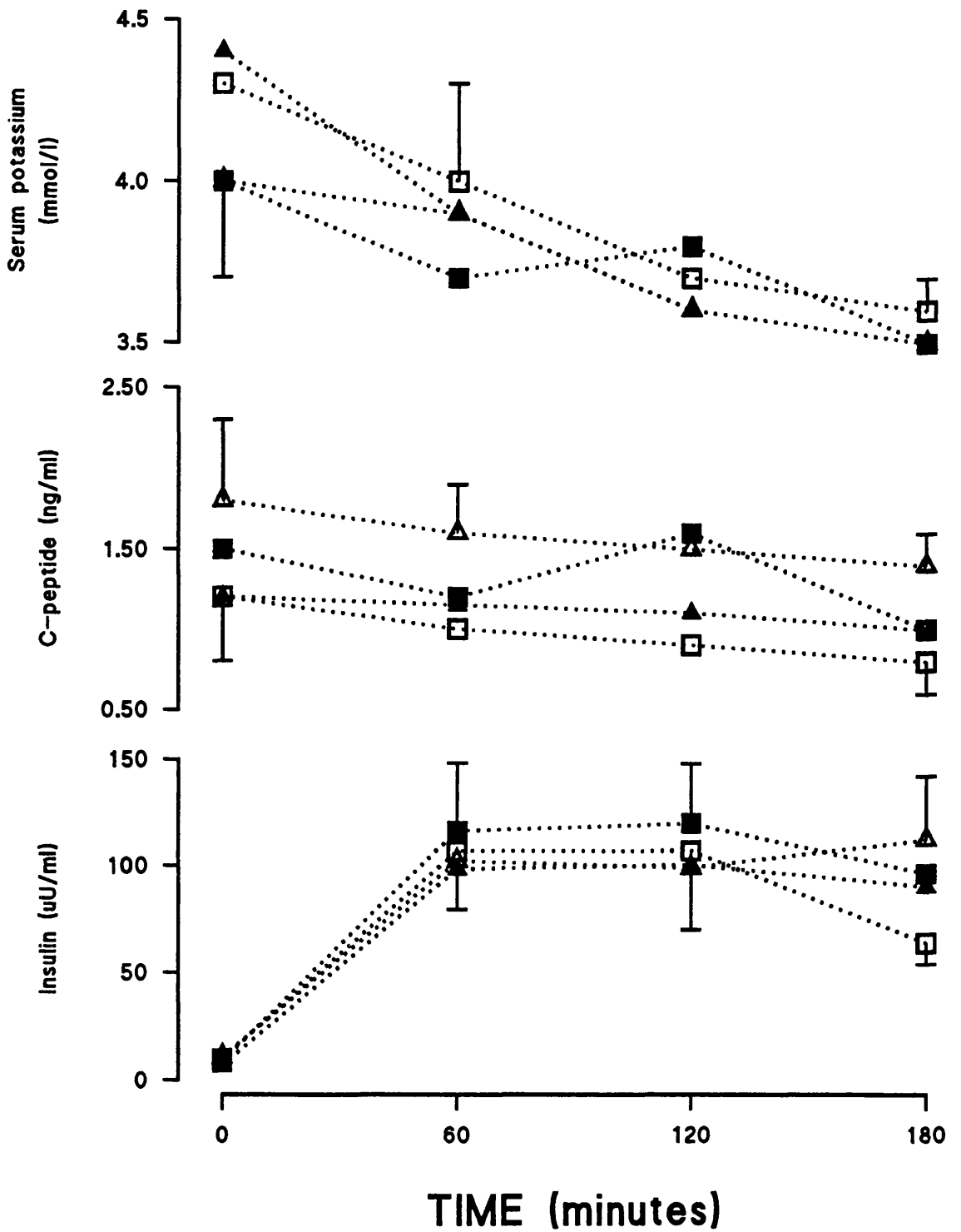


Figure 4.10: Mean profiles of serum insulin, C-peptide and potassium of hypertensive patients during the euglycaemic hyperinsulinaemic clamp.
 ■ = first dose placebo. ▲ = 2 weeks placebo.
 □ = first dose lacidipine. △ = 2 weeks lacidipine.

PATIENT	FIRST DOSE PLACEBO	2 WEEKS PLACEBO	FIRST DOSE LACIDIPINE	2 WEEKS LACIDIPINE
1	5.5	6.4	5.5	6.0
2	11.0	9.0	9.9	NR
3	2.1	3.0	3.3	2.3
4	8.3	8.0	7.1	8.0
5	6.9	6.6	7.1	7.0
6	6.0	6.0	6.6	5.3
MEAN	6.6 +/- 2.4	6.5 +/- 2.0	6.6 +/- 3.0	5.7 +/- 2.2

Table 4.3: Individual values for whole body insulin sensitivity of hypertensive patients in lacidipine study. Values expressed as mg glucose/kg/min.

(figure 4.11.). Correspondingly, potassium levels fell from 4.0 ± 0.3 to 3.5 ± 0.1 , and from 4.4 ± 0.3 to 3.4 ± 0.1 mmol/L, during the same study days (figure 4.10.). Lacidipine had no significant effect on these insulin-stimulated reductions in triglyceride and potassium concentrations.

4.4.6. Plasma noradrenaline, plasma renin activity and serum aldosterone.

There were no significant differences in plasma catecholamine concentrations (figure 4.12), plasma renin activity and serum aldosterone concentrations (figure 4.13) following 2 weeks treatment with lacidipine.

4.5. DISCUSSION.

Lacidipine had no effect on whole body insulin-stimulated glucose uptake in this group of insulin sensitive healthy male volunteers or in patients with essential hypertension. The confidence intervals for the effect of chronic lacidipine on insulin sensitivity in the healthy volunteers excludes a 10% decrease and a 15% increase in insulin-sensitive glucose transport, i.e. effects similar to those reported with hydrochlorothiazide and captopril respectively in patients with essential hypertension (Klauser et al, 1991; Pollare et al, 1989b). In the patient group, there was a tendency for whole body insulin sensitivity to decrease after 2 weeks lacidipine (5.7 vs 6.6 mg/kg/min) but the confidence intervals were relatively wide and a type II statistical error cannot be excluded. An additional finding in the healthy volunteer group was that, after chronic dosing, there was a significant reduction in fasting serum triglyceride concentrations.

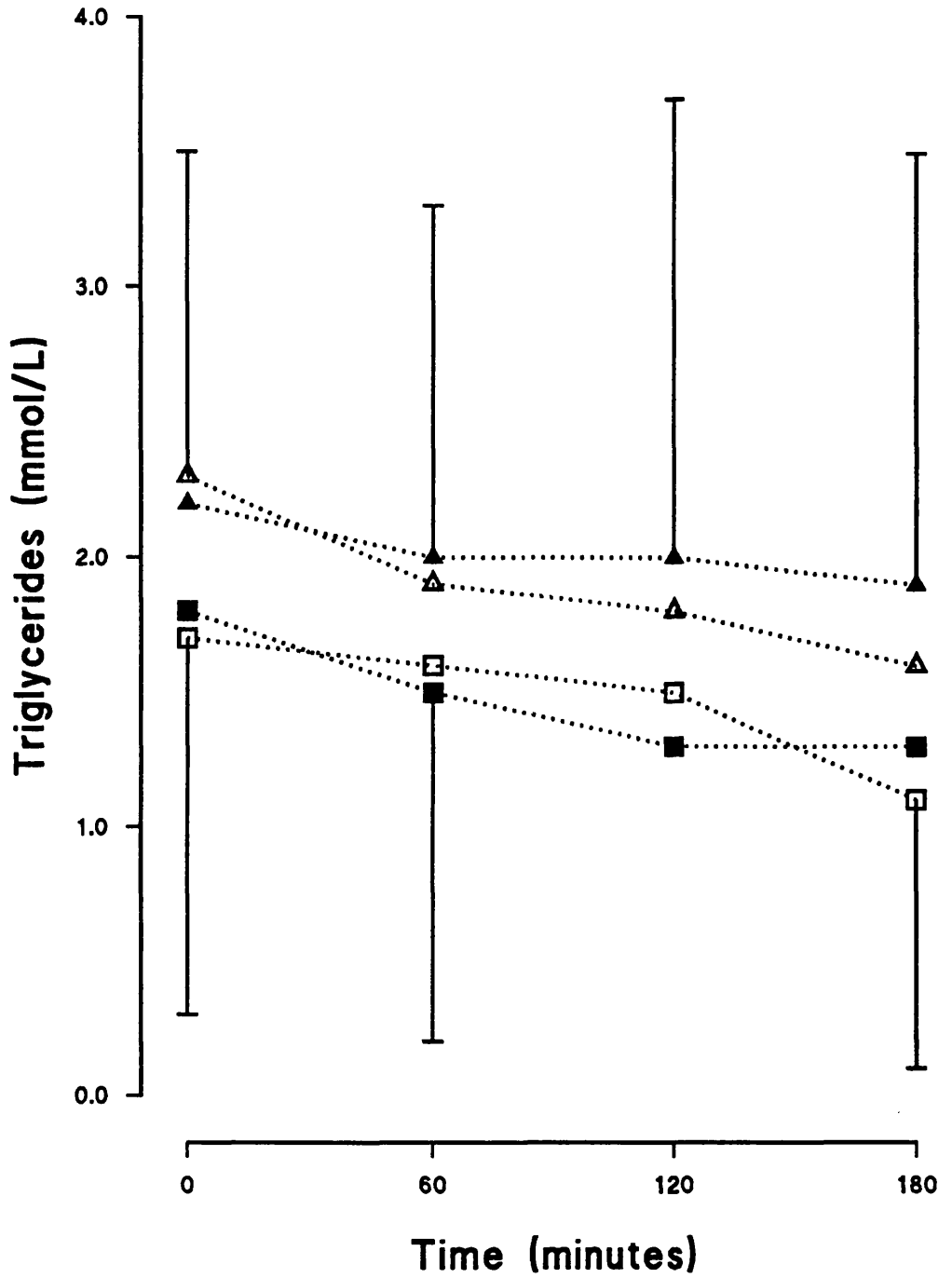


Figure 4.11: Mean profiles of triglyceride concentrations for hypertensive patients during euglycaemic clamp in lacidipine study

■ = first dose placebo. ▲ = 2 weeks placebo
 □ = first dose lacidipine. △ = 2 weeks lacidipine

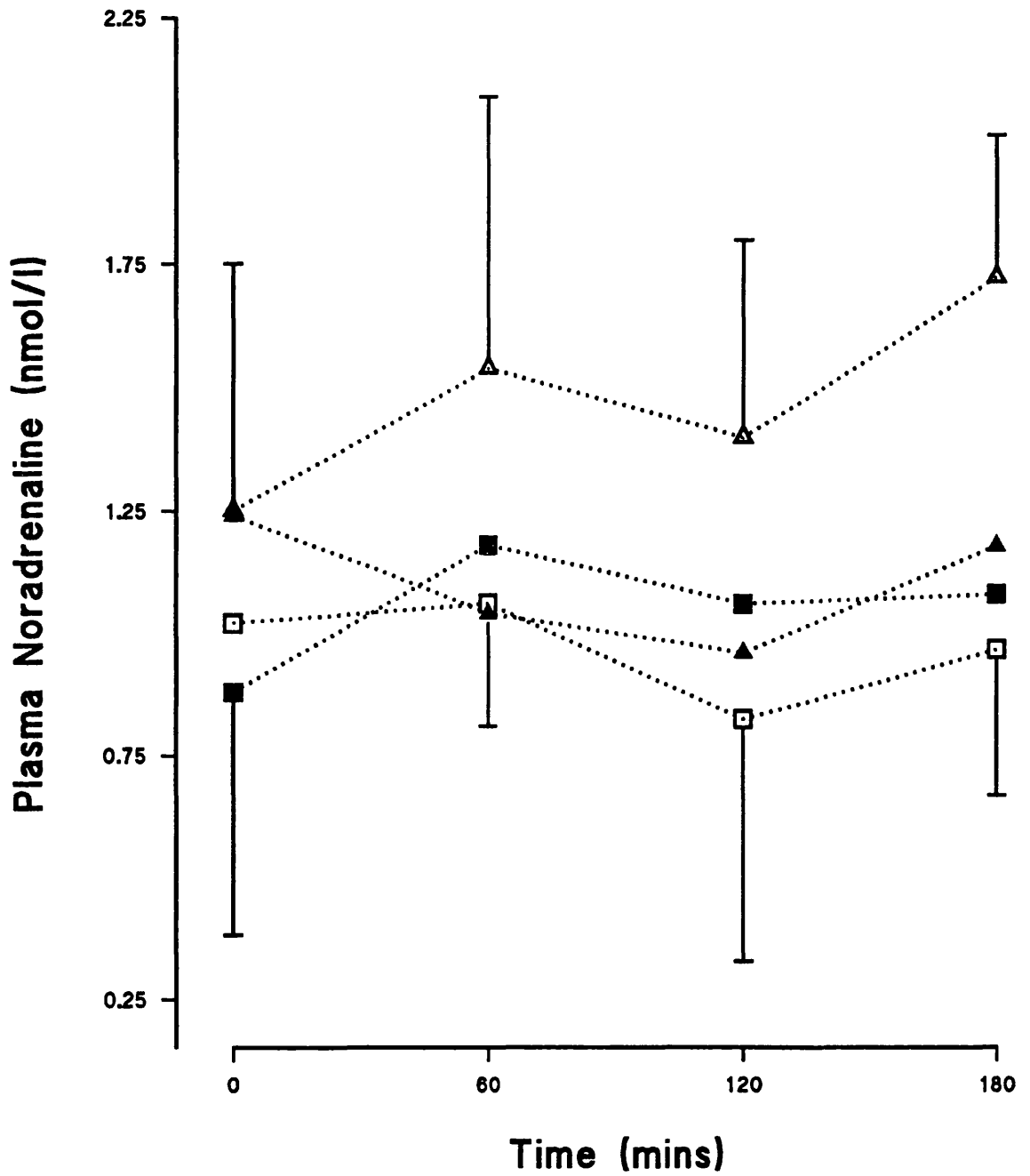


Figure 4.12: Mean profiles of noradrenaline concentrations of hypertensive patients during euglycaemic clamp in lacidipine study.

■ = acute placebo ▲ = 2 weeks placebo
 □ = acute lacidipine △ = 2 weeks lacidipine

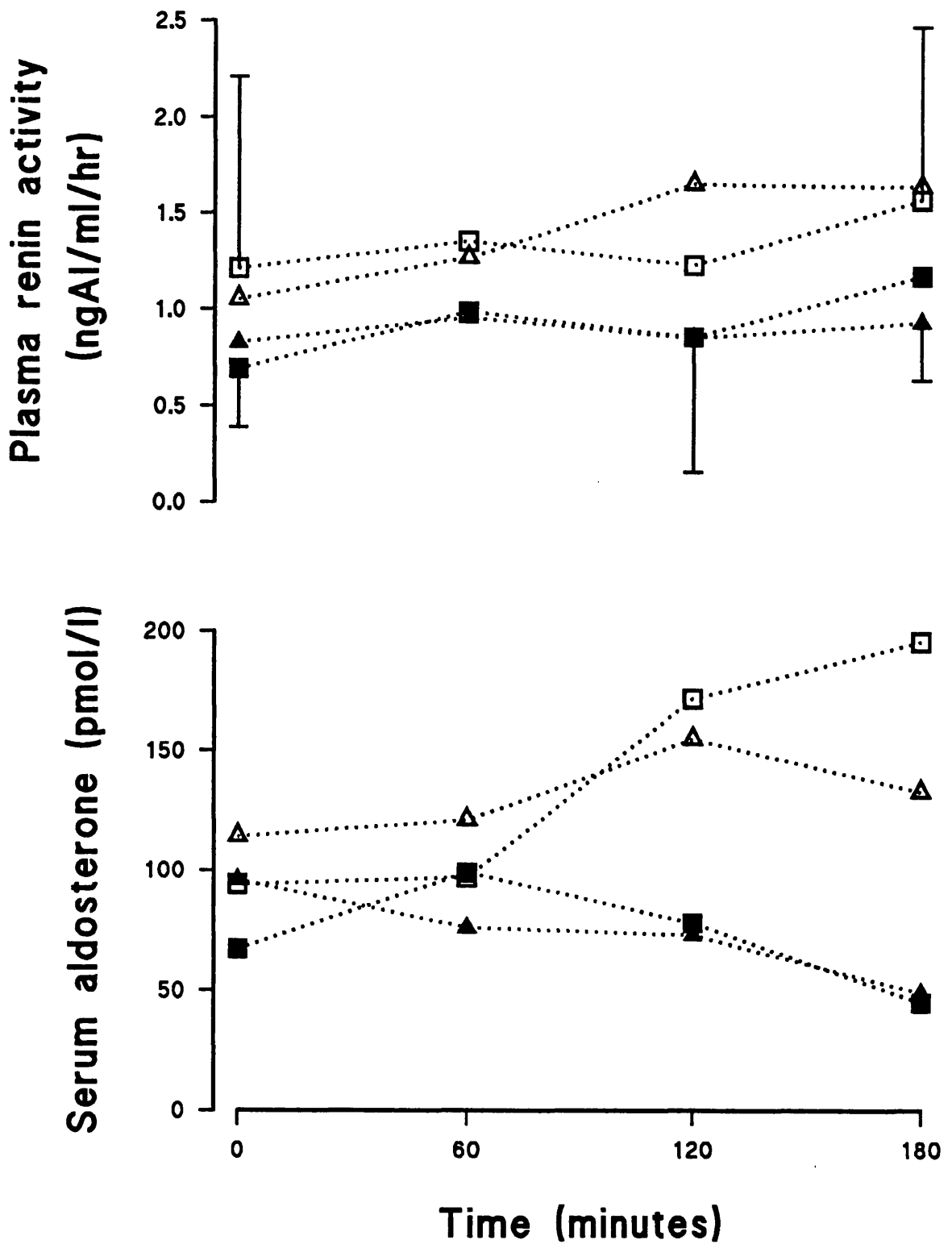


Figure 4.13: Mean profiles of aldosterone and plasma renin activity of hypertensive patients during euglycaemic clamp.
 ■ = acute placebo ▲ = 2 weeks placebo
 □ = acute lacidipine △ = 2 weeks lacidipine

It should be borne in mind, however, that the results of this 2 week "short-term" study may not necessarily reflect the metabolic effects of long term treatment with lacidipine in hypertensive patients.

The role of calcium in glucose-induced insulin release is well established but the significance of this intracellular cation in regulation of insulin mediated responses remains to be clearly defined (Levy et al, 1989). There are some data, however, that suggest that the intracellular calcium concentration correlates with *in vivo* insulin sensitivity (Resnick et al, 1993).

Calcium antagonists may affect glucose and insulin metabolism via a number of different mechanisms, e.g. decreased insulin secretion, enhanced release of glucagon and altered hepatic glucose output (Giugliano et al, 1980; Charles et al, 1981). In addition, since blood flow is an important determinant of glucose uptake into skeletal muscle (Baron et al, 1991b; Julius et al, 1991), vasodilatation *per se* may increase peripheral glucose utilisation. With dihydropyridines, however, the vasodilator response observed after acute dosing is often associated with reflex increases in plasma renin activity and catecholamines which might attenuate insulin-dependent glucose transport during short-term administration. This sympathetic activation often subsides during chronic treatment when baroreflex mechanisms reset. An interesting feature of this study in both the healthy subjects and patients with essential hypertension was that increases in plasma noradrenaline and significant increases in supine heart rate were observed following the first dose of lacidipine and also after 2 weeks of drug administration. This reflex tachycardia observed after 2 weeks would be entirely consistent with the once-daily administration of a dihydropyridine with a short half life, and in keeping with the observed plasma half-life of lacidipine of 1 - 3 hours (Hall et al, 1991). In addition, the lack of a significant reduction in supine blood pressure in the patient group 24 hours after the last dose compared with placebo does not suggest a sustained 24 hour duration of action of lacidipine, although the number of patients studied was small. Despite this

evidence of sympathetic activation after single dosing and after two weeks there was no change in insulin sensitivity compared with placebo administration.

Calcium antagonists have generally been reported to have neutral effects on glucose tolerance [chapter 1.3.2.] but there have been conflicting reports about the specific effect of dihydropyridines on insulin-stimulated glucose uptake by peripheral tissues. Diabetogenic effects of nifedipine (Bhatager et al, 1984) and enhanced insulin sensitivity with nitrendipine (Beer et al, 1993) are frequently cited, though uncontrolled studies. Many other investigators have reported contrasting effects of dihydropyridines on glucoregulatory control (Trost et al, 1984; Gill et al, 1987; Chellingsworth et al, 1989; Klauser et al, 1991; Oehman et al, 1985). The conflicting results may be due, in part, to limitations of study design (often a lack of placebo data), differences in methodology for assessing insulin sensitivity, and heterogeneous patient groups in whom the biochemical mechanisms of insulin resistance, and drug effects, may not be the same, e.g. in diabetic patients compared with hypertensive patients. Many of these uncontrolled studies have used oral glucose tolerance tests as an indirect measure of peripheral insulin sensitivity. The difficulty with this method is that while profiles of glucose and insulin concentration for groups of subjects are usually quite smooth, curves for individuals are often erratic and the interpretation is hampered because both dependent variables, glucose and insulin, are changing simultaneously [chapter 1.2.1]. To the best of my knowledge, the present study is the one of the first double-blind, placebo-controlled, crossover studies to evaluate both the acute and short-term metabolic effects of a calcium antagonist in healthy subjects and patients with uncomplicated essential hypertension.

In the healthy volunteer group, steady state treatment with lacidipine resulted in a significant reduction in fasting serum triglyceride concentrations at baseline, but this effect was not observed in the patients with essential hypertension, although the number of patients studied was small limiting the power of the study. Increased triglyceride tolerance has been reported previously with dihydropyridines (Marrotta et

al, 1989; Trost & Weidmann, 1987) but the mechanism of this action remains unclear. There is limited evidence that calcium antagonists may enhance secretion of lipoprotein lipase which is the principal enzyme involved in triglyceride removal (Pasanisi et al, 1988). In both groups, lacidipine had no effect on insulin-stimulated triglyceride uptake during the 180 minute clamp. Thus, there was no evidence from this study that lacidipine exerted an additive or synergistic effect on the effect of insulin on triglyceride disposal which is also mediated via lipoprotein lipase.

In summary, this placebo-controlled study has shown a reduction in fasting triglyceride levels with lacidipine in the healthy volunteer group and no adverse effects on insulin-stimulated uptake of glucose, potassium and triglyceride in both the patients with essential hypertension and the healthy volunteers. These data are relevant to the debate of the inter-relationship between insulin resistance, non-insulin dependent diabetes and essential hypertension. Thus, it is well established that lacidipine lowers blood pressure by vasodilatation of resistance arterioles, many of which perfuse skeletal muscle, yet in this study, despite significant cardiovascular effects (and presumably a modest increase in peripheral blood flow), there was no change in whole body insulin sensitivity. In order to draw any firm conclusions regarding regional tissue perfusion, however, it would clearly be necessary to repeat a similar study with direct measurements of skeletal muscle blood flow.

CHAPTER 5

**THE EFFECTS OF THE ACE INHIBITOR TRANDOLAPRIL
ON INSULIN SENSITIVITY IN PATIENTS WITH ESSENTIAL
HYPERTENSION AND IMPAIRED GLUCOSE TOLERANCE OR NIDDM**

5.1. GENERAL INTRODUCTION

Angiotensin converting enzyme (ACE), also known as kininase II, is responsible for the enzymatic conversion of angiotensin I to the potent vasoconstrictor peptide angiotensin II. The ACE inhibitor drugs, of which captopril and enalapril were the first to be licensed for clinical use, have become established in the treatment of hypertension (Brunner et al, 1981) and cardiac failure (CONSENSUS, 1987).

There is some debate about the metabolic effects of ACE inhibitors [chapter 1.3.2]. Anecdotal reports and small clinical studies have suggested increased insulin sensitivity following ACE inhibition, but few, if any, of these reports are based upon a placebo-controlled, cross-over design. Furthermore, most of the available evidence is based on indirect measurements of insulin sensitivity derived from oral or intravenous glucose tolerance tests. Thus, deficiencies in study design may be partly responsible for conflicting results about the effects of ACE inhibitors on insulin action and glycaemic control. For example, it has been shown that single doses of captopril increase insulin sensitivity in hypertensive diabetics (Torlone et al, 1991; Uehara et al, 1994; Rett et al, 1988), whereas others have found no effect (Helve et al, 1993). Likewise, there are confusing reports regarding metabolic changes after long-term administration of ACE inhibitors; while the studies of Pollare et al (1989a), Paolisso et al (1992) and Torlone et al (1993) report an increase in insulin sensitivity with captopril, other investigators have reported neutral effects of various ACE inhibitor drugs, including captopril (Seefeldt et al, 1990), perindopril (Bak et al, 1992), enalapril (Moore et al, 1988) and cilazapril (Santoro et al, 1992). However, none of these uncontrolled studies is entirely satisfactory. The present study, therefore evaluates the effects of a new ACE inhibitor, trandolapril, on whole body insulin sensitivity in patients with at least two cardiovascular risk factors, namely glucose intolerance or NIDDM, and essential hypertension, using a double-blind, placebo-controlled study design with the euglycaemic hyperinsulinaemic clamp.

5.1.1. Clinical pharmacology oftrandolapril

Trandolapril is a potent non-sulphydryl ACE inhibitor which, following oral administration is hydrolysed to the active diacid metabolite, trandolaprilat, which inhibits both serum and tissue ACE (figure 5.1). Since trandolapril is a pro-drug, it is subject to extensive first-pass metabolism and its absolute bioavailability is about 7.5% (De Ponti et al, 1991). The absolute bioavailability of trandolaprilat administered as trandolapril is 40-60%. The pharmacokinetics of trandolapril are linear whereas those of trandolaprilat are non linear; this may be explained by the saturation of binding to angiotensin converting enzyme (Bree et al, 1992). The effective half-life of trandolapril at steady state is between 16 and 24 hours, thus allowing once daily dosing. In terms of efficacy, results of double blind placebo-controlled comparative studies have shown that trandolapril administered at a single daily dose of 2 to 4mg is effective in lowering blood pressure for up to 24 hours in patients with mild to moderate hypertension (Mancia et al, 1992). Trandolapril is fully licensed in the U.K. (Knoll Pharmaceuticals).

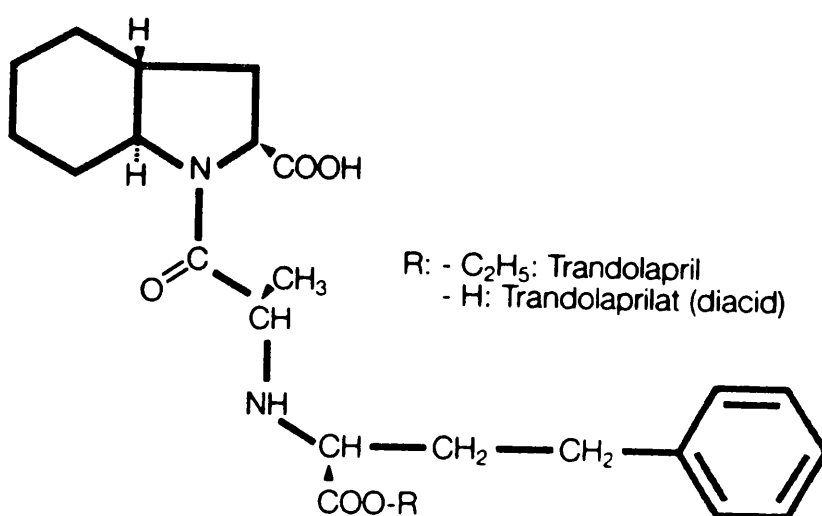


Figure 5.1: Chemical structure of trandolapril.

5.2. PATIENTS AND METHODS

The study group consisted of 12 male patients with untreated essential hypertension and either impaired glucose tolerance (IGT) or (diet-controlled) NIDDM. A further 10 patients were screened and randomised, but were excluded from the study after visit 2 because they failed to meet blood pressure or glucose tolerance entry criteria. On entry into the study, the median age was 54 years, mean BMI 31.7 ± 5.2 kg/m² and mean blood pressure $164/95 \pm 19/8$ mmHg. Four patients were smokers, with a mean consumption of 15 cigarettes daily. On the basis of the screening OGTT, 6 of the patients were diagnosed as having IGT and six had NIDDM according to standard WHO criteria [chapter 2.1.2]. All patients were asked to adhere to an isocaloric diabetic diet throughout the study consisting of approximately 55% carbohydrate, 25% fat and 20% protein [chapter 2.1.4]. They were asked to refrain from strenuous exercise and none of the subjects was receiving any proprietary or prescription medication. The clinical details of the patients are shown in table 5.1.

Following routine screening [chapter 2.1.1], the study consisted of a single blind placebo run-in period for 2 weeks followed by 2 double-blind crossover periods, each of 4 weeks, when patients were randomised to receive trandolapril 2mg once daily or matching placebo. A 2 week washout period was included between the crossover phases [figure 5.2].

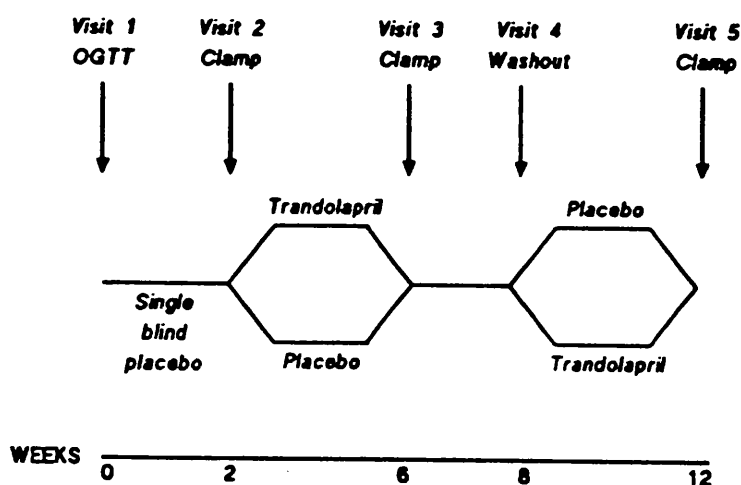


Figure 5.2: Schematic of study design.

PATIENT No.	AGE (years)	WEIGHT (kg)	BMI (cm)	SEX	SCREEN BP (mm Hg)	GLUCOSE fasting (mmol/l)	OGTT 2 hr. glucose (mmol/l)	Duration of hypertension	Previous Treatment
1	46	96	35.3	M	163/96	5.4	8.6	2 years	amlodipine 10 mg o.d.
2	65	84	29.8	M	164/96	9.0	17.1	5 years	amlodipine 5 mg o.d.
3	48	104	31.4	M	174/103	6.7	8.6	1 year	nil
4	62	115	35.9	M	168/88	6.0	10.9	15 years	nifedipine retard 20 mg b.d.
5	57	92	31.1	M	178/103	5.4	8.0	1 year	atenolol 25 mg o.d.
6	55	81	29.0	M	192/100	10.5	18.8	2 years	nil
7	42	66	25.1	M	141/97	5.8	9.1	1 year	nil
8	67	98	32.7	M	190/103	7.9	15.2	18 months	nil
9	54	131	43.7	M	169/96	5.6	11.5	6 months	nil
10	45	80	27.7	M	154/106	7.3	8.7	4 years	enalapril 20 mg o.d.
11	43	114	33.7	M	154/95	10.9	14.8	7 months	nifedipine retard 20 mg b.d.
12	72	57	24.7	M	181/84	6.9	13.5	10 months	nil

TABLE 5.1: DETAILS OF PATIENTS WITH HYPERTENSION AND IMPAIRED GLUCOSE TOLERANCE OR NIDDM IN TRANDOLAPRIL STUDY

Each subject attended three 5-hour study mornings (once after the single blind run in phase, and after each crossover period) to evaluate the metabolic effects of trandolapril and the corresponding placebo administrations. On each occasion, following an overnight fast, subjects attended the CIRU at 0745 am. After 20 minutes supine rest, baseline blood pressure and heart rate measurements were recorded (i.e. 24 hours after the last dose of trandolapril or placebo). Baseline blood samples were collected and trandolapril or placebo was administered orally with 100 mls water.

On each study day, whole-body insulin sensitivity was calculated using the euglycaemic hyperinsulinaemic clamp as described in chapter 2.3. The clamp was commenced 60 minutes after drug administration. Systolic and diastolic blood pressure and heart rate (HR) were recorded in duplicate 60 minutes before and at 15 minute intervals during the clamp. At baseline, and at 60-minute intervals during the 3-hour clamp, additional blood samples were collected for measurements of serum insulin, C-peptide, serum potassium, triglyceride, plasma noradrenaline, plasma renin activity, serum aldosterone concentrations and serum ACE activity.

