EFFECT OF METFORMIN TREATMENT 
ON ISOLATED CARDIAC FUNCTION AND BLOOD PRESSURE 
IN DIABETIC AND HYPERTENSIVE RATS 

by 

SUBODH VERMA 

B. Pharm., Gujarat University, India, 1991 

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF 
THE REQUIREMENTS FOR THE DEGREE OF 
MASTER OF SCIENCES 
in 
THE FACULTY OF GRADUATE STUDIES 
Faculty of Pharmaceutical Sciences 
Division of Pharmacology and Toxicology 

We accept this thesis as conforming 
to the required standard 

THE UNIVERSITY OF BRITISH COLUMBIA 
September 1993 
© Subodh Verma, 1993
In presenting this thesis in partial fulfilment of the requirements for an advanced
degree at the University of British Columbia, I agree that the Library shall make it
freely available for reference and study. I further agree that permission for extensive
copying of this thesis for scholarly purposes may be granted by the head of my
department or by his or her representatives. It is understood that copying or
publication of this thesis for financial gain shall not be allowed without my written
permission.

(Signature)

Department of Pharmaceutical Sci.
The University of British Columbia
Vancouver, Canada
Date October 12/1993
ABSTRACT

The general purpose of this thesis was to examine the effect of chronic metformin treatment on isolated cardiac function in rats made diabetic with STZ and secondly to explore the effect of metformin treatment on the hyperinsulinemic status of two rodent models of hypertension, the spontaneously hypertensive rat and the fructose-induced hypertensive rat.

In the first set of experiments, the effects of oral metformin administration were studied in isolated perfused working hearts from control and diabetic rats. Control (C) and streptozotocin (STZ) diabetic (D) rats were treated for 8 weeks with metformin hydrochloride. Treatment was initiated at 350 mg/kg/day and was gradually increased to a dose of 650 mg/kg/day which was maintained over a six week period. Isolated heart performance was assessed under conditions of increasing preload in order to evaluate the performance of each heart to "stress". Hearts from untreated D rats exhibited a depressed response to increases in left atrial filling pressure from 17.5 to 22.5 cm H2O in terms of left ventricular developed pressure (LVDP), ventricular contractility (+dP/dt) and ventricular relaxation (-dP/dt) when compared to age matched controls. The diabetic hearts also exhibited a delayed half time to relaxation (T1/2R) at filling pressures from 15 to 22.5 cm H2O. The function curves were performed at a constant heart rate of 300 beats/minute. These responses were restored to control values in D rats treated with metformin. Metformin treatment did not affect these ventricular indices in the C rats. Metformin reduced plasma glucose levels in the diabetic rats from 24.3 mM to 14.4 mM without any increase in the plasma insulin level. The D group had higher triglyceride levels than age matched untreated C rats and metformin administration in the D rat reduced triglyceride levels to control values but had no effect in C rats. In conclusion, metformin administration
improved cardiac performance in STZ-diabetic rats under conditions of increasing preload.

The second series of experiments aimed at exploring the relationship between elevated insulin levels and experimental hypertension using metformin as the experimental intervention. In particular, the effect of chronic metformin treatment was studied in a genetic and acquired model of hyperinsulinemia and hypertension, the Spontaneously hypertensive rat (SHR) and the fructose-induced hypertensive rat.

SHR and their genetic normotensive controls, the Wistar-Kyoto (WKY) rat, were procured at 5 weeks (when hypertension is not manifest in the SHR). The rats were divided into four groups: SHR untreated, SHR metformin-treated, WKY untreated and WKY metformin-treated. Baseline measurements of plasma insulin, plasma glucose and systolic blood pressure were performed from week 9 to 13. At week 16 insulin implants were surgically implanted in the treated animals to evaluate the effect of artificially raising plasma insulin and systolic blood pressure were measured.

The SHR exhibited full blown systolic hypertension by 9 weeks of age which persisted throughout the experiment. Paralleling the elevation in systolic blood pressure, the SHR exhibited sustained hyperinsulinemia by 9 weeks of age. Metformin treatment of the SHR prevented the rise in plasma insulin when compared to untreated SHR. The treatment also attenuated the systolic blood pressure by approximately 30-35 mm Hg. Metformin administration did not change the plasma glucose level in any group. Raising the plasma insulin level in the treated groups via insulin implants caused an elevation in the plasma insulin level in the SHR-treated group which was accompanied by an increase in systolic blood pressure.

The next step was to explore if the insulin-blood pressure relationship in the fructose-induced hypertensive rat, where hypertension is not genetically determined but is induced by feeding rats a fructose-enriched diet. Sprague Dawley rats were
divided into four groups: control untreated, control metformin-treated, fructose treated and fructose-fed metformin-treated. A baseline reading of plasma insulin, plasma glucose and systolic blood pressure was performed at week 6 following which chronic metformin treatment was initiated in the treated groups at the same dose as discussed in the previous experiment. At week 7, the animals in the fructose groups were started on a fructose-enriched diet. Weekly measurements of plasma insulin, plasma glucose and systolic blood pressure were performed from week seven to eleven.

The fructose-fed untreated rats exhibited an elevated plasma insulin level by week 9 as compared to control rats. This hyperinsulinemia was sustained over the experimental period. The fructose-fed untreated rats also exhibited an elevation in systolic blood pressure of about 20-25 mm Hg. As seen in the previous study, metformin treatment did not change the plasma glucose levels in any group. Treatment of Sprague Dawley rats with metformin prior to starting fructose, prevented the rise in plasma insulin when compared to fructose-fed untreated rats. This was also accompanied by a complete prevention in the rise of systolic blood pressure seen in the fructose fed untreated rats.

The fact that a specific drug intervention (metformin) caused a decrease in the plasma insulin level and systolic blood pressure, provides strong support to the notion that elevated insulin levels contribute at least in part to the development of hypertension in these two rodent models of hypertension. Further studies addressing this issue need to be carried out to clearly establish a cause-effect relationship of insulin and hypertension.
TABLE OF CONTENTS

ABSTRACT ii
TABLE OF CONTENTS v
LIST OF TABLES vii
LIST OF FIGURES viii
LIST OF ABBREVIATIONS ix
ACKNOWLEDGEMENTS x
DEDICATION xi

INTRODUCTION

A. Overview 1
B. Complications of chronic diabetes 3
C. Chronic diabetes and heart disease 3
D. Left ventricular systolic function in diabetes 4
E. Left ventricular diastolic function 4
F. Pathogenesis of diabetic heart disease 5
G. Models of cardiac dysfunction in diabetic rats 9
H. Prevention of diabetes-induced cardiac changes 10
I. Metformin 10
J. Glucose lowering effect of metformin 12
K. Effect of metformin on glucose utilization 13
L. Metformin and lipid metabolism 17
M. Hyperinsulinemia and hypertension 18
N. Hypertension and carbohydrate metabolism 19
O. Insulin-hypertension: possible mechanisms 20
P. Rodent models used to evaluate the interplay between hyperinsulinemia/insulin resistance and hypertension

Q. Rationale of the proposed experiments

SPECIFIC GOALS OF THE PRESENT INVESTIGATION

MATERIALS AND METHODS

STUDY 1: EFFECT OF METFORMIN ON ISOLATED CARDIAC FUNCTION

1. Animals and Methods
2. Heart Perfusion
3. Plasma analysis
4. Statistical analysis

STUDY 2: EFFECT OF METFORMIN TREATMENT ON THE HYPERINSULINEMIC STATUS OF RODENT MODELS OF HYPERTENSION

(A) Spontaneously hypertensive rat study
   Animals and Experimental design

(B) Fructose-induced hypertension study
   Animals and Research design

(C) Methods
   1. Blood Pressure Measurement
   2. Biochemical Measurement
   3. Statistical Analysis

RESULTS

DISCUSSION

CONCLUSIONS

REFERENCES
LIST OF TABLES

<table>
<thead>
<tr>
<th>Tables</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>General features of IDDM and NIDDM diabetes</td>
</tr>
<tr>
<td>2</td>
<td>General characteristics of the rats at termination in the isolated working heart study</td>
</tr>
<tr>
<td>3</td>
<td>Time to half relaxation from 15 to 22.5 cm H₂O in the isolated working heart study</td>
</tr>
<tr>
<td>4</td>
<td>Food and fluid intake in the SHR study</td>
</tr>
<tr>
<td>5</td>
<td>Plasma glucose in the SHR study</td>
</tr>
<tr>
<td>6</td>
<td>Plasma insulin levels pre- and post-implant, in the SHR study</td>
</tr>
<tr>
<td>7</td>
<td>Plasma glucose levels pre- and post-implant, in the SHR study</td>
</tr>
<tr>
<td>8</td>
<td>Systolic blood pressure pre- and post-implant, in the SHR study</td>
</tr>
<tr>
<td>9</td>
<td>Food and Fluid intake in fructose study</td>
</tr>
<tr>
<td>10</td>
<td>Plasma glucose in the fructose study</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figures</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Factors important in the development of diabetic cardiac dysfunction</td>
</tr>
<tr>
<td>2</td>
<td>Chemical Structure of Metformin</td>
</tr>
<tr>
<td>3</td>
<td>Possible links between hyperinsulinemia/insulin resistance and hypertension</td>
</tr>
<tr>
<td>4</td>
<td>Effect of metformin treatment on $+dP/dT$ in the isolated working heart study</td>
</tr>
<tr>
<td>5</td>
<td>Effect of metformin treatment on $-dP/dT$ in the isolated working heart study</td>
</tr>
<tr>
<td>6</td>
<td>Effect of metformin treatment on LVDP in the isolated working heart study</td>
</tr>
<tr>
<td>7</td>
<td>Body weight in the four experimental groups in the SHR study</td>
</tr>
<tr>
<td>8</td>
<td>Plasma insulin in the four experimental groups in the SHR study</td>
</tr>
<tr>
<td>9</td>
<td>Systolic blood pressure in the four experimental groups in the SHR study</td>
</tr>
<tr>
<td>10</td>
<td>Body weight in the experimental groups in the fructose study</td>
</tr>
<tr>
<td>11</td>
<td>Plasma insulin in the different groups in the fructose study</td>
</tr>
<tr>
<td>12</td>
<td>Systolic blood pressure in the different groups in the fructose study</td>
</tr>
</tbody>
</table>
## LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoA</td>
<td>Coenzyme A</td>
</tr>
<tr>
<td>-dP/dT</td>
<td>Rate of Left Ventricular Pressure Decline</td>
</tr>
<tr>
<td>+dP/dT</td>
<td>Rate of Left Ventricular Pressure Development</td>
</tr>
<tr>
<td>FFA</td>
<td>Free Fatty Acids</td>
</tr>
<tr>
<td>HGO</td>
<td>Hepatic Glucose Output</td>
</tr>
<tr>
<td>IDDM</td>
<td>Insulin Dependent Diabetes Mellitus</td>
</tr>
<tr>
<td>LVDP</td>
<td>Left Ventricular Developed Pressure</td>
</tr>
<tr>
<td>mg</td>
<td>milligrams</td>
</tr>
<tr>
<td>min</td>
<td>minutes</td>
</tr>
<tr>
<td>ms</td>
<td>milliseconds</td>
</tr>
<tr>
<td>SHR</td>
<td>Spontaneously Hypertensive Rats</td>
</tr>
<tr>
<td>SNS</td>
<td>Sympathetic nervous system</td>
</tr>
<tr>
<td>STZ</td>
<td>Streptozotocin</td>
</tr>
<tr>
<td>T1/2R</td>
<td>Time to half relaxation</td>
</tr>
<tr>
<td>u</td>
<td>micro</td>
</tr>
<tr>
<td>U</td>
<td>units</td>
</tr>
<tr>
<td>WKY</td>
<td>Wistar-Kyoto Rats</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

I am extremely grateful to Dr. John McNeill for not only providing me with an opportunity, but for being an excellent supervisor and an exceptional human being.

I would like to thank the members of my supervisory committee, Dr. Frank Abbott, Dr. Jack Diamond, Dr. Kath MacLeod and Dr. David Godin for their scientific input and advice.

My special thanks to Dr. Sanjay Bhanot for his knowledgeable guidance throughout my project and for being a sincere, trustworthy and truthful friend.

My thanks to our laboratory manager, Ms. Mary Battell for her cooperation throughout my masters. I would also like to thank Dr. Soter Dai for helping me with the working heart preparation.

My special thanks to Ms. Sylvia Chan for helping me with my thesis and for being a constant support.