It was calculated from previous data that this study would have greater than 80% power to detect a 15% change in insulin sensitivity.

5.3. **RESULTS**

5.3.1. **Tolerance**

The euglycaemic clamp was generally well tolerated and there were no adverse biochemical events. One patient (no.11) experienced an episode of micturition syncope on his final study day and the procedure was abandoned after 120 minutes. The plasma glucose concentration at the time was 5.5 mmol/L. As steady-state had not been reached, the biochemical data are included in the analyses but the insulin sensitivity data from visit 5 have been omitted.

5.3.2. Blood pressure and heart rate

Chronic treatment with trandolapril was associated with a significant decrease in baseline blood pressure (i.e. 24 hours after the last dose) compared with placebo. Mean supine values were $167/97 \pm 10/7$ mmHg after the placebo run-in phase and $160/93 \pm 17/9$ and $152/88 \pm 13/7$ mmHg after 4 weeks of placebo and trandolapril respectively ($p < 0.05$ ANOVA). At subsequent time points during the clamp, trandolapril was associated with significant decreases in supine blood pressure from 30 to 180 mins; for example, after 4 weeks treatment, supine blood pressure at 180 minutes was $138/72 \pm 12/7$ mm Hg compared with $153/82 \pm 13/12$ mm Hg after placebo (figure 5.3). There were no statistically significant differences in heart rate between the 3 study days (figure 5.4).

5.3.4. Serum insulin and C-peptide concentrations

Serum insulin levels increased to a plateau within the first 60 minutes of the infusion and there were no significant differences in insulin concentrations between the 3 study days (figure 5.5); for example, at 120 minutes, serum insulin concentrations were 143 ± 35 , 141 ± 47 and 151 ± 30 uU/ml after the placebo run-in, and after 4 weeks placebo and trandolapril respectively. The acute rise in serum insulin concentration was associated with a significant decrease in endogenous insulin release. C-peptide concentrations at 180 minutes were 30-40% lower than baseline fasting levels; for example, baseline values were 2.3 ± 1.1 ng/ml after 4 weeks placebo and 2.3 ± 1.1 ng/ml after 4 weeks trandolapril. The corresponding values at 180 minutes were 1.2 ± 0.1 ng/ml and 1.7 ± 1.3 ng/ml respectively (figure 5.5) ($p < 0.001$). Thus, trandolapril appeared to have no effect on insulin clearance.

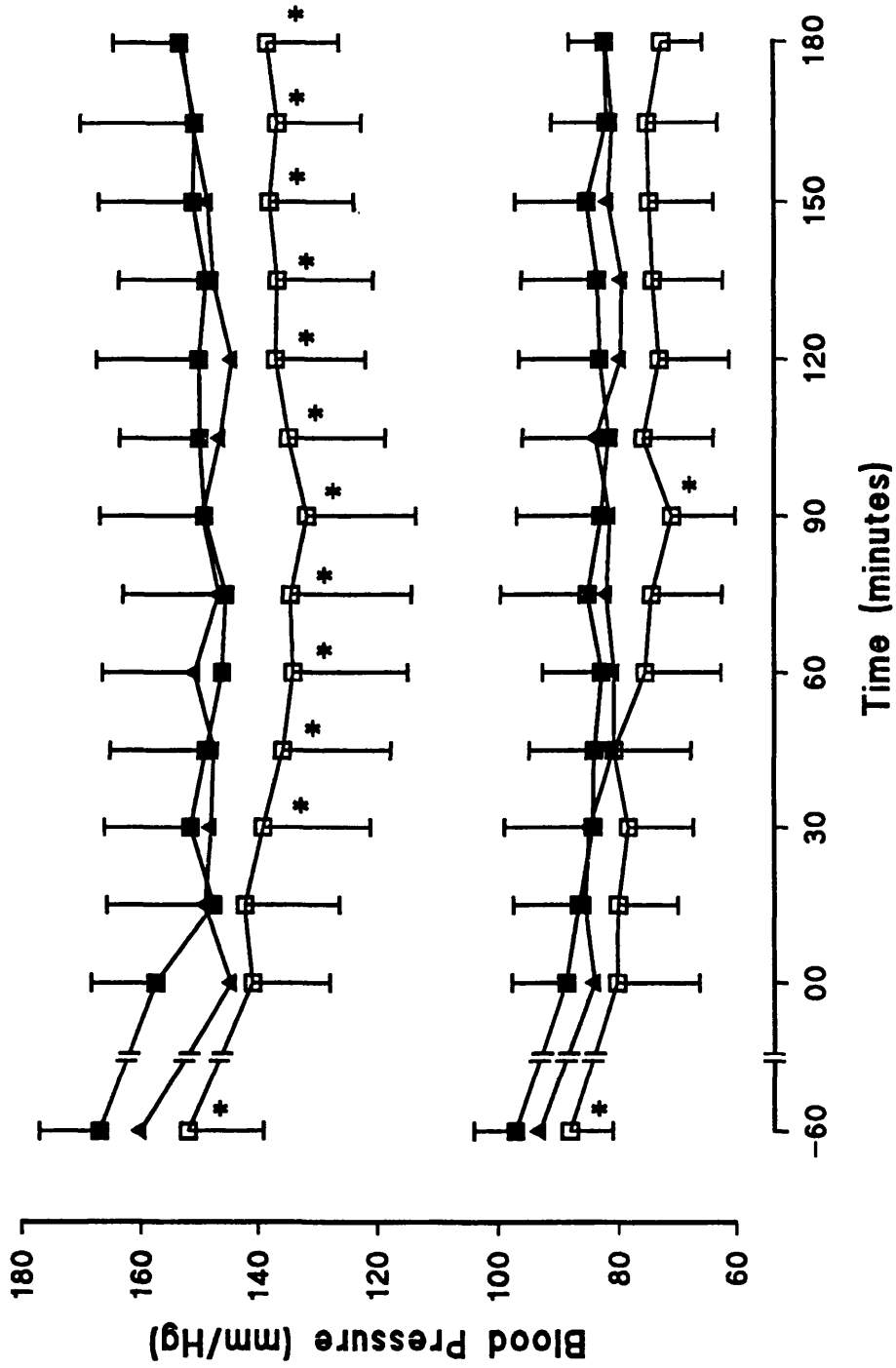


Figure 5.3: Mean profiles of supine systolic and diastolic blood pressure of patients during euglycaemic clamp in trandolapril study. ■ = run in placebo, ▲ = 4 weeks placebo, □ = 4 weeks trandolapril.

* = $p < 0.01$ 4 weeks trandolapril vs. 4 weeks placebo timepoint.

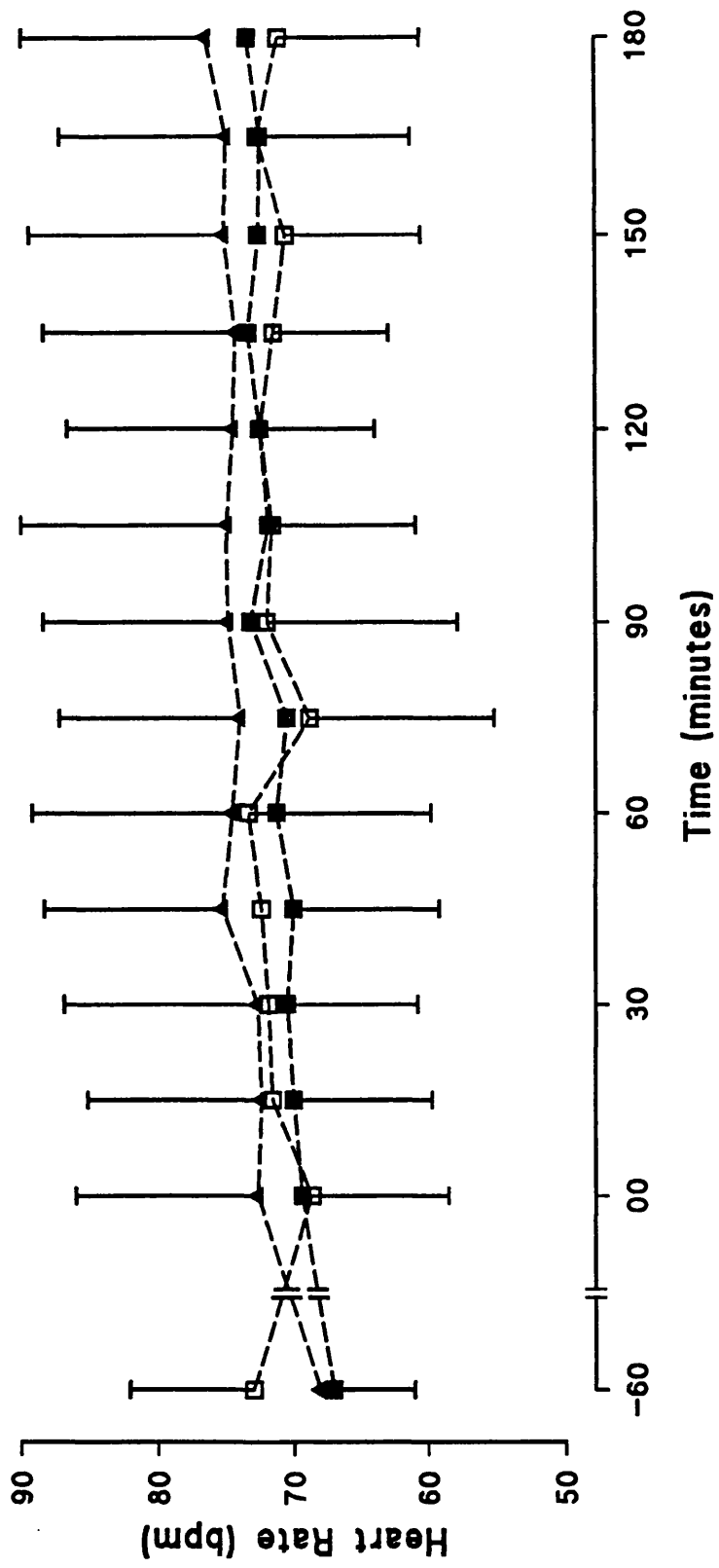


Figure 5.4: Mean profiles of supine heart rate of patients during euglycaemic clamp in trandolapril study.
 ■ = placebo run in, ▲ = 4 weeks placebo, □ = 4 weeks trandolapril

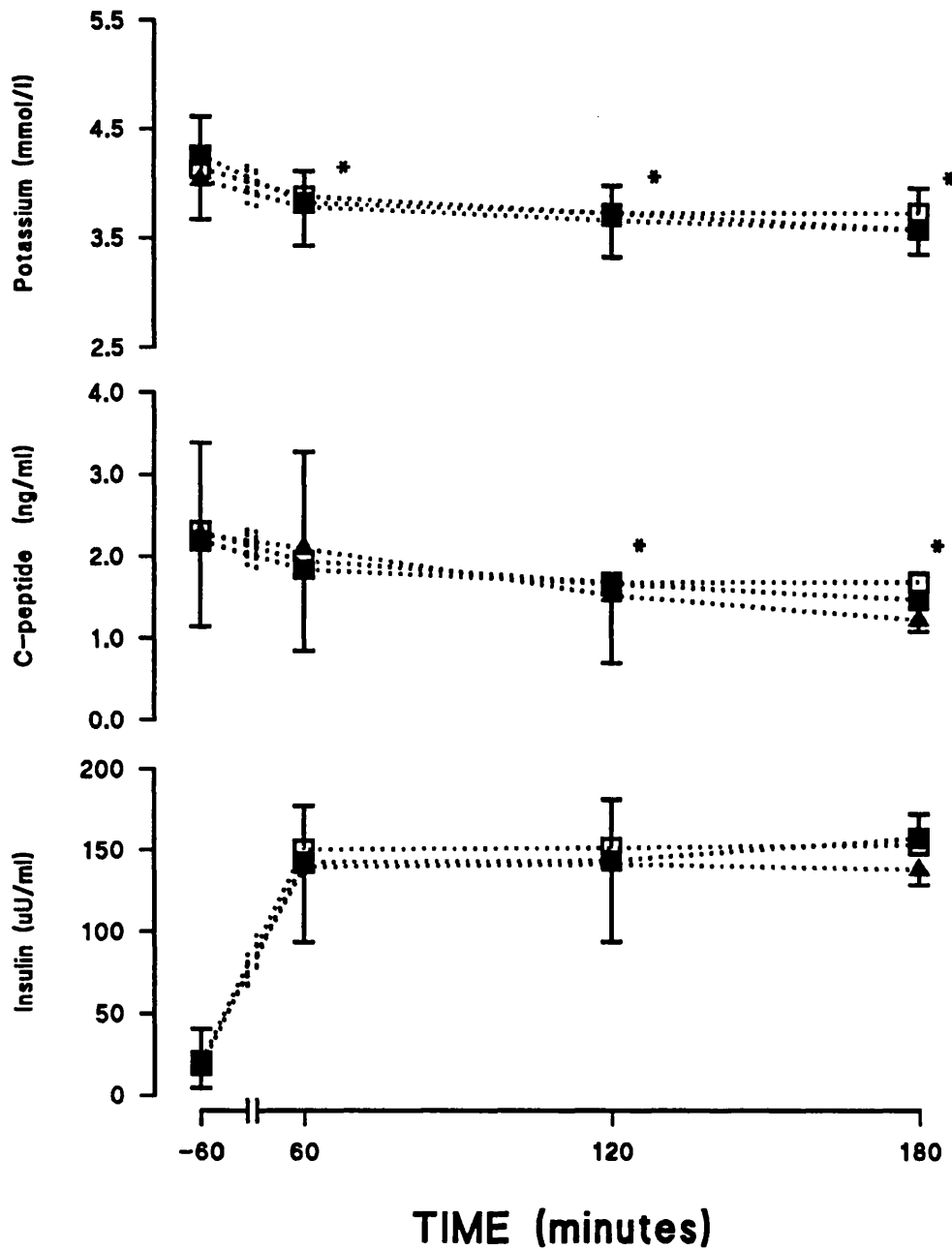


Figure 5.5: Mean profiles of serum insulin, potassium and C-peptide of patients during the euglycaemic clamp in trandolapril study.

■ = placebo run in, ▲ = 4 weeks placebo, □ = 4 weeks trandolapril.

* = $p < 0.001$ 4 weeks trandolapril or 4 weeks placebo vs. corresponding baseline (-60 mins) timepoint.

5.3.5. Plasma glucose and whole body insulin sensitivity

There was no significant difference in plasma glucose concentrations during the 3 clamps; mean fasting plasma glucose concentrations were 6.6 ± 1.2 mmol/L, 6.8 ± 1.4 and 6.8 ± 1.2 for the run-in, placebo andtrandolapril study days respectively. The corresponding mean plasma glucose concentrations for the last 40 minutes of each clamp were 5.2 ± 0.3 , 5.3 ± 0.3 and 5.2 ± 0.3 mmol/L.

There was no significant difference in insulin-stimulated glucose uptake between the 3 study days; mean values for whole body insulin sensitivity were 4.6 ± 1.7 , 4.7 ± 1.9 and 4.7 ± 1.3 mg glucose/kg/min on the run-in, placebo andtrandolapril study days respectively ($p=0.75$). Confidence intervals for this observation were (-0.6,1.1) for 4 weeks placebo versus 4 weeks trandolapril (table 5.2).

5.3.6 Plasma ACE activity

Trandolapril was associated with a significant decrease in baseline ACE activity (i.e. 24 hours after the last dose); mean values were 16.9 ± 6.3 EU/L and 23.4 ± 5.4 EU/L after 4 weeks trandolapril and 4 weeks placebo respectively ($p<0.01$) (figure 5.6). Following drug administration on the active study day, trandolapril was associated with a prompt decrease in plasma ACE activity. The mean percentage ACE inhibition at 120-180 minutes was $70 \pm 15\%$ (figure 5.6).

5.3.7. Serum potassium concentrations

The time course of changes in serum potassium concentrations during the clamp are depicted in figure 5.5. There was a significant reduction in serum potassium concentration during each clamp; the reduction was maximal within 60 minutes and

SUBJECT	RUN IN PLACEBO	4 WEEKS PLACEBO	4 WEEKS TRANDOLAPRIL
1	4.3	5.0	4.5
2	4.9	3.5	4.3
3	6.5	8.3	5.4
4	3.6	2.2	2.7
5	5.6	6.4	6.4
6	4.1	5.6	4.8
7	6.0	4.9	6.3
8	3.9	4.4	4.5
9	1.4	2.2	2.2
10	5.0	4.8	6.3
11	2.8	2.3	NR
12	7.5	5.2	5.2
MEAN	4.6 +/- 1.7	4.7 +/- 1.9	4.7 +/- 1.3

Table 5.2: Individual values of whole body insulin sensitivity of patients in trandolapril study.

Values expressed as mg glucose/kg/min.

There was no significant effect of trandolapril on insulin sensitivity

p = 0.75 4 weeks placebo vs. 4 weeks trandolapril (ANOVA).

95% confidence intervals -0.6,1.1.

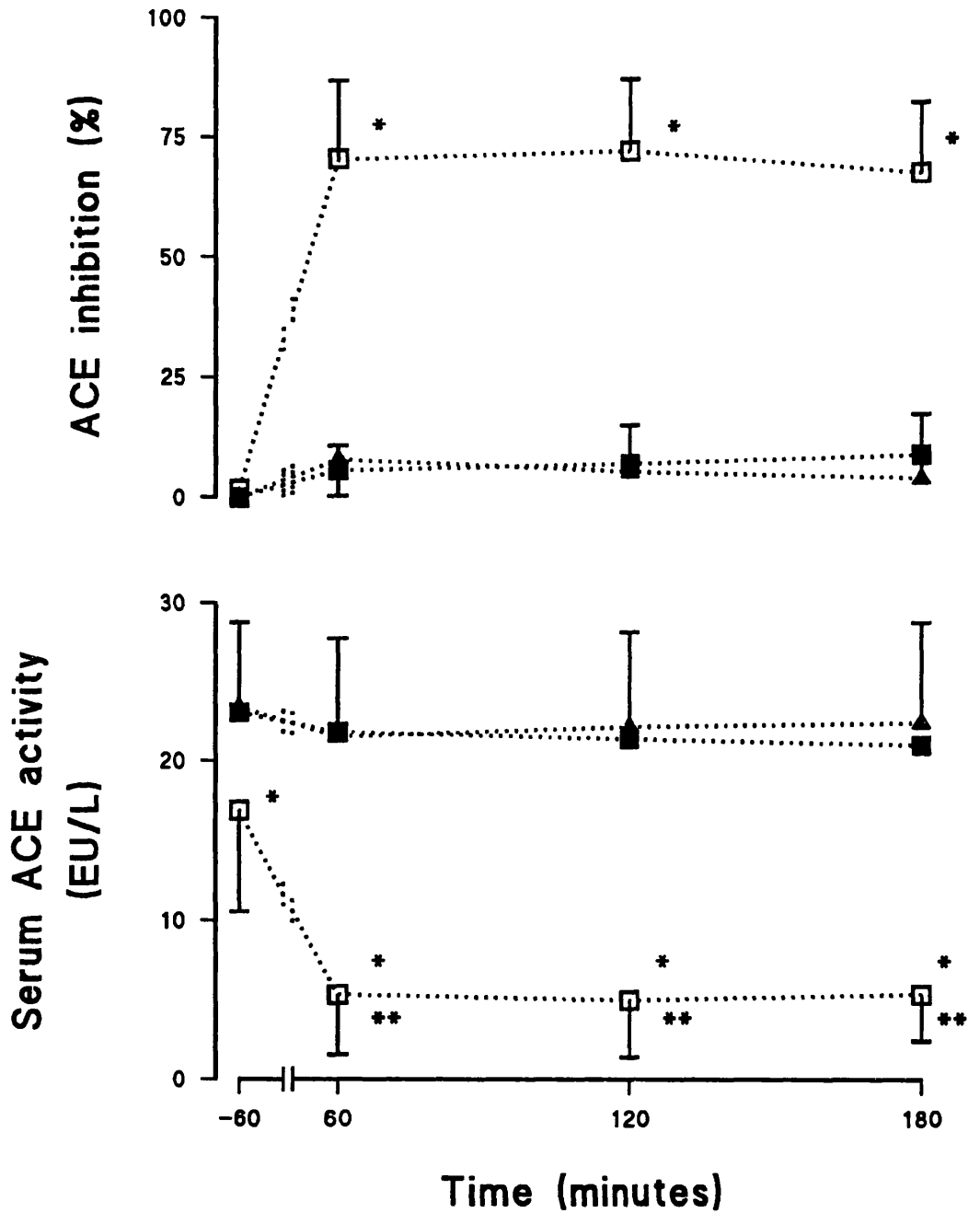


Figure 5.6: Mean profiles of serum ACE activity and percentage ACE inhibition of patients during euglycemic clamp in trandolapril study.

■ = run in placebo ▲ = 4 weeks placebo, □ = 4 weeks trandolapril.

* = $p < 0.001$ 4 weeks trandolapril vs. corresponding 4 weeks placebo timepoint

** = $p < 0.01$ trandolapril vs. baseline (-60 mins) trandolapril timepoint.

there were no further significant reductions thereafter. Treatment with trandolapril had no significant effect on insulin-stimulated reductions in serum potassium concentration during the clamp.

5.3.8. Serum aldosterone, plasma renin activity and plasma noradrenaline

The time course of changes in serum aldosterone concentrations during the clamp are shown in figure 5.7. Both trandolapril and placebo clamps were associated with a significant ($P < 0.004$) decline in plasma aldosterone, and chronic ACE inhibition had a further independent (20%) effect of lowering plasma aldosterone concentrations; for example, at 120 minutes aldosterone levels were 67 ± 30 pg/ml and 100 ± 64 pg/ml after 4 weeks trandolapril and placebo respectively.

There was a significant increase in plasma renin activity after trandolapril administration; for example, at 180 minutes plasma renin activity was 4.6 ± 3.7 ng AI/min after trandolapril compared with 1.6 ± 0.2 and 1.9 ± 1.5 ng AI/min after the run-in and placebo phases respectively ($p < 0.001$) (figure 5.7).

The acute rise in serum insulin was not associated with any significant change in plasma noradrenaline concentrations during any of the three study days (figure 5.8).

5.3.9. Serum triglyceride concentrations

Treatment with trandolapril was associated with a significant reduction in fasting triglyceride concentrations. Thus, baseline values were 2.1 ± 0.9 mmol/l and 2.0 ± 1.2 following placebo run-in and 4 weeks placebo, compared with 1.4 ± 0.6 mmol/l after 4 weeks trandolapril treatment ($p < 0.01$). Both the trandolapril and placebo clamps were associated with a significant ($P < 0.05$) decline in serum triglyceride concentrations during the procedure, but chronic ACE inhibition had no further independent effects on lowering plasma triglycerides during the clamp (figure 5.8).

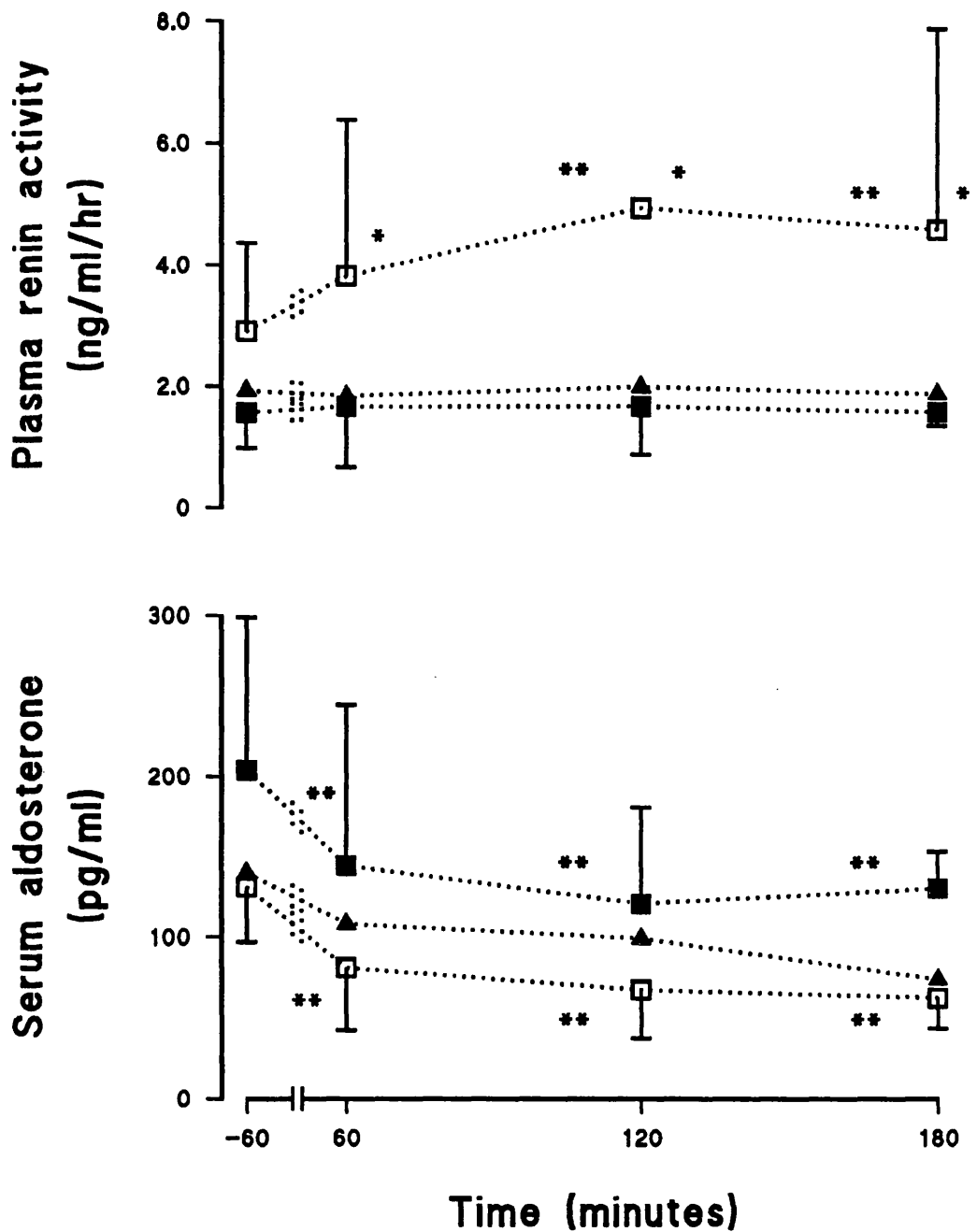


Figure 5.7: Mean profiles of serum aldosterone and plasma renin activity of patients during euglycemic clamp in trandolapril study.

■ = placebo run in, ▲ = 4 weeks placebo, □ = 4 weeks trandolapril.

* = $p < 0.01$ 4 weeks trandolapril vs. corresponding 4 weeks placebo timepoint

** = $p < 0.01$ trandolapril or placebo vs. corresponding baseline (-60 mins) timepoint

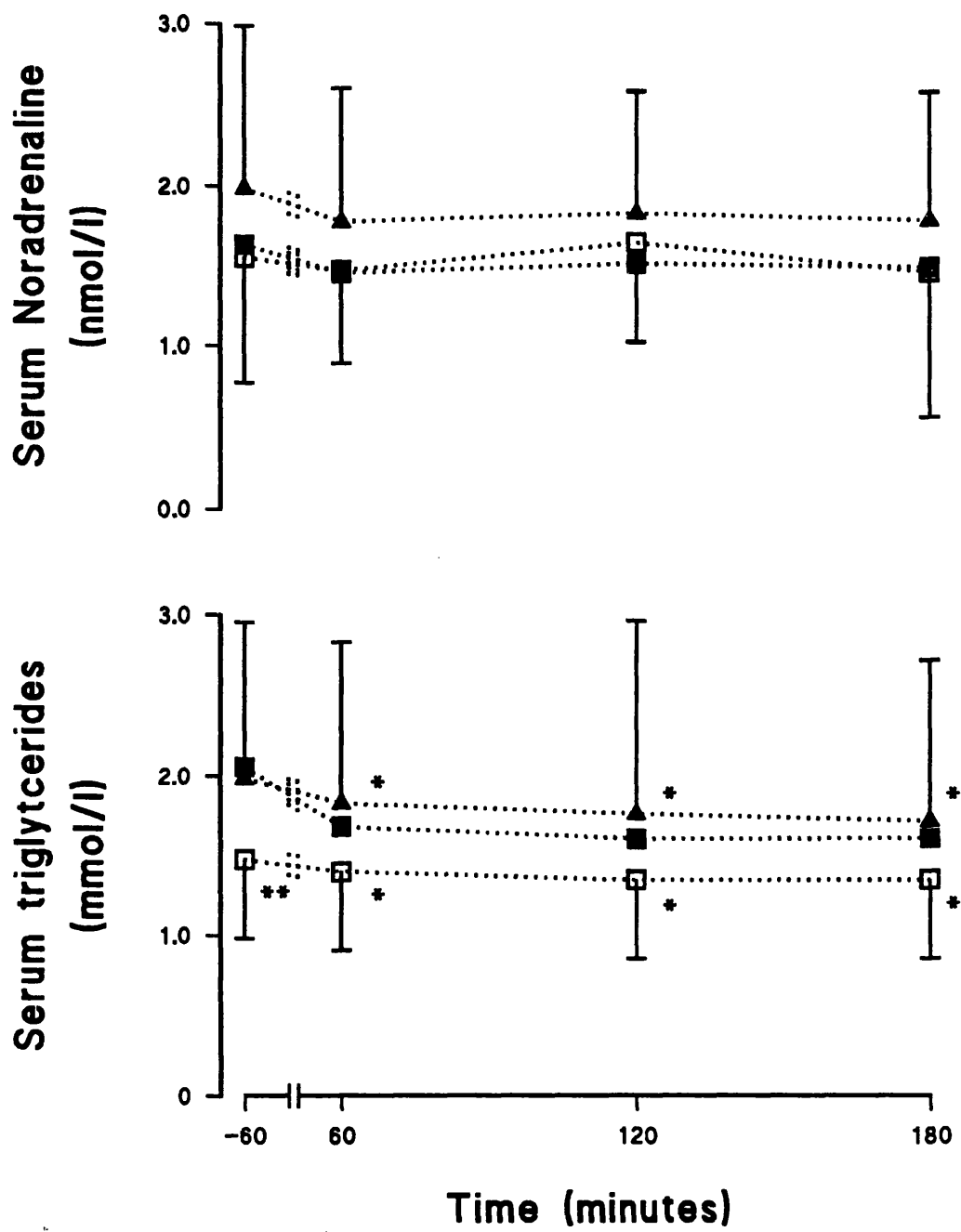


Figure 5.8: Mean profiles of triglyceride and noradrenaline concentrations of patients during euglycemic clamp in trandolapril study.

- = run in placebo ▲ = 4 weeks placebo, □ = 4 weeks trandolapril.
- * = $p < 0.05$ 4 weeks trandolapril or 4 weeks placebo vs. corresponding baseline (-60 mins) timepoint.
- ** = $p < 0.01$ 4 weeks trandolapril vs. 4 weeks placebo

5.4. DISCUSSION

This study has shown that in patients with essential hypertension and glucose tolerance ACE inhibition with trandolapril reduced blood pressure but had no effect on whole body insulin-stimulated glucose uptake. The confidence intervals for the effect of trandolapril (after 4 weeks) excludes a 12% increase or a 23% decrease in insulin sensitivity. The patients in this study were moderately hypertensive, obese (10 of the 12 patients had a BMI greater than 27) and glucose intolerant. These three factors are all recognised to have additive detrimental effects on insulin sensitivity; accordingly the group as a whole were markedly insulin resistant with a mean baseline insulin sensitivity of only 4.6 ± 1.3 mg glucose/min, although limited evidence suggests that hypertension does not add significantly to the magnitude of the insulin resistance that is already present in obesity and NIDDM (Bonora et al, 1993).

The primary aim of this study was to evaluate the effects of chronic ACE inhibition on insulin-stimulated glucose uptake. Much recent research has focused on mechanisms whereby ACE inhibition might contribute to enhanced glucose uptake by skeletal muscle. It has been suggested, for example, that microcirculatory changes in muscle, secondary to vascular hypertrophy and rarefaction, might contribute to insulin resistance, especially in essential hypertension (Julius et al, 1991) [chapter 1.2.1]. It is therefore reasonable to propose that any drug with vasodilatory actions may increase insulin sensitivity by virtue of improved regional perfusion. There are uncontrolled data suggesting that augmented forearm glucose uptake in diabetics receiving captopril is due to a modest increase in limb blood flow (Rett et al, 1986; Jauch et al, 1987; Kodama et al, 1990), although another possible mechanism is blockade of the RAS. ACE inhibitors reduce angiotensin II formation and there are *in vitro* data that suggest angiotensin II has some gluconeogenic and glycogenolytic actions (Kaley et al, 1967; Kneer & Lardy, 1983). Thus, activation of the RAS might be expected to have a deleterious effect on insulin sensitivity and conversely, ACE inhibition might have an opposite effect. This issue is addressed in chapter 6. The last possible mechanism

relates to elevation in systemic kinin levels associated with ACE inhibition. Jauch and colleagues (1988) reported that during a euglycaemic hyperinsulinaemic clamp, the administration of bradykinin was associated with a marked increase in forearm glucose uptake and a reduction in hepatic glucose output in post-surgical patients.

Despite various theoretical mechanisms whereby ACE inhibition might increase insulin-mediated glucose uptake, the principal finding of this study was that ACE inhibition failed to change whole body insulin sensitivity. This result is in agreement with previous studies examining the long term effects of ACE inhibition on whole body insulin sensitivity with cilazapril (Santoro et al, 1992), and captopril (Seghieri et al, 1992), but stands in contrast to well publicised reports that whole body insulin sensitivity is increased after chronic ACE inhibition with captopril (Pollare et al, 1989a; Torlone et al, 1993; Paolisso et al, 1992). Several explanations may account for the disparity in these observations. Firstly, the study of heterogeneous patient groups in whom the defect in insulin sensitivity may not be the same is a possible explanation. This appears unlikely as an improvement in insulin sensitivity has been reported in both glucose tolerant hypertensive patients (Pollare et al, 1989a) and diabetic hypertensive patients (Torlone et al, 1993; Paolisso et al, 1992). Secondly, differences in experimental design may be responsible. For example, in Pollare's study, a small but significant decrease in insulin sensitivity was observed when 3 months treatment with captopril was compared with 3 months of treatment with hydrochlorothiazide. There was no direct comparison with placebo and the study was analysed as parallel groups although it had been designed as a placebo-controlled crossover study. Torlone's study did utilise a placebo-controlled crossover design in 20 diabetic hypertensive patients, but the study was open, used an incremental infusion regimen of insulin to evaluate insulin sensitivity during a euglycaemic hyperinsulinaemic clamp, and patients were allowed to continue oral hypoglycaemic therapy (sulphonylureas and biguanides) throughout the study. And thirdly, it is possible that the effect reported is drug-specific, rather than class-specific. Uncontrolled studies have suggested that the acute improvement in insulin sensitivity

observed with captopril therapy is because it contains a sulphhydryl group (Jauch et al 1987; Uehara et al, 1994). Finally, a potentially important feature of the study design is whether the ACE inhibitor is administered on the morning of the euglycaemic hyperinsulinaemic clamp. As discussed above, it is well established that ACE inhibitor drugs acutely vasodilate forearm blood vessels. Such a mechanism of increasing insulin sensitivity would be common to any vasodilator and not specific to manipulation of the renin-angiotensin system. It is of interest that in the studies of Santoro and Seghieri, drug administration was omitted on each study day, whereas in the studies demonstrating a beneficial effect of captopril, drug treatment was administered immediately prior to the measurement of insulin sensitivity. Lastly, a possible explanation is that some studies have had inadequate power to detect a change in insulin sensitivity. With reference to my study, this appears unlikely, as the 95% confidence intervals exclude a 12% increase in insulin sensitivity with trandolapril treatment.

These results also clarify the response of the RAS to insulin administration in hypertensive diabetic patients. Trovatti and colleagues (1989) reported that during a 1.0 nmol/l euglycaemic hyperinsulinaemic clamp in healthy subjects, plasma renin activity and angiotensin II levels increased while reducing serum aldosterone concentrations. The activity of serum ACE was not affected. These hormonal changes were abolished by potassium supplementation. My results have confirmed adequate inhibition of plasma ACE in this group of hypertensive diabetic patients after once daily dosing of trandolapril; 4 weeks therapy was associated with a 30% reduction in serum ACE activity compared with placebo at baseline (i.e. 24 hours after the last dose) and maximal enzyme inhibition (65-70%) was achieved within 2-4 hours during the clamp. ACE activity was unaffected by the insulin stimulus alone. In this group of patients, trandolapril treatment was associated with a non significant increase in plasma renin activity at baseline and a further significant increase during the clamp. The insulin stimulus alone had no effect on plasma renin activity. The rise in plasma insulin concentrations induced by intravenous insulin caused marked hypokalaemia

with mean serum potassium decrements of 0.44 ± 0.05 and 0.33 ± 0.05 following 4 weeks placebo and 4 weeks trandolapril respectively; the corresponding decrements in serum aldosterone concentrations from baseline were 37% and 52% respectively.

In summary, this is one of the first double-blind, placebo-controlled crossover study to evaluate the effects of ACE inhibitor therapy on insulin dependent metabolic responses in hypertensive patients with impaired glucose tolerance or NIDDM. Despite conflicting evidence from previous uncontrolled studies, there was no evidence that sustained ACE inhibition affected whole body insulin sensitivity after 4 weeks. There has been much speculation as to potential mechanisms whereby ACE inhibitors may improve insulin action in both short-term and long-term studies, including increased bradykinin concentrations (Dietze 1982), increased blood flow to "insulin sensitive" tissues (Junichi et al, 1991), via an angiotensin II sparing effect [see chapter 6], or even a direct metabolic effect of the ACE inhibitor itself. The present data suggest no such beneficial effect with a non-sulphydryl group ACE inhibitor. The interpretation of results of studies of insulin sensitivity following treatment with ACE inhibitors are highly dependent upon the type of protocol used.

CHAPTER 6

EFFECTS OF SUB-PRESSOR AND PRESSOR DOSES OF
ANGIOTENSIN II ON INSULIN SENSITIVITY IN HEALTHY SUBJECTS
AND PATIENTS WITH NIDDM

6.1. GENERAL INTRODUCTION.

Peripheral and hepatic insulin resistance, affecting multiple pathways of glucose and lipid metabolism, plays a major role in the development of NIDDM (DeFronzo et al, 1992) [chapter 1.1.1]. In addition, resistance to insulin-mediated glucose transport and hyperinsulinaemia (short of overt diabetes) have been implicated as both slow-pressor and atherogenic mechanisms that might contribute to increased blood pressure and the long term development of hypertensive vascular complications (Reaven, 1988; Ferrannini et al, 1987; Stout, 1989). Thus, the significance of common associations among metabolic and haemodynamic abnormalities, not only in patients with diabetes but also in non-diabetic subjects, has attracted widespread interest [chapter 1.1.4]. Since hypertension is at least twice as common in patients with NIDDM (independent of body weight and renal dysfunction) and the combination of diabetes and hypertension exerts a greater-than-additive effect on coronary heart disease risk (Hypertension in Diabetes Study Group 1993), there are important reasons for pursuing the nature of this association and for identifying possible sites of interaction between mechanisms of blood pressure and endocrine regulation. Thus, at both *in vitro* and *in vivo* levels, numerous studies have suggested important pathophysiological links between glucose and insulin metabolism and the neuroendocrine systems involved in cardiovascular regulation, particularly the renin-angiotensin-aldosterone system (RAS) and the sympathetic nervous system (Trovatti et al, 1989; Ferrannini et al, 1992; Rowe et al, 1981). In particular, it has been proposed that changes in glucose and insulin metabolism might be secondary to hypertensive microvascular complications (Baron et al, 1993), and much discussion has focussed on the importance of skeletal muscle blood flow as a determinant of glucose disposal rate and the potential insulin-antagonist effects of neuroendocrine mechanisms involved in cardiovascular regulation (Baron et al, 1991a; Julius et al, 1991). Thus, the insulin antagonist effects of catecholamines are well established

(Deibert & DeFronzo, 1980), and insulin augments vascular reactivity to noradrenaline (Gans et al, 1991a).

More recently, attention has focussed on the role of the RAS in insulin resistant states. There is evidence that the circulating and tissue-based RAS may be implicated in the pathogenesis of common diabetic complications including hypertension, microalbuminuria, renal dysfunction and both micro- and macrovascular disease. Local generation of angiotensin peptides occurs in the microvascular network of skeletal muscle (Vicaut & Hou, 1993), and might potentially regulate blood flow distribution to muscle fibres of varying insulin sensitivity. In addition, there is limited evidence that components of the RAS directly affect metabolic control; *in vitro* studies have shown hyperglycaemic effects of angiotensin II on the liver, via increased glycogenolysis and decreased gluconeogenesis (Kaley et al, 1967). Hyperinsulinaemia enhances the aldosterone response to angiotensin II with no effect on the vasoconstrictor response (Gans et al, 1991a; Vierhapper et al, 1983). However, there are no *in vivo* data examining the direct metabolic effects of angiotensin II. The only evidence comes indirectly from the metabolic effects of renin-angiotensin system blockade with angiotensin converting enzyme inhibitor drugs (chapters 1.3.2. and 5.1.) and from studies evaluating insulin sensitivity after dietary salt modification; thus, there is evidence that angiotensin converting enzyme inhibitors may enhance insulin sensitivity in both diabetic and non-diabetic subjects (Pollare et al, 1989a; Prince et al, 1988; Gans et al, 1991b), even after single-dose administration (Jauch et al, 1987), and that dietary salt restriction in "salt-sensitive" normotensive volunteers may increase insulin sensitivity (Sharma et al, 1991; Donovan et al, 1993).

Therefore, there is evidence suggesting involvement of the RAS in some of the metabolic as well as the haemodynamic features of diabetes. Since the metabolic derangements associated with diabetes produce structural as well as functional changes in arteries and resistance arterioles, and since haemodynamic responses to angiotensin II (as well as other vasoconstrictor peptides) are reported to differ

significantly in diabetic compared with non-diabetic subjects (Tuck et al, 1980; Christlieb, 1976), the present study was designed to evaluate the effects of two doses of angiotensin II on blood pressure and insulin sensitivity in both healthy subjects and in patients with NIDDM.

6.2. SUBJECTS AND METHODS.

Twelve healthy normal volunteers, median age 25 years with mean BMI 24.0 ± 2.0 , and eleven diet-controlled patients with NIDDM, median age 56 years, BMI 27.5 ± 2.0 , with mean duration of diabetes of 4 years, gave written informed consent to participate in this study. Clinical details of the individual subjects are shown in table 6.1.

Each subject attended three 5-hour study mornings, one week apart, in the CIRU to evaluate the effects of placebo, subpressor (1 ng/kg/min) and pressor (5 ng/kg/min) doses of angiotensin II on insulin stimulated metabolic responses in a randomised, double blind, crossover design. On each occasion, following an overnight fast, subjects attended the CIRU at 0745 hours. After 20 minutes supine rest, baseline blood pressure and heart rate measurements were recorded. On each study day, whole body insulin sensitivity was assessed using the euglycaemic hyperinsulinaemic clamp [chapter 2.3]. In addition to the infusions of insulin and 20% dextrose, a third constant rate infusion of either saline, low (1 ng/kg/min), or high (5 ng/kg/min) dose angiotensin II was administered for 180 minutes. The angiotensin II or placebo infusion was prepared in 50 mls of saline by an independent investigator in the Pharmacy Department at the Western Infirmary, Glasgow and administered intravenously into the left antecubital fossa using a Braun perfusor pump.

Systolic and diastolic blood pressure and heart rate were recorded at 15 minute intervals during the euglycaemic clamp. At baseline, and at 60 minute intervals

<u>VOLUNTEER</u> <u>No.</u>	<u>AGE</u> <u>(years)</u>	<u>BMI</u> <u>(kg/m²)</u>	<u>SEX</u>
1	22	26.5	M
2	27	24.0	M
3	18	23.5	M
4	35	23.6	M
5	27	23.9	M
6	20	22.7	M
7	24	26.5	M
8	30	29.4	M
9	28	23.2	M
10	26	20.1	M
11	26	21.7	M
12	24	22.6	M

<u>PATIENT</u> <u>No.</u>	<u>AGE</u> <u>(years)</u>	<u>BMI</u> <u>(kg/m²)</u>	<u>SEX</u>	<u>SCREEN BP</u> <u>(mm Hg)</u>	<u>GLUCOSE</u> <u>(fasting mmol/l)</u>	<u>HbA_{1c}</u> <u>(%)</u>
1	33	20.7	M	96/60	5.8	9.0
2	44	27.6	M	146/88	8.4	8.2
3	67	23.7	M	170/90	5.8	8.8
4	53	24.0	F	143/70	7.4	7.8
5	46	27.8	M	126/88	9.1	7.8
6	52	25.5	M	131/80	9.7	8.0
7	56	20.1	F	137/75	18.9	11.2
8	56	34.8	M	131/79	10.2	9.0
9	70	36.3	M	147/74	6.6	8.2
10	65	23.7	M	123/74	9.3	8.8
11	56	39.5	F	134/64	8.9	9.0

TABLE 6.1: DETAILS OF HEALTHY SUBJECTS AND PATIENTS WITH NON-INSULIN DEPENDENT DIABETES IN ANGIOTENSIN II INFUSION STUDY

additional blood samples were collected for measurements of serum insulin, C-peptide, plasma renin activity, serum aldosterone and plasma catecholamine concentrations. A single 10ml blood sample for the measurement of plasma angiotensin II concentration was collected at 120 minutes.

It was calculated from previous work that these studies would have greater than 80% power to detect a 15% change in insulin sensitivity in both the healthy volunteers and patients with NIDDM.

6.3. RESULTS (I) - HEALTHY VOLUNTEERS.

6.3.1. Tolerance.

The euglycaemic clamp was well tolerated; there were no adverse biochemical events and there were no withdrawals from the study.

6.3.2. Blood pressure.

The high dose infusion of angiotensin II was associated with a significant increase in supine systolic and diastolic blood pressure from 15 to 180 mins; for example, at 150 minutes, supine blood pressure was $131/65 \pm 13/11$ mmHg compared with $118/57 \pm 14/10$ for the corresponding placebo infusion. The subpressor infusion of ANG II was not associated with any significant change in blood pressure (figure 6.1).

6.3.3. Heart rate.

There were no statistically significant differences in heart rate between the 3 study days (figure 6.2).

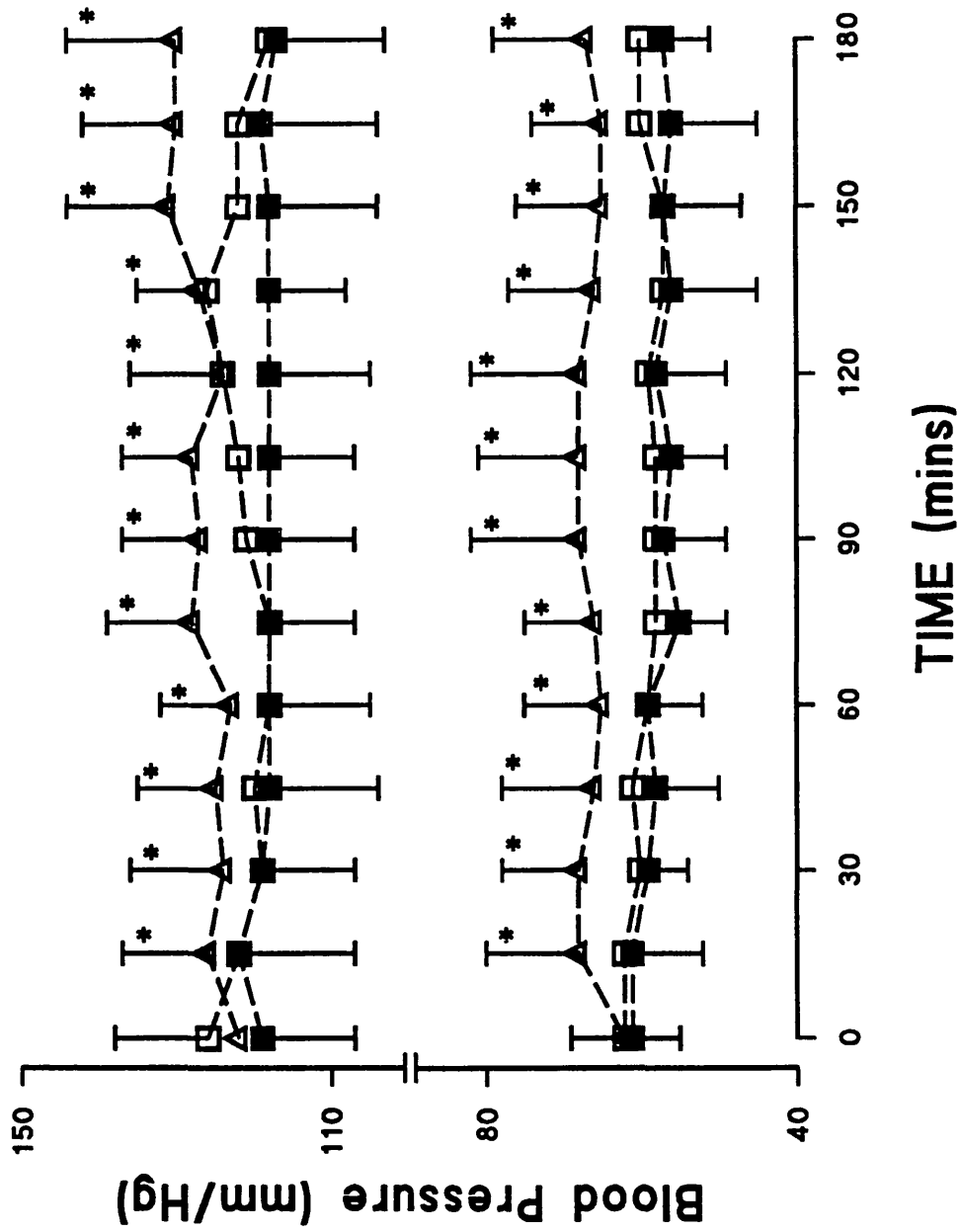
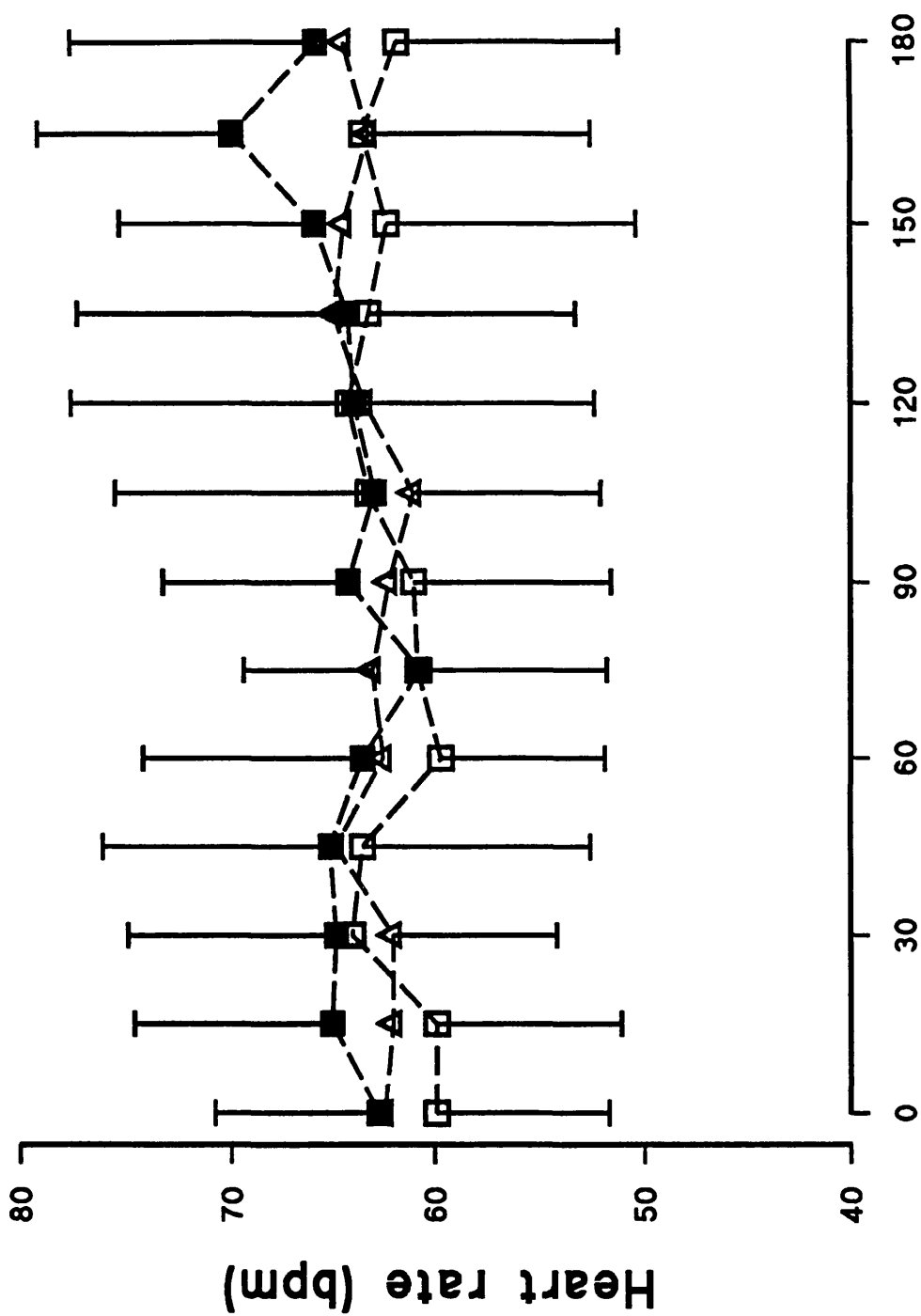


Figure 6.1: Mean profiles of supine systolic and diastolic blood pressure of healthy subjects during euglycaemic clamp in angiotensin II infusion study.

■ = placebo, □ = sub-pressor ANG II, △ = pressor ANG II
 * = $p < 0.01$ pressor ANG II versus placebo



Time (minutes)

Figure 6.2: Mean profiles of supine heart rate of healthy subjects during euglycaemic clamp in angiotensin II infusion study.

■ = placebo, □ = subpressor ANG II, Δ = pressor ANG II.

6.3.4. Serum insulin and C-peptide concentrations.

There were no significant differences in insulin concentrations during the clamp on the 3 study days; a "steady-state" insulin concentration was achieved within the first 60 minutes of the clamp (figure 6.3). For example, at 120 minutes, serum insulin concentrations were 122 ± 22 , 129 ± 43 and 126 ± 38 uU/ml after placebo, subpressor angiotensin II and pressor ANG II respectively. The acute rise in serum insulin was associated with a significant decrease in C-peptide concentrations; for example, at 180 minutes values were 20-25% lower than baseline fasting levels (figure 6.3) ($p < 0.01$).

6.3.5. Plasma glucose and whole body insulin sensitivity.

Angiotensin II had no significant effect on insulin-stimulated glucose uptake. Mean values for whole body insulin sensitivity were 10.5 ± 2.0 , 10.5 ± 2.2 and 10.9 ± 3.4 mg glucose/kg/min on the placebo, sub-pressor angiotensin II and pressor angiotensin II study days respectively (table 6.2). Confidence intervals for this observation were (-1.9, 1.1) for placebo versus high dose angiotensin II. There was no significant difference in plasma glucose concentrations between the clamps on the 3 study days; mean plasma glucose concentration for the last 40 minutes of the clamp were 5.2 ± 0.4 , 5.1 ± 0.3 and 5.2 ± 0.3 mmol/l for placebo, low and high dose angiotensin II respectively. The mean c.v. of plasma glucose concentrations during all the clamps was 8%.

6.3.6. Plasma angiotensin II concentrations.

Plasma angiotensin II levels at 120 minutes were 11 ± 5 , 27 ± 9 and 125 ± 28 pg/ml after placebo, low dose and high dose infusions of angiotensin II respectively. Individual values of plasma angiotensin II concentrations are shown in table 6.3.

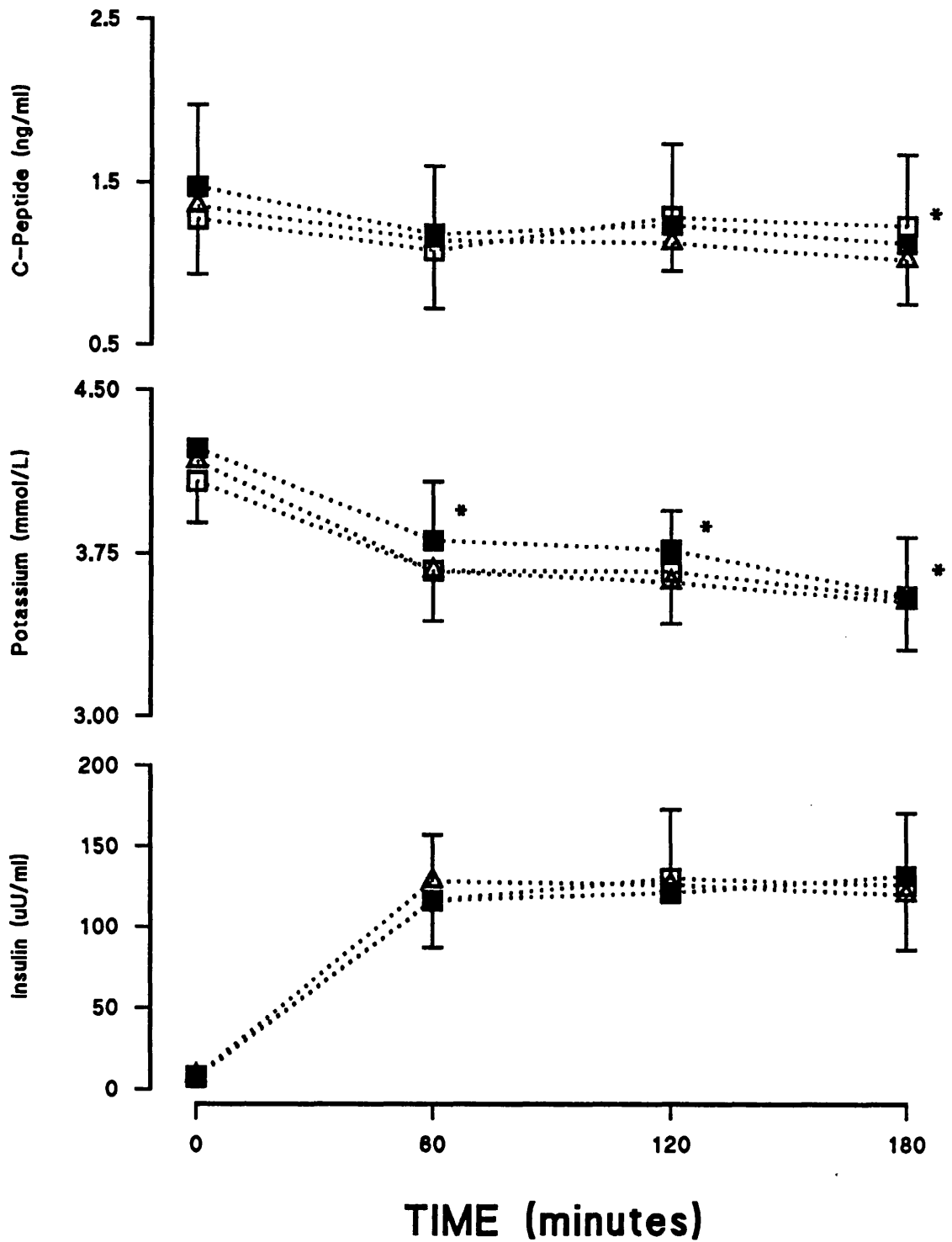


Figure 6.3: Mean profiles of serum insulin, C-peptide and serum potassium of healthy subjects during euglycaemic clamp in angiotensin II infusion study

■ = placebo, □ = sub-pressor ANG II, △ = pressor ANG II
 * = p < 0.01 from baseline fasting values

SUBJECT	PLACEBO	LOW DOSE ANG II	HIGH DOSE ANG II
1	10.2	10.0	10.5
2	8.8	5.0	5.0
3	9.3	10.0	10.7
4	12.0	12.0	12.2
5	12.5	11.8	13.5
6	9.5	11.5	10.0
7	9.0	11.0	9.0
8	7.5	9.3	6.5
9	8.8	11.9	9.0
10	13.0	13.1	12.8
11	11.3	9.5	12.0
12	14.0	13.0	18.2
MEAN	10.5 ± 2.0	10.7 ± 2.2	10.9 ± 3.4

Table 6.2: Individual values of whole body insulin sensitivity of healthy subjects in angiotensin II study.
Low dose ANG II = 1ng/kg/min. High dose ANG II = 5 ng/kg/min.
Values expressed as mg glucose/kg/min.

SUBJECT	PLACEBO	LOW DOSE ANG II	HIGH DOSE ANG II
1	NR	35.0	20.0
2	9.8	NR	106.4
3	14.4	24.6	140.8
4	8.8	26.7	150.0
5	4.6	20.8	131.5
6	6.8	11.2	159.6
7	4.6	23.6	91.0
8	10.9	24.0	148.2
9	13.2	27.8	139.2
10	17.6	27.0	135.7
11	10.3	46.4	83.2
12	10.7	31.8	85.0
MEAN	11 ± 5	27 ± 9	125 ± 28

Table 6.3: Individual values of plasma angiotensin II concentrations in healthy subjects at 120 minutes in angiotensin II infusion study. NR = not recorded. Low dose ANG II = 1ng/kg/min. High dose ANG II = 5 ng/kg/min Values expressed as pg/ml.

6.3.7. Serum potassium concentrations.

There was a significant reduction in serum potassium concentration during each clamp; the reduction was maximal within 60 minutes and there were no further significant reductions thereafter (figure 6.3). Angiotensin II had no significant effect on insulin stimulated reductions in serum potassium concentration during the clamp.

6.3.8. Plasma noradrenaline, plasma renin activity and serum aldosterone.

The high dose of angiotensin II was associated with a significant increase in plasma aldosterone concentration at all time points after starting the infusion; for example, at 120 minutes, 314 ± 161 pg/ml compared with 62 ± 19 pg/ml during the placebo infusion ($p < 0.001$) (figure 6.4). There was no significant change in plasma renin activity after angiotensin II administration; for example, after the high dose angiotensin II infusion; at 180 minutes the plasma renin activity was 1.62 ± 0.97 ng AI/min compared with 1.65 ± 0.63 ng AI/min after the placebo infusion. The mean profiles of plasma catecholamines are shown in figure 6.5. There was no significant change in the concentrations of plasma noradrenaline or adrenaline during the euglycaemic hyperinsulinaemic clamp.

6.4. RESULTS (II) - PATIENTS WITH NIDDM.

6.4.1. Tolerance.

In the patients with NIDDM, the clamp was well tolerated and there were neither any adverse biochemical events nor any withdrawals from the study.