I would also like to thank all my laboratory colleagues for their constant support and encouragement.
DEDICATION

I dedicate this thesis

in the fond memory of my father
who would have been the happiest man today

my mother
for leading her children into intellectual pursuits

my sister
who makes everything worthwhile

my friends
Baljeet and Sanjay
who were always there

and to time
for allowing me to get this far.
INTRODUCTION

A. OVERVIEW

Diabetes mellitus is a group of syndromes characterized by hyperglycemia, relative insulin deficiency, altered lipid, carbohydrate and protein metabolism and by long term complications involving the eyes, kidney, cardiovascular system and nerves (Kahn and Shechter 1990). Virtually all forms of diabetes mellitus are due to either a decrease in the circulating concentration of insulin (insulin deficiency) or a decrease in the response of the peripheral tissues to insulin (insulin resistance) in association with an excess of hormones with actions opposite to those of insulin (for example, glucagon, growth hormone, cortisol and catecholamines). These hormonal alterations lead to abnormal carbohydrate, lipid, fat and ketone metabolism with the central feature of the syndrome being hyperglycemia. Insulin lowers the concentration of glucose in the blood by inhibiting hepatic glucose production (gluconeogenesis and glycogenolysis) and by stimulating the uptake of glucose into the peripheral tissues like muscle and fat (Kahn and Shechter 1990; Foster 1991). Most patients can be classified as having either insulin-dependent diabetes mellitus (IDDM) or non insulin-dependent diabetes mellitus (NIDDM). There are genetic and environmental components involved in the pathogenesis of both IDDM and NIDDM. Some general characteristics of IDDM and NIDDM diabetes are listed in Table 1.
**TABLE 1**

**GENERAL FEATURES OF IDDM AND NIDDM DIABETES**

<table>
<thead>
<tr>
<th></th>
<th>IDDM</th>
<th>NIDDM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genetic locus</strong></td>
<td>Chromosome 6</td>
<td>Chromosome 11 (?)</td>
</tr>
<tr>
<td><strong>Age of onset</strong></td>
<td>&lt;40</td>
<td>&gt;40</td>
</tr>
<tr>
<td><strong>Body habitus</strong></td>
<td>Normal to wasted</td>
<td>Obese</td>
</tr>
<tr>
<td><strong>Plasma insulin</strong></td>
<td>Low to absent</td>
<td>Normal to high</td>
</tr>
<tr>
<td><strong>Plasma glucagon</strong></td>
<td>High, suppressible</td>
<td>High, resistant</td>
</tr>
<tr>
<td><strong>Acute complication</strong></td>
<td>Ketoacidosis</td>
<td>Hyperosmolar coma</td>
</tr>
<tr>
<td><strong>Insulin therapy</strong></td>
<td>Responsive</td>
<td>Responsive to resistant</td>
</tr>
<tr>
<td><strong>Sulfonylurea therapy</strong></td>
<td>Unresponsive</td>
<td>Responsive</td>
</tr>
</tbody>
</table>

FROM FOSTER D.W. 1991
B. COMPLICATIONS OF CHRONIC DIABETES

Diabetes mellitus is a multifaceted syndrome which affects various systems and organs. The two major acute complications of insulin deficiency include ketoacidosis and hyperosmolar non-ketotic coma (Carroll and Matz, 1983; Foster and McGarry, 1983). The former is a complication of IDDM while the later is generally seen in NIDDM diabetes. More importantly, the diabetic patient has an increased risk of developing serious long-term pathophysiology that leads to morbidity and premature mortality. These include cardiovascular alterations, increased risk of coronary artery disease (CAD), increased frequency of silent myocardial infarction (Brownlee et al., 1988), diabetic retinopathy (Ramsay et al., 1988), nephropathy (Sequist et al., 1989), neuropathy (Mogenesen and Christensen, 1984) and diabetic foot disease (LoGerfo and Coffmann, 1984).

C. CHRONIC DIABETES AND HEART DISEASE

Several clinical and experimental studies have shown that the incidence of cardiac disease is much greater in the diabetic population compared to the non-diabetic population and accounts for almost 80% of all diabetic deaths (Pierce et al., 1988).

The causal role of diabetes mellitus in the development of congestive heart failure was most conclusively demonstrated in the Framingham Study, where the relative risk of developing cardiac failure during the 18-year follow-up was 2.4 times higher in male and 5.1 times higher in female diabetic subjects than in the respective non-diabetic subjects (McGee et al., 1971; Kannel et al., 1974). More importantly, this greater risk persisted after taking into account the age, blood pressure, weight and serum cholesterol concentrations of the subjects. The Framingham Study also showed
that the association of diabetes and congestive heart failure was confined to IDDM subjects. However, other studies, such as that of Hamby et al. (1974) showed that congestive heart failure is also more prevalent in NIDDM.

D. LEFT VENTRICULAR SYSTOLIC FUNCTION IN DIABETES

Early studies using systolic time intervals to evaluate left ventricular systolic function at rest revealed a prolonged pre-ejection period (PEP) and a shorter left ventricular ejection time (LVET) and a higher PEP/LVET ratio in diabetic subjects (Ahmed et al., 1975). Similar findings have been reported in several other studies on diabetic patients who had not been assigned to a specific diagnostic classification of diabetes and in patients on insulin treatment or with verified IDDM (Shapiro et al., 1981; Jermendy et al., 1983).

As left ventricular function disturbances are more evident upon exercise than at rest, several studies focussed on the performance of diabetic hearts upon exercise. Despite normal left ventricular ejection fraction (LVEF) at rest, Vered et al. (1984) demonstrated an abnormally low LVEF response to exercise in 43% of diabetic patients but in none of the control subjects. Similar findings have been reported by Mildenberger et al. (1984). It is important to note that in most of the studies the abnormal LVEF response to exercise could not be related to the duration of diabetes, metabolic control, autonomic nervous function or diabetic microvascular complications.

E. LEFT VENTRICULAR DIASTOLIC FUNCTION

Diastolic abnormalities have been extensively studied in diabetes. This is mainly because diastolic filling alterations are one of the earliest signs of left
ventricular dysfunction (Inouye et al., 1984). The diastolic abnormalities observed include: an increased atrial contribution to left ventricular filling, prolonged isovolumic relaxation time and slowing of the thinning of the left ventricular wall (for review see Matti et al., 1990).

The abnormal diastolic function of the diabetic heart is indicative of diminished left ventricular compliance and prolonged left ventricular relaxation. Support for the view that chamber stiffness (decreased ventricular compliance) contributes to diastolic dysfunction in the diabetic heart is provided by a study in which 12 NIDDM patients with chest pain but no significant coronary artery narrowing were catheterized and studied. An elevated left ventricular end diastolic pressure and a reduced stroke volume were noted in these patients supporting the view of a decreased ventricular compliance in diabetes (Regan et al., 1977).

F. PATHOGENESIS OF DIABETIC HEART DISEASE

The pathogenesis of diabetic heart disease is believed to be due to several factors and multiple mechanisms (Giles and Sander, 1989; Rodrigues and McNeill, 1992). A summary of the established and potential factors important in the development of cardiac dysfunction in diabetes are illustrated in Figure 1. Until 20 years ago, it was assumed that the increased risk of congestive heart failure in the diabetic population reflected the increased incidence of coronary atherosclerosis and myocardial infarctions in these patients. However, beginning with the study of Rubler et al. (1972) clinical, epidemiological and pathological data have mounted to support the existence of a specific cardiomyopathy in the absence of coronary artery disease, macroangiopathy, microangiopathy, autonomic neuropathy or hypertension in the diabetic population (for review see Zarich and Nesto, 1989). Clinical evidence for the existence of diabetic cardiomyopathy was provided by Hamby et al. (1974), who noted
an increased incidence of diabetes in patients with idiopathic cardiomyopathy. Sixteen of 73 patients with idiopathic cardiomyopathy were diabetic, compared with only 11% in an age and sex-matched cohort without cardiomyopathy. More important was the finding that only 1 out 16 diabetic patients with cardiomyopathy was insulin-dependent. This study coupled with the Framingham study clearly demonstrated that diabetes per se, either IDDM or NIDDM, is associated with cardiac abnormalities. Regan et al. (1983) described angiographic and hemodynamic findings in a group of 17 patients with NIDDM in the absence of hypertension or valvular disease. D'Elia et al. (1979) found diastolic and systolic dysfunction in 59% of a diabetic cohort with renal failure in the absence of coronary artery disease. The fact that diabetic heart disease can occur both in IDDM and NIDDM despite their divergence in respect to the pathogenesis of hyperglycemia, suggests that raised blood glucose and/or metabolic changes associated with hyperglycemia probably have a key role in the pathogenesis of diabetic heart muscle disease.

Not only has cardiac disease in diabetes been described in clinical settings, it is now well characterized in animal models of chemically induced diabetes. Isolated working hearts from streptozotocin (STZ)- or alloxan-induced diabetic rats show a decreased ability to respond to increases in left-atrial filling pressure in terms of left ventricular developed pressure (LVDP), rate of contraction (+dP/dt), rate of relaxation (-dP/dt), cardiac and aortic output (Vadlamudi et al., 1982). The lack of ability of the hearts to respond to increases in filling pressure suggests that the diabetic heart, although capable of functioning like controls under normal conditions, does not adequately respond to "stress" simulated by increases in preload. A diminished ability of diabetic hearts to respond to increases in afterload has also been demonstrated (Ingebreston et al., 1980, Nichol et al., 1992). Moreover, isolated papillary muscles from STZ-induced diabetic rat hearts exhibit a depressed velocity of shortening and a delayed onset of relaxation (Fein et al., 1980). Regan et al. (1974) using one-year old
FIGURE 1. FACTORS IMPORTANT IN THE DEVELOPMENT OF DIABETIC CARDIAC DYSFUNCTION

TAKEN FROM: Giles T.D. et al., 1989
alloxan-diabetic dogs, showed ventricular stiffness associated with shortening of left ventricular ejection time. In isolated papillary muscle from rabbits made diabetic with alloxan, Fein et al. (1985) found a prolonged duration of isotonic and isometric contraction.

In addition to the factors depicted in Figure 1, in the last few years considerable attention has been directed towards the role of altered myocardial energetics in the development of diabetic cardiomyopathy (For review see Rodrigues and McNeill, 1992). Under normal conditions, an estimated 60-70% of myocardial energy is derived from the metabolism of lipids; the remainder is derived from non-lipid sources including carbohydrates, ketone bodies and amino acids. In diabetes, the circulating levels of free fatty acids (FFA) are increased as determined by their mobilization and synthesis by the adipose tissue and liver. As a result, there is an increased uptake, oxidation and storage of fatty acids by the myocardial cell. Thus, there is an almost exclusive dependence on FFA oxidation as a source of myocardial energy, which increases from about 60-70% to about 90% in diabetes. Elevated FFA oxidation leads to a build up of intermediates such as long chain acyl carnitines which have deleterious effects on the myocardial cell through a variety of mechanisms. Moreover, the inability of the diabetic heart to utilize glucose leads to an elevated oxygen demand per molecule of ATP produced. Support for this hypothesis comes from studies in which perfusion of diabetic rat hearts with dichloroacetate (a glucose oxidation stimulator) was shown to acutely reverse the depression of cardiac function. Thus, it is now believed that the inability of the diabetic heart to utilize glucose as a substrate for energy production and the total reliance on FFA oxidation as a source of ATP may be a potential factor important in the development of diabetic cardiomyopathy (for review see Rodrigues and McNeill, 1992).

Several studies from our laboratory point towards alterations in lipid metabolism as being an important determinant of cardiac dysfunction in diabetes. For
example, in a study in which Wistar and Wistar-Kyoto (WKY) rats were injected with identical doses of STZ, although both groups of rats exhibited elevated plasma glucose levels, only the Wistar rats exhibited elevated levels of circulating lipids. Interestingly, depression of the myocardial function was seen in the group of Wistar rats while the cardiac function in the STZ-injected WKY rats remained unaffected (Rodrigues and McNeill, 1986). In another study, STZ-diabetic rats treated with hydralazine showed elevated blood glucose but normal circulating lipids. The function of the hearts from diabetic rats treated with hydralazine was similar to that of non-diabetic controls, supporting the notion that hyperlipidemia may be an important determinant of cardiac disease in the diabetic rat (Rodrigues et al. 1986).