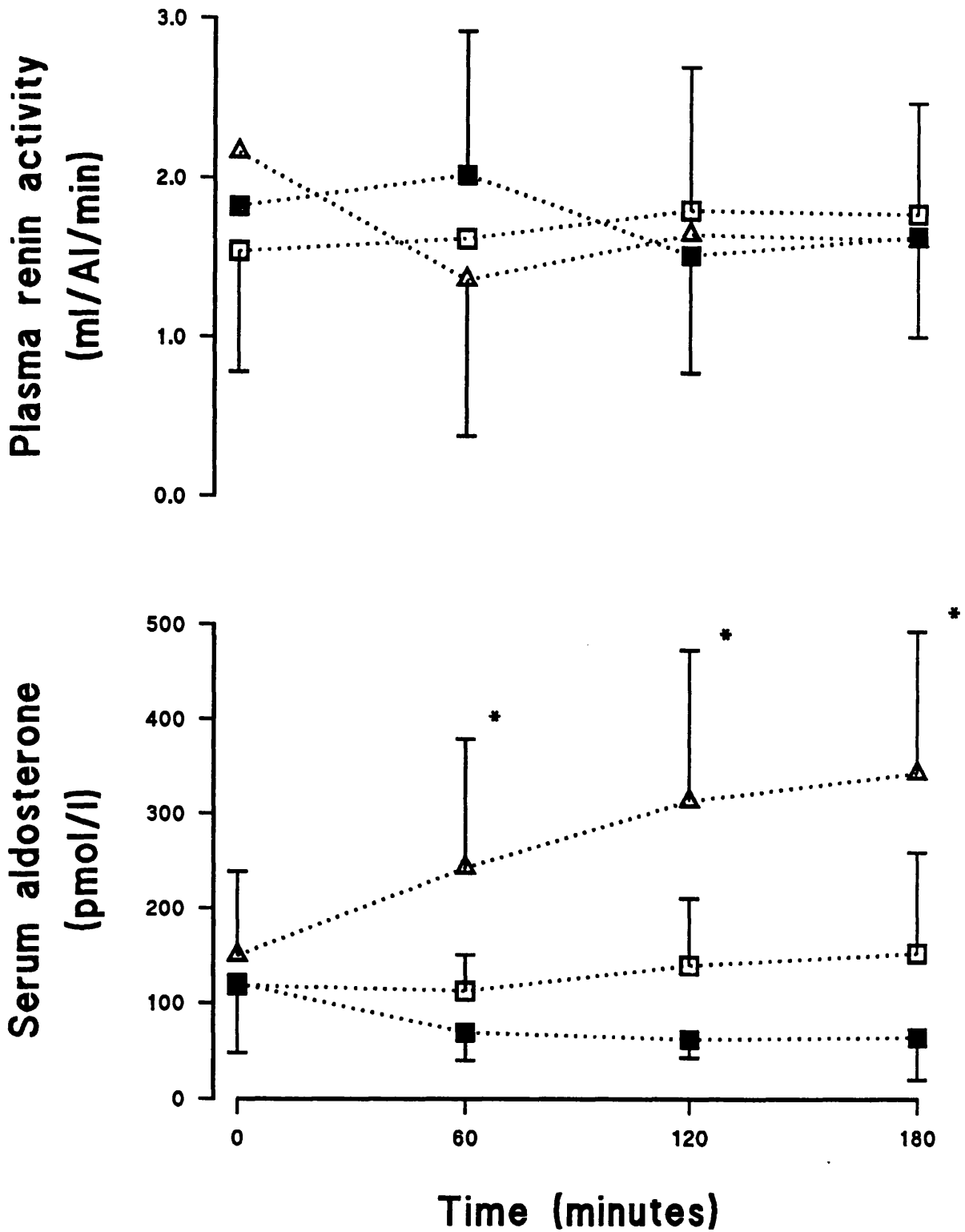


Figure 6.4: Mean profiles of serum aldosterone and plasma renin activity of healthy subjects during euglycaemic clamp in angiotensin II infusion study
 ■ = placebo, □ = sub-pressor ANG II, △ = pressor ANG II
 * = $p < 0.001$ ANG II vs. placebo.

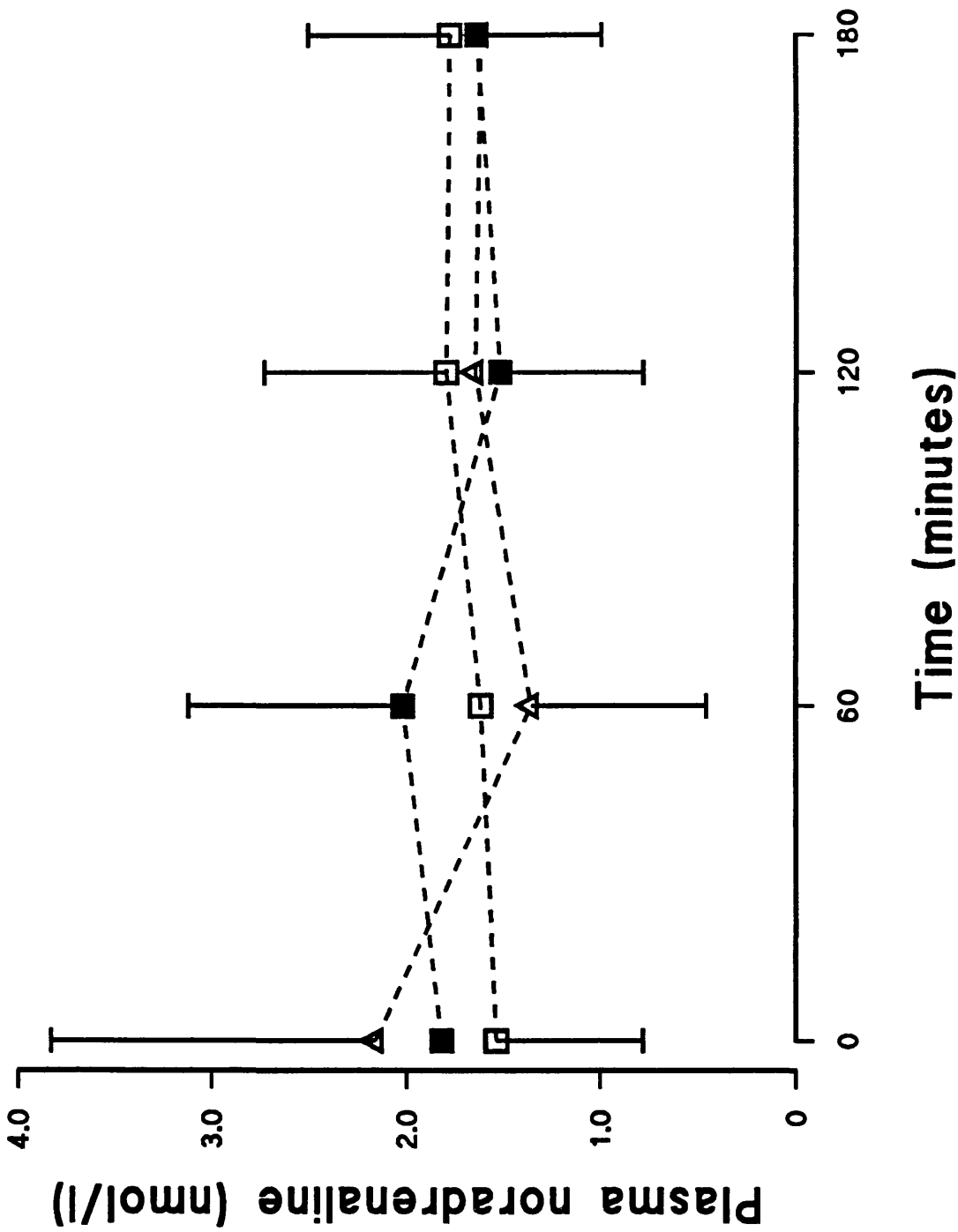


Figure 6.5: Mean profiles of plasma noradrenaline of healthy subjects during euglycaemic clamp in angiotensin II infusion study.

■ = placebo, □ = sub-pressor ANG II, Δ = pressor ANG II.

6.4.2. Blood pressure

The high dose infusion of angiotensin II was associated with a significant increase in supine systolic and diastolic blood pressure from 15 to 180 mins; for example, at 180 minutes, supine blood pressure was $138/81 \pm 23/13$ mmHg compared with $122/68 \pm 16/12$ for the corresponding placebo infusion. The subpressor infusion of angiotensin II had no significant effect on blood pressure (figure 6.6).

6.4.3. Heart rate.

There were no differences in heart rate between the 3 study days (figure 6.7).

6.4.4. Serum insulin and C-peptide concentrations.

The profiles of serum insulin and C-peptide concentrations on the 3 study days are depicted in figure 6.8. Serum insulin levels increased to a plateau within the first 60 minutes of the infusion and there were no significant differences between the placebo and angiotensin II study days. The hyperinsulinaemic stimulus was associated with a decrease in C-peptide concentrations; at 180 minutes values were 30-40% lower than baseline fasting levels (figure 6.8.) ($p < 0.001$).

6.4.5. Plasma glucose and whole body insulin sensitivity.

There was no significant difference in fasting plasma glucose concentrations or the glucose concentration during the clamps; mean fasting plasma glucose values on the three study days were 8.8 ± 3.2 mmol/L, 9.0 ± 3.6 mmol/L and 8.8 ± 3.2 mmol/L for placebo, sub-pressor angiotensin II and pressor angiotensin II respectively. The corresponding mean plasma glucose concentrations for the last 40 minutes of the clamp were 5.3 ± 0.3 , 5.2 ± 0.3 and 5.3 ± 0.3 mmol/L.

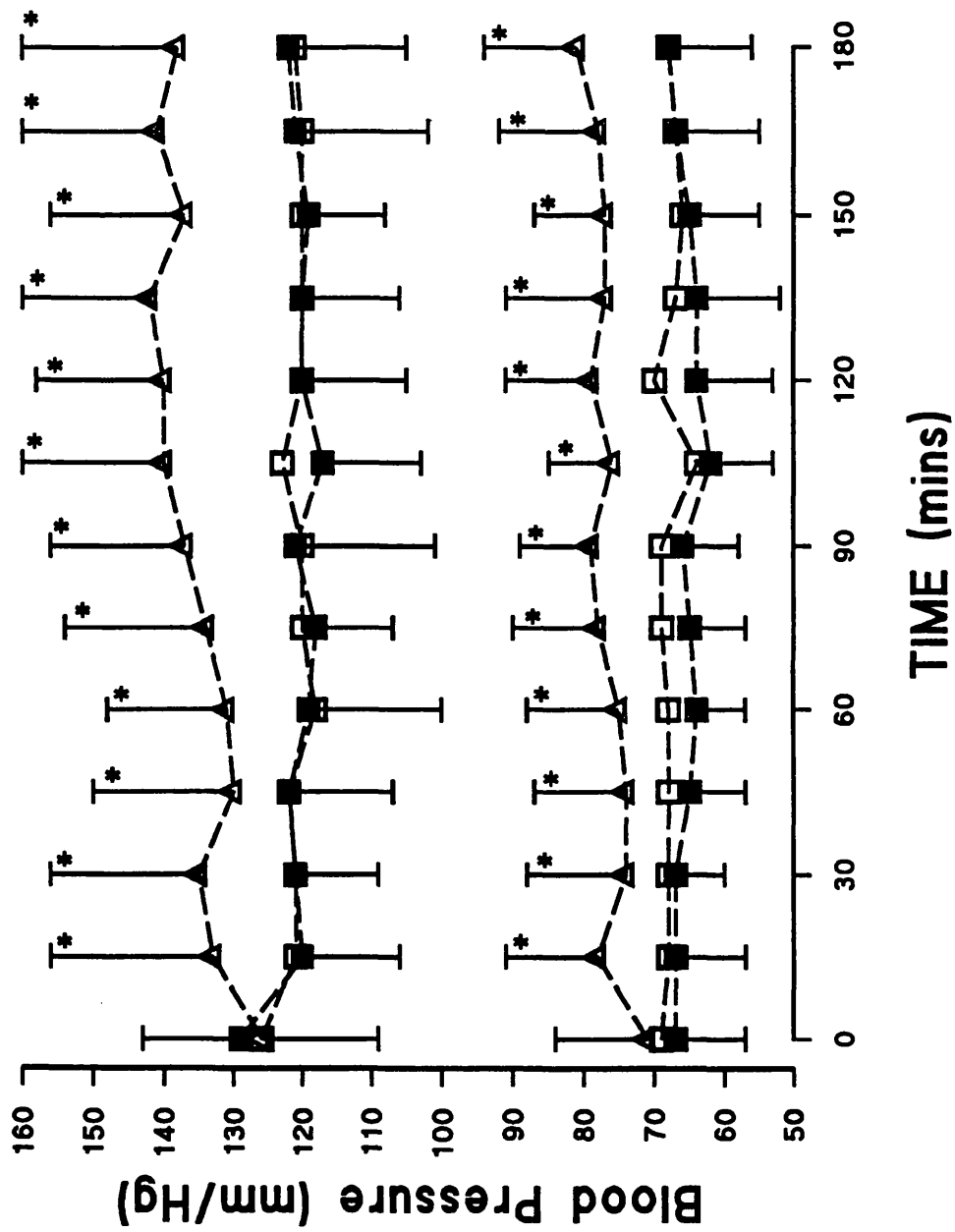


Figure 6.6: Mean profiles of supine systolic and diastolic blood pressure of diabetic patients during euglycaemic clamp in angiotensin II infusion study
 ■ = placebo, □ = sub-pressor ANG II, Δ = pressor ANG II.
 * = p<0.01 pressor ANG II vs. placebo

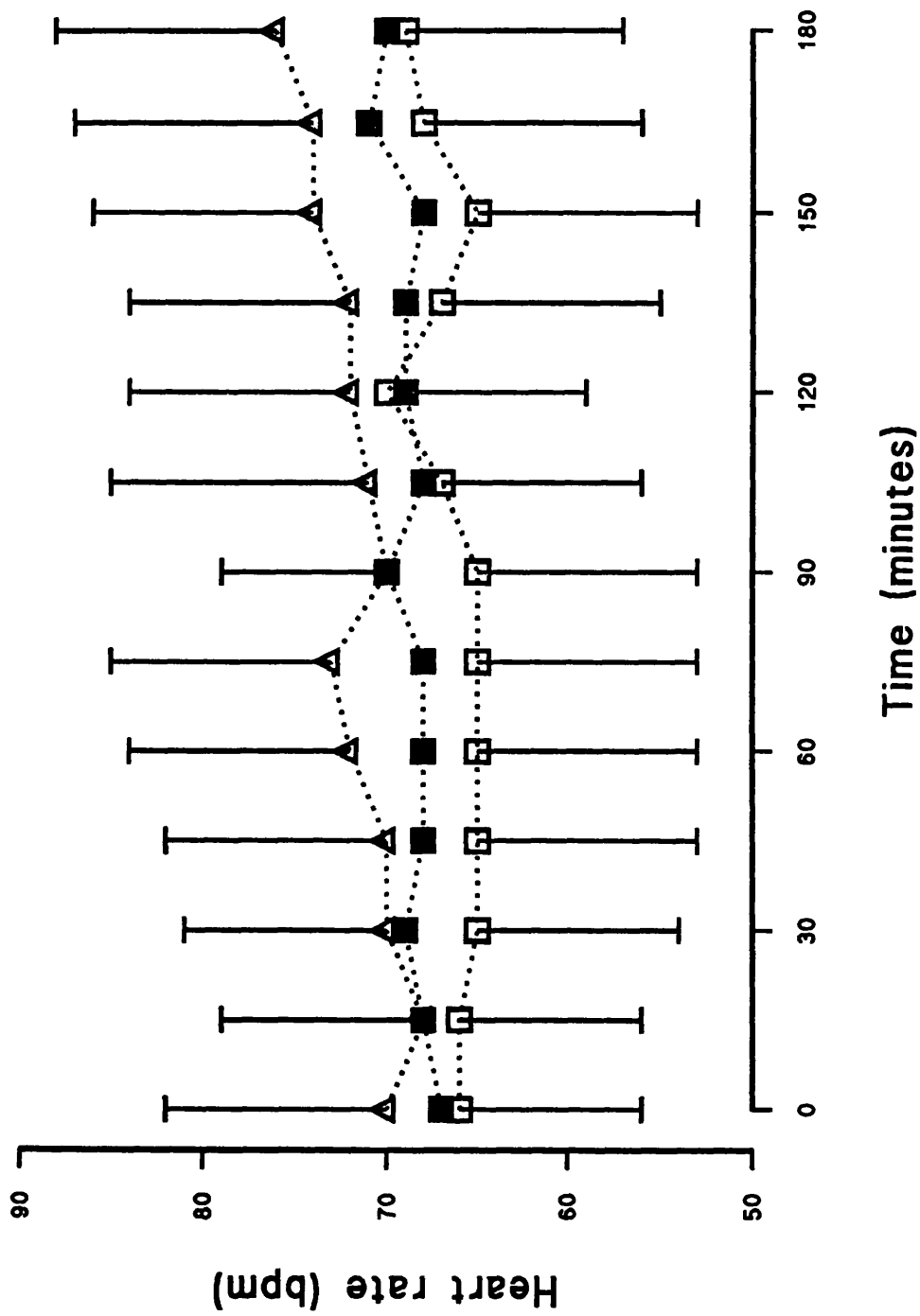


Figure 6.7: Mean profiles of supine heart rate of diabetic patients during euglycaemic clamp in angiotensin II infusion study.

■ = placebo, □ = sub-pressor ANG II, △ = pressor ANG II.

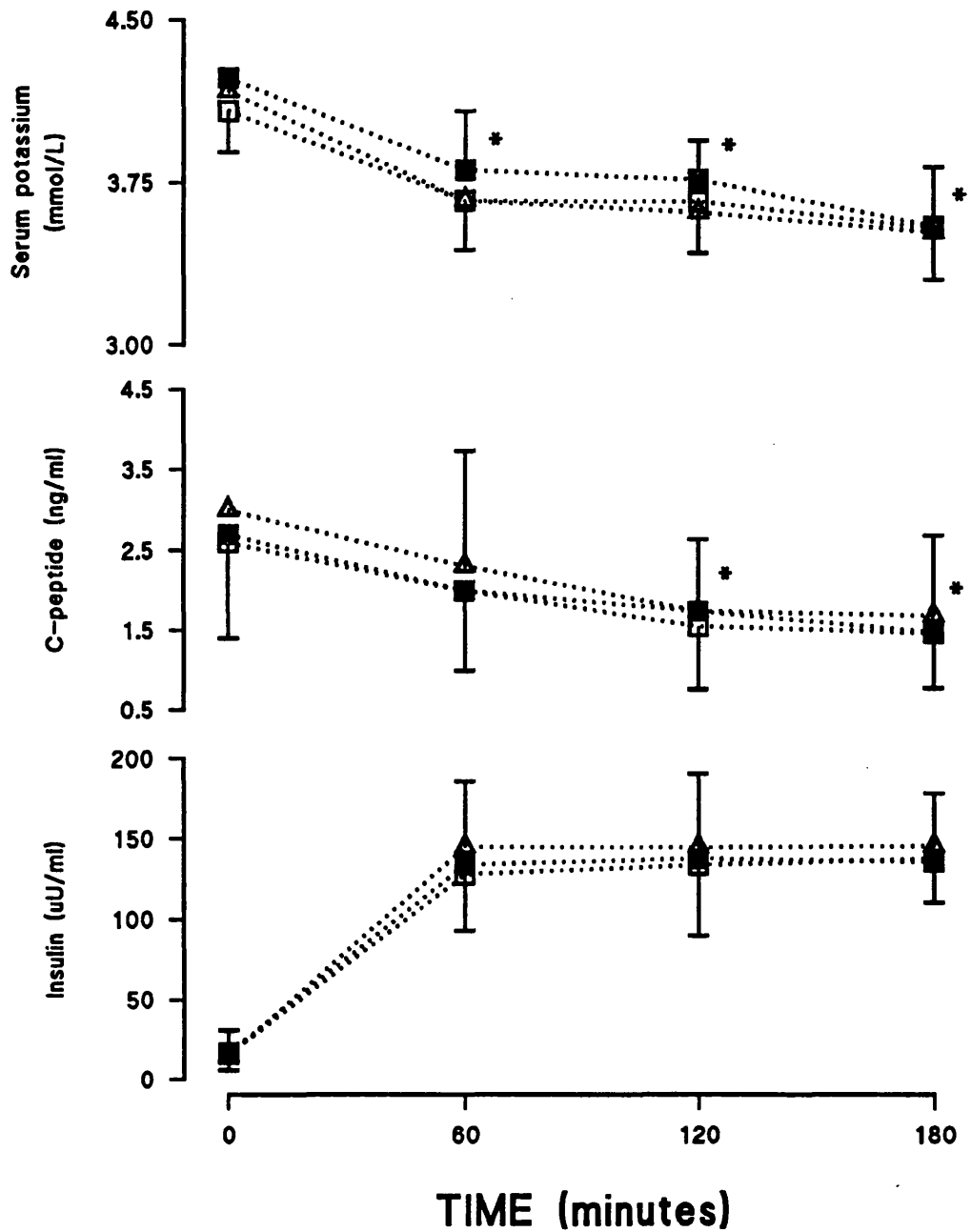


Figure 6.8: Mean profiles of serum insulin, C-peptide and serum potassium concentrations of diabetic patients during euglycaemic clamp in angiotensin II infusion study

■ = placebo, □ = sub-pressor ANG II, ▲ = pressor ANG II.

* = p < 0.001 from corresponding baseline value.

Angiotensin II increased insulin-stimulated glucose uptake; mean values for whole body insulin sensitivity were 4.3 ± 2.3 , 5.5 ± 2.3 and 4.9 ± 2.4 mg glucose/kg/min on the corresponding study days ($p < 0.05$). Confidence intervals for this observation were (-0.1, -1.7) for placebo versus angiotensin II (table 6.3).

6.4.6 Plasma angiotensin II concentrations.

Plasma angiotensin II levels at 120 minutes were 8 ± 4 , 28 ± 9 and 162 ± 45 pg/ml after placebo, low dose and high dose infusions of angiotensin II respectively. The individual values of plasma angiotensin II concentrations are shown in table 6.4.

6.4.7. Serum potassium concentrations.

There was a significant reduction in serum potassium concentration on each study day which was maximal within 60 minutes after starting the clamp. There were no further significant reductions thereafter (figure 6.8). Angiotensin II had no significant effect on insulin stimulated reductions in serum potassium during the clamp.

6.4.8. Plasma noradrenaline, plasma renin activity and serum aldosterone concentrations.

The high dose of angiotensin II was associated with a significant increase in plasma aldosterone concentration at all time points after starting the infusion; for example, at 120 minutes, 325 ± 128 pg/ml compared with 79 ± 25 pg/ml during the placebo infusion ($p < 0.01$) (figure 6.9.). There was no significant change in plasma renin activity after angiotensin II administration; for example, after the high dose angiotensin II infusion; at 180 minutes the plasma renin activity was 1.53 ± 1.58

PATIENT	PLACEBO	LOW DOSE ANG II	HIGH DOSE ANG II
1	4.7	6.6	6.2
2	2.6	4.1	2.8
3	5.2	6.6	4.9
4	8.9	9.5	8.1
5	1.5	2.1	0.6
6	2.8	3.5	5.2
7	5.2	5.5	7.0
8	3.4	3.5	3.3
9	4.1	5.5	5.7
10	7.4	8.2	8.0
11	1.1	5.1	2.1
MEAN	4.3 ± 2.3	5.5 ± 2.3	4.9 ± 2.4

Table 6.4: Individual values of whole body insulin sensitivity of patients with diabetes in angiotensin II study.
 Low dose ANG II = 1ng/kg/min. High dose ANG II = 5 ng/kg/min
 Values expressed as mg glucose/kg/min.
 Angiotensin II significantly increased insulin sensitivity
 p<0.05; 95% confidence intervals -0.1,-1.7

PATIENT	PLACEBO	LOW DOSE ANG II	HIGH DOSE ANG II
1	NR	NR	NR
2	9.3	23.0	203.0
3	6.7	18.3	235.0
4	6.0	27.5	164.0
5	9.3	46.0	198.8
6	3.0	24.5	105.6
7	15.2	42.3	NR
8	10.8	30.0	161.3
9	9.1	23.8	168.8
10	3.5	16.0	111.8
11	11.8	29.7	112.8
MEAN	8.5 \pm 4.0	28.0 \pm 9.0	162.1 \pm 45.6

Table 6.5: Individual values of plasma angiotensin II concentrations in patients with diabetes at 120 minutes in angiotensin II infusion study.
 Low dose ANG II = 1ng/kg/min. High dose ANG II = 5 ng/kg/min
 NR = not recorded.
 Values expressed as pg/ml.

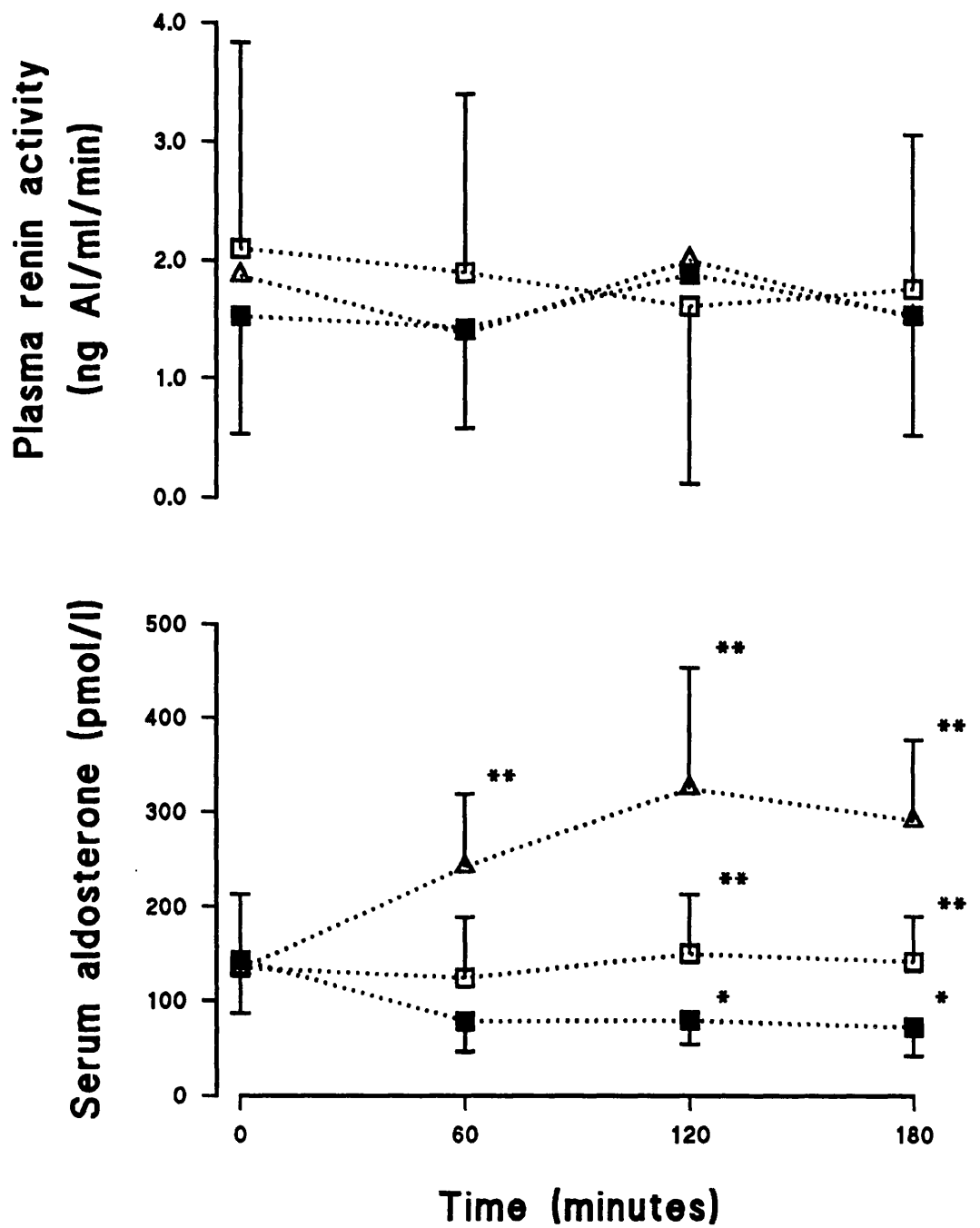


Figure 6.9: Mean profiles of serum aldosterone and plasma renin activity of diabetic patients during euglycaemic clamp in angiotensin II infusion study

■ = placebo, □ = sub-pressor ANG II, △ = pressor ANG II.

* = $p < 0.05$ from baseline.

** = $p < 0.01$ from corresponding placebo value.

ng/AI/min compared with 1.55 ± 1.08 ng/AI/min after the placebo infusion.

The acute rise in serum insulin was associated with a significant increase in plasma noradrenaline and adrenaline concentrations ($p < 0.01$) (figure 6.10). Angiotensin II had no significant effect on the increase in plasma catecholamine concentrations during the clamp.

6.5. DISCUSSION.

This placebo-controlled study has shown that a 3-hour infusion of angiotensin II in healthy volunteers (producing plasma angiotensin II concentrations within the pathophysiological range), with or without an increase in blood pressure, has no effect on insulin-stimulated uptake of glucose, potassium and triglycerides under euglycaemic hyperinsulinaemic conditions. However, in patients with NIDDM who were normotensive and had no evidence of microvascular complications, the results clearly demonstrate that under euglycaemic hyperinsulinaemic conditions both low and high dose angiotensin II infusions were associated with increases in whole-body insulin-stimulated glucose uptake of 28% and 14% respectively, while only the higher dose of angiotensin II increased blood pressure.

In both groups of subjects a reproducible insulin stimulus (within the physiological range) was obtained on each of the 3 study days; the intra-subject variation in plasma insulin concentrations during the last 60 minutes was less than 8% in the healthy subjects and 6% in the patients with NIDDM. There was no evidence of an effect of angiotensin II on insulin clearance in either group. As with most clamp studies, the glucose infusion rate, the critical determinant of glucose disposal, was adjusted manually (by myself) in response to frequent measurements of plasma glucose at the bedside. It is often recognised but seldom acknowledged that this can introduce subjective bias, even though glucose levels are maintained within reasonable

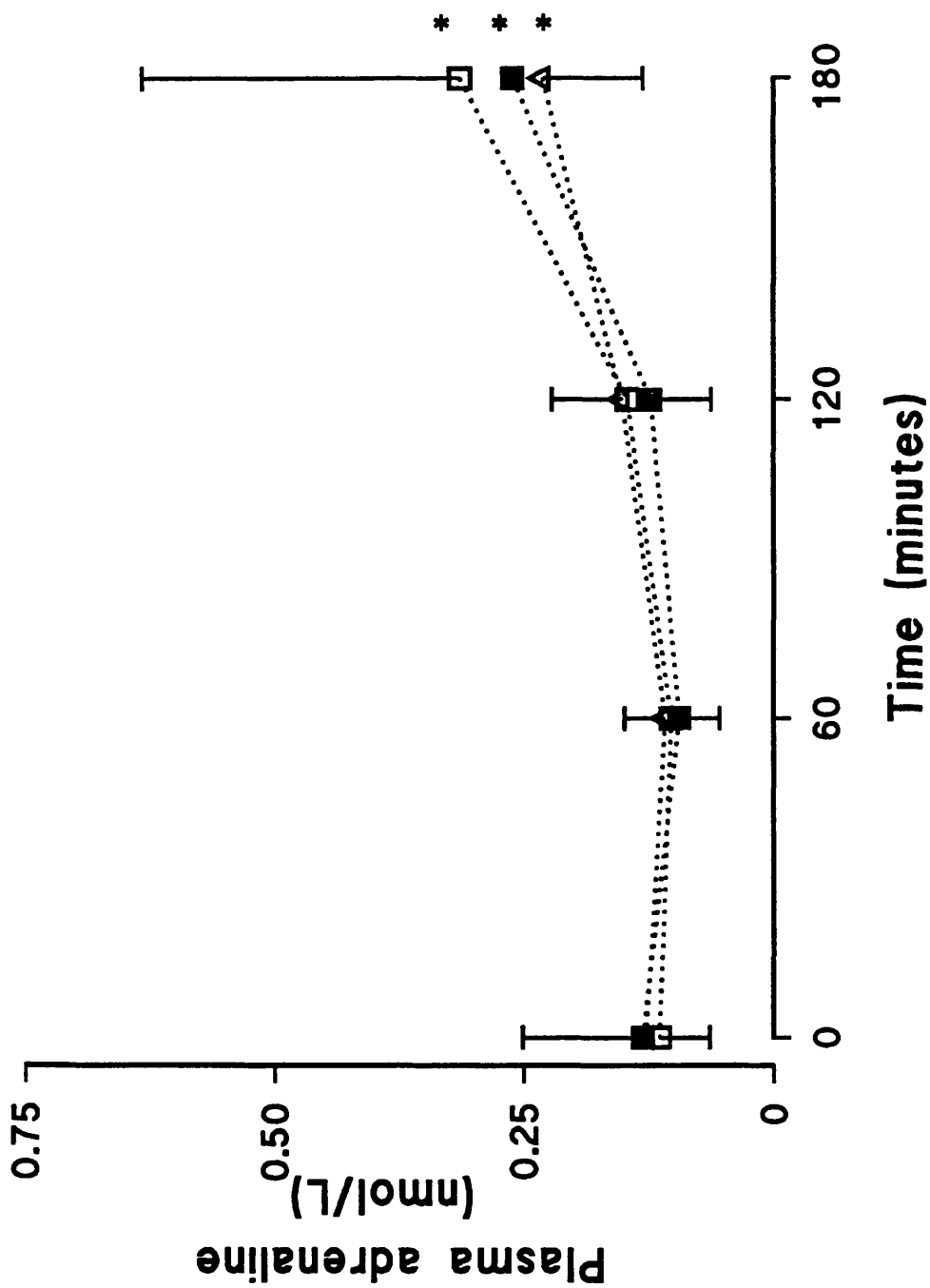


Figure 6.10: Mean profiles of plasma adrenaline concentrations of diabetic patients during euglycaemic clamp in angiotensin II infusion study
 ■ = placebo, □ = sub-pressor ANG II, Δ = pressor ANG II.
 * = $p < 0.01$ from baseline fasting value.

limits of "euglycaemia". A notable feature of this study was that the investigator performing the clamp remained blind to angiotensin II administration. Blood pressure recordings were monitored automatically and then stored by the Datascope Accutorr semiautomatic sphygmomanometer [chapter 2.2.3], and the changes in arterial pressure with high dose angiotensin II were sufficiently small and variable that a pressor effect was not immediately obvious. The results in healthy subjects and patients with NIDDM will now be discussed independently.

(i) Results in healthy subjects

These observations in healthy subjects have recently been extended by other investigators who have reported the effects of various doses of angiotensin II on insulin sensitivity in lean, healthy male volunteers (Townsend et al, 1993; Buchanan et al, 1993; Townsend & DiPette 1993).

My study has used a placebo-controlled double blind design and has demonstrated that the higher dose of angiotensin II (5 ng/kg/min) produced a small, sustained increase in blood pressure (11/9 mmHg) but neither this dose nor 1ng/kg/min of ANG II affected glucose uptake under hyperinsulinaemic conditions.

Townsend and colleagues (1993a) in an uncontrolled study of 8 normotensive young men, also found that a 3 hour infusion of subpressor (0.3ng/kg/min and 1ng/kg/min) doses of angiotensin II had no effect on insulin sensitivity in normal subjects during a euglycaemic clamp. However, the same group using higher doses of angiotensin II (10-20 ng/kg/min) have more recently reported increases in glucose transport and oxidation in skeletal muscle in 8 healthy subjects which generally tended to parallel the associated increments in blood pressure (Townsend & DiPette 1993b). This study however was not placebo-controlled.

Buchanan and colleagues (1993), in a study of 7 healthy males, performed a 270 minute euglycaemic hyperinsulinaemic clamp on 3 occasions each, one week

apart. On each study day, for the final 90 minutes of the clamp, they infused one of three dose pairs of angiotensin II: 0.5 and 2.0, 2.5 and 10.0, 5.0 and 20 ng/kg/min. The lower of each pair was given for 30 minutes followed by the higher dose for 60 minutes. Despite the fact that there was no placebo control in this study which would have confirmed that the clamp had indeed reached steady state prior to the angiotensin II infusion, they reported a convincing dose-related increase in insulin-mediated glucose disposal of 41% with the 10ng/kg/min infusion and 72% with the 20ng/kg/min infusion (Buchanan et al, 1993). Thus, the available data suggest that in healthy subjects angiotensin II increases glucose disposal in a dose-dependent fashion.

(ii) Results in patients with NIDDM

To the best of my knowledge, the effects of angiotensin II on glucose and insulin metabolism in patients with NIDDM have not been previously reported. In comparison with the above studies in normal subjects, the data from the patients with NIDDM suggest that these patients are more sensitive to the metabolic as well as the haemodynamic effects of angiotensin II. Perhaps the most significant finding in this study is that the metabolic response to angiotensin II was completely independent of its effect on blood pressure. Both active infusions increased insulin sensitivity and the effect on glucose uptake tended to be greater with the low-dose infusion which had no effect on arterial pressure. This dissociation of the haemodynamic and metabolic effects of angiotensin II was not observed in healthy volunteers and is difficult to reconcile with the recently proposed mechanisms of angiotensin II-induced insulin sensitisation (Townsend & DiPette, 1993; Buchanan et al, 1993). In particular, my results suggest that a haemodynamic (i.e. vasoconstrictor) effect of angiotensin II, presumably involving redistribution of blood flow from the splanchnic and visceral (especially renal) circulation towards skeletal muscle, might not be the sole (or even principal) underlying mechanism in patients with NIDDM. Three specific features of

the present data suggest, albeit indirectly, that mechanisms in addition to haemodynamic changes might be contributing to the observed increase in insulin sensitivity in patients with NIDDM:

(1) Although blood flow was not directly measured, it seems unlikely that the metabolic response to such a low dose of angiotensin II (1 ng/kg/min) could be entirely accounted for by an increase in blood flow through large conduit arteries, e.g. to the limbs, in the absence of an effect on blood pressure.

(2) Both Buchanan and colleagues (1993) and Townsend and DiPette (1993b) have shown that high doses of angiotensin II (10-20 ng/kg/min) significantly decrease insulin clearance due to a reduction in renal plasma flow. Since there was no evidence of this effect in the present study, it would appear that the 25% increase in glucose uptake occurred without evidence of significant vasoconstriction in renal tissues.

(3) If changes in muscle perfusion were solely responsible for the increase in glucose extraction, it seems somewhat surprising that there was not a similar increase in potassium and triglyceride disposal under euglycaemic hyperinsulinaemic conditions.

Thus several features of the my study suggest that in patients with NIDDM mechanisms in addition to haemodynamic changes and simple redistribution of cardiac output might be contributing to angiotensin-II induced insulin sensitisation.

These data would be consistent with a direct biochemical effect of angiotensin II and since diabetic individuals are more sensitive to both the endocrine (i.e. aldosterone) and cardiovascular effects of the peptide (Tuck et al, 1990; Christlieb 1980) it is conceivable that such a response is much less evident in normal subjects. An additional mechanism that has not been considered relates to local vascular effects

of angiotensin II within skeletal muscles. Buchanan and colleagues (1993) have postulated that the metabolic effects of angiotensin II are due to large vessel redistribution of blood flow away from relatively insulin-resistant tissues (particularly the kidney) towards muscular areas (e.g. the limbs), but it is also possible that angiotensin II produces significant vascular effects (independent of major changes in blood pressure) at the level of the microcirculation. This is consistent with recent observations that local generation of angiotensin peptides occurs in the microvascular network of skeletal muscles (Vicaut & Hou 1993) and this might serve a paracrine role in regulating the perfusion of muscle fibres of varying insulin sensitivity (James et al, 1986). Thus, subpressor doses of angiotensin II, while having little effect on larger arterioles, might still redistribute blood flow within skeletal muscles of mixed fibre-type composition, producing a net increase in perfusion of more insulin-sensitive (type 1) fibres. Such an effect might also serve a useful function in "fight or flight" reactions, since angiotensin II increases during the stress response.

The present study has not excluded an effect of angiotensin II on hepatic glucose production. However, it has been shown that angiotensin II has no effect on hepatic glucose production in normal subjects (Buchanan et al, 1993), and limited data from *in vitro* experiments suggest a tendency for angiotensin II to increase glycogenolysis and decrease gluconeogenesis (Kaley et al, 1967), which would have tended to give an opposite result.

(iii) General discussion

The results of this study contribute information on the role of neuroendocrine regulation in insulin resistant states. The insulin antagonist effects of catecholamines and cortisol are well established. Reflex activation of the sympathetic nervous system has been directly associated with the development of metabolic complications in cardiovascular disease. For example, peripheral and hepatic insulin resistance in

patients with congestive heart failure is directly related to increased plasma concentrations of noradrenaline (Paolisso et al, 1991). Thus, sympathetic activation clearly worsens insulin sensitivity but in contrast the present data suggest that the vasoconstrictor angiotensin II increases peripheral insulin sensitivity. This is somewhat unexpected, especially in view of some *in vitro* evidence showing that angiotensin II might increase plasma glucose levels via increased glycogenolysis and decreased gluconeogenesis (Kaley et al, 1967).

These data are also relevant to the present debate concerning the metabolic effects of ACE inhibitors, and specifically the reports that ACE inhibitors improve various parameters of glycaemic control in both diabetic and non-diabetic subjects (Pollare et al, 1989; Prince et al, 1988) [chapters 1.3.2 and chapter 5]. In the past it has been suggested that removal of angiotensin II-mediated effects on potassium and glucose transport are important (Ferrannini et al, 1992) but, conversely, the present studies suggest that the metabolic effects of ACE inhibition are probably independent of angiotensin II. Indeed, if the purported effects of ACE inhibitors were mediated via an angiotensin II sparing effect, the present data would suggest that angiotensin converting enzyme inhibitors would exert a deleterious effect on insulin sensitivity. However, the results of this acute study may not necessarily reflect the long term metabolic effects of ACE inhibitors described in hypertensive and diabetic (insulin resistant) patients.

An interesting biochemical feature of this study is that plasma renin activity did not show the expected decrease during angiotensin II administration. This may be explained by an interaction with the clamp; previous studies have demonstrated that under euglycaemic conditions insulin induced hypokalaemia increases plasma renin activity (Trovatti et al, 1989). Furthermore, the lower dose of angiotensin II was only associated with a modest increase in aldosterone concentration; this is perhaps surprising since insulin has been shown to enhance angiotensin II induced aldosterone secretion (Rocchini et al, 1990). This may be related to hypokalaemia induced by the

clamp, since the weight of evidence indicates that serum potassium concentration is the key determinant to the aldosterone response to other stimuli (Young et al, 1984).

Another interesting finding was the increase in plasma catecholamines observed at the end of the clamp in the diabetic patients. A possible explanation for this observation is that when plasma glucose is maintained at 5.2 mmol/L, these patients with NIDDM (who are accustomed to a fasting glucose concentration of 8.0 to 12.0 mmol/L) experience "relative" hypoglycaemia which induces an adrenergic response.

In conclusion, acute administration of angiotensin II at physiological plasma concentrations did not alter whole-body insulin sensitivity in healthy subjects but increased whole-body insulin sensitivity in patients with NIDDM. In contrast to recent observations in healthy volunteers which report a dose-related increase in glucose disposal with higher doses of angiotensin II than used in this study, the metabolic effects of angiotensin II in patients with NIDDM were observed at lower doses of angiotensin II and were independent of changes in blood pressure. While angiotensin II-induced redistribution of blood flow is likely to be an important underlying mechanism, these data suggest that additional effects of angiotensin II (including biochemical and/or microvascular changes within skeletal muscle) might be contributing to the metabolic response in NIDDM.

CHAPTER 7

DETERMINANTS OF INSULIN SENSITIVITY IN HEALTH AND DISEASE

7.1. GENERAL INTRODUCTION

Despite attempts at standardisation, recent studies have demonstrated that the oral glucose tolerance test (OGTT) has poor reproducibility and therefore does not constitute a controlled physiological stimulus. This casts further doubt on the test's validity not only in the clinical research setting but also in the accurate classification of glucose intolerance (Yudkin et al, 1990). In order to investigate the complex interplay of insulin secretion and insulin action in pathophysiological states, standardised experimental techniques have been devised to quantify pancreatic insulin secretion and the sensitivity of target tissues to insulin [chapter 1.2]. Thus, the euglycaemic hyperinsulinaemic clamp, the minimal model technique and the insulin suppression test were devised as reproducible methods of determining the sensitivity of insulin-mediated responses, whereas the hyperglycaemic clamp can be used to quantify pancreatic sensitivity to glucose-induced insulin secretion. Whilst these tests define specific aspects of glucoregulatory control, some authors claim they are "unphysiological" (Ng et al, 1988). Furthermore, because these techniques are relatively labour intensive, expensive and generally impractical for large numbers of subjects, epidemiological studies have relied on the simpler OGTT to infer abnormalities in insulin secretion and insulin resistance. For example, many of the large population-based studies linking insulin resistance to hypertension (for example, Modan et al, 1985; Asch et al, 1991) [see chapter 1.1.2] used fasting plasma insulin concentrations as a surrogate marker of peripheral insulin resistance. The problem with this approach is that few, if any, previous studies have properly validated the OGTT (or indeed other morphometric or biochemical indices) against direct measurements of insulin sensitivity derived from the euglycaemic hyperinsulinaemic clamp, which is arguably the "gold standard" for determining insulin-stimulated responses.

Having evaluated the methodology of the euglycaemic hyperinsulinaemic clamp and characterised insulin sensitivity not only in hypertensive, diabetic and

diabetic hypertensive patients but also in healthy subjects, analysis of the entire data base is suitable for identifying factors which may account for the large inter-subject variability in insulin sensitivity. Thus, the principal aim of this study was to determine which clinical measurements, e.g. body weight, BMI, blood pressure etc. were the major determinants of insulin sensitivity in both normal individuals and in a range of patients with hypertension, impaired glucose tolerance and NIDDM. The following biochemical and morphometric indices were included: basal plasma glucose and insulin concentrations, weight, BMI, blood pressure, sex, waist hip-ratio, fasting cholesterol and triglycerides.

7.2. PATIENTS AND METHODS

7.2.1. General Methods.

Four clinical research studies have been described in this thesis which, in total, include measurements of whole body insulin sensitivity by means of the euglycaemic hyperinsulinaemic clamp in 65 individuals. In addition, 10 patients who were screened but excluded from thetrandolapril study [chapter 5] were also included for the purpose of the present analysis. These patients underwent an OGTT and had insulin sensitivity measured during the placebo run-in, but they failed to meet the entry criteria for the study; 7 had impaired glucose tolerance or NIDDM but were not hypertensive, and 3 were hypertensive but not glucose intolerant. Thus, by including data from these subjects, OGTT's and euglycaemic clamps were performed in a total of 75 individuals.

Each subject underwent a standard 75g OGTT at screening [chapter 2.2.4]; blood samples were collected for serum insulin and plasma glucose profiles at baseline and at 30 minute intervals until 120 minutes. On at least one occasion thereafter (while on placebo therapy), each individual attended the CIRU when a euglycaemic hyperinsulinaemic clamp was performed using arterialised blood samples to derive the

calculation of whole-body insulin sensitivity. The clinical protocol is described in detail in chapter 2.3, and the same method was used in all subjects to derive insulin sensitivity (M) in mg glucose/kg/min.

7.2.2. Statistical analysis

In each subject, a number of metabolic, demographic, morphometric, neuro-endocrine and biochemical variables which may influence insulin sensitivity were recorded. The measurement techniques are described in chapter 2.

(i) Metabolic variables

- Fasting serum insulin concentration.
- Fasting plasma glucose concentration.

(ii) Haemodynamic variables.

- Screening supine systolic blood pressure.
- Screening supine diastolic blood pressure.
- Screening supine mean arterial blood pressure.

(iii) Biochemical variables.

- Serum total cholesterol.
- Serum triglycerides.
- Serum potassium.

(iv) Demographic and morphometric variables.

- Age
- Body mass index
- Waist hip ratio

(v) Neuro-endocrine variables

- Baseline noradrenaline concentrations.
- Baseline aldosterone concentrations.
- Baseline plasma renin activity.

Data from the OGTT were used to calculate the ratio of fasting insulin to fasting glucose and the ratio of the 120 minute postload insulin to the fasting insulin (Saad et al, 1988). The area under the insulin and glucose curves in the OGTT were calculated by the trapezoidal rule.

The relationship between one or more of these variables and insulin sensitivity (M) was investigated in two ways; firstly, the relationship of individual parameters to insulin sensitivity was assessed by simple correlation analysis of the form:

$$y = AX + Z$$

where X is the independent variable, e.g. BMI or fasting plasma glucose concentration, and y is the dependent variable, i.e. insulin sensitivity in mg glucose/kg/min. All scatterplots were inspected to detect non-linear associations and outliers. Thus, a square array of each pair of variables was constructed and this correlation matrix was then used as a starting point for stepwise linear regression analysis.

Stepwise linear regression analysis was performed by fitting a hierarchy of linear models to the distribution of values for insulin sensitivity. Thus, M is defined as the dependent variable and the independent variables are modelled separately and in all combinations with each other to find the best overall fit. The R² value obtained for each model represents the percentage variability in insulin sensitivity which can be accounted for by the variable or variables in the model. One, 2, and 3 variable models were fitted sequentially to the data, e.g.:

$$y = AX_1 + Z \quad (1)$$

$$y = AX_1 + BX_2 + Z \quad (2)$$

$$y = AX_1 + BX_2 + CX_3 + Z \quad (3)$$

where y is the M value, X_1 , X_2 and X_3 are the independent variables, e.g. BMI, fasting glucose, triglycerides, etc., and A, B, and C are the coefficients. A forward stepping selection procedure was utilised. Thus, one variable was selected and each remaining variable was considered individually; the one with the highest F-to-enter statistic was then included in the model. To guard against overfitting, the value of R^2 for the best subset of each size was plotted against the size of the subset; and the "optimal" number of regressors was identified where the plot began to level off. Because of the large number of comparisons, only p values of less than 0.001 were taken to indicate statistical significance.

The correlation coefficients and multiple linear regression analyses were firstly performed for the group as a whole ($n=75$). Subsequently, sub-group analysis was performed for the "insulin resistant" patient group with impaired glucose tolerance or NIDDM ($n=30$).

In an attempt to evaluate which variables (either those that *influence* insulin sensitivity, e.g. BMI and blood pressure, or those variables that *reflect* insulin sensitivity, e.g. fasting insulin concentrations), were of the greatest value in the prediction of insulin sensitivity in an individual, 95% confidence intervals for predicted values of M and the prediction error were calculated.

7.3. RESULTS

7.3.1. General

Table 7.1 shows the mean age, sex, BMI and blood pressure together with fasting and 2 hour post-load plasma glucose concentrations, in each of the subject categories. The healthy volunteers were all male, significantly younger, less heavy and had significantly lower blood pressures compared with the patient groups. Thus, direct comparison between the three groups (normal, hypertensive and glucose intolerant) was not feasible. On analysis of the group as a whole, there was a wide range in insulin sensitivity as derived from the euglycaemic hyperinsulinaemic clamp; whole body glucose uptake ranged from 1.1 mg/kg/min in the most insulin resistant individual to 16.2 mg/kg/min in the most insulin sensitive subject. The number of cigarette smokers (22%) in the whole group was too small to allow any formal statistical analysis.

7.3.2. Correlation analysis

(i) All subjects (n=75)

Table 7.2 shows the correlation coefficients for one-variable analysis of insulin sensitivity versus the various demographic, metabolic, haemodynamic and biochemical measurements for all 75 subjects. A number of measurements correlated with insulin sensitivity. Strongest negative correlations were for identified for age ($r=-0.64$), BMI ($r=-0.68$), fasting plasma glucose concentration ($r=-0.68$) and diastolic blood pressure ($r=-0.62$), as shown in figure 7.1. There were also significant negative correlations with fasting insulin and total cholesterol concentrations, but there was no correlation with fasting triglycerides. Similarly, there was no significant correlation between serum aldosterone, serum potassium, plasma renin activity or plasma noradrenaline concentrations and insulin sensitivity.

	Healthy Subjects (n=36)	NIDDM or IGT Patients (n=30)	Hypertensive Patients (n=9)
Sex	36 males	27 males 3 females	4 males 5 females
Age (median)	26 years range 18-43	56 years range 33-73	50 years range 33-66
BMI (kg/m ²)	23.8 +/- 3.2	30.0 +/- 5.5	26.5 +/- 2.4
Blood pressure (mm/Hg)	118/65 +/- 11/8	157/89 +/- 21/11	174/93 +/- 16/7
Fasting glucose (mmol/L)	5.0 +/- 0.4	7.9 +/- 2.3	5.1 +/- 0.8
120 min glucose (OGTT)	4.9 +/- 1.0	12.8 +/- 4.0	5.3 +/- 1.1
Fasting insulin (uU/ml)	9.0 +/- 3.7	20.1 +/- 13.4	8.1 +/- 4.3
120 min insulin (OGTT)	25.0 +/- 13.1	90.1 +/- 66.0	48.7 +/- 18.2
AUC glucose (OGTT)	810 +/- 128	1512 +/- 35.8	935 +/- 234
AUC insulin (OGTT)	5500 +/- 2957	8806 +/- 6297	8466 +/- 4211
Triglycerides (mmol/L)	1.19 +/- 0.88	2.01 +/- 0.88	1.46 +/- 1.59
Cholesterol (mmol/L)	4.41 +/- 1.18	5.70 +/- 0.84	6.20 +/- 0.50
WHR	0.94 +/- 0.09	1.00 +/- 0.07	0.78 +/- 0.5
M Value (mg/kg/min)	10.4 +/- 2.3	4.5 +/- 1.9	7.6 +/- 2.1
M value range (mg/kg/min)	6.2 - 16.2	1.1 - 8.8	5.8 - 10.0

Table 7.1: Demographic, metabolic and biochemical characteristics of normal subjects, glucose intolerant patients and hypertensive patients studied. Data shown are mean +/- S.D. unless otherwise stated IGT = impaired glucose tolerance, AUC = area under the curve BMI = body mass index.

	<u>r value</u>	<u>pvalue</u>
Age	-0.64	<0.001
Weight	-0.50	<0.001
Height	0.32	N.S
BMI	-0.68	<0.001
Systolic BP	-0.50	<0.001
Diastolic BP	-0.62	<0.001
Mean Arterial BP	-0.59	<0.001
Fasting glucose	-0.68	<0.001
Fasting insulin	-0.58	<0.001
AUC glucose (OGTT)	-0.67	<0.001
AUC insulin (OGTT)	-0.38	N.S.
Triglycerides	-0.25	N.S.
Cholesterol	-0.54	<0.001
Noradrenaline	-0.38	N.S.
Plasma renin activity	-0.05	N.S.
Potassium	-0.07	N.S.
Aldosterone	-0.38	N.S.
Waist Hip Ratio	-0.52	<0.001

Table 7.2: Correlation between demographic, metabolic and haemodynamic measurements and insulin sensitivity derived from the euglycaemic hyperinsulinaemic clamp in the 75 individual subjects studied.

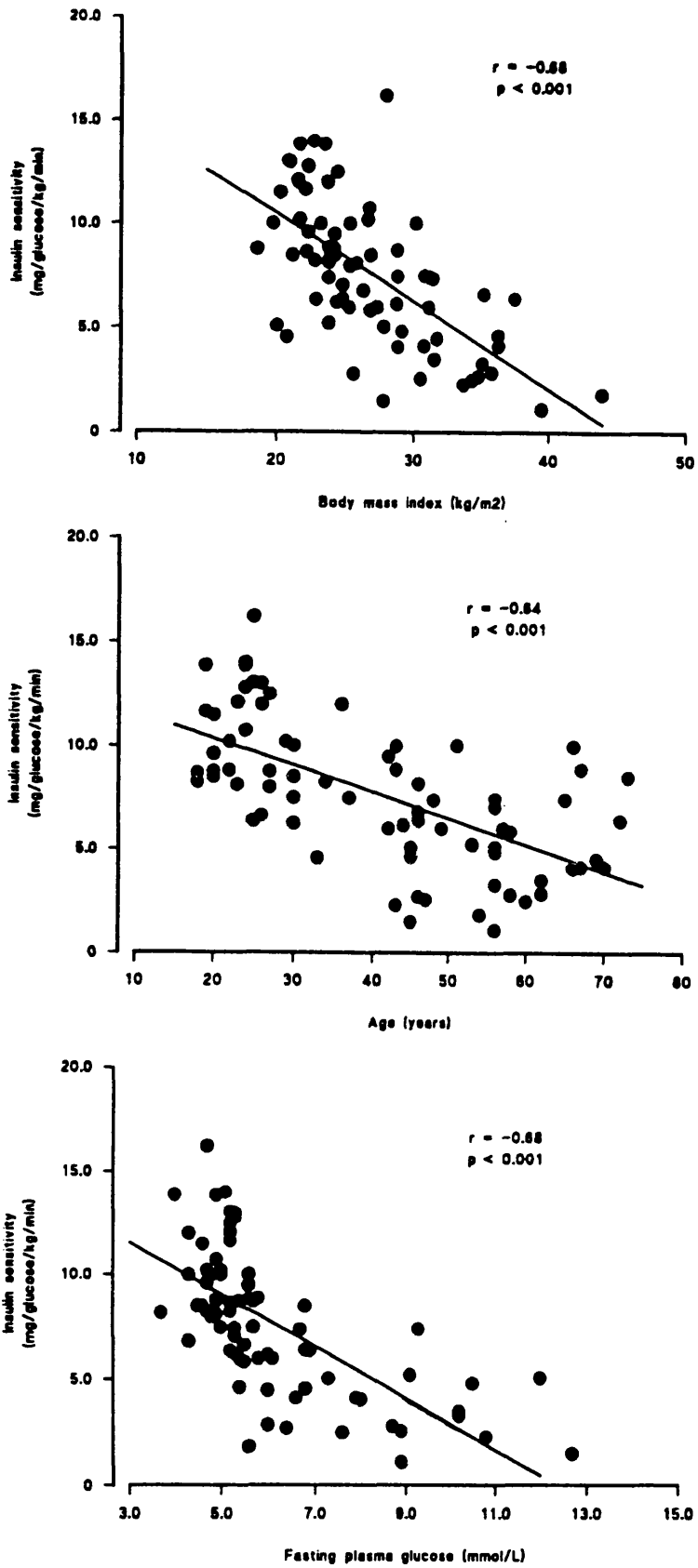


Figure 7.1: Correlations of body mass index (top panel), age (middle panel) and fasting plasma glucose (lower panel) to whole body insulin sensitivity in all 75 subjects studied.

- (ii) Patients with impaired glucose tolerance or NIDDM (n=30)

Table 7.3 shows the correlations between insulin sensitivity and the various individual parameters for the 30 patients with either impaired glucose tolerance or NIDDM. In these subjects, the strongest negative correlations were with waist hip ratio ($r=-0.81$), BMI ($r=-0.59$), fasting insulin ($r=-0.53$) and the ratio of the 120 minute post load insulin to the fasting insulin ($r=0.60$). These correlations are represented diagrammatically in figure 7.2. There was also a significant correlation for fasting cholesterol and fasting triglyceride concentrations.

7.3.2. Multiple linear regression analysis

For the group as a whole and the subgroup of patients with impaired glucose tolerance or NIDDM, insulin sensitivity was modelled with independent variables using stepwise least squares linear regression analysis [section 7.2.2]. The results are summarised in table 7.4, which presents the R^2 values for each of the one-variable analyses and the combination of variables that was most appropriate for predicting the inter-subject differences in insulin sensitivity in the 2 groups. Thus, for all subjects, BMI was singularly the best predictor of insulin sensitivity, accounting for 45% of the variability in M, while age and diastolic blood pressure accounted for 41% and 38% respectively (table 7.4). Incorporating additional variables in more complex models improved the correlation; for example, the 3 variable model shown below explained 67% of the variability in insulin sensitivity:

$$M \text{ value} = 36.4 - 0.254 \text{ BMI} - 0.126 \text{ DBP} - 12.7 \text{ WHR}$$

The prediction error for this equation was 1.8 ± 1.6 mg glucose/kg/min. Using regression analysis, fasting insulin alone explained only 27% of the variability in M values observed. The prediction error was 2.8 ± 2.0 mg glucose/kg/min.

	<u>r value</u>	<u>pvalue</u>
Age	-0.31	N.S.
Weight	-0.54	<0.001
Height	-0.13	N.S.
BMI	-0.59	<0.001
Systolic BP	-0.16	N.S.
Diastolic BP	0.06	N.S.
Mean Arterial BP	0.12	N.S.
Fasting glucose	-0.29	N.S.
Fasting insulin	-0.62	<0.001
AUC glucose (OGTT)	-0.11	N.S.
AUC insulin (OGTT)	-0.24	N.S.
Fast. glucose/fast. insulin	-0.34	N.S.
120 min insulin/fast insulin	0.60	<0.001
30 min insulin/30 min glucose	-0.21	N.S.
Triglycerides	-0.54	<0.001
Cholesterol	-0.54	<0.001
Noradrenaline	-0.38	N.S.
Plasma renin activity	-0.19	N.S.
Potassium	-0.17	N.S.
Aldosterone	-0.24	N.S.
Waist Hip Ratio	-0.81	<0.001

Table 7.3: Correlation between demographic, metabolic and haemodynamic measurements and insulin sensitivity derived from the euglycaemic hyperinsulinaemic clamp in the 30 patients with impaired glucose tolerance or NIDDM.

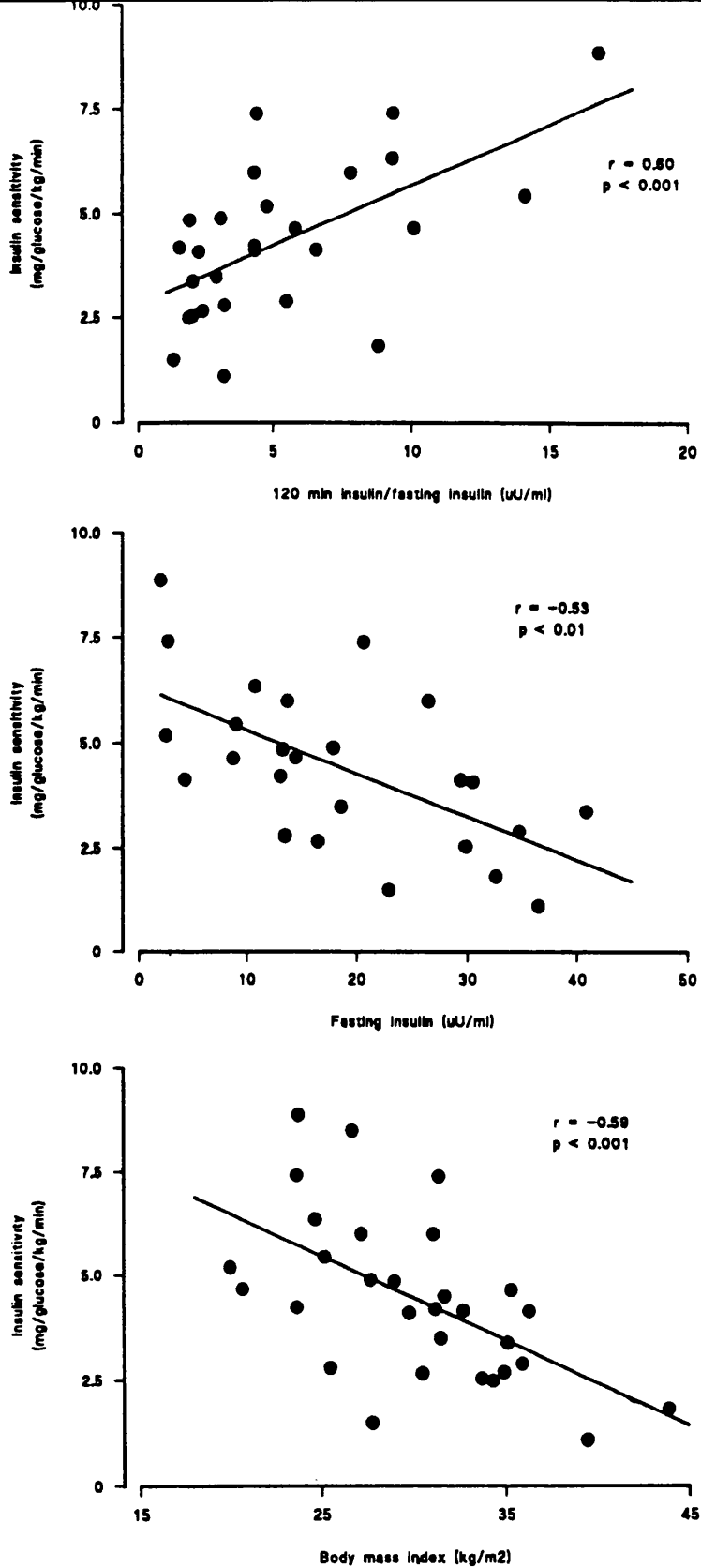


Figure 7.2: Correlations of the ratio of the 120 minute postload to the fasting insulin (top panel), fasting insulin (middle panel) and body mass index (lower panel) to whole body insulin sensitivity in the 30 patients studied with either impaired glucose tolerance or NIDDM.

	R ² FOR 1-VARIABLE MODELS			BEST MODEL (R ²)
	BMI	Age	Diastolic B.P.	
All subjects (n=75)	45%	41%	38%	3-variable: age, BMI, diastolic BP (67%)
IGT & NIDDM subjects (n=30)	WHR	BMI	Cholesterol	Trigs
	65%	35%	27%	27%
	2-Variable: WHR, BMI, (79%)			

Table 7.4: Multiple linear regression analysis using independent variables for all subjects (n=75) and patients with impaired glucose tolerance or NIDDM (n=30). R² represents the percentage of the variability in insulin sensitivity accounted for by the variables in the model.

For the patients with impaired glucose tolerance or NIDDM, waist hip ratio was singularly the best predictor of insulin sensitivity with a R^2 value of 65%. However, the most appropriate model to describe the variability in insulin sensitivity was a 2 variable model incorporating waist hip ratio and BMI. This model accounted for 79% and was defined by the equation:

$$M \text{ value} = 26.7 - 0.137 \text{ BMI} - 18.3 \text{ WHR}$$

The prediction error for this equation was 1.1 ± 1.4 mg glucose/kg/min. Using regression analysis, fasting insulin alone explained only 24% of the variability in M values observed. The prediction error was 1.9 ± 1.4 mg glucose/kg/min.

7.4. DISCUSSION

When studying insulin sensitivity it is necessary for the investigator to have a precise measure of quantifying glucose disposal in response to insulin. Many recent epidemiological studies have inferred the presence of insulin resistance from OGTT data but few studies have validated the OGTT against formal measurements of insulin sensitivity derived from the euglycaemic hyperinsulinaemic clamp. The purpose of this study was therefore to investigate which variables, if any, are the best determinants of insulin sensitivity in an individual.