G. MODELS OF CARDIAC DYSFUNCTION IN DIABETIC RATS

Two chemicals have been extensively used to produce diabetes in laboratory animals: alloxan and streptozotocin (STZ). Both these agents produce beta cell necrosis after a single dose in laboratory animals, thereby causing marked hyperglycemia and hypoinsulinemia, the severity of which can be varied by altering the dose of the agent. The methylnitrosourea analog STZ has now largely replaced alloxan as a primary agent for the induction of diabetes in animals because of its higher selectivity for the β-cells of the pancreas and longer half life (Rakienten et al., 1963).

Animals injected with STZ exhibit typical signs of diabetes, including polydypsia, polyphagia, polyuria, decreased body weight gain, hyperglycemia, hypoinsulinemia, hyperlipidemia and depressed cardiac performance. The depression of the cardiac function is noticed as early as 6-8 weeks after the induction of diabetes with STZ or alloxan (McNeill and Tahiliani, 1986)
H. PREVENTION OF DIABETES-INDUCED CARDIC CHANGES

Insulin treatment is successful in preventing or retarding the cardiac abnormalities in diabetes. The effectiveness of in vivo insulin treatment of STZ diabetic rats on various functional and biochemical cardiac parameters has been demonstrated (Tahiliani and McNeill, 1986; Dillmann 1980; Lopaschuck et al., 1983). Insulin treatment of diabetic rats seems to be less effective in preventing and reversing cardiac alterations in the more chronic stages (five months) versus the early stages (6-8 weeks). Treatment of 5 month old diabetic animals with insulin was successful in controlling plasma glucose however, only a partial reversal of cardiac performance was noticed (Tahiliani et al., 1983). In a chronic canine model of diabetes, cardiac function was unaffected by insulin control of plasma glucose (Regan et al., 1981). In conjunction with these experimental studies, myocardial abnormalities have also been reported in clinical settings despite insulin treatment and tight glucose control (The University Group Diabetes Program, 1975). It would, therefore, be desirable to have drug treatments, in addition to insulin, that have (a) an insulin-like/insulin-enhancing effect and (b) a lipid-lowering effect, to prevent the diabetes-induced myocardial alterations.

I. METFORMIN

The biguanides, metformin and phenformin, were introduced in 1957 as glucose lowering agents. Phenformin initially received greater use but was withdrawn in many countries during the 1970's because of its association with lactic acidosis. Metformin is now accepted as the biguanide of choice. It is used extensively worldwide, except in the U.S., where it is undergoing clinical trials. Metformin is readily distinguishable from sulfonylureas because it ameliorates hyperglycemia.
FIGURE 2. CHEMICAL STRUCTURE OF METFORMIN

\[
\begin{align*}
\text{NH} & \quad \text{NH} \\
\| & \quad \| \\
(CH_3)_2 \text{N-C-NH-C-NH}_2
\end{align*}
\]
without stimulating insulin, promoting weight gain, or causing clinical hypoglycemia (Bailey, 1985). The chemical structure of metformin is given in Figure 2.

Absorption of metformin occurs mainly from the small intestine. The absorption half life of the drug is 0.9-2.6 h and the bioavailability is about 50-60%. Concentrations of metformin in peripheral plasma reach a maximum of about 2 ug/ml about 2 hours after an oral dose. Metformin is stable and does not bind to the plasma proteins. The drug is excreted in the urine apparently unchanged. The elimination is rapid, with about 90% being cleared in 12 hours (Tucker et al., 1981).

J. GLUCOSE LOWERING EFFECT OF METFORMIN

Metformin is better described as an antihyperglycemic rather than an hypoglycemic, as it rarely causes hypoglycemia (Bailey 1985, 1988). Early studies showing the antihyperglycemic effect of metformin have been thoroughly reviewed by Hermann (1979). Metformin appears to be as effective in non-obese as in obese NIDDM subjects (Clarke and Duncan 1968; Clark and Campbell, 1977). The drug is given in combination with sulfonylureas to patients in which sulfonylureas alone do not achieve an acceptable level of glycemic control or in IDDM patients in conjunction with insulin.

The antihyperglycemic effect of metformin cannot be attributed to an increase in insulin concentration. Basal insulin concentrations and concentrations after an oral glucose load are typically unchanged or slightly reduced during metformin therapy (Lord et al., 1983; Prager and Schernthaner, 1983; Fantus and Brosseau, 1986; Barzilai and Simonson, 1988; Wu et al., 1990; Prager et al., 1986; Rizkalla et al., 1986; Campbell et al., 1987; Nosadini et al., 1987; Pederson et al., 1989; Benzi et al., 1990). Similar observations have been made in animal models of obese and non-obese hyperinsulinemic and hypoinsulinemic diabetes (Lord et al., 1983; Penicaud et
The drug has little or no effect on the secretion of glucagon, somatostatin, growth hormone or cortisol (Penicaud et al., 1989).

The main mechanism of the antihyperglycemic effect of metformin is thought to be via an enhancement of insulin-mediated glucose utilization in the peripheral tissues such as the muscle and fat. The effect of metformin on basal hepatic glucose production (HGP) has also been extensively evaluated, and it is observed that a modest decrease in basal hepatic glucose production (HGP) is seen when metformin produces a substantial decrease in basal glycemia, which may involve a modest reduction in gluconeogenesis (for review see Bailey, 1992).

K. EFFECT OF METFORMIN ON GLUCOSE UTILIZATION

In the basal state, glucose utilization is largely independent of insulin and the main tissues utilizing glucose are the brain, blood cells, kidney medulla, intestine and skin (Felig et al., 1990). Metformin does not affect aerobic or anaerobic glucose utilization by the brain, kidney medulla or skin of normal mice (Wilcock and Bailey, 1990). Also basal glucose utilization by hepatocytes of normal and STZ-treated rats and mice was not affected by therapeutic concentrations of metformin. The intestine, which accumulates much higher concentrations of metformin than other tissues, has recently been identified as an important site of metformin-stimulated glucose oxidation. In obese fa/fa rats, metformin (350 mg/kg/day) increased basal glucose utilization in the jejunum (Penicaud et al., 1989). Administration of metformin (250 mg/kg/day) to fasted rats also increased anaerobic glycolysis by the intestine (Bailey et al., 1989).

The antihyperglycemic effect of metformin is evident after a glucose challenge (Lord et al., 1983; Prager and Schernthaner, 1983; Fantus and Brosseau, 1986; Barzilai and Simonson, 1988; Frayn et al., 1971; Wu et al., 1990). Glucose disposal is
shared by the liver and peripheral tissues, particularly muscle. Glucose uptake by the liver is an insulin-independent process and studies have shown that metformin has little effect on hepatic glucose uptake after an oral glucose challenge (Bailey, 1992). Diabetes is characterized by an increased hepatic glucose output (HGO) which is accounted for mainly by an increased gluconeogenesis (Consoli et al., 1989). Several studies have investigated whether metformin can suppress gluconeogenesis. Metformin reduced gluconeogenesis in normal guinea pigs (Meyer et al., 1967). High concentrations of the drug reduced basal and glucagon-stimulated gluconeogenesis by animal liver and kidney in vitro in the absence of added insulin (Meyer et al., 1967; Alengrin et al., 1987). A synergistic reduction in gluconeogenesis by isolated rat hepatocytes for a range of substrates (lactate, pyruvate, glutamine, alanine, glycerol) was observed when physiological concentrations of insulin were added to therapeutic concentrations of metformin (Wollen and Bailey, 1988). In vivo studies are not consistent with the antigluconeogenic effect of metformin. For example, in a study in which $^{14}$C-lactate was administered to rats via the hepatic portal vein, the appearance of $^{14}$C-glucose in the plasma was not affected by metformin (Bailey 1992). It is speculated that as metformin increases the supply of gluconeogenic substrate to the liver in the form of lactate produced by the intestine, it is probable that even a small increase of gluconeogenic substrate can override the inherent antigluconeogenic action of the drug. This has been proposed as an important mechanism that prevents the drug from causing clinical hypoglycemia because the supply of lactate from the intestine to the liver ensures that gluconeogenesis is not critically impaired (Bailey 1992).

The fact that metformin improves glucose tolerance without increasing insulin secretion suggests that the drug may enhance insulin-mediated glucose disposal. Several lines of evidence now lend support to this concept. Prager et al (1986) showed a 23% increase in glucose disposal in obese and non-obese NIDDM subjects
receiving metformin. A similar increase (43%) has been reported in obese NIDDM subjects receiving metformin (Nosadini et al., 1987). Data from experimental studies are consistent with clinical findings. During a steady-state hyperglycemic-hyperinsulinemic clamp in normal rats, 250 mg/kg/day metformin increased glucose disposal by 20% (Bailey, 1992). Similar findings have been reported in mildly diabetic STZ rats given metformin (Rossetti et al., 1990). Studies documenting a reduction in insulin requirement by metformin in overweight insulin-treated NIDDM and IDDM patients lends further support to the idea that metformin's antihyperglycemic effect is mediated via an improvement in insulin action (Leblanc et al., 1987). Of importance is the study by Gin et al. (1982) in which addition of metformin (1.7g/day for 2 days) reduced postprandial insulin requirement of IDDM patients by 26%. Gin et al (1985) also showed that IDDM patients receiving metformin supplement (1.7 g/day for 7 days) had an 18% improvement in glucose uptake assessed during a euglycemic hyperinsulinemic clamp.

Two major insulin responsive peripheral tissues include the muscle and the adipose tissue, the skeletal muscle being quantitatively the major site of insulin mediated glucose uptake. Several studies have consistently demonstrated that metformin improves insulin-stimulated glucose uptake in the muscle of both humans and animals (for review see Bailey, 1992). Soleus muscle isolated from STZ-induced diabetic mice treated with metformin at a dose of 250 mg/kg/day showed a 20% increase in insulin-stimulated glucose uptake and oxidation (Bailey and Pauh, 1986). When metformin was incubated with hemidiaphramas of alloxan-induced diabetic rats, insulin-mediated glucose uptake was increased by 27%, suggesting that metformin can directly act on diabetic muscle (Frayn and Adnitt, 1972). In muscle strips isolated from insulin-resistant humans, metformin increased insulin-mediated glucose uptake (Galuska et al., 1991).

The effect of metformin on insulin-mediated glucose utilization in adipose
tissue remains unclear. In normal rat adipocytes, metformin increased basal and insulin-stimulated glucose uptake by 19-43% (Jacobs et al., 1986; Matthaei et al., 1991). This increase was associated with an increase in insulin-induced translocation of glucose transporters from microsomes into the plasma membrane. In a study in which adipose tissue biopsies from normal human subjects were utilized, metformin (2 ug/ml) increased insulin-stimulated glucose oxidation and incorporation into triglyceride (Cigolini et al., 1984); however, a similar study failed to detect any effect (Pedersen et al., 1989). Also, in a study using adipocytes from obese NIDDM subjects treated with metformin, no alterations in glucose transport, oxidation or lipogenesis could be seen in spite of clear evidence that the drug decreased glycemia (Pedersen et al., 1989). Thus, it appears that the chief mechanism through which metformin enhances insulin action is via an increase in insulin mediated glucose utilization in muscle.

The cellular mechanism of action of metformin could potentially involve insulin receptor and/or post-receptor effects. There appears to be a poor correlation between the effects of metformin on insulin receptor binding and glucose metabolism. There are many reports that metformin can improve glucose homeostasis in diabetic states without any alteration in insulin binding (Prager and Schernthaner, 1983; Fantus and Brosseau, 1986). Moreover, in view of the large number of spare receptors, increased binding does not appear to have a significant effect on glucose homeostasis at normal circulating insulin concentrations. It thus appears that effects of metformin distal from the receptor may play a significant role in defining metformin's insulin-enhancing ability.
L. METFORMIN AND LIPID METABOLISM

Metformin therapy is associated with a decrease in circulating triglyceride concentrations in non-diabetic and NIDDM patients. Reductions of 10-20% are often found in non-hypertriglyceridemic subjects and up to 50% in patients with elevated triglycerides (Gustafson et al., 1971; Fedele et al., 1976; Sirtori et al., 1977; Sirtori et al., 1984; Montaguti et al., 1979; Janka, 1985). The decrease in the triglycerides is believed to be via a decrease in very low density lipoproteins (VLDL-triglyceride) (Zavaroni et al., 1984).

In animals with hypertriglyceridemia, a significant hypocholesterolemic effect can be noted (Sirtori et al., 1977). This effect on cholesterol is not evident in animals with lipid parameters within the normal range (Billingham et al., 1980). The decrease in cholesterol is believed to be via a decrease in low density lipoprotein (LDL) cholesterol or VLDL cholesterol. In patients, metformin treatment has been associated with a small decrease in cholesterol (10%) in non-diabetic and NIDDM subjects (Wu et al., 1990). The effect of metformin treatment on high density lipoprotein cholesterol concentrations is unclear. In one study, metformin modestly increased (10%) HDL concentrations in non-diabetic and NIDDM subjects (Sirtori, 1988). Other clinical and animal studies do not support this finding (Rains et al., 1988; Billingham et al., 1980). In alloxan-diabetic rats, metformin administration led to a decrease in hydroxymethyl-glutaryl-CoA (HMG CoA) reductase activity, suggesting that the drug may decrease cholesterol biosynthesis in these rats.

In the past several years, there has been an increased awareness that abnormalities in lipid and lipoprotein abnormalities contribute to the premature development of arterial vascular disease. In conjugation with this, a substantial body of evidence now implicates peripheral hyperinsulinemia as an important risk factor for arterial vascular disease. Bearing in mind the ability of metformin to improve
glycemia without increasing insulin concentrations and the beneficial antihypertriglyceridemic effect, one can observe a potentially favorable profile emerging that might help to delay or ameliorate some chronic complications of diabetes.

M. HYPERINSULINEMIA AND HYPERTENSION

Essential hypertension in man is a multifactorial condition in which environmental influences act upon an unknown number of genetic factors. Although hypertension remains the major risk factor for the development of coronary artery disease (CAD), it is difficult to demonstrate an improvement in morbidity and mortality rates from CAD despite successful programs to pharmacologically control hypertension (Reaven et al., 1991). In the past several years, a growing wealth of data suggest that a subset of hypertension is associated with metabolic abnormalities involving hyperinsulinemia and/or insulin insensitivity, obesity and lipid abnormalities (for review see Reaven, 1988; Zavaroni et al., 1987). Of particular concern is the finding that these metabolic abnormalities persist with conventional antihypertensive therapy like thiazides and β-blockers (Skarfors et al., 1989; Pollare et al., 1989; Weinberger 1986; Frishman, 1988). Central to this concept, evidence recently has accumulated showing that hyperinsulinemia, suggestive of reduced insulin sensitivity or insulin resistance, is frequently found in essential hypertensive patients even when they have normal body weight and glucose tolerance. Evidence, both from clinical and experimental studies, now suggests that hyperinsulinemia and insulin resistance may be central in the development of hypertension, dyslipidemia and atherosclerosis (Reaven, 1991; DeFronzo and Ferrannini, 1991; Ferrannini et al., 1991; Haffiner et al., 1992).
N. HYPERTENSION AND CARBOHYDRATE METABOLISM

Parillo et al. (1988) investigated differences in carbohydrate metabolism between normotensive and hypertensive subjects using an oral glucose tolerance test. They found that hypertensive subjects had a higher serum glucose level and serum insulin levels 60-180 minutes after ingestion of oral glucose than did normotensive subjects. Perhaps the earliest publication demonstrating the presence of higher than normal plasma insulin concentrations in patients with high blood pressure was that of Welborn et al. (1966). They studied 19 patients diagnosed as having essential hypertension, 10 of whom were not being treated, and found that the group with high blood pressure had significantly higher plasma insulin concentrations. More importantly, the hyperinsulinemia was noted before, and at every time point measured after an oral glucose load, and was found in both the treated and untreated patients with high blood pressure. Modan et al. (1985) in a survey of about 2500 patients found that hypertensive patients exhibited fasting and postprandial hyperinsulinemia independent of obesity, age, or magnitude of glucose tolerance. Several clinical and epidemiological studies confirm these observations (for review see Kannel et al., 1991; Ferrannini and Natali, 1991). More direct evidence for the involvement of hyperinsulinemia and hypertension comes from a study in which physical exercise training of obese hyperinsulinemic subjects led to a decrease in insulin level and blood pressure without a change in weight (Rocchini et al., 1989).

Not only have elevated insulin levels been documented in human essential hypertensives, they have also been shown in rodent models of hypertension. The interplay between elevated insulin levels and hypertension in rats is best elucidated in the fructose-fed hypertensive rat model. In this model insulin resistance, hyperinsulinemia and hypertension are induced in normal Sprague Dawley rats by fructose feeding (Hwang et al., 1987). In these rats, exercise training resulted in an
improvement in insulin sensitivity and a resultant decrease in blood pressure (Reaven et al., 1988). Furthermore, somatostatin administration led to a decrease in hyperinsulinemia and an attenuation in blood pressure in these rats (Reaven et al., 1989). Further support for the involvement of insulin in the development of hypertension comes from data obtained in spontaneously hypertensive rat (SHR) in which hyperinsulinemia precedes the development of hypertension (Reaven and Chang, 1991).

In summary, data from clinical, epidemiological and animal studies are consistent with the hypothesis that changes in insulin metabolism are related to hypertension. The next necessary question to ask is how do insulin levels lead to hypertension?

O. INSULIN - HYPERTENSION: POSSIBLE MECHANISMS

In an effort to maintain normal glycemia in the presence of insulin insensitivity, the pancreatic beta cells try to offset the insulin resistance by secreting more insulin. In other words, the trade off in maintaining euglycemia in the presence of insulin insensitivity is hyperinsulinemia. High insulin levels can lead to hypertension through several mechanisms. Elevated insulin levels could lead to sympathetic stimulation and hypertension (Rowe et al., 1981; Liang et al., 1982; Young, 1988). Another possible link between hyperinsulinemia and hypertension involves the effect of insulin on the handling of sodium and water by the kidney. There is evidence that insulin can act to promote renal tubular sodium resorption in man (DeFronzo et al., 1985). More recently it has been demonstrated that insulin acts at the level of the proximal tubule to increase volume reabsorption (Mondon and Reaven, 1988). Thus, elevated insulin levels have the potential to cause volume overload and high blood pressure. In addition, elevated insulin levels can increase
intracellular sodium and calcium concentrations (Mahnensmith and Aroson, 1985; Canessa et al., 1987; Wiedmann et al., 1985), which in turn may lead to increased vascular reactivity to pressor amines and finally may cause an increase in vascular smooth muscle proliferation (Stout, 1990). Another suggested mechanism involves resistance of the vascular smooth muscle cells to the vasodilatory actions of insulin, i.e. in the face of insulin insensitivity/insulin resistance the vascular smooth muscle (VSM) is more responsive to the actions of vasoconstrictor amines. This would in turn lead to an increase in peripheral vascular resistance and an increase in blood pressure (Sowers et al., 1991; Zemel et al., 1991). Figure 3 illustrates the possible links between hyperinsulinemia/insulin resistance and high blood pressure. All of these events may potentially act independently or in an additive fashion to lead to hypertension.

P. RODENT MODELS USED TO EVALUATE THE INTERPLAY BETWEEN HYPERINSULINEMIA/INSULIN RESISTANCE AND HYPERTENSION

The Spontaneously Hypertensive Rat (SHR)

The SHR has a genetic propensity to develop hypertension. These rats have also been shown to be insulin-resistant and hyperinsulinemic when compared to their genetic controls, the Wistar Kyoto rats (WKY) (Mondon and Reaven, 1988). Studies on adipocytes isolated from SHR indicate that insulin-stimulated glucose uptake is lower when compared to adipocytes isolated from WKY rats (Reaven et al., 1989). Reaven and Chang (1991) found a positive correlation (r=0.6) between the degree of insulin resistance in isolated adipocytes and the degree of blood pressure. A significant relationship (P<0.001) was seen between plasma insulin concentration and blood pressure in the same rats. Although two recent reports do not support the
FIGURE 3. POSSIBLE LINKS BETWEEN HYPERINSULINEMIA / INSULIN RESISTANCE AND HYPERTENSION

FROM: ROCCHINI, 1992
presence of insulin resistance in the SHR, the presence of hyperinsulinemia was confirmed even in those studies (Buchanan et al., 1992; Buchanan et al., 1992). It has been proposed that nutrient-stimulated hyperinsulinemia may play a role in the development and regulation of blood pressure in the SHR (Buchanan et al., 1992). These studies support the notion that hyperinsulinemia may play a role in the regulation of blood pressure in this animal model.

**The Fructose-Fed Hypertensive Rat**

Zavoroni et al. (1980) showed that substituting fructose for the carbohydrate conventionally present in rat chow led to insulin resistance and hyperinsulinemia in Sprague Dawley rats independent of obesity. More recently Hwang et al. (1987) demonstrated that this dietary manipulation also led to an increase in blood pressure by about 20 mm Hg in these animals. Furthermore, administration of clonidine to these rats inhibited the hypertension but did not improve the associated metabolic defects, suggesting that the defects in glucose metabolism may not be secondary to an increase in sympathetic outflow. Fructose-induced hypertension is not accompanied by any changes in plasma renin or angiotensin levels (Hwang 1989); however, it is characterized by elevated atrial natriuretic peptide levels and decreased plasma aldosterone levels suggestive of volume overload (Hwang, 1989). As hyperinsulinemia can potentially lead to sodium and water retention, it is possible that fructose hypertension is caused by volume overload secondary to hyperinsulinemia. In contrast to the SHR, the fructose-induced hyperinsulinemic, insulin resistant and hypertensive rat represents an acquired form of hypertension.
Q. RATIONALE OF THE PROPOSED EXPERIMENTS

The general purpose of this thesis was to examine the effect of metformin treatment on isolated cardiac responses in STZ-diabetic rats and secondly to explore the effect of metformin treatment on the hyperinsulinemic status of two rodent models of hypertension, the SHR and the fructose-induced hypertensive rat.

In the first set of experiments, we hypothesized that if metformin improves glucose homeostasis and has beneficial antihypertriglyceridemic actions, it should prevent the development of cardiac dysfunction in the diabetic rat. The experimental design involved treatment of STZ-induced diabetic rats with metformin hydrochloride and measurement of isolated cardiac function under conditions of increasing preload. Four indices of ventricular function were evaluated, i.e. rate of contraction (+dP/dt), rate of relaxation (-dP/dt), left ventricular developed pressure (LVDP) and time to half relaxation (T1/2R). Recent data indicate that insulin itself may be incapable of preventing cardiac disease in the chronic diabetic. In light of these findings, if metformin treatment could prevent the development of functional cardiac abnormalities, it may serve as a novel metabolic approach for the treatment of serious and frequently fatal cardiovascular complications of chronic diabetes.

Our next series of experiments were aimed at exploring the relationship between elevated insulin levels (hyperinsulinemia) and experimental hypertension. We hypothesized that if defects in carbohydrate metabolism manifested as hyperinsulinemia/insulin insensitivity were responsible for the development and regulation of hypertension, drugs which improve insulin sensitivity (e.g. metformin) and decrease insulin levels may potentially ameliorate hypertension. Particularly relevant was the study by Landin et al. (1991) in which metformin treatment of non-obese, non-diabetic untreated hypertensives led to improved glucose tolerance and lower insulin levels. Surprisingly, the treatment also lowered their blood pressure.
light of this recent and interesting finding, we decided to examine the effect of metformin treatment on (a) a genetic model of hyperinsulinemic hypertension, the SHR and (b) an acquired model of hyperinsulinemia and hypertension, the fructose-fed hypertensive rat. In an effort to establish causality between insulin levels and hypertension, we proposed to examine the effect of artificially raising the plasma insulin (via insulin implants) and studying the resultant effect on blood pressure in SHR animals treated with metformin. If metformin lowers plasma insulin and blood pressure in SHR animals, then artificially increasing plasma insulin levels should increase blood pressure. As mentioned earlier, the aim of these studies was directed towards providing insight into the role of hyperinsulinemia in the development of experimental hypertension and secondly to examine the potential antihypertensive effect of metformin.
SPECIFIC GOALS OF THE PRESENT INVESTIGATION

1. To study the effect of chronic treatment of STZ-induced diabetic rats with metformin on various indices of ventricular function, i.e., \( +dP/dt \), \( -dP/dt \), LVDP and \( T_{1/2}R \).

2. To examine the effect of metformin treatment on the hyperinsulinemic status of the SHR and the fructose-fed hypertensive rat.

3. To study the effect of metformin treatment on the hypertensive status of the SHR and the fructose-fed hypertensive rat.

4. To evaluate the inter-relationship between hyperinsulinemia and hypertension by attempting to correlate changes in plasma insulin with changes in blood pressure in these two models of experimental hypertension and hyperinsulinemia.