A number of haemodynamic, morphometric, metabolic and biochemical markers were examined in relation to insulin sensitivity; the results clearly indicate that insulin sensitivity is correlated with age independently of BMI or blood pressure. This is consistent with previous reports that insulin resistance is a characteristic feature of the normal ageing process (Rowe et al, 1983). Likewise, blood pressure was correlated with insulin insensitivity independently of age and BMI, and glucose tolerance. However, for the whole group, by far the most important determinants of insulin sensitivity as derived by the euglycaemic hyperinsulinaemic clamp was the BMI. Of the data derived from the OGTT, the fasting insulin concentration showed the

best correlation with insulin sensitivity; more complex calculations of AUC's of insulin or glucose profiles, or ratios of insulin and glucose concentrations conferred no advantage.

In the patients with NIDDM or impaired glucose tolerance, fasting insulin concentrations correlated with insulin sensitivity but better predictors of insulin sensitivity were BMI and waist hip ratio. The insulin and glucose responses to an OGTT were poor predictors of insulin sensitivity. A possible explanation for this is the well recognised "Starling's curve of the pancreas" in patients with NIDDM, whereby, in normal weight individuals with impaired glucose tolerance and in mild diabetics, the plasma insulin response to ingested glucose increases progressively until the fasting glucose reaches approximately 6.8 mmol/L when progressive increases in plasma glucose levels are associated with a progressive decline in insulin secretion (DeFronzo, 1988). Another possible explanation of the poor correlation of plasma insulin profiles during an OGTT is the possible confounding effect of defective insulin processing or secretion in patients with established NIDDM tending to discrepant measurements of insulin in these subjects (Temple et al, 1991) [chapter 1.1.3]. This is supported by a recent report that fasting levels of split proinsulin correlated well with insulin sensitivity (as measured by an insulin tolerance test) in both healthy subjects and patients with impaired glucose tolerance or NIDDM (Phillips et al, 1994).

In summary, these data suggest good correlation between BMI, WHR and fasting insulin concentrations and derived measurements of insulin sensitivity. Thus, these variables may individually explain up to 50% of the inter-subject variability in the M value. Unfortunately however, when these variables are used individually, they are insufficiently robust to *predict* insulin sensitivity in an individual subject. This is demonstrated by the prediction errors of the regression equations [section 7.3.2]. For example, if an attempt is made to predict the M value of an individual [e.g. volunteer no.1 of the lacidipine study] in whom the M value as determined by the euglycaemic hyperinsulinaemic clamp was 13.9 mg glucose/kg/min, the regression equation incorporating BMI, diastolic blood pressure and waist hip ratio [section 7.3.2] predicts

an M value of 11.4 mg glucose/kg/min. The 95% confidence intervals of this prediction are wide (6.0,16.8 mg glucose kg/min). Thus, these variables are imprecise when used as predictors of insulin sensitivity in an individual as they may underestimate or overestimate the M value by up to 50%.

In conclusion, I have demonstrated that factors such as BMI, blood pressure and waist hip ratio may explain up to 50% of the observed inter-subject variability in M as determined by the euglycaemic hyperinsulinaemic clamp. It is evident, however, that these factors are of little value when used to predict the insulin sensitivity of an individual. Thus, an accurate measurement of insulin sensitivity requires a sophisticated technique such as the euglycaemic clamp; estimates of insulin resistance derived from OGTT and morphometric data in epidemiological studies of glucose tolerance should be viewed with caution.

CHAPTER 8
GENERAL DISCUSSION

8.1. DISCUSSION

Reports from epidemiological studies (Modan et al, 1985) that patients with essential hypertension are hyperinsulinaemic when compared with controls, and from glucose clamp studies demonstrating that hypertensive patients are resistant to insulin stimulated glucose uptake (Ferrannini et al, 1987; Pollare et al, 1990) have fuelled an increasing recognition that high blood pressure is only one facet of a metabolic syndrome that represents a cluster of risk factors for long-term development of cardiovascular disease. Thus, glucose intolerance, hyperinsulinaemia, altered cell membrane structure and function, hypertriglyceridaemia and altered concentrations of cholesterol subfractions are frequently found in patients with primary hypertension, and may contribute to the risk of atherosclerosis conferred by high blood pressure. Failure to address these metabolic aspects may partly explain the shortfall in the reduction in morbidity and mortality from coronary heart disease which might have been anticipated in several of the large studies of the treatment of mild-to-moderate hypertension. Thus, in terms of the treatment of hypertension, much recent research has focussed on the metabolic as well as the haemodynamic effects of pharmacological intervention.

This thesis is comprised of a series of studies which have utilised the euglycaemic hyperinsulinaemic clamp to measure insulin sensitivity in normal subjects, patients with hypertension and patients with impaired glucose tolerance or NIDDM.

(i) Evaluation of the euglycaemic hyperinsulinaemic clamp

A comprehensive understanding of the prevalence of insulin resistance and the ability to evaluate the effects of pharmacological and non-pharmacological interventions on insulin sensitivity is dependent upon a precise and reproducible technique to quantify the body's response to insulin. The simplest, and perhaps the crudest, estimation of insulin sensitivity is provided by the OGTT. However, the resultant plasma insulin and glucose concentrations following an OGTT are the result

of insulin secretion as well as insulin action of an individual; thus, mean curves for a population are smooth but individual curves are notoriously erratic. Thus, techniques have been devised which provide a more accurate measurement of insulin action (euglycaemic hyperinsulinaemic clamp, IVGTT minimal model technique, CIGMA) and insulin secretion (hyperglycaemic technique and IVGTT).

It was recognised, however, that despite widespread use of the euglycaemic clamp in clinical research, key aspects of the technique remained to be validated. Thus, the intra- and inter- subject variability, the time for insulin mediated glucose disposal to reach steady state, the need to arterialise venous blood, and the haemodynamic and metabolic effects of hand warming were evaluated in 24 healthy male subjects and 6 patients with essential hypertension.

My data suggest that M values derived from clamps of 120 minute duration are 10% less than those derived from 180 minute clamps. The intra-subject variability is less than 10% in both healthy subjects and patients with essential hypertension, whereas the inter subject variability is much greater at 20-30% even in a group of apparently homogeneous healthy subjects. Thus, the clamp lends itself to studies where subjects act as their own controls.

The haemodynamic and metabolic effects of a hand-warming box, an integral part of the clamp procedure designed to arterialise venous blood sampled from a dorsal hand vein, were evaluated in 12 healthy subjects. The box was shown to effectively arterialise venous blood with oxygen saturations of greater than 95%. Hand warming was associated with an increase in the derived measurement of insulin sensitivity, with an increase in heart rate and decrease in blood pressure. Thus, the hand warming device should be used with caution, as it may be associated with a confounding increase in the derived measurement of whole body insulin sensitivity from the euglycaemic hyperinsulinaemic clamp.

(ii) Anti hypertensive therapy and insulin sensitivity

The fact that blood pressure tends to increase, and glucose tolerance and

insulin sensitivity decrease throughout the middle to late years of life even in the absence of anti hypertensive treatment (Skarsfors et al, 1991) renders uncontrolled studies and epidemiological observations ill suited for the evaluation of the effects of anti hypertensive drugs in causing insulin resistance, impaired glucose tolerance or NIDDM. On review of the literature, however, there are few controlled studies of the effects of anti hypertensive drugs on insulin sensitivity and few direct comparisons with placebo. Despite well publicised studies of anti hypertensive drugs improving insulin sensitivity (Pollare et al, 1989a), many conflicting reports have been published. This thesis has incorporated two studies which have evaluated the effects of two anti hypertensive drugs, the dihydropyridine calcium antagonist, lacidipine, and the ACE inhibitor, trandolapril, on insulin sensitivity using a randomised, double-blind placebo-controlled crossover design.

Despite indirect evidence of vasodilatation as reflected by an increase in supine heart rate, there was no significant effect of 2 weeks treatment with lacidipine on insulin sensitivity in neither healthy male subjects nor patients with essential hypertension; the confidence intervals excluded a 15% change in insulin sensitivity in the volunteer group. This is one of the first placebo-controlled crossover studies to evaluate the effects of a calcium antagonist on insulin sensitivity.

The second study evaluated the effects of 4 weeks treatment with trandolapril on insulin sensitivity in patients with at least 2 cardiovascular risk factors, namely, glucose intolerance and essential hypertension. The hypothesis under test was that ACE inhibition would increase insulin sensitivity via a vasodilatory mechanism and angiotensin II "sparing" effect. Despite evidence of 70% ACE inhibition and blood pressure lowering effects of trandolapril, there was no evidence that trandolapril altered insulin sensitivity when compared with placebo. Confidence intervals excluded a 12% increase in insulin sensitivity.

Thus, my results suggest that treatment (2 to 5 weeks) with either a calcium antagonist or an ACE inhibitor exert neutral effects on whole-body insulin sensitivity. These results contrast with previous reports from uncontrolled studies of

heterogeneous patient groups.

Despite the theoretical concern that certain classes of antihypertensive agent are associated with the exacerbation of insulin resistance, the available data suggest that any such effect, at best, is modest especially when compared with the magnitude of the pre-existing insulin resistance observed in NIDDM, obesity and essential hypertension. The pivotal concern is therefore: "Do these adverse metabolic effects matter?" Only when there are data demonstrating that long-term anti hypertensive therapy with different drug classes is associated with differential effects on coronary events will we be able to assess the overall importance of biochemical abnormalities in the treatment of the individual hypertensive patient.

(iii) Effects of angiotensin II on insulin sensitivity

Having evaluated the effects of pharmacological removal of angiotensin II withtrandolapril, the angiotensin II infusion study was performed to test the hypothesis that acute administration of the vasoconstrictor would reduce insulin sensitivity in healthy subjects and patients with NIDDM.

It has been proposed that changes in glucose and insulin metabolism might be secondary to hypertensive microvascular complications, and much discussion has focussed on the importance of skeletal muscle blood flow as a determinant of glucose disposal rate and the potential insulin-antagonist effects of neuroendocrine mechanisms involved in cardiovascular regulation. Although activation of the sympathetic nervous system (via release of catecholamines) is diabetogenic, the effects of other vasoactive hormones on glucose and lipid metabolism have been poorly documented.

Using doses that produced systemic angiotensin II concentrations within the pathophysiological range, (20 to 140 pg/ml), angiotensin II had no effect of insulin sensitivity in healthy subjects. When the identical experimental protocol was repeated in patients with NIDDM, angiotensin II significantly increased whole body insulin sensitivity. Other investigators (Buchanan et al, 1993; Townsend & DiPette, 1993),

using much higher doses of the octapeptide in healthy subjects, have reported a dose-related increase in insulin sensitivity.

The dissociation of metabolic and blood pressure effects of angiotensin II suggests that haemodynamic alterations and redistribution of cardiac output might not be the sole (or principal) underlying mechanism explaining the observed increase in insulin mediated glucose uptake in patients with NIDDM.

(iv) Determinants of insulin sensitivity

Having evaluated the methodology of the euglycaemic hyperinsulinaemic clamp and characterised insulin sensitivity not only in hypertensive, diabetic and diabetic hypertensive patients but also in healthy subjects, analysis of the entire data base was performed to identify factors which may account for the large inter-subject variability in insulin sensitivity.

The most important determinant of insulin sensitivity as derived by the euglycaemic hyperinsulinaemic clamp was the BMI. Of the data derived from the OGTT, the fasting insulin concentration showed the best correlation with insulin sensitivity; more complex calculations of AUC's of insulin or glucose profiles, or ratios of insulin and glucose concentrations conferred no advantage.

In patients with NIDDM or impaired glucose tolerance, the fasting insulin concentrations correlated with insulin sensitivity but the best predictors of insulin sensitivity were BMI and waist hip ratio. The insulin and glucose responses to an OGTT were poor predictors of insulin sensitivity.

Thus, my data suggest good correlation between BMI, WHR and fasting insulin concentrations and derived measurements of insulin sensitivity, and these variables may individually explain up to 50% of the inter-subject variability in the M value. When these variables are analysed on an individual basis, however, they are insufficiently robust to *predict* insulin sensitivity in an individual subject. Thus, estimates of insulin resistance derived from OGTT or morphometric data in epidemiological studies of glucose tolerance should be viewed with caution.

(v) Summary

The last ten years has seen an increase in interest and research into the phenomenon of insulin resistance and hyperinsulinaemia and their importance in the pathogenesis of disease. Not only is insulin resistance and hyperinsulinaemia of primary importance in the development of NIDDM, it also occurs in other conditions, most notably obesity and essential hypertension.

This thesis has evaluated the euglycaemic clamp as a method of reproducibly measuring insulin-mediated metabolic responses. In two separate studies, two anti hypertensive agents have been shown to exert neutral effects on insulin-stimulated responses, casting further doubt on the importance of iatrogenic "worsening" of insulin sensitivity associated with the treatment of hypertension. The effects of angiotensin II on whole-body insulin sensitivity have been evaluated in both healthy subjects and patients with NIDDM. Contrary to general expectations, the vasoconstrictor increased insulin sensitivity in patients with NIDDM, suggesting a possible dissociation of haemodynamic and metabolic effects of angiotensin II. Lastly, using the entire database of all 75 subjects studied, major determinants of insulin sensitivity in man have been evaluated.

In conclusion, there is increasing recognition that high blood pressure is only one facet of a metabolic syndrome that represents a cluster of risk factors for long-term development of cardiovascular disease. The true impact of insulin resistance and hyperinsulinaemia on the promotion of an atherogenic lipid profile, elevation of blood pressure and acceleration of the atherosclerotic process remains to be clearly defined.

REFERENCES

Abramson, D.I., Tuck, S., Chu, L.S.W., Lee, S.W., Gibbon, C., Richardson, G. (1965) Indirect vasodilatation in thermotherapy. *Archives of Physiology and Medical Rehabilitation*, **46**, 412-420.

Amery, A., Berthaux, P., Bulpitt, C., Deruyttere, M., de Schaepdryver, A., Dollery, C.T., Fagard, R., Forette, F., Lund-Johansen, J., Tuomilehto, J. (1978). Glucose intolerance during diuretic therapy: results of trial by the European Working Party on high blood pressure in the elderly. *Lancet*, **1**, 681-683.

Amiel, S. (1993) Amylin and diabetes. *Lancet*, **341**, 1249-1253.

Anderson E.A., Hoffman R.P., Babon T.W., Sinkey C.A., Mark A.L. (1991) Hyperinsulinaemia produces both sympathetic neural activation and vasodilation in normal humans. *Journal of Clinical Investigation*, **87**, 2246-2252.

Andersson D.E.H., Rodjmark S. (1981) Modulation by verapamil of insulin and glucagon secretion in man. *Acta Medica Scandinavia*, **210**, 27-33.

Andres, R., Swerdloff, R., Pozefsky, T., Coleman, D. (1966) Manual feedback technique for the control of blood glucose concentration. In: Skeggs Jr, LT (ed). *Automation in Analytical Chemistry*, Medicaid Inc, New York, pp486-497.

Andrews, J., Klimes, I., Vasquez, B., Nagulesparan, M., Reaven, G.M. (1984) Can mixed venous blood be used to measure insulin action during the hyperinsulinaemic clamp? *Hormone and Metabolic Research*, **16**, 164-166.

Asch S., Wingard D.L., Barrett-Connor E.L. (1991) Are insulin and hypertension independently related? *Annals of Epidemiology*, **1**, 231-244.

Bak, J.F., Gerdes, L.U., Sorensen, N.S., Pedersen, O. (1992) Effects of perindopril on insulin sensitivity and plasma lipid profile in hypertensive non-insulin-dependent diabetic patients. *American Journal of Medicine*, **92**, 69-72.

Banskota, N.K., Taub, R., Zellner, K., King, G.L. (1989) Insulin, insulin like growth factor-1 and PDGF interact additively in the induction of the proto-oncogene c-myc and cellular proliferation in cultured bovine aortic smooth muscle cells. *Molecular Endocrinology*, **3**, 1183-1190.

Barbieri, R.L., Smith, S., Ryan, K.J. (1988) The role of hyperinsulinaemia in the pathogenesis of ovarian hyperandrogenism. *Fertility and Sterility*, **50**, 197-212.

Barker, D.J., Gluckman, P.D., Godfrey, K.M., Harding, J.E., Owens, J.A., Robinson, J.S. (1993a) Foetal nutrition and cardiovascular disease in adult life. *Lancet*, **341**, 938-941.

Barker, D.J., Hales, C.N., Fall, C.H., Osmond, C., Phipps, K., Clark, P.M. (1993b) Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia*, **36**, 62-67.

Barnes, A.J., Garbien, K.J.T., Crowley, M.F., Bloom, A. (1974) Effects of short and long term chlorpropamide treatment on insulin release and blood glucose. *Lancet*, **2**, 69-72.

Baron, A.D., Laakso, M., Brechtel, G., Hoit, B., Watt, C., Edelman, S.V. (1990) Reduced postprandial skeletal muscle blood flow contributes to glucose intolerance in human obesity. *Journal of Clinical Endocrinology and Metabolism*, **70**, 1525-1533.

Baron, A.D., Laakso, M., Brechtel, G., Edelman, S.V. (1991a) Reduced capacity and affinity of skeletal muscle for insulin mediated glucose uptake in non-insulin dependent diabetic subjects. Effects of insulin therapy. *Journal of Clinical Investigation*, **87**, 1186-1194.

Baron, A.D., Laakso, M., Brechtel, G., Edelman, S.V. (1991b). Mechanism of insulin resistance in insulin-dependent diabetes mellitus: A major role for reduced skeletal muscle blood flow. *Journal of Clinical Endocrinology and Metabolism*, **73**, 637-643.

Baron, A.D., Brechtel-Hook, G., Johnston, A., Hardin, D. (1993) Skeletal muscle blood flow: a possible link between insulin resistance and blood pressure. *Hypertension*, **21**, 129-135.

Baum, M. (1987) Insulin stimulates volume absorption in the proximal convoluted tubule. *Journal of Clinical Investigation*, **56**, 335-340.

Beatty, O.L., Harper, R., Sheridan, B., Atkinson, A.B., Bell, P.M. (1993) Insulin resistance in offspring of hypertensive parents. *British Medical Journal*, **307**, 92-96.

Beck-Nielsen, H., Pedersen, O., Lindskov, H.O. (1979) Increased insulin sensitivity and cellular insulin binding in obese diabetics following treatment with glibenclamide. *Acta Endocrinologica*, **90**, 451-462.

- Beer, N.A., Jakubowicz, D.A., Beer, R.M., Arocha, I.R., Nestler, J.E. (1993) Effects of nitrendipine on glucose tolerance and serum insulin and dehydroepiandrosterone sulfate levels in insulin-resistant obese and hypertensive men. *Journal of Clinical Endocrinology and Metabolism*, **76**, 178-83.
- Benediktsson, R., Lindsay, R.S., Noble, I., Seckl, J.R., Edwards, C.R.W. (1993) Glucocorticoid exposure in utero: new model for adult hypertension. *Lancet*, **341**, 339-341.
- Bengtson, C., Blohmé, G., Lapidus, L., Lindquist, O., Lundgren, H., Nyström, E., Petersen, K., Sigurdsson, J.A. (1984) Do antihypertensive drugs precipitate diabetes? *British Medical Journal*, **298**, 1495-1497.
- Beretta-Piccoli C., Davies, D.L., Boddy, K., Brown, J.J., Cumming, A.M.M., East, B.W., Fraser, R., Lever, A.F., Padfield, P.L., Semple, P.F., Robertson, J.I.S., Weidmann, P., Williams, E.D. (1982) Relation of arterial pressure with body sodium, body potassium and plasma potassium in essential hypertension. *Clinical Science*, **63**, 257-270.
- Berglund, G., Larsson, B., Andersson, O., Larsson, O., Svardsudd, K., Bjorntorp, P., Wilhelmsen, L. (1976) Body composition and glucose metabolism in hypertensive middle-aged men. *Acta Medica Scandinavica*, **200**, 163-169.
- Bergman, R.N., Prager, R., Volund, A., Olefsky, J.M. (1987) Equivalence of the insulin sensitivity index in man derived by the minimal model method and the euglycaemic glucose clamp. *Journal of Clinical Investigation*, **79**, 790-800.
- Bergman, R.N., Hope, I.D., Yang, Y.J., Watanabe, R.M., Meador, M.A., Young, J.H., Ader, M. (1989) Assessment of insulin sensitivity in vivo: a critical review. *Diabetes/Metabolism Reviews*, **5**, 411-429.
- Berne, C., Pollare, T., Lithell, H. (1991) Effects of antihypertensive treatment on insulin sensitivity. *Diabetes Care*, **14**, 25-29.
- Bhathagar, S.K., Amin, M.M.A., Al-Yusuf, A.R. (1984). Diabetogenic effects of nifedipine (letter). *British Medical Journal*, **289**, 19.
- Bland, M. (1987) *An Introduction to Medical Statistics*. Oxford University Press, Oxford. pp277-280.

Bogardus, C., Ravussin, E., Robbins, D.C., Wolfe, R.R., Horton, E.S., Sims, E.A.H. (1984) Effects of physical training and diet therapy on carbohydrate metabolism in patients with glucose intolerance and non-insulin dependent diabetes mellitus. *Diabetes*, **33**, 311-318.

Bogardus, C., Lillioja, S., Stone, K., Mott, D. (1987) Correlation between muscle glycogen synthase activity and in vivo insulin action in man. *Journal of Clinical Investigation*, **80**, 415-425.

Bonadonna, R.C., Groop, L.C., Kraemer, N., Ferrannini, E., Del-Prato, S., De Fronzo, R.A. (1990) Obesity and insulin resistance in humans: a dose-response study. *Metabolism*, **39**, 452-459.

Bonora, E., Bonadonna, R.C., Del-Prato, S., Gulli, G., Solini, A., Matsuda, M., DeFronzo, R.A. (1993) In-vivo glucose metabolism in obese and type II diabetic subjects with or without hypertension. *Diabetes*, **42**, 764-772.

Brands, M.W., Mizelle, H.L., Gaillard, D.A., Hildebrandt, D.A., Hall, J.E. (1991) The haemodynamic response to chronic hyperinsulinaemia in conscious dogs. *American Journal of Hypertension*, **4**, 164-168.

Bree, F., Nguyen, P., Tillement, J.P., Johnson, C., Tremblay, D. (1992) How do trandolapril, a new ACE inhibitor, and trandolaprilat, the major and active metabolite, bind to human blood constituents? (abstract) *Journal of Hypertension*, **10** (Suppl 4), 188.

Bressler, R., Johnson, D. (1992) New pharmacological approaches to therapy of NIDDM. *Diabetes Care*, **15**, 792-805.

Brunner, D.B., Despond, G., Biollaz, J. (1981) Effect of a new angiotensin converting enzyme inhibitor, MK421, and its lysine analogue on the components of the renin angiotensin system in healthy subjects. *British Journal of Clinical Pharmacology*, **11**, 461-467.

Buchanan, T.A., Sipos, G.F., Gadalam, S., Yip, K.P., March, D.J., Hsueh, W. Bergman, R.N. (1991) Glucose tolerance and insulin action in rats with renovascular hypertension. *Hypertension*, **18**, 341-347.

Buchanan, T.A., Thawani, H., Kades, W., Modrall, J.G., Weaver, F.A., Laurel, C., Poppiti, R., Xiang, A., Hseuh, W. (1993) Angiotensin II increases glucose utilization during acute hyperinsulinaemia via a hemodynamic mechanism. *Journal of Clinical Investigation*, **92**, 720-726.

Bursztyn, M., Ben-Ishay, D., Gutman A. (1992) Insulin resistance in spontaneously hypertensive rats but not in DOCA-salt or renal vascular hypertension. *Journal of Hypertension*, **10**, 137-42.

Calle Pascual, A.L., Rodriguez, C., Martin Alvarez, P.J., Camacho, F., Calle, J.R., Yuste, E., Hildago, I., Diaz, R.J., Martin-Vaquero, P., Santiago, M. (1991) Effect of weight loss on insulin sensitivity and cardiovascular risk factors in glucose tolerant and intolerant obese subjects. *Diabetologia*, **17**, 404-409.

Calver, A., Collier, J., Vallance, P. (1992) Inhibition and stimulation of nitric oxide synthesis in the human forearm arterial bed of patients with insulin-dependent diabetes. *Journal of Clinical Investigation*, **90**, 2548-2554.

Cambien, F., Warner, J-M., Eachwege, E., Jacqueson, A., Richard, J.L., Rosselin, G. (1987) Body mass, blood pressure, glucose and lipids: does plasma insulin explain their relationships? *Arteriosclerosis*, **7**, 197-202.

Canessa, M., Adragna, N., Solomon, H.S., Connolly, T.M., Tosteson, D.C. (1980) Increased sodium-lithium countertransporter in red cells of patients with essential hypertension. *New England Journal of Medicine*, **302**, 772-776.

Capaldo, B., Lembo, G., Napoli, R., Rendina, V., Albano, G., Sacca, L., Trimarco, B. (1991) Skeletal muscle metabolism is a primary site of insulin resistance in essential hypertension. *Metabolism*, **40**, 1320-1322.

Charles, S., Ketelslegers, J.M., Buysschaert, M., Lambert, A.E. (1981). Hyperglycaemic effect of nifedipine. *British Medical Journal*, **283**, 19-20.

Chellingsworth, M., Kendall, M., Wright, A., Sugh, B., Pasi, J. (1989) The effects of verapamil, diltiazem, nifedipine and isopropranolol on metabolic control in hypertensives with non-insulin dependent diabetes mellitus. *Journal of Human Hypertension*, **3**, 35-39.

Chen, Y-D.I., Golay, A., Swislocki, A., Reaven, G.M. (1987) Resistance to insulin suppression of plasma free fatty acid concentration and insulin stimulation of glucose uptake in NIDDM. *Journal of Clinical Endocrinology and Metabolism*, **64**, 17-21.

Chiariello, M. (1991) A double-blind comparison of the efficacy and safety of lacidipine and hydrochlorothiazide in essential hypertension. *Journal of Cardiovascular Pharmacology*, **17** (suppl 4), S35-S37.

Chiknas, S.G. (1979) Liquid chromatography assisted assay for angiotensin converting enzyme in serum. *Clinical Chemistry*, **25**, 1259-1262.

Christlieb, A.R. (1976) Vascular reactivity to angiotensin II and to norepinephrine in diabetic subjects. *Diabetes*, **25**, 268-274.

Colca, J.R., Morton, D.R. (1990) Antihyperglycaemic thiazolidinediones. In: *Antidiabetic drugs*, Eds: Bailey, C.J., Flatt, P.R. London, Smith-Gordon, p255-261.

Collins, R., Peto, R., MacMahon, S., Hebert, P., Fiebach, N.H., Eberlein, K.H., Godwin, I., Qizilbach, N., Taylor, J.O., Hennekens, C.H. (1990) Blood pressure, stroke and coronary heart disease. Part 2. Short-term reductions in blood pressure: overview of randomised drug trials in their epidemiological context. *Lancet*, **335**, 827-838.

Collins, W.C.J., Cullen, M.J., Felly, J. (1986) The effect of therapy with dihydropyridine calcium channel blockers on glucose tolerance in non-insulin dependent diabetes. *British Journal of Clinical Pharmacology*, **21**, 568.

Cooper, G.J.S., Leighton, B., Dimitriadis, G.D., Parry-Billings, M., Kowalchuk, J.M., Howland, K., Rothbard, J.B., Willis, A.C., Reid, K.B. (1988) Amylin found in amylin deposits in human type 2 diabetes may be a hormone that regulates glycogen metabolism in skeletal muscle. *Proceedings of the National Academy of Science of America*, **85**, 7763-7766.

CONSENSUS Trial Study Group. (1987) Report. *New England Journal of Medicine*, **316**, 1429-1435.

Conway, G.S., Clark, P.M.S., Wong, D. (1993) Hyperinsulinaemia in the polycystic ovary syndrome confirmed with a specific immunoradiometric assay for insulin. *Clinical Endocrinology*, **38**, 219-222.

Creager, M.A., Cooke, J.P., Mendelson, M.D., Gallagher, S.G., Coleman, S.M., Loscalzo, J., Dzau, V.J. (1990) Impaired vasodilatation of forearm resistance vessels in hypercholesterolaemic humans. *Journal of Clinical Investigation*, **86**, 228-234.

- Creager, M.A., Liang, C-S., Coffman, J.D. (1985) Beta adrenergic-mediated vasodilator response to insulin in the human forearm. *Journal of Pharmacology and Experimental Therapeutics*, **235**, 709-714.
- Cullen, K., Stenhouse, N.S., Wearne, K.L., Welborn, T.A. (1983) Multiple regression analysis of risk factors for cardiovascular disease and cancer mortality in Busselton, Western Australia - 13 year study. *Journal of Chronic Diseases*, **36**, 371-377.
- Czyzyk, A., Lawecki, J., Sadowski, J., Ponikowksa, I., Szczepanik, Z. (1968) Effect of biguanides on intestinal absorption of glucose. *Diabetes*, **17**, 492-498.
- Daly, P.A., Landsberg, L. (1991) Hypertension in obesity and NIDDM: role of insulin and the sympathetic nervous system. *Diabetes Care*, **14**, 240-248.
- da Prada, M., Zurcher, G. (1976). Simultaneous determination of plasma adrenaline, noradrenaline and dopamine in the femtomole range. *Life Sciences*, **19**, 1161.
- Deedwania, P., Shah, J., Robison, J. (1984) Effects of nifedipine on glucose tolerance and insulin release in man. *Journal of American College of Cardiology*, **3**, 577.
- DeFronzo, R.A., Cooke, C.R., Andres, R., Faloona, G.R., David, P.J. (1975) The effect of insulin on renal handling of sodium, potassium, calcium and phosphate in man. *Journal of Clinical Investigation*, **55**, 845-855.
- DeFronzo, R.A., Tobin, J., Andres, R. (1979). Glucose clamp technique: a method for quantifying insulin secretion and resistance. *American Journal of Physiology*, **237**, E214-E223.
- DeFronzo, R.A., Felig, P., Ferrannini, E., Wahren, J. (1980) Effect of graded doses of insulin on splanchnic and peripheral metabolism in man. *American Journal of Physiology*, **238**, E421-E427.
- DeFronzo, R.A. (1981) The effects of insulin on renal sodium metabolism. *Diabetologia*, **21**, 165-171.
- DeFronzo, R.A., Sherwin, R.S., Kraemer, N. (1987) Effect of physical training on insulin action in obesity. *Diabetes*, **36**, 1379-1385.
- DeFronzo, R.A., Barzilai, N., Simonson, D.C. (1991) Mechanism of metformin action in obese and lean non-insulin-dependent diabetic subjects. *Journal of Clinical Endocrinology and Metabolism*, **73**, 1294-1301.

DeFronzo, R.A., Bonadonna, R.C., Ferrannini, E. (1992) Pathogenesis of NIDDM: a balanced overview. *Diabetes Care*, **15**, 318-368.

Deibert, D.C., DeFronzo, R. (1980) Epinephrine-induced insulin resistance in man. *Journal of Clinical Investigation*, **65**, 717-721.

De Marinis, L., Barbarino, A. (1980) Calcium antagonists and hormone release. Effects of verapamil on insulin release in normal subjects and patients with islet-cell tumours. *Metabolism*, **29**, 599-604.

De Ponti, F., Marelli, C., D'Angelo, L., Caravaggi, M., Bianco, L., Lecchini, S., Figo, G.M., Crema, A. (1991) Pharmacological activity and safety of trandolapril (RU 44570) in healthy volunteers. *European Journal of Clinical Pharmacology*, **40**, 149-153.

Derkx, F.H.M., Tan-Tjong, H.L., Man in't Veld, A.J., Schalekamp, M.P.A., Schalekamp, M.A.D.H. (1979). Activation of inactive plasma renin by renin and plasma kallikreins. *Clinical Science*, **57**, 351-357.

Després, J.P., Moorjani, S., Lupien, P.J., Tremblay, A., Nadeau, A., Bouchard, C. (1990) Regional distribution of body fat, plasma lipoproteins and cardiovascular disease. *Arteriosclerosis*, **10**, 497-511.

Dietze, G. (1982) Modulation of the action of insulin in relation to the energy state in skeletal muscle. Possible involvement of kinins and prostaglandins. *Molecular and Cellular Endocrinology*, **25**, 127-149.

Doberne, L., Greenfield, M.S., Schulz, B., Reaven, G.M. (1981) Enhanced glucose utilization during prolonged glucose clamp studies. *Diabetes*, **30**, 829-835.

Dohm, G.L., Elton, C.W., Friedman, J.E., Pilch, P.F., Porles, W.J., Atkinson, S.M., Caro, J.F. (1991) Decreased expression of glucose transporter in muscle from insulin resistant patients. *American Journal of Physiology*, **260**, E459-E463.