5. To evaluate the effect on blood pressure of artificially raising plasma insulin levels in the SHR.
MATERIALS AND METHODS

STUDY 1: EFFECT OF METFORMIN TREATMENT ON ISOLATED CARDIAC FUNCTION

1. Animals and Methods

Forty four male Wistar rats (175-200g) obtained locally were used in this study. The rats were divided into two groups. One group received a single tail vein injection of streptozotocin (STZ, obtained from Sigma, St. Louis, MO) at a dose of 50mg/kg and served as the diabetic group. Previous studies from our laboratory have indicated that animals so treated develop hyperglycemia, hypoinsulinemia and depressed cardiac function. The other group was injected with 0.9% sodium chloride solution and served as age-matched controls. The rats injected with STZ were checked for hyperglycemia at 48 hours and 72 hours. Only those rats with plasma glucose values greater than 15 mM were included in the study. The control and diabetic groups were then further divided into two groups: untreated control (n=10), metformin-treated control (n=10), untreated diabetic (n=12) and metformin-treated diabetic(n=12). The rats were housed two to three per cage and received food and water ad libitum. The treated groups received metformin hydrochloride (a gift from Nordic Pharmaceuticals Inc.) dissolved in the drinking water. Metformin treatment was initiated at a dose of 350 mg/kg/day and was gradually increased over a two week period to a final dose of 650 mg/kg/day. The treated groups received this final dose for a 6 week period. At the end of 8 weeks, hearts were isolated and perfused as described in the next section. At termination, whole blood (arterial and venous) was collected in heparinized tubes and the plasma separated by centrifugation (3000Xg)
for 10 minutes. The plasma samples were stored at -80°C until analyzed for glucose, insulin and triglyceride.

2. **Heart Perfusion**

Hearts were removed from control and diabetic rats anesthetized with sodium pentobarbital and perfused by the Langendorff procedure for approximately 5 minutes and subsequently perfused in the working heart mode as previously described by Rodrigues et al. (1988). The hearts were perfused with oxygenated (95% O2, 5% CO2) Chenoweth-Koelle (CK) buffer maintained at 37±1 °C. The CK buffer composition was: NaCl 120 mM, KCl 5.6 mM, CaCl2 2.18 mM, MgCl2 2.1 mM, NaHCO3 19 mM, glucose 10 mM. The left ventricular developed pressure (LVDP) was measured using a Statham P23 AA transducer (Statham Gould) attached to a 3 cm piece of polyethylene glycol (PE-90) tubing which in turn was attached to a 20 gauge needle which was inserted into the left ventricle through the apex of the heart. Cardiac work was initiated by switching the perfusion from the Langendorff to the working heart mode. Briefly, in the working heart mode, the CK buffer entered the left ventricle through the left atrium and was pumped out through the aorta. The aortic outflow was subjected to an afterload of a 75-mm column of water. The left ventricular pressure and first derivative of the left ventricular pressure was recorded on a Grass model 79D polygraph. A platinum electrode from a Grass Model SD9D stimulator was connected to the left atrium of each heart and stimulated at twice the threshold voltage with square wave pulses of 5 ms to give a rate of 300 beats/minute. The cardiac function data [left ventricular developed pressure (LVDP), rate of force development (+dP/dt) and rate of relaxation (-dP/dt)] were collected and analyzed using a computer program. The pressure transducer signal from the polygraph was sampled at 667 Hz over 1.5 sec at each function curve. This resulted in data being
collected for six complete cardiac pulses. Three of the six were analyzed with curve fitting techniques in order to determine pulse height, area, start and finish. The values of these three pulses were averaged to give the data at that point. This was all calculated by the computer program. Each heart was equilibrated at 15 cm H$_2$O for 10-15 minutes before the function curves were performed. The function curves were performed by estimating the left ventricular function against varying left atrial filling pressures. The filling pressures were altered by changing the height of the atrial filling reservoirs from 7.5 to 22.5 cm in 2.5 cm increments. The filling pressure was first reduced stepwise from 15 to 7.5 cm H$_2$O after which it was increased stepwise to 22.5 cm and finally decreased to 15 cm H$_2$O. At each point, the pressure developed was allowed to stabilize before it was recorded. In general, stable pressure development was achieved within 2 minutes after the left atrial filling pressure was altered. A complete function curve required about 20-30 minutes. Time to half relaxation (T$_{1/2}$R) was calculated manually from each hard copy of the tracing. A perpendicular line was drawn from the peak of the function curve intersecting the baseline of the tracing. The horizontal line from the midpoint of this perpendicular line was drawn to intersect the pressure decline portion of the curve and the distance was noted in millimeters (mm). With the help of the chart speed the distance in mm was converted into milliseconds (ms), and expressed as T$_{1/2}$R.

3. **Plasma Analysis**

The plasma samples were analyzed for glucose and triglycerides using diagnostic kits from Boehringer Mannheim Diagnostic (Dorval, Quebec, Canada). Plasma insulin was assayed using a double antibody radioimmunoassay kit from ICN Biomedicals, Costa Mesas, CA, USA.
4. **Statistical Analysis:**

Values are indicated as mean±S.E. of the mean. "n" indicates the number of animals in each group. Statistical analysis was performed with a two way analysis of variance followed by a Newman-Keuls test. A probability of P<0.05 was taken as the level of statistical significance.

**STUDY 2: EFFECT OF METFORMIN TREATMENT ON HYPERINSULINEMIC STATUS IN TWO RODENT MODELS OF HYPERTENSION**

(A) **Spontaneous Hypertensive Rat Study**

**Animals and Experimental Design:**

As discussed in the introduction, the SHR has been characterized as a model of insulin resistant and hyperinsulinemic hypertension, similar to human essential hypertension. The purpose of this study was to examine if metformin treatment of SHR can prevent the development of hyperinsulinemia and hypertension as compared to untreated SHR.

Male SHR and WKY rats were procured at 4 weeks of age from Charles River, Montreal Canada. The rats were allowed one week to adapt and were then divided into the following experimental groups: SHR-untreated (n=9), SHR metformin-treated (n=9), WKY-untreated (n=7) and WKY metformin-treated (n=7). Basal values of systolic blood pressure, plasma glucose and insulin were recorded at week 5 (weeks denote age of the animals). Following the initial measurements, chronic metformin treatment was initiated at a dose of 350mg/kg/day and gradually titrated to a final dose of 500mg/kg/day over a two week period. The treated groups received this final dose
throughout the experiment. This dose was chosen based on our previous study. Starting week 8, weekly measurements of systolic blood pressure, plasma glucose and plasma insulin (all samples collected from 5 hour fasted rats) were performed until week 13. Food intake, fluid intake and body weight of all the animals was recorded once a week. In order to examine the effect of increasing exogenous plasma insulin concentration on blood pressure, insulin implants were surgically placed in the treated animals at week 16. The implants were placed subcutaneously on the dorsal side of each rat. The implant delivered insulin at a dose of 1 unit/rat/day. At week 18 (post-implant) systolic blood pressure, plasma insulin and glucose was measured.

**B) Fructose-induced Hypertension Study**

*Animals and Research Design:*

As previously discussed, in normal Sprague Dawley rats substituting fructose for the starch usually present in the rat chow results in hyperinsulinemia, insulin resistance and hypertension. The model thus represents an acquired form of elevated systolic blood pressure. This study was designed to examine the effect of metformin treatment on elevated insulin levels and blood pressure in the fructose-induced hypertensive Sprague Dawley rat.

Male Sprague Dawley rats were procured locally (180-200g body weight) at 6 weeks of age. The animals were divided into four experimental groups: control untreated (n=8), control treated (n=8), fructose untreated (n=9) and fructose metformin treated (n=10) and basal values of plasma insulin, glucose and systolic blood pressure were recorded. Starting at week 7 (weeks signify the age of the animal) chronic metformin treatment was initiated in the control treated and the fructose treated groups. Treatment was initiated at a dose of 350 mg/kg/day and
500mg/kg/day over a two week period. One week after initiating metformin treatment, the animals in the fructose and fructose treated group were started on a 66% fructose diet. The fructose diet (66% fructose, 12% fat and 22% protein, Teklad Labs, Madison, WI, U.S.A.) has an electrolyte, protein and fat content very comparable to the standard rat chow. The only difference is that the 60% vegetable starch present in normal rat chow is replaced by 66% fructose in the fructose diet. Starting at week 8, blood pressure, plasma insulin and plasma glucose were measured each week for the next four weeks. In addition, the food intake, fluid intake and body weights of the animals were recorded each week. Results of this study would indicate if starting metformin prior to the administration of the fructose diet prevents fructose-induced metabolic changes and hypertension in this rodent model of hypertension.

(C) METHODS

I. Blood Pressure Measurement

Indirect systolic BP was measured in conscious rats using the indirect tail cuff method without external preheating (Bunag 1973). The animals were preconditioned to the experimental procedure before conducting the actual measurements. The apparatus used includes a BP sensor cuff, a BP amplifier and an analog/digital recorder and printer (Model 179 semi-automatic BP analyzer, IITC INC., Woodland Hills, CA, USA). The various parameters, such as cycling interval and inflation/deflation rate, are kept constant by the semi-automatic equipment. In this method, the reappearance of pulsations on gradual deflation of the BP cuff are detected by a photoelectric sensor and are amplified and recorded digitally as the systolic BP. An average of 5 such readings was taken to obtain the individual systolic BP. The major advantage of this method is that the recordings are carried out at a low
ambient temperature, thus eliminating the heat stress typical of other BP measuring devices.

2. **Biochemical Measurements**

Plasma glucose was measured with the glucose oxidase method using a diagnostic kit from Boehringer Mannheim. Plasma insulin was assayed using the double antibody radioimmunoassay using a kit from ICN biomedicals, Costa Mesa, CA, USA.

3. **Statistical Analysis**

In the series of experiments outlined above, the independent variable was the drug intervention (treated vs. control). Since there were several dependent variables e.g. glucose, blood pressure, insulin, the differences among groups were evaluated using multivariate analysis of variance (MANOVA), using a Number Cruncher statistical Program (NCSS). MANOVA is the most powerful statistical procedure available for this type of analysis. In MANOVA, the mean vector (made up of the individual variate means) is examined for any difference. In the current study, a probability of \( P<0.05 \) was taken to indicate a significant difference between the means. When the MANOVA detected a significant difference in the mean vector, the individual variables were analyzed employing the Newman-Keuls test for multiple comparisons.
RESULTS

STUDY 1: EFFECT OF METFORMIN TREATMENT ON ISOLATED HEART FUNCTION IN STZ-DIABETIC RATS

General Features of Experimental Animals

The general features of the experimental animals are presented in Table 2. Induction of diabetes with STZ resulted in symptoms characteristic of the diabetic state. The untreated diabetic animals had a lower body weight, higher food and fluid intake, higher plasma glucose and triglyceride levels and lower plasma insulin values when compared to age-matched control rats.

Treatment of the diabetic rats with metformin resulted in a significant reduction in plasma glucose levels to near control values. The insulin levels in the diabetic treated and untreated groups were similar indicating that the glucose lowering effect occurred without any increase in the insulin levels. Metformin treatment also lowered the elevated plasma triglyceride levels in the diabetic group to control level. Treatment of the control group with metformin resulted in no changes in the glucose and triglyceride values when compared to age matched untreated control rats. However, metformin significantly decreased the plasma insulin values in control-treated rats. Metformin's antihyperglycemic action is believed to be mediated via an enhancement of insulin action (Bailey 1985, 1992). In the diabetic group, this was manifested as a decrease in glucose levels without any increase in the insulin level, whereas in the control-treated animals metformin allowed similar glucose levels in the face of lower plasma insulin levels. This effect of a decrease in the plasma insulin levels in control animals has not only been seen with metformin but has also been noticed with other insulin enhancing agents like vanadyl sulphate (Cam et al., 1993).
Effect of metformin on heart function of experimental animals

Cardiac performance of control and diabetic hearts was assessed by measuring left ventricular responses to changing left atrial filling pressures in terms of left ventricular developed pressure (LVDP), rate of contraction (+dP/dt) and rate of relaxation (-dP/dt). Figures 4-6 illustrate the left ventricular responses measured in the various experimental groups. The time to half relaxation (T1/2R), an index of beginning heart failure, was calculated at left atrial filling pressures of 15 to 22.5 cm H2O (Table 3). The myocardial performance in control, metformin-treated control, diabetic and metformin-treated diabetic groups was similar at atrial filling pressures from 7.5-15 cm H2O. However, hearts from untreated diabetic animals exhibited a depressed response to increases in atrial filling pressure from 17.5 to 22.5 cm H2O in terms of +dP/dT, -dP/dT and LVDP. More importantly, the diabetic hearts exhibited a delayed time to half relaxation at filling pressures of 15 to 22.5 cm H2O. Metformin treatment completely restored the ability of the diabetic hearts to respond to increases in atrial filling pressure from 17.5 to 22.5 cm H2O. The treatment had no effect on the control animals.