Donnelly, T., Harrower, A.D.B. (1980) Effect of nifedipine on glucose tolerance and insulin secretion in diabetic and non-diabetic patients. *Current Medical Research and Opinion*, **6**, 690-693.

- Donovan D.S., Solomon, C.G., Seely, E.W., Williams, G.H., Simonson, D.C. (1993) Effect of sodium intake on insulin sensitivity. *American Journal of Physiology*, **264**, E730-E734.
- Dowse, G.K., Collins, V.R., Alberti, K.G.M., Zimmet, P.Z., Tuomilehto, J., Chitson, P., Gareeboo, H. (1993) Insulin and blood pressure levels are not independently related in Mauritians of Asian Indian, Creole or Chinese origin. *Journal of Hypertension*, **11**, 297-307.
- Ducimetiere, P., Eschwege, E., Papoz, L., Richard, J.L., Claude, J.R., Roselin, G. (1980) Relationship of plasma insulin levels to the incidence of myocardial infarction and coronary heart disease mortality in a middle-aged population. *Diabetologia*, **19**, 205-210.
- Ducsterdieck, G., McElwee, G. (1971) Estimation of angiotensin II in human plasma by radioimmunoassay. Some applications to physiological and clinical states. *European Journal of Clinical Investigation*, **2**, 32-38.
- Dulbecco, A., Albenga, C., Borretta, G., Vacca, G., Milanese, G., Lavezzori, M. (1989) Effect of acipimox on plasma glucose levels in patients with non-insulin-dependent diabetes mellitus. *Current Therapeutic Research*, **46**, 478-483.
- Ebeling, P., Bourey, R., Koranyi, L., Tuominen, J.A., Groop, L.C., Henriksson, J., Mueckler, M., Sovijärvi, A., Koivisto, V.A. (1993) Mechanism of enhanced insulin sensitivity in athletes: increased blood flow, muscle glucose transport protein (GLUT-4) concentration and glycogen synthase activity. *Journal of Clinical Investigation*, **92**, 1623-1631.
- Edwards, C.R.W., Benediktsson, R., Lindsay, R., Seckl, J.R. (1993) Dysfunction of placental glucocorticoid barrier: link between fetal environment and early hypertension? *Lancet*, **341**, 355-357.
- Elliott, T.G., Cockcroft, J.R., Groop, P-H., Viberti, G-C., Ritter, J.M. (1993) Inhibition of nitric oxide synthesis in forearm vasculature of insulin-dependent diabetic patients: blunted vasoconstriction in patients with microalbuminuria. *Clinical Science*, **85**, 687-693.
- Eschwege, E., Richard, J.L., Thibault, N., Ducimetiere, P., Warnet, J.M., Claude, J.R., Rosselin, G.E. (1985) Coronary heart disease mortality in relation with diabetes, blood glucose and plasma insulin levels: the Paris Prospective study, ten years later. *Hormone and Metabolic Research*, **14**, 41-46.

Evans, D.J., Hoffman, R.G., Kalkhoff, R.K., Kissebah, A.H. (1984) Relationship of body fat topography to insulin sensitivity and metabolic profiles in premenopausal women. *Metabolism*, **33**, 68-75.

Faberberg, B., Berglund, A., Holme, E., Wilhelmsen, L., Elmfeldt, D. (1990) Metabolic effects of controlled-release metoprolol in hypertensive men with impaired or diabetic glucose tolerance: a comparison with atenolol. *Journal of Internal Medicine*, **227**, 37-43.

Faberberg, B., Berglund, A., Andersson, O.K., Berglund, G. (1992) Weight reduction versus antihypertensive drug therapy in obese men with high blood pressure: effects upon plasma insulin levels and association with changes in blood pressure and serum lipids. *Journal of Hypertension*, **10**, 1053-1061.

Facchini, F.S., Hollenbeck, C.B., Jeppesen, J. Chen, Y-D.I., Reaven, G.M. (1992) Insulin resistance and cigarette smoking. *Lancet*, **339**, 1128-31.

Fantus, G.I., Brousseau, R. (1986) Mechanism of action of metformin: insulin receptor and postreceptor effects in vitro and in vivo. *Journal of Clinical Endocrinology and Metabolism*, **63**, 898-905.

Feldman, J.M., Lebovitz, H.E. (1969) Appraisal of the extrapancreatic actions of sulphonylureas. *Archives of Internal Medicine*, **123**, 314-322.

Ferlito, S., Fichera, C., Carr, G. (1980) Effect of nifedipine on blood sugar, insulin and glucagon levels after an oral glucose load. *Panminerva Medicus*, **23**, 75-80.

Ferlito, S., Modica, L., Romano, F. (1982) Effect of verapamil on glucose insulin and glucagon levels after oral glucose load in normal and diabetic subjects. *Journal of Internal Medicine*, **24**; 221-226.

Ferrannini E., Buzzicoli G., Bonadonna R., Giorico, M.A., Oleggini, M., Graziadei, L., Pedrinelli, R., Brandi, L., Bevilacqua, S. (1987). Insulin resistance in essential hypertension. *New England Journal of Medicine*, **317**, 350-357.

Ferrannini, E. (1989) The theoretical basis for indirect calorimetry: a review. *Metabolism*, **37**: 287-301.

- Ferrannini, E., Haffner, A.M., Stern, M.P. (1990) Essential hypertension: an insulin resistant state. *Journal of Cardiovascular Pharmacology* **15** (suppl 5), 518-525.
- Ferrannini, E., Galvan, A.Q., Santoro, D., Natali, A. (1992) Potassium as a link between insulin and the renin-angiotensin-aldosterone system. *Journal of Hypertension*, **10** (Suppl. 1), S5-S10.
- Ferrara, L.A., Soro, S., Fasano, M.L. (1985) Effects of nitrendipine on glucose and serum lipid concentrations. *Current Therapeutic Research*, **37**, 614-618.
- Ferrari, P., Weidmann, P. (1990). Insulin, insulin sensitivity and hypertension. *Journal of Hypertension*, **8**, 491-500.
- Ferrari, P., Weidmann, P., Shaw, S., Giachino, D., Riesen, W., Allemann, Y., Heynan, G. (1991a) Altered insulin sensitivity, hyperinsulinaemia and dyslipidaemia in individuals with a hypertensive parent. *American Journal of Medicine*, **91**, 589-596.
- Ferrari, P., Giachino, D., Weidmann, P., Shaw, S., Riesen, W., Neuner, N., Keller, U., Heynan, G. (1991b). Unaltered insulin sensitivity during calcium channel blockade with amlodipine. *European Journal of Clinical Pharmacology*, **41**, 109-113.
- Ferriere, M., Bringer, J., Richard, J., Rodier, M., Jaffiol, C., Mirouze, J. (1985) Captopril and insulin sensitivity. *Annals of Internal Medicine*, **102**, 134-135.
- Finegood, D.T., Bergman, R.N., Vranic, M. (1987) Estimation of endogenous glucose production during euglycaemic hyperinsulinaemic glucose clamps: comparison of unlabelled and labelled exogenous glucose infusates. *Diabetes*, **36**, 914-924.
- Foley, J.E. (1992) Rationale and application of fatty acid oxidation inhibitors in treatment of diabetes mellitus. *Diabetes Care*, **15**, 773-784.
- Fontbonne, A., Charles, M.A., Thibault, N., Richaer, J.L., Claude, J.R., Warnet, J.M., Rosselin, G.E., Eschwege, E. (1991) Hyperinsulinaemia as a predictor of coronary heart disease mortality in a healthy population: the Paris Prospective Study. *Diabetologia*, **34**, 356-361.
- Forster, H.V., Dempsey, J.A., Thompson, J., Vidruk, E., doPico, G.A. (1972) Estimation of arterial pO₂, pCO₂, pH and lactate from arterialised venous blood. *Journal of Applied Physiology*, **32**, 134-137.

Frank, R., Rougier, R.F.I., Tonet, J.L., Harding, S., Bons, J., Fontaine, G., Grosseat, Y. (1990) Effect of the new once-daily calcium antagonist, lacidipine in controlling 24-hour ambulatory blood pressure. *American Journal of Cardiology*, **66**, 1228-1230.

Fruncilo, R.J., Tomensch, H.H., Vlasses, P.H., Koplin, J.R., Monsell, K., Clementini, R.A., Fergusson, R.A. (1983) Effects of captopril and hydrochlorothiazide on the pressor responses to norepinephrine and angiotensin II in hypertensives. *Clinical Pharmacology and Therapeutics*, **33**, 226-229.

Fuccella, L.M., Goldaniga, G., Lovisolo, P., Maggi, E., Musatti, L., Mandelli, V., Sirtori, C.R. (1980) Inhibition of lipolysis by nicotinic acid and acipimox. *Clinical Pharmacology and Therapeutics*, **28**, 790-795.

Fujita, N., Baba, T., Tomiyami, T., Kodama, T., Kako, N. (1992) Hyperinsulinaemia and blood pressure in patients with insulinoma. *British Medical Journal*, **304**, 1157.

Fukagawa, N.K., Anderson, J.W., Hageman, G., Young, V.R., Minaker, K.L. (1990) Low carbohydrate, high fiber diets increase peripheral insulin sensitivity in healthy young and old adults. *American Journal of Clinical Nutrition*, **52**, 524-528.

Gallen, I.W., Macdonald, I.A. (1990) Effect of two methods of hand heating on body temperature, forearm blood flow, and deep venous oxygen saturation. *American Journal of Physiology*, **259**, E639-E643.

Galloway, J.A., Hooper, S.A., Spradlin, C.T., Howey, D.C., Frank, B.H., Bousher, R.R., Anderson, J.H. (1992) Biosynthetic human pro-insulin. *Diabetes Care*, **15**, 666-692.

Gans, R.O., Bilo, H.J.G., Maarschalkerweerd, W.W.A., Heine, R.J., Nauta, J.J.P., Donker, A.J.M. (1991a) Exogenous insulin augments in healthy volunteers the cardiovascular reactivity to noradrenaline but not to angiotensin II. *Journal of Clinical Investigation*, **88**, 512-518.

Gans, R.O.B., Bilo, H.J.G., Nauta, J.J.P., Popp-Snijders, C., Heine, R.J., Donker, A.J.M. (1991b) The effect of angiotensin-I converting enzyme inhibition on insulin action in healthy volunteers. *European Journal of Clinical Investigation*, **21**, 527-533.

Gill, J.S., Al-Hussary, N., Zezulka, A.V., Pasi, K.J., Atkins, T.W., Beevers, D.G. (1987). Effect of nifedipine on glucose tolerance, serum insulin and serum fructosamine in diabetic and nondiabetic subjects. *Clinical Therapeutics*, **9**, 304-310.

Gin, H., Messerchnitt, C., Brottier, E., Aubertin, J. (1983) Metformin improves insulin resistance in type I diabetes. *Diabetologia*, **24**, 351-354.

Giugliano, D., Torella, R., Cacciapuot, F., Gentile, S., Verza, M., Varricchio, M. (1980). Impairment of insulin secretion in man by nifedipine. *European Journal of Clinical Pharmacology*, **18**, 395-398.

Giugliano, D., Gentile, S., Verza, M. Passariello, N., Giannetti, G., Varricchio, M. (1981) Modulation by verapamil of insulin and glucagon secretion in man. *Acta Diabetologia*, **18**, 163-171.

Gliemann, J., Sonne, O. (1978) Binding and receptor-mediated degradation of insulin in adipocytes. *Journal of Biological Chemistry*, **253**, 7857-7863.

Greenfield, M.S., Doberne, L., Kraemer, F., Tobey, T., Reaven, G. (1981) Assessment of insulin resistance with the insulin suppression test and the euglycaemic clamp. *Diabetes*, **30**, 387-392.

Groop, L.C., Pelkonen, R. (1984) Treatment failures: a common problem in the management of patients with type II diabetes. *Acta Endocrinologia*, **105** (Suppl 262), 131-135.

Grootenhuis, P.A., Mooy, J.M., Popp-Snijders, C., Bouter, L.M., Heine, R.J. (1993) Proinsulin like molecules in relation to blood lipids and blood pressure in a general caucasian population. (abstract) *Diabetologia*, **36**, A270.

Gupta, A.K., Clark, R.V., Kirchner, K.A. (1992) Effects of insulin on renal sodium excretion. *Hypertension*, **19** (suppl I), I67-I71.

Haffner, S.M., Ferrannini, E., Hazuda, H.P., Stern. M.P. (1992) Clustering of cardiovascular risk factors in confirmed prehypertensive individuals. *Hypertension*, **20**, 38-45.

Haffner, S.M. (1993) Insulin and blood pressure - fact and fantasy. *Journal of Clinical Endocrinology and Metabolism* **76**, 541-543

Hale, P.J., Singh, B.M., Crase, J., Baddeley, R.M., Nattrass, M. (1988) Following weight loss in massively obese patients correction of the insulin resistance of fat metabolism is delayed relative to the improvement in carbohydrate metabolism. *Metabolism*, **37**, 411-417.

Halkin, H., Or, J., Fuchs, Z., Lusky, A., Chetrit, A., Modan M. (1989) Smoking accounts for the adverse effects of antihypertensive medication on plasma lipids: a population based study. *Hypertension*, **14**, 210-217.

Hall, J.E., Coleman, T.G., Mizelle, H.L., Smith, M.J. (1990) Chronic hyperinsulinaemia and blood pressure regulation. *American Journal of Physiology*, **258**, F722-F731.

Hall, S.T., Harding, S.M., Evans, G.L., Pellegatti, M., Rizzini, P. (1991) Clinical pharmacology of lacidipine. *Journal of Cardiovascular Pharmacology*, **17** (suppl 4), S9-S13.

Harano, Y., Hidaka, H., Takatsuki, K., Ohgaku, S., Haneda, M., Motoi, S., Kawagoe, K., Shigeta, Y., Abe, H. (1978) Glucose, insulin and somatostatin infusion for the determination of insulin sensitivity in vivo. *Metabolism*, **27**, 1449-56.

Hardin, D., Dominguez, J.H., Garvey, W.T. (1993) Muscle group-specific regulation of GLUT 4 glucose transporters in control, diabetic and insulin-treated diabetic rats. *Metabolism*, **42**, 1310-1315.

Hargreaves, A.D., Logan, R.L., Elton, R.A., Buchanan, K.D., Oliver, M.F., Riermersma, R.A. (1992) Glucose tolerance, plasma insulin, HDL cholesterol and obesity: 12 year follow-up and development of coronary artery disease in Edinburgh men. *Atherosclerosis*, **94**, 61-69.

Harper, R., Ennis, C., Atkinson, B., Johnston, D., Bell, P. (1993) Effect of low and conventional dose bendrofluazide on insulin action in essential hypertension (abstract) *Diabetes*, **42**, 165A.

Heath, G.W., Gavin, J.R.III., Hinderliter, J.M. (1983) Effects of exercise and lack of exercise on glucose tolerance and insulin sensitivity. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*, **55**, 512-517.

Heber, M.E., Broadhurst, P.A., Brigden, G.S., Raftery, E.B. (1990) Effectiveness of the once-daily calcium antagonist, lacidipine, in controlling 24-hour ambulatory blood pressure. *American Journal of Physiology*, **66**, 1228-1232.

Heding, L.G. (1977) Specific and direct radioimmunoassay for human proinsulin in serum. *Diabetologia*, **13**, 467-474

- Hedner, T., Thulin, T., Gustaffson, S., Olsson, S.O. (1990) A comparison of diltiazem and metoprolol in hypertension. *European Journal of Clinical Pharmacology*, **39**, 859-865.
- Helgeland, A., Leren, P., Foss, O.P., Hjermann, I., Holme, I., Lund-Larsen, I. (1984) Serum glucose levels during long-term observation of treated and untreated men with mild hypertension: the Oslo study. *American Journal of Medicine*, **76**, 802-805.
- Helgeland, A., Strommen, R., Hagelund, C.H., Trett, S. (1986) Enalapril, atenolol and hydrochlorothiazide in mild-to-moderate hypertension, *Lancet*, **1**, 872-876.
- Helve, E., Tuominen, J.A., Koivisto, V.A. (1993) Captopril does not acutely enhance insulin sensitivity. *Journal of Internal Medicine*, **234**, 41-44.
- Herbette, L.G., Gaviraghi, G., Tulenko, T., Mason, R.P. (1993) Molecular interaction between lacidipine and biological membranes. *Journal of Hypertension*, **11** (suppl), S13-S19.
- Himsworth, H.P. (1936) Diabetes mellitus; its differentiation into insulin-sensitive and insulin-insensitive types. *Lancet*, **1**, 127-130.
- Himsworth, H.P., Kerr, R.B. (1939) Insulin-sensitive and insulin-insensitive types of diabetes mellitus. *Clinical Science*, **4**, 119-123.
- Hodsman, G.P., Brown, J.J., Davies, D.L. (1982) Converting enzyme inhibitor enalapril in the treatment of hypertension with renal artery stenosis. *British Medical Journal*, **285**, 1697-1699.
- Hofmann, C.A., Lorenz, K., Colca, J.R. (1991) Glucose transport deficiency in diabetic animals is corrected by treatment with the oral antihyperglycaemic agent pioglitazone. *Endocrinology*, **129**, 1915-1925.
- Hofmann, C.A., Colca, J.R. (1992) New oral thiazolidinedione antidiabetic agents act as insulin sensitisers. *Diabetes Care*, **15**, 1075-1078.
- Hosker, J.P., Matthews, D.R., Rudenski, A.S., Burnett, M.A., Darling, P., Bown, E.G., Turner, R.C. (1985) Continuous infusion of glucose with model assessment: measurement of insulin resistance and β -cell function in man. *Diabetologia*, **28**, 401-411.

- Howes, L.G., Millar, S.K., Reid, J.L. (1985) The simultaneous assay of 3,4 dihydroxyphenylethylene glycol and norepinephrine in human plasma by HPLC with electrochemical detection. *Journal of Chromatography*, **338**, 401-403.
- Hughes, T.A., Gwynne, J.T., Switzer, B.R., Herbst, C., White, G. (1984) Effects of caloric restriction and weight loss on glycaemic control, insulin release and resistance, and atherosclerotic risk in obese patients with type II diabetes mellitus. *Kidney International*, **77**, 7-17.
- Hunt, S.C., Wu, L.L., Hopkins, P.N., Williams, R.R. (1989) Sodium-lithium countertransport and fasting insulin in patients with familial combined hyperlipidaemia and hypertension. (abstract) *Hypertension*, **14**, 345.
- Hunt, S.C., Stephenson, S.H., Hopkins, P.N., Hasstedt, S.J., Williams, R.R. (1991) A prospective study of sodium-lithium countertransporter and hypertension in Utah. *Hypertension*, **17**, 1-7.
- Hwang, I-S., Ho, H., Hoffman, B.B., Reaven, G.M. (1987) Fructose induced insulin resistance and hypertension in rats. *Hypertension*, **10**, 512-516.
- Hypertension in Diabetes Study Group (1993). Hypertension in Diabetes Study (HDS): I. Prevalence of hypertension in newly presenting type 2 diabetic patients and the association with risk factors for cardiovascular and diabetic complications. *Journal of Hypertension*, **11**, 309-317.
- Iversen, E., Jeppesen, D., Steensgard-Hansen F. (1990) Direct diabetogenic effects of diltiazem? *Journal of Internal Medicine*, **227**, 285-286.
- Iwamoto, Y., Kuzuya, T., Matsuda, A. Awata, T., Kumakura, S., Inooka, G., Shiraishi, I. (1991) Effect of the new oral antidiabetic agent CS-045 on glucose tolerance and insulin secretion in patients with NIDDM. *Diabetes Care*, **14**, 1083-1086.
- Jamerson, K.A., Julius, S., Gudbrandsson, T., Andersson, O., O'Brant, D. (1993) Reflex sympathetic activation induces acute insulin resistance in the human forearm. *Hypertension*, **21**, 618-623.
- James, D.E., Burleigh, K.M., Storlien, L.H., Bennett, S.P., Kraegen, E.W. (1986) Heterogeneity of insulin action in muscle: influence of blood flow. *American Journal of Physiology* **251**: E422-E430.

- Jauch, K.W., Hartl, W., Guenther, B., Wicklmays, M., Rett, K., Dietze, G. (1987). Captopril enhances insulin responsiveness of forearm muscle tissue in non-insulin-dependent diabetes mellitus. *European Journal of Clinical Investigation*, **17**, 448-454.
- Jauch, K.W., Hartl, W.H., Georgeieff, M., Wolfe, R.R., Dietze, G., Gunther, B. (1988). Low-dose bradykinin infusion reduces endogenous glucose production in surgical patients. *Metabolism*, **37**, 185-190.
- Jiang, X., Srinivasa, S.R., Bao, W., Berenson, G.S. (1993) Association of fasting insulin with longitudinal changes in blood pressure in children and adolescents. The Bogalusa Heart Study. *American Journal of Hypertension*, **6**, 564-569.
- Joffe, B.I., Lamprey, J.M., Shires, R., Baker, S., Viljoen, M., Seftel, H.C. (1983) Lack of hormonal effects of a single dose of nifedipine in healthy young men. *Journal of Cardiovascular Pharmacology*, **5**, 599-604.
- Johlin-Dannfelt, A., Frisk-Holmberg, M., Karlsson, J., Tesch, P. (1979) Central and peripheral circulation in relation to muscle fibre type composition in normotensive and hypertensive man. *Clinical Science*, **56**, 335-340.
- Johnson, C.I.J., Kerr, J.H. (1985) Automatic blood pressure recorders: a clinical evaluation of five models in adults. *Anaesthesia*, **40**, 471-478.
- Julius, S., Gudbrandsson, T., Jamieson, K., Shabab, S.T., Andersson, O. (1991) The haemodynamic link between insulin resistance and hypertension. *Journal of Hypertension*, **9**, 983-986.
- Junichi, K., Shigehiro, K., Kiyishi, T., Akira, I., Shyoji, K., Jun, I. (1991) Effects of captopril on glucose concentration: possible role of augmented postprandial forearm blood flow. *Diabetes Care*, **13**, 1109-1111.
- Kadish, A.H., Little, R.L., Sternberg, J.C. (1968) A new and rapid method for the determination of glucose by measurement of rate of oxygen consumption. *Clinical Chemistry*, **14**, 116.
- Kaley, G., Donshik, P.C., Schlissler, G.L. (1967) Metabolic effects of angiotensin. *Federation Proceedings*, **26**, 439-443.
- Kannel, W.B., McGhee, D.L. (1979) Diabetes and cardiovascular risk factors: the Framingham Study. *Circulation*, **59**, 8-13.

Kannell, W.B., Wolf, P.A., Castelli, W.P., D'Agostini, R.B. (1987) Fibrinogen and risk of cardiovascular disease. *Journal of American Medical Association*, **258**, 1183-1186.

Kannell, W.B., Wilson, P.W.F., Zhang, T-J. (1991) The epidemiology of impaired glucose tolerance and hypertension. *American Heart Journal*, **121**, 1268-1273.

Kashiwagi, A., Verso, M.A., Andrews, J., Vasquez, B., Reaven, G.M., Foley, J.E. (1983) In vitro insulin resistance of human adipocytes isolated from subjects with non-insulin dependent diabetes mellitus. *Journal of Clinical Investigation*, **72**, 1246-1254.

Kendall, M.J., Lewis, H., Griffith, M., Barnett, A.H. (1988) Drug treatment of the hypertensive diabetic. *Hypertension*, **1**, 249-258.

Kjellstrom, T., Blychert, E., Lindgarde, F. Felodipine in the treatment of hypertensive type II diabetics: effect on glucose homeostasis. *Journal of Internal Medicine*, **229**, 233-239.

Klauser, R., Prager, R., Gaube, S., Gisinger, C., Schnack, C., Kuenburg, E., Schernthaner, G. (1991). Metabolic effects of isradipine versus hydrochlorothiazide in diabetes mellitus. *Hypertension*, **17**, 15-21.

Kneer, N.M., Lardy, H.A. (1983) Regulation of gluconeogenesis by norepinephrine, vasopressin and angiotensin II: a comparative study in the absence and presence of extracellular calcium. *Archives of Biochemistry and Biophysics*, **225**, 187-195.

Kolterman, O.G., Gray, R.S., Shapiro, G., Scarlett, J.A., Griffin, J., Olefsky, J.M. (1984) The acute and chronic effects of sulphonylurea therapy in type II diabetic subjects. *Diabetes*, **33**, 346-354.

Krone, W., Naegle, H., Behnke, B., Greten H. (1988) Opposite effects of insulin and catecholamines on LDL-receptor activity in human mononuclear leucocytes. *Diabetes*, **37**, 1386-1391

Kuzuya, T., Iwamoto, Y., Kosaka, K., Takebe, K., Yamanouchi, T., Kasuga, M., Kajinuma, H., Akanuma, Y., Yoshida, S., Shigeta, Y. (1991) A pilot clinical trial of a new oral hypoglycaemic agent, CS-045, in patients with NIDDM. *Diabetes Research and Clinical Practice*, **11**, 147-153.

- Landkin, K., Tengborn, L., Smith, U. (1990) Elevated fibrinogen and plasminogen activator inhibitor (PAI-1) in hypertension are related to metabolic risk factors for cardiovascular disease. *Journal of Internal Medicine*, **60**, 491-494.
- Landmark, K. (1985) Antihypertensive and metabolic effects of long-term therapy with nifedipine slow-release tablets. *Journal of Cardiovascular Pharmacology*, **7**, 12-17.
- Lang, R., Klein, H.O., Shapiro, M.S., Kaplinsky, E. (1981) Verapamil therapy and blood glucose concentration. *Archives of Internal Medicine*, **141**, 1724.
- Lembo, G., Rendina, V., Iaccarino, G., Lamenza, F., Massimo, V., Trimarco, B. (1993) Insulin reduces sympathetic vasoconstriction in healthy humans. *Hypertension*, **21**, 1015-1019.
- Leonetti, G., Fogari, R., Mazzola, C., Mangiameli, S., Castello, C. (1991a) Comparison of the effects on blood pressure and left ventricular hypertrophy of lacidipine and hydrochlorothiazide in hypertensive patients. *Journal of Hypertension*, **9** (suppl 3), S29-S31.
- Leonetti, G. (1991b) Comparative study of lacidipine and nifedipine SR in the treatment of hypertension: an Italian multicenter study. *Journal of Cardiovascular Pharmacology*, **17** (suppl 4), S31-S34.
- Levy, J., Zemel, M.B., Sowers, J.R. (1989) Role of cellular calcium metabolism in abnormal glucose metabolism and diabetic hypertension. *American Journal of Medicine*, **87**(suppl. 6A), 7S-16S.
- Lichtenstein, M.J., Yarnell, J.W.G., Elwood, P.C., Beswick, A.D., Sweetnam, P.M., Marks, V., Teale, D., Riad-Fahmy, D. (1987) Sex hormones, insulin, lipids and prevalent ischaemic heart disease. *American Journal of Epidemiology*, **126**, 647-57.
- Lillioja, S., Young, A.A., Culter, C.L., Ivy, J.L., Abbott, W.G., Zawadzki, J.K., Yki-Jarvinen, H., Christin, L., Secomb, T.W., Bogardus, C. (1987) Skeletal muscle capillary density and fibre type are possible determinants of in-vivo insulin resistance in man. *Journal of Clinical Investigation*, **80**, 415-425.
- Lind, L., Lithell, H., Wide, L., Ljunghall, S. (1992) Metabolic cardiovascular risk factors and the renin-angiotensin-aldosterone system in essential hypertension. *Journal of Human Hypertension*, **6**, 27-29.

- Liu, D., Moberg, E., Kollind, M., Lins, P-E., Adamson, W., MacDonald, I.A. (1992). Arterial, arterialised venous, venous and capillary blood glucose measurements in normal man during hyperinsulinaemic euglycaemia and hypoglycaemia. *Diabetologica*, **35**, 287-290.
- Lund, S., Vestergaard, H., Andersen, P.H., Schmitz, O., Gotzsche, L.B.H., Pedersen, O. (1993) GLUT-4 content in plasma membrane of muscle from patients with non-insulin dependent diabetes mellitus. *American Journal of Physiology*, **265**, E889-E897.
- Lundgren, H., Bjorkman, L., Keiding, P., Lundmark, S., Bengtsson, C. (1988) Diabetes in patients with hypertension receiving pharmacological treatment. *British Medical Journal*, **297**, 1512.
- Lyons, D., Fowler, G., Webster, J., Hall, S.T., Petrie, J.C. (1994) An assessment of lacidipine and atenolol in mild to moderate hypertension. *British Journal of Clinical Pharmacology*, **37**, 45-51.
- MacKenzie, J.K., Clements, J.A. (1974). Simplified radioimmunoassay for serum aldosterone utilising increased antibody specificity. *Journal of Clinical Endocrinology and Metabolism*, **38**, 622-627.
- MacMahon, S.W., MacDonald, G.J., Blacket, R.B. (1985) Plasma lipoprotein levels in treated and untreated hypertensive men and women. *Arteriosclerosis*, **5**, 391-396.
- Mancia, G., De Cesaris, R., Fogari, R., Lattuada, S., Montemurro, G., Palombo, C., Porcellati, C., Ranieri, G., Tettamanti, F., Verdecchia, P. (1992) Evaluation of once-a-day trandolapril by 24-hour ambulatory blood pressure monitoring. The Italian Trandolapril Study Group. *American Journal of Cardiology*, **70**, 60D-66D.
- Marigliano, A., Teddle, R., Sechi, L.A., Pala, A., Pisanu, G., Pacifico, A. (1990) Insulinaemia and blood pressure relationships in patients with primary and secondary hypertension, and with and without glucose metabolism impairment. *American Journal of Hypertension*, **3**, 521-526.
- Marquie, G. (1978) Effect of insulin in the induction and regression of experimental cholesterol atherosclerosis in the rabbit. *Postgraduate Medical Journal*, **54**, 80-85.
- Marrotta, T., Ferrara, L.A., Pasanisi, F., Rubba, P., Iovine, C., Mancini, M. (1989). Enhancement of exogenous triglyceride removal following calcium channel blockade. *Artery*, **16**, 312-326.

- Matthews, D.R., Hosker, J.P., Rudenski, J.S., Naylor, B.A., Treacher, B.F., Turner, R.C. (1985) Homeostasis model assessment: insulin resistance and β -cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, **28**, 412-419.
- Mattiasson, I., Berntrop, K., Lindgarde, F. (1992) Sodium-lithium countertransport and platelet cytosolic free calcium concentration in relation to peripheral insulin sensitivity in postmenopausal women. *Clinical Science*, **83**, 319-324.
- Mbanya, J.C., Thomas, T.H., Wilkinson, R., Alberti, K.G.M.M., Taylor, R. (1988) Hypertension and hyperinsulinaemia: a relation in diabetes but not in essential hypertension. *Lancet*, **1**, 733-744.
- McGuire, E.A.H., Helderman, J.H., Tobin, R.D., Andres, R., Bergman, R. (1976) Effects of arterial versus venous sampling on analysis of glucose kinetics in man. *Journal of Applied Physiology*, **41**, 565-573.
- McMurray, J., Fraser, D.M. (1986) Captopril, enalapril and blood glucose. (letter) *Lancet*, **1**, 1035.
- McVeigh, G.E., Brennan, G.M., Johnston, G.D., McDermott, B.J., McGrath, L.T., Henry, W.R., Andrews, J.W., Hayes, J.R. (1992) Impaired endothelium-dependent vasodilation in patients with type 2 (non-insulin dependent) diabetes mellitus. *Diabetologia*, **35**, 771-776.
- Medical Research Council Working Party. (1985) MRC trial of treatment of mild hypertension: principal results. *British Medical Journal*, **291**, 97-104.
- Meistas, M.T., Margolis, S., Kowarski, A.A. (1983) Hyperinsulinaemia of obesity is due to decreased clearance of insulin. *American Journal of Physiology*, **245**, E155-E159.
- Minaker, K.L., Rowe, J.W., Tonino, R., Pallotta, J.A. (1982) Influence of age on clearance of insulin in man. *Diabetes*, **31**, 851-855.
- Mitchell, B.D., Stern, M.P., Haffner, S.M., Hazuda, H.P., Patterson, J.K. (1990) Risk factors for cardiovascular mortality in Mexican Americans and non-Hispanic whites: the San Antonio Heart Study. *American Journal of Epidemiology*, **131**, 423-433.

Modan, M., Halkin, H., Almog, S., Lusky, A., Eshkol, A., Shefi, M., Shitrit, A., Fuchs, Z. (1985) Hyperinsulinaemia; A link between hypertension, obesity and glucose intolerance. *Journal of Clinical Investigation*, **75**, 809-817.