STUDY 2: EFFECT OF METFORMIN ON THE HYPERINSULINEMIC STATUS IN EXPERIMENTAL HYPERTENSION

Metformin treatment of the SHR

Table 4 illustrates the food and fluid intake of the rats in the four experimental groups. Treatment of the SHR and WKY rats with metformin led to a 20-25% decrease in the food and fluid intake when compared to untreated animals. Paralleling the decrease in food and fluid intake the SHRM and WKYM rats exhibited a
decreased weight gain when compared to untreated animals (Figure 7). Another noteworthy observation was that as the rats grow older, the WKY appear to gain significantly more than age matched SHR. At week 16 the WKY weigh approximately 40 g more than the SHR.

Figure 8 illustrates the plasma insulin level (uU/ml) in the various experimental groups. The SHR group exhibited sustained hyperinsulinemia by week 9 when compared to the WKY group. Treatment of the SHR rats with metformin (SHRM) prevented the development of hyperinsulinemia. The treatment had no effect on the insulin levels in the WKYM group. Table 5 illustrates the plasma glucose level in the four experimental groups. Metformin treatment did not affect the plasma glucose profile in either the SHR or WKY groups.

Figure 9 illustrates the systolic blood pressure (mm Hg) in the various experimental groups. Paralleling the elevation in plasma insulin, the SHR exhibited systolic hypertension by week 9 when compared to the normotensive WKY. Treatment of the SHR with metformin (SHRM) led to a 30-35 mm Hg decrease in systolic blood pressure. This effect was maintained during the entire treatment time frame. Treatment with metformin had no effect on the systolic blood pressure in the WKYM rats.

Table 6 indicates the plasma insulin levels in the four experimental groups prior (week 16) to and two weeks post (week 18) the insulin implants. The insulin implants were placed only in the treated animals i.e. WKYM and SHRM. The SHRM group exhibited lower plasma insulin levels than the SHR prior to the implant (week 16). The insulin implants caused a marked increase in the plasma insulin level in the SHRM group but had no effect in the WKYM group. However, artificially raising the plasma insulin also caused a decrease in plasma glucose in the SHRM and WKYM rats (Table 7). Table 8 illustrates the blood pressure in the animals before and two week after the implants. Paralleling the elevation in plasma insulin in the SHRM
group, an increase in systolic blood pressure was also observed which was similar to the SHR group. The implants did not affect the blood pressure in the WKYM group.

**Metformin treatment of the fructose-fed Sprague Dawley rat**

Table 9 illustrates the food and fluid intake in the various experimental groups. As noticed in the SHR experiment, treatment of the control and fructose animals with metformin (con+m and fruc+m) led to a 20-25% decrease in food and fluid intake. Figure 10 illustrates the body weight in all the groups. The treated groups exhibited a lower body weight gain, which was significant at weeks 10 and 11. Table 10 illustrates the plasma glucose levels in the four experimental groups. Metformin treatment did not affect the plasma glucose level in either the control or the fructose groups.

Figure 11 illustrates the plasma insulin level (uU/ml) in the various groups. Fructose feeding of control animals led to elevated insulin levels when compared to control animals. Metformin treatment of the fructose group (fruc+m) prevented the increase in plasma insulin when compared to the fructose group. The treatment also led to a significant decrease in the plasma insulin level in control treated animals (con+m).

The systolic blood pressure in the four groups is shown in figure 12. The fructose group exhibited a higher systolic blood pressure when compared to the control group. This elevation in systolic blood pressure was sustained over the entire experiment. This increase in blood pressure induced by fructose feeding was completely prevented by pre-treatment with metformin (fruc+m). Metformin treatment had no effect on the blood pressure of the control treated group.
TABLE 2
GENERAL CHARACTERISTICS OF THE RATS IN THE FOUR EXPERIMENTAL GROUPS AT TERMINATION (8 WEEKS) IN THE ISOLATED WORKING HEART STUDY

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experimental Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>468±12</td>
</tr>
<tr>
<td>Fluid Intake (ml/day)</td>
<td>47±1</td>
</tr>
<tr>
<td>Food Intake (g/day)</td>
<td>32±1</td>
</tr>
<tr>
<td>Plasma Glucose (mM)</td>
<td>9.9±0.3</td>
</tr>
<tr>
<td>Plasma Insulin (μU/ml)</td>
<td>57.2±5.0</td>
</tr>
<tr>
<td>Plasma Triglyceride (mM)</td>
<td>2.5±0.5</td>
</tr>
</tbody>
</table>

* P < 0.05 different from control
# P < 0.05 different from diabetic
+ P < 0.05 different from diabetic and diabetic–treated

Statistical differences were determined by ANOVA followed by a Newman–Keuls test.

[Control (C, n=10), Control-treated (CT, n=10), Diabetic (D, n=11), Diabetic Treated (DT, n=12)]. Rats were injected with streptozotocin (50mg/kg/day) to induce diabetes. The treated groups received metformin hydrochloride in the drinking water for 8 weeks. At the end of 8 weeks of treatment, isolated working heart performance was assessed under conditions of increasing preload.
FIGURE 4

Effect of metformin treatment on rate of contraction (+dP/dT) in the isolated working heart preparation at left atrial filling pressures from 7.5 to 22.5 cm H₂O. Hearts from the four experimental groups [Control ▼ (n=7), Control metformin-treated ▼ (n=6), Diabetic ○ (n=8) and Diabetic metformin-treated ● (n=8)] were isolated and perfused following 8 weeks of metformin treatment. Values are expressed as mean±SE. "n" indicates the number of rats in each group. * P<0.05, ANOVA, different from diabetic.
FIGURE 5

Effect of metformin treatment on rate of relaxation (-dP/dt) in the isolated working heart preparation at left atrial filling pressures from 7.5 to 22.5 cm H₂O. Hearts from the four experimental groups [Control ▼ (n=7), Control metformin-treated▼(n=6), Diabetic○(n=8) and Diabetic treated●(n=8)] were isolated and perfused following 8 weeks of metformin treatment. Values are expressed as mean±SE. "n" represents the number of rats per group. * P<0.05, ANOVA, different from diabetic.
FIGURE 6

Effect of metformin treatment on left ventricular developed pressure (LVDP) in the isolated working heart preparation at left atrial filling pressures from 7.5 to 22.5 cm H$_2$O. Hearts from the four experimental groups [Control \( \nabla \) (n=7), Control metformin-treated \( \downarrow \) (n=6), Diabetic \( \bigcirc \) (n=8) and Diabetic metformin-treated \( \bullet \) (n=8)] were isolated and perfused following 8 weeks of treatment. Values are expressed as mean±SE. "n" indicates the number of rats in each group. * P<0.05 different from diabetic.
TABLE 3
TIME TO HALF RELAXATION (T1/2R mseconds) IN THE FOUR EXPERIMENTAL GROUPS AT PRELOADS OF 15 TO 22.5 CM H2O IN THE ISOLATED WORKING HEART EXPERIMENT

[Control (C, n=7), control metformin-treated (CT, n=6), diabetic (D, n=8) and diabetic metformin-treated (DT, n=8)]. Time to half relaxation expressed in msec from filling pressures of 15 to 22.5 cm H2O. * P<0.05, ANOVA, different from C, CT, and DT.

<table>
<thead>
<tr>
<th>Filling Pressure (cm H2O)</th>
<th>C</th>
<th>CT</th>
<th>D</th>
<th>DT</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>34.0±4</td>
<td>28.8±4</td>
<td>64.0±8*</td>
<td>32.0±4</td>
</tr>
<tr>
<td>17.5</td>
<td>29.6±4</td>
<td>28.0±4</td>
<td>56.0±8*</td>
<td>25.6±8</td>
</tr>
<tr>
<td>20</td>
<td>20.8±4</td>
<td>27.2±4</td>
<td>56.0±4*</td>
<td>29.6±4</td>
</tr>
<tr>
<td>22.5</td>
<td>25.6±8</td>
<td>22.4±4</td>
<td>56.0±8*</td>
<td>31.2±8</td>
</tr>
<tr>
<td>Group</td>
<td>Week 5 (baseline)</td>
<td>Week 9</td>
<td>Week 11</td>
<td>Week 13</td>
</tr>
<tr>
<td>-----------</td>
<td>-------------------</td>
<td>--------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>WKY</td>
<td>17.0±0.4</td>
<td>26.7±1.1</td>
<td>21.1±0.8</td>
<td>25.0±0.9</td>
</tr>
<tr>
<td>WKY-M</td>
<td>18.0±0.7</td>
<td>26.7±0.7</td>
<td>15.6±0.4*</td>
<td>17.3±0.6*</td>
</tr>
<tr>
<td>SHR</td>
<td>21.3±0.2</td>
<td>22.6±1.0</td>
<td>20.6±0.4</td>
<td>23.0±0.4</td>
</tr>
<tr>
<td>SHR-M</td>
<td>21.6±0.4</td>
<td>26.0±1.4</td>
<td>19.7±0.3</td>
<td>18.3±0.5*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Week 5 (baseline)</th>
<th>Week 9</th>
<th>Week 11</th>
<th>Week 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>34±1.3</td>
<td>41±0.7</td>
<td>46±1.7</td>
<td>49±1.2</td>
</tr>
<tr>
<td>WKY-M</td>
<td>32±0.5</td>
<td>30±0.1*</td>
<td>39±2.9*</td>
<td>32±2.3*</td>
</tr>
<tr>
<td>SHR</td>
<td>37±0.5</td>
<td>39±1.0</td>
<td>45±2.1</td>
<td>48±2.0</td>
</tr>
<tr>
<td>SHR-M</td>
<td>35±0.5</td>
<td>30±1.4*</td>
<td>33±1.0*</td>
<td>31±1.0*</td>
</tr>
</tbody>
</table>

WKY (n=7), WKY metformin-treated (WKYM, n=7), SHR (n=9) and SHR metformin-treated (SHRM, n=9). "n" indicates the number of rats in each group. Values expressed as mean±SE.

* P<0.05 MANOVA different from WKY

■ P<0.05 MANOVA different from SHR
TABLE 5
PLASMA GLUCOSE (mM) IN THE FOUR EXPERIMENTAL GROUPS
IN THE SHR STUDY

<table>
<thead>
<tr>
<th>Groups</th>
<th>Week 5</th>
<th>Week 9</th>
<th>Week 11</th>
<th>Week 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR(n=7)</td>
<td>6.1±0.3</td>
<td>6.7±0.2</td>
<td>6.1±0.3</td>
<td>6.2±0.5</td>
</tr>
<tr>
<td>SHRM(n=7)</td>
<td>6.5±0.1</td>
<td>6.4±0.1</td>
<td>5.8±0.2</td>
<td>5.7±0.2</td>
</tr>
<tr>
<td>WKY(N=7)</td>
<td>6.9±0.2</td>
<td>6.6±0.4</td>
<td>6.6±0.3</td>
<td>6.5±0.3</td>
</tr>
<tr>
<td>WKYM(N=8)</td>
<td>6.6±0.3</td>
<td>5.9±0.2</td>
<td>5.7±0.1</td>
<td>6.0±0.2</td>
</tr>
</tbody>
</table>

SHRM= SHR metformin-treated, WKYM=WKY metformin-treated. "n" indicates the number of rats in each group.
FIGURE 7

Body weight (g) in the four experimental groups in the SHR study. [WKY ○ (n=7), WKYM ● (n=7), SHR ▽ (n=9) and SHRM ▼ (n=9)]. Metformin treatment was initiated at week 6 (week denotes age of animal).

* P<0.05, MANOVA different from WKYM and SHRM

P<0.05, MANOVA different from SHR
FIGURE 8

Plasma insulin (uU/ml) in the four experimental groups in the SHR study. [WKY ○ (n=7), WKYM ● (n=7), SHR △ (n=9), SHRM ▼ (n=9)]. Metformin treatment initiated at week 6 (week denotes age of animal). "n" indicates the number of rats in each group. Values expressed as mean±SE.

★ P<0.001, MANOVA, different from WKY, WKYM and SHRM.
FIGURE 9

Systolic blood pressure (mm Hg) in the four experimental groups in the SHR study. [WKY ○ (n=7), WKYM ● (n=7), SHR ▽ (n=9), SHRM ▼ (n=9)]. Metformin treatment was initiated at week 6 (week denotes age of animal). "n" indicates the number of rats in each group. Values are expressed as mean±SE.

@ P<0.001, MANOVA different from WKY, WKYM and SHRM

* P<0.001, MANOVA different from WKY, WKYM and SHR
PLASMA INSULIN LEVELS (µU/ml) DURING THE IMPLANT EXPERIMENT

<table>
<thead>
<tr>
<th>GROUP</th>
<th>PRE-IMPLANT (16 WEEKS OLD)</th>
<th>POST-IMPLANT (18 WEEKS OLD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY (n=7)</td>
<td>52.6±3.5</td>
<td>59.2±1.2</td>
</tr>
<tr>
<td>WKYM (n=7)</td>
<td>58.1±5.0</td>
<td>63.9±2.9</td>
</tr>
<tr>
<td>SHR (n=7)</td>
<td>57.5±3.6</td>
<td>63.7±1.3</td>
</tr>
<tr>
<td>SHRM (n=8)</td>
<td>36.5±1.2</td>
<td>66.1±2.6 *</td>
</tr>
</tbody>
</table>

* P<0.001 ANOVA, DIFFERENT FROM SHRM

At week 16 implants were surgically implanted in the dorsal neck region of the WKYM and SHRM groups. The untreated groups served as the controls (without implant) to evaluate the effect of artificially raising plasma insulin in the treated groups on blood pressure.
TABLE 7

PLASMA GLUCOSE LEVELS (mM) DURING THE IMPLANT EXPERIMENT

<table>
<thead>
<tr>
<th>GROUP</th>
<th>PRE-IMPLANT (16 WEEKS OLD)</th>
<th>POST-IMPLANT (18 WEEKS OLD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY (n=7)</td>
<td>6.6±0.5</td>
<td>6.8±0.7</td>
</tr>
<tr>
<td>WKYM (n=7)</td>
<td>5.9±0.4</td>
<td>4.4±0.7 *</td>
</tr>
<tr>
<td>SHR (n=7)</td>
<td>6.1±0.4</td>
<td>6.3±0.3</td>
</tr>
<tr>
<td>SHRM (n=8)</td>
<td>6.4±0.7</td>
<td>5.1±0.4 *</td>
</tr>
</tbody>
</table>

* P<0.05, ANOVA, different from pre-implant WKYM and SHRM

At week 16 implants were surgically implanted in the dorsal neck region of the WKYM AND SHRM GROUPS. The untreated groups served as the controls (without implant) to evaluate the effect of artificially raising plasma insulin in the treated groups on blood pressure.
<table>
<thead>
<tr>
<th>GROUP/B.P</th>
<th>WKY</th>
<th>WKYM</th>
<th>SHR</th>
<th>SHRM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-implant</td>
<td>143±3.6</td>
<td>148±1.8</td>
<td>212±6.3</td>
<td>168±2.3</td>
</tr>
<tr>
<td>Post-implant</td>
<td>150±3.1</td>
<td>156±2.4</td>
<td>208±4.6</td>
<td>189±3.1</td>
</tr>
</tbody>
</table>

WKY (n=7), WKYM (n=7), SHR(n=9), SHRM(n=9). Values are expressed as mean±SE. "n" indicates the number of rats in each group.

# P<0.05, ANOVA different from SHRM (pre-implant)
TABLE 9

Food (g/day) and fluid (ml/day) intake in the various experimental groups. The experimental groups consisted of control (n=8), control metformin-treated (con+M, n=8), fructose (n=9) and fructose metformin-treated (n=10). Metformin was initiated at week 6 and fructose was started at week 7. Weeks denote the age of the rats. Values are expressed as mean±SE. "n" indicates the number of rats in each group.

**FOOD INTAKE IN THE FOUR EXPERIMENTAL GROUPS (g/day)**

<table>
<thead>
<tr>
<th>Group/Week</th>
<th>6 (baseline)</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>18.0±0.3</td>
<td>26.9±0.3</td>
<td>29.1±0.3</td>
<td>29.8±0.2</td>
</tr>
<tr>
<td>CON+M</td>
<td>16.4±0.2</td>
<td>24.5±0.2</td>
<td>24.5±0.4$</td>
<td>24.9±0.2@</td>
</tr>
<tr>
<td>FRUCTOSE</td>
<td>18.6±0.3</td>
<td>24.6±0.6</td>
<td>25.2±0.3</td>
<td>30.1±0.3</td>
</tr>
<tr>
<td>FRUC+M</td>
<td>17.6±0.3</td>
<td>24.1±0.0</td>
<td>24.2±0.2</td>
<td>25.4±0.4#</td>
</tr>
</tbody>
</table>

**FLUID INTAKE IN THE FOUR EXPERIMENTAL GROUPS (ml/day)**

<table>
<thead>
<tr>
<th>Group/Week</th>
<th>6 (baseline)</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>34.5±0.7</td>
<td>39.6±0.4</td>
<td>43.6±0.4</td>
<td>45.3±0.4</td>
</tr>
<tr>
<td>CON+M</td>
<td>31.4±0.3</td>
<td>36.9±0.3</td>
<td>36.0±0.3$</td>
<td>37.9±0.3@</td>
</tr>
<tr>
<td>FRUCTOSE</td>
<td>33.8±0.3</td>
<td>38.0±0.8</td>
<td>39.7±0.8</td>
<td>40.3±0.1</td>
</tr>
<tr>
<td>FRUC+M</td>
<td>33.4±0.6</td>
<td>35.6±0.3</td>
<td>35.3±0.3</td>
<td>34.2±0.4#</td>
</tr>
</tbody>
</table>

@ P<0.05 different from Control(Week 11), MANOVA
# P<0.05 different from Fructose(Week 11), MANOVA
$ P<0.05 different from Control(Week 10), MANOVA
<table>
<thead>
<tr>
<th>Group/Week</th>
<th>6</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con (n=8)</td>
<td>6.6±0.3</td>
<td>6.4±0.2</td>
<td>6.0±0.4</td>
<td>6.3±0.1</td>
</tr>
<tr>
<td>Con+m(n=8)</td>
<td>5.9±0.5</td>
<td>6.1±0.3</td>
<td>6.7±0.5</td>
<td>5.8±0.5</td>
</tr>
<tr>
<td>Fruc (n=9)</td>
<td>6.0±0.1</td>
<td>6.8±0.5</td>
<td>6.6±0.2</td>
<td>6.1±0.3</td>
</tr>
<tr>
<td>Fruc+m(n=10)</td>
<td>5.8±0.5</td>
<td>6.1±0.5</td>
<td>6.2±0.2</td>
<td>6.3±0.4</td>
</tr>
</tbody>
</table>

Con = control; Con+m = control metformin-treated; fruc = fructose; fruc+m = fructose metformin-treated. Metformin initiated at week 6 and fructose started at week 7 in the treated and fructose groups respectively. Values expressed as mean±SE. "n" = number of rats in each group.
FIGURE 10

Body weight (g) in the four experimental groups in the fructose study. [Control (con n=8), control metformin-treated (con+m n=8), fructose (fruc n=9) and fructose metformin treated (fruc+m n=10)] Metformin treatment was started at week 6 and fructose was initiated at week 7 in the treated and fructose groups respectively. Weeks denote the age of the rats. Values are expressed as mean±SE. "n" indicates the number of rats in each group.

# P<0.001, MANOVA different from CON+M and FRUC+M.
FIGURE 11

Plasma insulin (uU/ml) in the four experimental groups in the fructose study. [Control (con, ○ n=8), control metformin-treated (con+m ● n=8), fructose (fruc ▼ n=9), fructose metformin-treated (fruc+m ▼ (n=10)]. Metformin treatment was initiated at week 6 and fructose was started at week 7 in the treated and fructose groups respectively. Weeks denote the age of the rats. "n" indicates the number of rats in each group. Values are expressed as mean ± SE.

* P<0.001, MANOVA, different from CON, CON+M, FRUC+M

# P<0.001, MANOVA, different from CON
FIGURE 12

Systolic blood pressure (mm Hg) in the four experimental groups in the fructose study. [Control (con, ○ n=8), control metformin-treated (con+m, ● n=8), fructose (fruc, ▼ n=9), fructose metformin-treated (fruc+m, ▼ n=10)]. Metformin treatment was initiated at week 6 and fructose was started at week 7 in the treated and fructose groups respectively. Weeks denote the age of the rats. Values are expressed as mean±SE. "n" denotes the number of rats in each group.  
* P<0.001, MANOVA, different from con, con+m and fruc+m.
DISCUSSION

The work outlined in this thesis examined the effect of an oral antihyperglycemic agent, metformin, on isolated cardiac function and blood pressure in diabetic and hypertensive rats, respectively. In the first series of experiments, we examined the effect of metformin treatment on various functional cardiac parameters in rats made diabetic with STZ. The second part of the project explored the relationship between elevated insulin levels and hypertension in two rodent models of hyperinsulinemia and hypertension, the SHR and the fructose-fed rat using metformin as the experimental intervention.

The association between chronic diabetes and cardiac dysfunction has been demonstrated in both human and animal studies (Ahmed et al., 1975; Fein et al., 1985; Penpargul et al., 1980; Regan et al., 1977; Vadlamudli et al., 1982). The cardiac problems in diabetes include a decreased ventricular contractility manifested as a low stroke volume, cardiac index and ejection fraction and a diminished ventricular compliance manifested as an elevated left ventricular end diastolic pressure (D'Elia et al., 1979; Hamby et al., 1974; Ledet et al., 1979). The presence of these functional abnormalities, independent of atherosclerosis of the coronary vessels, suggests the existence of a specific cardiac muscle disease (cardiomyopathy) in diabetes.

The first study describes the effects of metformin treatment on isolated cardiac performance in the STZ-diabetic rat. Induction of diabetes with STZ resulted in typical symptoms of diabetes; the rats exhibited hyperglycemia, hypoinsulinemia, elevated food and fluid intake and a decreased weight gain when compared to non-diabetic rats. Treatment of the diabetic rats with metformin on a chronic basis decreased plasma glucose to near control values (Table 2). The improvement in glucose levels was seen without any increase in the plasma insulin profile, supporting previous work that showed that the antihyperglycemic effect of metformin is not
mediated by an increase in insulin secretion but via enhancement of insulin-mediated glucose uptake in peripheral tissues (Bailey and Puah, 1986; Galuska et al., 1991). Similar observations have been made in animal models of obese and non-obese hyperinsulinemic and hypoinsulinemic diabetes (Lord et al., 1983; Penicaud et al., 1985; Rossetti et al., 1990).

STZ-induced diabetic rats exhibited elevated triglyceride levels (Table 2) when compared to non-diabetic rats and these were restored to control values in the diabetic-treated group, again demonstrating the insulin enhancing effects of metformin.

Hearts from control and diabetic rats were isolated and cardiac performance was evaluated under physiological and super-physiological filling pressures, simulated by increases in left atrial filling pressure from 7.5 to 22.5 cm H2O. This allowed assessment of cardiac function under normal and stressful conditions.

Paralleling the elevation of plasma glucose and triglycerides, the diabetic group also exhibited a decreased cardiac performance as assessed by their inability to respond to increases in left atrial filling pressure from 17.5 to 22.5 cm H2O (Figures 4-6). The diabetic rat hearts exhibited an elevated T1/2R at filling pressures of 15 to 22.5 cm H2O. Although the diabetic heart performance differed significantly from controls only at filling pressures of 17.5 to 22.5 cm H2O when measured in terms of ±dP/dt and LVDP, calculation of the T 1/2R revealed that the diabetic hearts had a delayed onset of relaxation even at filling pressures of 15 cm H2O (Table 3). This is consistent with the fact that T 1/2R is a highly sensitive index of beginning heart failure. Metformin treatment restored the ability of the diabetic animals to respond to increases in filling pressure for all the functional indices studied.