Modan, M., Halkin, H. (1991) Hyperinsulinaemia or increased sympathetic drive as links for obesity and hypertension *Diabetes Care*, **14**, 470-487.

Moller, D.E., Flier, J.S. (1991) Insulin resistance - mechanisms, syndromes and implications. *New England Journal of Medicine*, **325**, 938-948.

Moore, M.P., Elliott, T.W., Nicholls, M.G. (1988) Hormonal and metabolic effects of enalapril treatment in hypertensive subgroups with NIDDM. *Diabetes Care*, **11**, 397-401.

Morton, J.J., Webb, D.J. (1985) Measurement of plasma angiotensin II. *Clinical Science*, **68**, 483-484.

Multiple Risk Factor Intervention Research Group (1982) Multiple Risk Factor Intervention Trial: Risk factor changes and mortality results. *Journal of American Medical Association*, **248**, 1465-1477.

Murphy, M.B., Lewis, P.J., Kohner, E., Schumer, B., Dollery, C.T. (1982) Glucose intolerance in hypertensive patients treated with diuretics; a fourteen-year follow-up. *Lancet*, **2**, 1293-1295.

Nakano, J., Kusakari, T. (1966) Effects of synthetic angiotensin on plasma FFA and blood glucose levels. *Nature*, **209**, 922-923.

Natali, A., Santoro, D., Palambo, C., Cerri, M., Ghione, S., Ferrannini, E. (1991). Impaired insulin action on skeletal muscle metabolism in essential hypertension. *Hypertension*, **17**, 170-178.

Neahring, J.M., Stepniakowski, K., Greene, A.S., Egan, B.M. (1993) Insulin does not reduce forearm alpha-vasoreactivity in obese hypertensive or lean normotensive men. *Hypertension*, **22**, 584-590.

Ng, L.L. (1988) Application of modelling techniques to the assessment of insulin sensitivity in man. *Diabetic Medicine*, **5**, 217-223.

Odigwe, C.O., McCulloch, C.J., Williams, D.O., Tunbridge, W.M. (1986). A trial of the calcium antagonist nisoldipine in hypertensive non-insulin diabetic patients. *Diabetic Medicine*, **3**, 463-467.

Oehman, K.P., Weiner, L., Schenck, K., Karlberg, B.E. (1985). Antihypertensive and metabolic effects of nifedipine and labetalol alone and in combination in primary hypertension. *European Journal of Clinical Pharmacology*, **29**, 149-154.

Ohno, Y., Suzuki, H., Yamakawa, H., Nakamura, M., Otsuka, K., Saruta, T. (1993) Impaired insulin sensitivity in young, lean normotensive offspring of essential hypertensives: possible role of disturbed calcium metabolism. *Journal of Hypertension*, **11**, 421-426.

O'Hare, J.A., Ferriss, J.B., Brady, D., Twomey, B., O'Sullivan, D.J. (1985) Exchangeable sodium and renin in hypertensive diabetic patients with and without nephropathy. *Hypertension*, **7** (suppl. II), II43-II48.

Olefsky, J., Reaven, G.M., Farquhar, J.W. (1974) Effects of weight reduction on obesity: studies of lipid and carbohydrate metabolism in normal and hyperlipoproteinaemic subjects. *Journal of Clinical Investigation*, **53**, 64-76.

Oliver, F.J., De la Rubia, G., Feener, G., Feener, E.P., Lee, M.E., Loeken, M.R., Shiba, T., Quertermans, T., King, G.L. (1991) Stimulation of endothelin-1 gene expression by insulin in endothelial cells. *Journal of Biological Chemistry*, **266**, 23251-23256.

Pandit, M.K., Burke, J., Gustafson, A.B., Minocha, A., Peiris, A.N. (1993) Drug-induced disorders of glucose tolerance. *Annals of Internal Medicine*, **118**, 529-539.

Panza, J.A., Quyyumi, A.A., Brush, J.E., Epstein, S.E. (1990) Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *New England Journal of Medicine*, **323**, 22-27.

Paolisso, G., De Riu, S., Marrazzo, G., Verza, M., Varricchio, M., D'Onofrio, F. (1991) Insulin resistance and hyperinsulinaemia in patients with chronic congestive heart failure. *Metabolism*, **40**, 972-977.

Paolisso, G., Gambardella, A., Verza, M., D'Amore, A., Sgambato, S., Varricchio, M. (1992) ACE inhibition improves insulin sensitivity in aged insulin resistant hypertensive patients. *Journal of Human Hypertension*, **6**, 175-179.

Parillo, M., Coulston, A., Hollenbeck, C., Reaven, G.M. (1988) Effect of a low fat diet on carbohydrate metabolism in patients with essential hypertension. *Hypertension*, **11**, 244-248.

Pasanisi, F., Ferrara, A.L., Iovine, C. (1986) Effects of nifedipine on insulin secretion and plasma lipids in hypertensive patients. *Current Therapeutic Research*, **39**, 894-899.

Pasanisi F., Marotta T., Ferrara A., Grasso A., Cortese C., Mancini M. (1988). Acute and chronic effects of dihydropyridines on triglycerides in humans. *Journal of Cardiovascular Pharmacology*, **12**, S140-S141.

Peiris, A.N., Struve, M.F., Mueller, R.A., Lee, M.B., Kissebah, A.H. (1988) Glucose metabolism in obesity: influence of body fat distribution. *Journal of Clinical Endocrinology and Metabolism*, **67**, 760-767.

Perelman, M. (1991) Selection of initial and maintenance dosages for lacidipine, a new once-daily calcium antagonist for the treatment of hypertension. *Journal of Cardiovascular Pharmacology*, **17**, S14-S19.

Perez, G.O., Lespier, L., Jacobi, J. (1977) Hyporeninaemia and hypoaldosteronism in diabetes mellitus. *Archives of Internal Medicine*, **137**, 852-855.

Phillips, D.I.W., Clark, P.M., Hales, C.N., Osmond, C. (1994) Understanding oral glucose tolerance: comparison of glucose or insulin measurements during the oral glucose tolerance test with specific measurements of insulin resistance and insulin secretion. *Diabetic Medicine*, **11**, 293-298.

Pollare, T., Lithell, H., Selinus, I., Berne, C. (1988). Application of prazosin is associated with an increase of insulin sensitivity in obese patients with hypertension. *Diabetologia*, **31**, 415-420.

Pollare, T.G., Lithell, H., Berne, C. (1989a). A comparison of the effects of hydrochlorothiazide and captopril on glucose and lipid metabolism in patients with hypertension. *New England Journal of Medicine*, **321**, 686-673.

Pollare, T., Lithell, H., Hvarfner, A., Morlin, C., Prantare, H., Ljunghall, S. (1989b) Metabolic effects of diltiazem and atenolol: results from a randomised, double-blind study with parallel groups. *Journal of Hypertension*, **7**, 551-59.

Pollare, T.G., Lithell, H., Selinus, I., Berne, C. (1989c). Sensitivity to insulin treatment with atenolol and metoprolol: a randomised, double-blind study of effects on carbohydrate and lipoprotein metabolism in hypertensive patients. *British Medical Journal*, **297**, 1147-1152.

Pollare, T.G., Lithell, H., Berne, C. (1990) Insulin resistance is a characteristic feature of primary hypertension independent of obesity. *Metabolism*, **39**, 167-174.

Pool, P.E., Seagren, S.C., Salel, A.F. (1985) Effects of diltiazem on serum lipids, exercise tolerance and blood pressure: randomised, double blind, placebo-controlled evaluation for systemic hypertension. *American Journal of Cardiology*, **56**, 86H-91H.

Ponchner, M., Heine, R.J., Pernet, A., Hanning, I., Francis, A.J., Cook, D., Orskov, H., Alberti, K.G. (1984) A comparison of the artificial pancreas (glucose controlled insulin infusion system) and a manual technique for assessing insulin sensitivity during euglycaemic clamping. *Diabetologia*, **26**, 420-425.

Porte, D. (1967) A receptor mechanism for the inhibition of insulin release by epinephrine in man. *Journal of Clinical Investigation*, **46**, 86-91.

Poulter, N., Sever, P., Thom, S. (1990) Antihypertensive drugs and adverse biochemical effects of bendrofluzide. (letter) *British Medical Journal*, **300**, 1465.

Prince, M.J., Stuart, C.A., Padia, M., Bandi, Z., Holland, O.B. (1988) Metabolic effects of hydrochlorothiazide and enalapril during treatment of the hypertensive patient. *Archives of Internal Medicine*, **148**, 1363-1368.

Pyölä, K. (1979) Relationships of glucose intolerance and plasma insulin to the incidence of coronary artery disease: results from two population studies in Finland. *Diabetes Care*, **2**, 131-141.

Randle, P.J., Hales, C.N., Garland, P.B., Newsholme, E.A. (1963) The glucose fatty-acid cycle: its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet*, **i**, 785-789.

Ratheiser, K., Schneeweiss, B., Waldhausl, W., Farching, P., Korn, A., Nowotny, P., Rohac, M., Wolf, H.P.O. (1991) Inhibition by etomoxir of CPT 1 reduces hepatic glucose production and plasma lipids in NIDDM. *Metabolism*, **40**, 1185-1190.

Reaven, G.M., Miller, R. (1968) Study of the relationship between glucose and insulin responses to an oral glucose load in man. *Diabetes*, **17**, 560-565.

Reaven, G.M. (1983) Insulin resistance in non-insulin dependent diabetes mellitus: Does it exist and can it be measured? *American Journal of Medicine*, **74**, 2-17.

Reaven, G.M. (1988a) Role of insulin resistance in human disease. *Diabetes*, **37**, 1495-1507.

Reaven, G.M., Moore, J., Greenfield, M. (1988b) Quantification of insulin secretion and *in vivo* insulin action in non-obese and moderately obese individuals with normal glucose tolerance. *Diabetes*, **32**, 600-604.

Reaven, G.M., Chang, H., Hoffman, B.B., Azhar, S. (1989a) Resistance to insulin-stimulated glucose uptake in adipocytes isolated from spontaneously hypertensive rats. *Diabetes*, **38**, 1155-1160.

Reaven, G.M., Ho, H., Hoffman, B.B. (1989b) Somatostatin inhibition of fructose-induced hypertension. *Hypertension*, **14**, 117-120.

Reaven, G.M., Twersky, J., Chang, H. (1991a) Abnormalities of carbohydrate and lipid metabolism in Dahl rats. *Hypertension*, **18**, 630-635.

Reaven, G.M., Chang, H. (1991b) Relationship between blood pressure, plasma insulin and triglyceride concentration, and insulin action in SHR and WKY rats. *American Journal of Hypertension*, **4**, 34-38.

Reaven, G.M., Chen, Y-D.I., Hollenbeck, C.B., Sheu, W.H.H., Ostrega, D., Polansky, K.S. (1993) Plasma insulin, C-peptide and proinsulin concentrations in obese and non-obese individuals with varying degrees of glucose tolerance. *Journal of Clinical Endocrinology and Metabolism*, **76**, 44-48.

Resnick, L.M. (1993) Ionic basis of hypertension, insulin resistance, vascular disease, and related disorders. The mechanism of "syndrome X". *American Journal of Hypertension*, **6**, 123S-134S.

Rett, K., Jauch, K.W., Wicklmayr, M., Dietze, G., Fink, E., Mehnert, H. (1986) Angiotensin converting enzyme inhibitors in diabetes: experimental and human experience. *Postgraduate Medical Journal*, **62** (Suppl. 1), 59-64.

Rett, K., Jauch, K.W., Wicklmayr, M. (1988) Hypoglycaemia in hypertensive diabetic patients treated with sulphonylureas, biguanides and captopril. *New England Journal of Medicine*, **319**, 1609.

Richardson, J.M., Balon, T.W., Treadway, J.L., Pessin, J.E. (1991) Differential regulation of glucose transport activity and expression in red and white skeletal muscle. *Journal of Biological Chemistry*, **266**, 12690-12694.

Rocchini, A.P., Moorehead, C., DeRemer, S., Bondie, D. (1989) Pathogenesis of weight related changes in blood pressure in the dog. *Hypertension*, **13**, 922-928.

Rocchini, A.P., Moorehead, C., DeRemer, S., Goodfriend, T.L., Ball, D.L. (1990) Hyperinsulinaemia and the aldosterone and pressor responses to angiotensin II *Hypertension*, **15**, 861-866.

Roddie, I.C., Shepherd, J.T., Whelan, R.F. (1956) Evidence from venous oxygen saturation measurements that the increase in forearm blood flow during body heating is confined to the skin. *Journal of Physiology*, **134**, 444-450.

Rodjmark, S., Andersson, D.E.H., Hed, R. (1981) Does verapamil influence glucose-induced insulin release in man? *Acta Medica Scandinavica*, **210**, 501-505.

Rodjmark, S., Andersson, D.E.H., Hed, R. (1980) Effect of verapamil on glucose response to intravenous injection of glucagon and insulin in healthy subjects. *Hormone and Metabolic Research*, **12**, 285-300.

Rodnick, K.J., Haskell, W.L., Swislocki, A.L.M., Foley, J.E., Reaven, G.M. (1987) Improved insulin action in muscle, liver and adipose tissue in physically trained human subjects. *American Journal of Physiology* **253**, E489-E495.

Rogers, M.A., Yamamoto, C., King, D.S., Hagberg, J.M., Ehsani, A.A., Holloszy, J.O. (1988) Improvement in glucose tolerance after one week of exercise in patients with mild NIDDM. *Diabetes Care*, **11**, 613-618.

Ronnemaa, T., Laasko, M., Pyorala, K., Kallio, V., Puukka, P. (1991) High fasting plasma insulin is an indicator of coronary artery disease in non-insulin dependent diabetic patients and non-diabetic patients. *Arteriosclerosis and Thrombosis*, **11**, 80-90.

Rosenthal, M., Haskell, W.L., Solomon, R., Widstrom, A., Reaven, G.M. (1983) Demonstration of a relationship between level of physical training and insulin-stimulated glucose utilization in normal humans. *Diabetes*, **32**, 408-411.

- Rowe, J.W., Young, J.B., Mimaker, K.L., Stevens, A.L., Pallotta, J., Landsberg, L. (1981) Effect of insulin and glucose infusions on sympathetic nervous system activity in normal man. *Diabetes*, **30**, 219-225.
- Rowe, J.W., Minaker, K.L., Pallotta, J.A., Flier, J.S. (1983) Characterization of the insulin resistance of aging. *Journal of Clinical Investigation*, **71**, 1523-1535.
- Saad, M.F., Knowler, W.C., Pettitt, D.J., Nelson, R.G., Mott, D.M., Bennett, P.H. (1988) The natural history of impaired glucose tolerance in the Pima Indians. *New England Journal of Medicine*, **319**, 1500-1506.
- Saad, M.F., Lillioja, S., Nyomba, B.L., Castillo, C., Ferraro, R., De Gregorio, M., Ravussin, M., Knowler, W.C., Bennett, P.H., Howard, B.V. (1991) Racial differences in the relation between blood pressure and insulin resistance. *New England Journal of Medicine*, **324**, 733-739.
- Saito, I., Nishino, M., Kawabe, H., Wainai, H., Hasegawa, C., Saruta, T., Nagano, S., Sekihara, T. (1992) Leisure time physical activity and insulin resistance in young obese students with hypertension. *American Journal of Hypertension*, **5**, 915-918.
- Sakai, K., Imaizumi, T., Masaki, H., Takeshita, A. (1993) Intra-arterial infusion of insulin attenuates vasoreactivity in the human forearm. *Hypertension*, **22**, 67-73.
- Salomaa, V.V., Strandberg, T.E., Vanhanen, H., Naukkarinen, V., Sarna, S., Miettinen, T.A. (1991) Glucose tolerance and blood pressure: long term follow up in middle aged men. *British Medical Journal*, **302**, 493-496.
- Sando, H., Katagiri, H., Okada, M. (1983) The effect of nifedipine and nicardipine on glucose tolerance, insulin and C-peptide. (abstract) *Diabetes*, **32**, 66A.
- Santoro, D., Natali, A., Palomba, C., Brandi, L.S., Piatti, M., Ghione, S., Ferrannini, E. (1992) Effects of chronic angiotensin converting enzyme inhibition on glucose tolerance and insulin sensitivity in essential hypertension. *Hypertension*, **20**, 181-191.
- Seefeldt, T., Orskov, L., Rasmussen, O., Pedersen, M.M., Moller, N., Christiansen, J.S., Schmitz, O. (1990) Lack of effects of angiotensin-converting enzyme inhibitors on glucose metabolism in insulin-dependent diabetes mellitus. *Diabetic Medicine*, **7**, 700-704.

Seghieri, G., Yin, W., Boni, C., Sanna, G., Anichini, R., Bartolmei, G., Ferrannini, E. (1992) Effect of chronic ACE inhibition on glucose tolerance and insulin sensitivity in hypertensive type II diabetic patients. *Diabetic Medicine*, **9**, 732-738.

Shamiss, A., Carroll, J., Rosenthal, T. (1992) Insulin resistance in secondary hypertension. *American Journal of Hypertension*, **5**, 26-28.

Sharma, A.M., Ruland, K., Spies, K-P., Distler, A. (1991) Salt sensitivity in young normotensive subjects is associated with a hyperinsulinaemic response to oral glucose. *Journal of Hypertension*, **9**, 329-335.

Shen, D.C., Shieh, S.M., Fuh, M.T., Wu, D-A., Chen, Y-D.I., Reaven, G.M. (1988) Resistance to insulin stimulated glucose uptake in patients with hypertension. *Journal of Clinical Endocrinology and Metabolism*, **66**, 580-583.

SHEP Cooperative Research Group. (1991) Prevention of stroke by antihypertensive drug treatment in older patients with isolated systolic hypertension. Final results of the Systolic Hypertension in the Elderly Programme (SHEP). *Journal of the American Medical Association*, **265**, 3255-3264.

Shieh, S-M., Shen, M., Fuh, M.M-T., Chen, Y-D.I., Reaven, G.M. (1987) Plasma lipid and lipoprotein concentrations in Chinese males with coronary artery disease, with and without hypertension. *Atherosclerosis*, **67**, 49-55.

Shultz, T.A., Lewis, S.B., Westbie, D.K., Wallin, J.D. (1977) Glucose delivery: a modulator of glucose uptake in contracting skeletal muscle. *American Journal of Physiology*, **2**, E514-E518.

Simonson, D.C., Ferrannini, E., Bevilacqua, S., Smith, D., Barrett, E., Carlson, R., De Fronzo, R.A. (1984) Mechanism of improvement in glucose metabolism after chronic glyburide therapy. *Diabetes*, **33**, 838-845.

Singer, P., Godicke, W., Voigt, S., Hajdu, I., Weiss, M. (1985) Post-prandial hyperinsulinaemia in patients with mild essential hypertension. *Hypertension*, **7**, 182-186.

Skarfors, E.T., Lithell, H.O., Selinus, I., Aberg, H. (1989) Do antihypertensive drugs precipitate diabetes in predisposed men? *British Medical Journal*, **298**, 1147-1152.

Skarsfors, E.T., Selinus, I., Lithell, H.O. (1991) Risk factors for developing non-insulin dependent diabetes: a 10 year follow up of men in Uppsala. *British Medical Journal*, **303**, 493-496.

Spradlin, C.T., Galloway, J.A., Anderson, J.H. (1990) Apparent increase in coronary heart disease with human proinsulin (HPI) - randomisation failure or HPI effect? (abstract) *Diabetologia*, **33**, A60.

Stacpoole, P.W., Moore, G.W., Kornhauser, D.M. (1978) Metabolic effects of dichloroacetate in patients with diabetes mellitus and hyperlipoproteinaemia. *New England Journal of Medicine*, **298**, 526-530.

Stacpoole, P.W., Gonzalez, M.G., Vlasak, J., Oshiro, Y., Bodor, N. (1987) Dichloroacetate derivatives: metabolic effects and pharmacodynamics in normal rats. *Life Science*, **41**, 2167-2176.

Stacpoole, P.W., Greene, Y.J. (1992) Dichloroacetate. *Diabetes Care*, **15**, 785-791.

Stamler, R., Stamler, J., Reidlinger, W.F., Algera, G., Roberts, R.H. (1978) Weight and blood pressure. *Journal of the American Medical Association*, **240**, 1607-1610.

Stein, P.P., Black, H.R. (1991) Drug treatment of hypertension in patients with diabetes mellitus. *Diabetes Care*, **14**, 425-448.

Steiner, K.E., Lien, E.L. (1987) Hypoglycaemic agents which do not release insulin. *Progress in Medical Chemistry*, **24**, 209-248.

Stern, M.P., Morales, P.A., Haffner, S.M., Valdez, R.A. (1992) Hyperdynamic circulation and the insulin resistance syndrome ("Syndrome X"). *Hypertension*, **20**, 802-808.

Stout, R.W. (1970) Development of arterial lesions in insulin treated animals fed a normal diet. *British Medical Journal*, **37**, 685-687.

Stout, R.W., Bierman, E.L., Ross, R. (1975) The effect of insulin on the proliferation of cultured primate arterial smooth muscle cells. *Circulation Research*, **36**, 319-327.

Stout, R.W. (1989). Insulin and atheroma. *Diabetes Care*, **13**, 631-654.

Strong, P., Anderson, R., Coates, J., Ellis, F., Evans, B., Gurden, M.F., Johnston, M., Kennedy, I., Martin, D.P. (1993) Suppression of non-esterified fatty acids and triacylglycerol in experimental animals by the adenosine analogue GR79236. *Clinical Science*, **84**, 663-669.

Struthers, A.D., Murphy, M.B., Dollery, C.T. (1985) Glucose tolerance during antihypertensive therapy in patients with diabetes mellitus. *Hypertension*, **7**, II95-II101.

Suter, S.L., Nolan, J.J., Wallace, P., Gumbiner, B., Olefsky, J.M. (1992) Metabolic effects of the new oral hypoglycaemic agent CS-045 in NIDDM subjects. *Diabetes Care*, **15**, 193-203.

Swislocki, A.L., Hoffman, B.B., Reaven, G.M. (1989) Insulin resistance, glucose intolerance and hyperinsulinaemia in patients with hypertension. *American Journal of Hypertension*, **2**, 419-423.

Temple, R.C., Carrington, C.A., Luzio, S.D., Owens, D.R., Schneider, A.E., Sobey, W.J., Hales, C.N. (1989) Insulin deficiency in non-insulin dependent diabetes. *Lancet*, **i**, 293-295.

Temple, R.C., Clark, P.M.S., Nagi, D.K., Schneider, A.E., Yudkin, J.S., Hales, C.N. (1990) Radioimmunoassay may overestimate insulin in non-insulin dependent diabetes. *Clinical Endocrinology*, **32**, 689-693.

Tentoria, A., Ghilardi, G., Pedroncelli, A., Benco, R., Stroppa, S., Adib, S., Gianola, D., Pagani, G. (1989). Insulin secretion and glucose tolerance in non-insulin dependent diabetic patients after chronic nifedipine treatment. *European Journal of Clinical Pharmacology*, **36**, 311-313.

Teuscher, A., Egger, M., Herman, J.B. (1989) Diabetes and hypertension. Blood pressure in clinical diabetic patients and a control population. *Archives of Internal Medicine*, **149**, 1942-1945.

Torlone, E., Rambotti, A.M., Perriello, G., Botta, G., Santeusano, F., Brunetti, P., Bolli, G.B. (1991) ACE-inhibition increases hepatic and extra-hepatic sensitivity to insulin in patients with type 2 (non-insulin dependent) diabetes mellitus and arterial hypertension. *Diabetologia*, **34**, 119-125.

Torlone, E., Britta, M., Rambotti, A.M., Perriello, G., Santeusanio, F., Brunetti, P., Bolli, G.B. (1993) Improved insulin action and glycaemic control after long-term angiotensin-converting enzyme inhibition in subjects with arterial hypertension and type II diabetes. *Diabetes Care*, **16**, 1347-1355.

Townsend, R.R., DiPette, D., Lieux, T.R., Wolfe, R.R. (1993) The role of the renin angiotensin system in insulin sensitivity in normotensive subjects. *American Journal of Medical Sciences*, **305**, 67-71.

Townsend, R.R., DiPette, D. (1993) Pressor doses of angiotensin II increase insulin-mediated glucose uptake in normotensive men. *American Journal of Physiology* **265**, E362-E366.

Trost, B.N., Weidmann, P. (1984). Effects of nitrendipine and other calcium antagonists on glucose metabolism in man. *Journal of Cardiovascular Pharmacology*, **6**, 986-995.

Trost, B.N., Weidmann, P. (1987) Nitrendipine in patients with hypertension and diabetes mellitus. *Journal of Cardiovascular Pharmacology*, **9** (suppl 4) S280-S285.

Trovatti, M., Massuco, P., Anfossi, G., Caralot, F., Mularoni, E., Mattiello, L., Rocca, G., Emanuelli, G. (1989) Insulin influences the renin-angiotensin-aldosterone system in humans. *Metabolism*, **38**, 501-503.

Trujillo, A., Eggena, P., Barrett, J., Tuck, M. (1989) Renin regulation in type II diabetes mellitus: influence of dietary sodium. *Hypertension*, **13**, 200-205.

Tuck, M., Corry, D., Trujillo, A. (1990) Salt-sensitive blood pressure and exaggerated vascular reactivity in the hypertension of diabetes mellitus. *American Journal of Medicine*, **88**, 210-216.

Turner, R.C., Holman, R.R., Matthews, D.R., Hockaday, T.D.R., Peto, J. (1979) Insulin deficiency and insulin resistance interaction in diabetes: estimation of their relative contribution by feedback analysis from basal insulin and glucose concentrations. *Metabolism*, **28**, 1086-1096.

Uehara, M., Kishikawa, H., Isami, S., Kisanuki, K., Ohkubo, Y., Miyamura, N., Miyata, T., Yano, T., Shichiri, M. (1994) Effect on insulin sensitivity of angiotensin converting enzyme inhibitors with or without a sulphhydryl group: bradykinin may improve insulin resistance in dogs and humans. *Diabetologia*, **37**, 300-307.

United Kingdom Lacidipine study Group. (1991) A double-blind comparison of the efficacy and safety of lacidipine with atenolol in the treatment of essential hypertension. *Journal of Cardiovascular Pharmacology*, **17** (suppl 4), S27-S30.

Vicaut, E., Hou, X. (1993) Arteriolar constriction and local renin-angiotensin system in rat microcirculation. *Hypertension*, **21**, 491-497.

Vierhapper, H., Waldhausl, W., Nowotny, P. (1983) The effect of insulin on the rise in blood pressure and plasma aldosterone after angiotensin II infusion in normal man. *Clinical Science*, **64**, 383-386.

Wada, S., Makayama, M., Masaki, K. (1982) Effects of diltiazem hydrochloride on serum lipids: comparison with beta-blockers. *Clinical Therapeutics*, **5**, 163-173.

Wahab, P.J., Rijnsburger, A.W.E., Oolbekkink, M., Heine, R.J. (1992) Venous versus arterialised blood for assessment of blood glucose levels during glucose clamping: comparison in healthy men. *Hormone and Metabolic Research*, **24**, 576-579.

Watt, G.C.M., Harrap, S.B., Foy, C.J.W., Holton, D.W., Edwards, H.V., Davidson, H.R., Connor, J.M., Lever, A.F., Fraser, R. (1992) Abnormalities of glucocorticoid metabolism and the renin-angiotensin system: a four corner approach to the identification of genetic determinants of blood pressure. *Journal of Hypertension*, **10**, 473-482.

Weaver, J.U., Hitman, G.A., Kopelman, P.G. (1992) An association between a BclI restriction fragment length polymorphism of the glucocorticoid receptor locus and hyperinsulinaemia in obese women. *Journal of Molecular Endocrinology*, **9**, 295-300.

Weidmann P., Ferrarri P. (1991) Central role of sodium in hypertension in diabetic subjects. *Diabetes Care*, **14**, 220-32.

Welborn, T.A., Breckenridge, A., Dollery, C.T., Rubinstein, A.H., Russell Fraser, T. (1966) Serum insulin in essential hypertension and in peripheral vascular disease. *Lancet*, **1**, 1336-1337.

Welin, L., Eriksson, H., Larsson, B., Ohlson, L.O., Svardsudd, K., Tobblin, G. (1992) Hyperinsulinaemia is not a major coronary risk factor in elderly men: the study of men born in 1913. *Diabetologia*, **35**, 766-770.

Whitcroft, I., Thomas, J., Davies, I.B. (1986) Calcium antagonists do not impair long-term glucose control in hypertensive non-insulin dependent diabetic patients. (abstract) *British Journal of Clinical Pharmacology*, **22**, 208P.

Widen, E., Blomqvist, A-C., Eriksson, J., Groop, L. (1991) Effect of glibenclamide on clearance of insulin in type II diabetes. (abstract) *Acta Endocrinology*, **124** (Suppl 3), 40.

Widen, E.I.M., Eriksson, J.G., Groop, L.C. (1992) Metformin normalises non-oxidative glucose metabolism in insulin resistant normoglycaemic first-degree relatives of patients with NIDDM. *Diabetes*, **41**, 354-358.

Winocour, P.H., Gordon, C., Waldek, C., Anderson, D.C. (1986) The effect of nifedipine on glucose tolerance and insulin secretion in insulin-treated diabetics with varying endogeneous insulin reserve. (abstract) *European Journal of Clinical Investigation*, **16**, A23.

Wolf, H.P.O. (1990) Aryl-substituted 2-oxirane carboxylic acids: a new group of antidiabetic compounds. In: *New Antidiabetic Drugs*, Bailey, C.J., Flatt, P.R. (Eds), London, p217-229.

Wollen, N., Bailey, C.J. (1988) Inhibition of hepatic gluconeogenesis by metformin. *Biochemical Pharmacology*, **37**, 4353-4358.

Wolpert, H.A., Steen, S.N., Istfan, N.W., Simonson, D.C. (1993) Insulin modulates circulating endothelin levels in humans. *Metabolism*, **42**, 1027-1030.

WHO Study Group (1987). Diabetes Mellitus: Report of a *WHO Technical Reports*, **727**, 1-113.

Yagi, S., Takata, S., Kiyokawa, H., Yamamoto, M., Noto, Y., Ikeda, T., Hattori, N. (1988) Effects of insulin on vasoconstrictive responses to norepinephrine and angiotensin II in rabbit femoral artery and vein. *Diabetes*, **37**, 1064-1067.

Yamamoto, M., Asayama, K., Kanai, M., Sugimoto, T., Yagi, S., Takata, S., Ikeda, T., Hattori, N. (1990) Effects of insulin on vasoconstrictor responses to alpha agonist and tilting. *Angiology*, **41**, 394-400.

- Yang, Y.J., John, J.H., Bergman, R.N. (1987) Modified protocols improve insulin sensitivity estimation using the minimal model. *American Journal of Physiology*, **16**, E595-E602.
- Yip, J., Mattock, M.B., Morocutti, A., Sethi, M., Trevisan, R., Viberti, G-C. (1993) Insulin resistance in insulin-dependent patients with microalbuminuria. *Lancet*, **342**, 883-887.
- Young, D.B., Smith, M.J., Jackson, T.E., Scott, R.E. (1984) Multiplicative interaction between angiotensin II and potassium concentration in stimulation of aldosterone. *American Journal of Physiology*, **247**, E328-E335.
- Yoshioka, S., Nishino, H., Shiraki, T., Ikeda, K., Koike, H., Okuno, A., Wada, M., Fujiwara, T., Horikoshi, H. (1993) Antihypertensive effects of CS-045 treatment in obese Zucker rats. *Metabolism*, **42**, 75-80.
- Yudkin, J.S., Alberti, K.G.M.M., McLarty, D.G., Swai, A.B.M. (1990) Impaired glucose tolerance. Is it a risk factor for diabetes or a diagnostic ratbag? *British Medical Journal*, **301**, 397-402.
- Yudkin, J.S. (1991) Hypertension and non-insulin dependent diabetes. *British Medical Journal*, **303**, 730-731.
- Zavaroni, I., Mazza, S., Dall'Aglio, E., Gasparini, P., Passeri, M., Reaven, G.M. (1992) Prevalence of hyperinsulinaemia in patients with high blood pressure. *Journal of Internal Medicine* **231**, 235-40.
- Zemel, M.B., Johnston, B.A., Ambrozy, S.A. (1992) Insulin stimulated vascular relaxation: role of Ca²⁺-ATPase. *American Journal of Hypertension*, **40**, 2111-2125.
- Zimmermann, S., Phillips, R.A., Dunaif, A., Finegood, D.T., Wilkenfeld, C., Ardeljan, M., Gorlin, R., Krakoff, L.R. (1992) Polycystic ovary syndrome: lack of hypertension despite profound insulin resistance. *Journal of Clinical Endocrinology and Metabolism*, **75**, 508-513.