The mechanisms by which metformin improved diabetic cardiac performance are not known. Several studies have attempted to correlate plasma and myocardial lipid levels to the cardiac dysfunction seen in the diabetic rat (Heyliger et al., 1986;
Shipp et al., 1973). The involvement of lipids is suggested by a study from our laboratory, in which Wistar and Wistar-Kyoto (WKY) rats were injected with identical doses of STZ (Rodrigues and McNeill, 1986). Although both group of rats exhibited elevated plasma glucose levels, only the Wistar rats exhibited elevated levels of circulating lipids. Interestingly, depression of myocardial function was seen in the group of Wistar rats while the cardiac function in the STZ-injected WKY rats remained unaffected. In another study, treatment of STZ-diabetic rats with hydralazine normalized lipid levels and restored cardiac function to non-diabetic control values (Rodrigues et al., 1986). In addition, other studies have shown that diabetic rats pretreated with L-carnitine and choline, and methionine did not show elevated plasma lipid levels and exhibited an improved cardiac performance when compared with untreated Wistar rats (Heyliger et al., 1986; Rodrigues et al., 1988). It thus appears that the improvement in the glucose homeostasis and normalization of triglycerides may be contributing, at least in part, to the improvement in cardiac function in the diabetic rats pretreated with metformin.

Several subcellular organelles and myocardial enzyme systems have been shown to be defective in diabetes (Dilmann 1980; Heyliger et al., 1987; Lopaschuck et al., 1983; Malhotra et al., 1981; Penpargul et al., 1981). One of the more prominent systems affected, which is known to regulate contractility, is the sarcoplasmic reticulum. In diabetic rat hearts, the ability of the sarcoplasmic reticulum to take up calcium is impaired, which explains the impairment of relaxation in the myocardial muscles (Lopaschuck et al., 1983). Also, as a consequence of lowered uptake, the amount of calcium available for release during the following beats may be lower than normal and thus lead to impaired contraction. The mechanism by which diabetes alters sarcoplasmic reticulum calcium uptake is believed to be via an elevation of long-chain acyl carnitine, a metabolic intermediate which is responsible for the transport of fatty acids into the mitochondria. This intermediate is a strong inhibitor
of sarcoplasmic reticulum calcium uptake *in-vitro* and also inhibits other enzymes such as Na+/K+ATPase. Along with the elevation of long-chain acyl carnitines, the levels of long-chain acyl CoA are also elevated, which may have detrimental effects on the myocardium (Dilman 1980; Heyliger et al., 1987; Lopaschuck et al., 1983; Malhotra et al., 1981; Penpargul et al., 1981). It would be interesting to examine if pre treatment of diabetic rats with metformin could restore such subcellular defects.

As outlined earlier in the introduction, in the last several years considerable attention has been directed towards investigating the role of altered myocardial energetics in the development of diabetic heart disease (Rodrigues and McNeill 1992). A view which is gaining acceptance is that diabetes causes metabolic changes and that these metabolic changes precede the development of overt cardiac failure in a chronic setting (Matti et al., 1990). Under normal conditions, an estimated 60-70% of myocardial energy is derived from the metabolism of lipids; the remainder is derived from non-lipid sources. Diabetes is associated with elevated triglyceride and FFA levels and as a result there is an increased uptake, oxidation and storage of fatty acids by the myocardium. Thus under conditions of diabetes the heart exhibits an exclusive dependence on FFA oxidation as a source of energy, which increases from 60-70% to almost 90% in diabetes. Elevated FFA oxidation has deleterious metabolic effects on the myocardium. Firstly, the inability of the diabetic heart to utilize glucose leads to an elevated oxygen demand per molecule of ATP produced. Secondly, increased FFA oxidation leads to an accumulation of intermediates of FFA oxidation, such as long chain acyl carnitines, which have deleterious effects on the myocardial cell via different mechanisms (Rodrigues and McNeill, 1992). One may speculate that the improvement in cardiac function seen in the present study may be secondary to a decrease in the triglyceride level and a switch in myocardial metabolism from predominantly FFA to glucose.
Other studies from our laboratory have shown that vanadium treatment of STZ-diabetic rats also prevents the development of cardiac dysfunction (Heyliger et al., 1986; Yuen et al., 1993). There appears to be some similarity between the effects of inorganic and organic vanadium compounds and metformin with respect to experimental diabetes. Both vanadium compounds and metformin enhance the action of insulin (Lord et al., 1983; Penicaud et al., 1985; Rossetti et al., 1990; Yuen et al., 1993) and prevent the development of secondary cardiovascular complications of diabetes. The exact mechanism(s) of action of biguanides and vanadium is not known and some overlap may exist. Although the effect of vanadium and metformin in preventing cardiac disease in humans remains to be determined, these agents may provide a unique metabolic approach to the treatment of cardiovascular complications of chronic diabetes.

The second series of experiments was aimed at exploring the inter-relationship between hyperinsulinemia, insulin resistance and essential hypertension in a genetic model of hypertension, the SHR, and an acquired model of hypertension, the fructose-induced hypertensive rat. We hypothesized that if elevated insulin levels and/or insulin resistance play a role in the development of hypertension, an agent such as metformin, which enhances the action of endogenous insulin and causes a decrease in the plasma insulin level, may alleviate or prevent the development of high blood pressure.

Treatment of the SHR and the WKY rats with metformin led to a 20-25% decrease in their food and fluid intake. This was accompanied by a decreased body weight gain in the treated groups which was significant at week 11, 13, 16 and 18. The decreased body weight gain in the SHRM and WKYM group could be attributed to the decrease in food and fluid intake seen in these rats. Moreover, metformin treatment often promotes weight loss (Hermann 1979), notably in obese and energy-restricted NIDDM patients (Wales, 1980) and animals (Bailey et al., 1986), possibly
via an increased thermogenic activity of brown adipose tissue and increased "futile" cycling of substrates (Leslie et al., 1986). Therefore, the decreased body weight gain in the treated groups may also be due to a direct action of the drug.

As discussed earlier, the SHR has a genetic propensity for hypertension and have also been shown to be insulin resistant and hyperinsulinemic when compared to their genetic controls, the WKY (Mondon and Reaven, 1988; Finch et al., 1990). As illustrated in figure 8, by week 9 the SHR group exhibited hyperinsulinemia when compared to the WKY. Paralleling the elevation in plasma insulin, the SHR exhibited full-blown systolic hypertension by week 9 (Figure 9). Treatment of SHR with metformin at a dose of 500 mg/kg/day prevented the development of hyperinsulinemia in these rats. The treatment also decreased the blood pressure in the SHRM group to near control values. Metformin did not completely restore the blood pressure to control levels; however, it was effective in alleviating the rise in blood pressure when compared to untreated SHR rats. This is not unexpected as plasma insulin is not the only determinant of hypertension in the SHR; it may be one contributing factor. It can be speculated that if elevated plasma insulin is one of the determinants of hypertension in the SHR, then improving this cause (preventing hyperinsulinemia) alleviated hypertension in the SHRM animals. In an attempt to further explore the relationship between insulin and blood pressure, we artificially raised plasma insulin in the WKYM and SHRM groups via insulin implants and studied the resultant effect on blood pressure. Table 6 illustrates the pre- and post-implant plasma insulin levels of the four experimental groups. It is clear on observation that in response to the implants the SHRM group exhibited marked elevations in the plasma insulin level. This was accompanied by an increase in systolic blood pressure (Table 8) in the SHRM group but not in the WKYM. In other words pretreatment of the SHR with metformin before hypertension is manifested, prevents the development of hyperinsulinemia and alleviates hypertension. Artificially raising the plasma insulin
level in these rats (SHRM) leads to restoration of hyperinsulinemia and an elevation in systolic blood pressure. Both these observations cannot confirm a causal relationship of insulin and hypertension yet indicate that these parameters may be related. Moreover, as we did not measure insulin resistance (using a hyperinsulinemic clamp), it is difficult to comment on the association of insulin resistance and hypertension in this rodent model of hypertension. The question that arises is why do changes in plasma insulin correlate with changes in blood pressure in the SHR but not in the WKY rats? It has been suggested that the SHR may have an increased genetic susceptibility towards certain insulin effects such as sodium retention, vascular hypertrophy and increased sympathetic nervous activity (Buchanan et al., 1992; also discussed later) when compared to the WKY. This could result in the development of high blood pressure selectively in the SHR. However, artificially raising plasma insulin in the WKYM and SHRM groups did cause a decrease in the plasma glucose level. A noteworthy observation from our data was the fact that from weeks 9 to 13 the SHR demonstrated hyperinsulinemia when compared to the WKY rats; however, the pre-implant plasma insulin values taken at week 16 were identical between the SHR and the WKY. A plausible explanation for this lies in the difference in the body weights between the WKY and the SHR at week 16 (pre-implant). At week 16 the WKY rats weighed about 40 g more than the SHR which may explain their higher plasma insulin levles. This observation highlights the importance of weight-matched animals in accurate interpretation of data on carbohydrate metabolism.

The next step was to examine if the insulin-blood pressure hypothesis was valid in another model of hyperinsulinemic hypertension, the fructose-induced rat. The fructose rat model represents an acquired form of systolic hypertension, wherein the rise in blood pressure is not genetically determined but is diet-induced (Hwang et al., 1987; Reaven et al., 1988). Given the fact that we still do not know the relative contribution of genetic and acquired factors towards the insulin resistance observed in
hypertension, we thought it would be very pertinent to examine the validity of the insulin-blood pressure hypothesis in this model.

Fructose feeding of normotensive Sprague Dawley rats led to sustained hyperinsulinemia and elevations in blood pressure when compared to control rats (Figure 11 and 12) This effect was apparent about 2 weeks after beginning the fructose-diet. Treatment with metformin prior to the administration of the fructose diet (fruc+m) prevented fructose-induced hyperinsulinemia and hypertension. The treatment did not affect the blood pressure in the control rats; however, metformin treatment of the control group caused a reduction in the plasma insulin level which was significant at weeks 9 and 11. This was not unexpected as in our first study on isolated working heart, a decrease in plasma insulin was observed in control treated animals also. This is consistent with the idea that metformin's action is not mediated via an increase in insulin secretion but via an enhancement of insulin mediated glucose disposal in the peripheral tissues. The question which comes to mind is why decreases in plasma insulin correlate with decreases in blood pressure in the fructose-treated group but not in the control treated group? The first point to consider here is that it is difficult to compare the action of an insulin-enhancing agent in an insulin-sensitive (control) and insulin resistant (fructose) tissue. As outlined in the introduction, one potential mechanism through which insulin resistance can lead to hypertension is thought to be via resistance to the vasodilator effects of insulin. It can be postulated that the insulin-resistant rats (in our case the fructose group) have attenuated vasodilator responses to hyperinsulinemia. An insulin enhancing agent like metformin may potentially improve the vasodilator responses to hyperinsulinemia and thereby decrease blood pressure.

The fact that an experimental intervention which decreased insulin levels caused a decrease in blood pressure in two models of hyperinsulinemia and hypertension certainly does not establish a causal role for insulin in the development
of hypertension, but strongly suggest a strong association. Further support for the interplay between insulin and hypertension is provided in the first part of this study where artificially raising plasma insulin levels led to an increase in blood pressure in the SHRM group. Other agents which improve insulin sensitivity and decrease plasma insulin levels have been shown to both prevent and reverse the development of hypertension in both the SHR and fructose rat model. These agents include vanadyl sulphate, an organic vanadium compound, bismaltolato (oxo) vanadium IV (Bhanot and McNeill 1993), and lastly pioglitazone (Bhanot and McNeill unpublished observations). The fact that multiple and specific drug interventions which enhance the action of insulin lead to a decrease in the plasma insulin and are accompanied by a fall in systolic blood pressure provide strong support to the notion that elevated insulin levels contribute at least in part to the development of hypertension in these two models of rodent hypertension. As mentioned earlier, two interventions in fructose-induced hypertensive rats provide indirect evidence for the hypothesis under study. Exercise training of fructose fed rats improved insulin sensitivity, decreased plasma insulin levels and decreased blood pressure (Mondon et al., 1980). Somatostatin administration to these rats prevented hyperinsulinemia and decreased blood pressure (Reaven et al., 1989). The observation that hyperinsulinemia and/or insulin resistance occur not only in untreated human hypertensives, but also in rodent models of hypertension and that preventing hyperinsulinemia via interventions like metformin, vanadyl sulphate and pioglitazone alleviates hypertension, strengthens our contention that these abnormalities are intrinsically linked with hypertension and are not mere coincidental findings.

As outlined earlier, hyperinsulinemia can lead to hypertension through three well documented mechanisms. Firstly, insulin and insulin like growth factors are mitogens capable of stimulating smooth muscle proliferation (Stout et al., 1975; Nako et al., 1985; King et al., 1985; Pfeifle and Ditschuneit, 1981; Sinha et al., 1989).
Therefore, hyperinsulinemia could result in vascular smooth muscle hypertrophy, narrowing of the lumen of resistance vessels and ultimately in the development of hypertension. With respect to the relationship between changes in vascular structure and hyperinsulinemia, Rocchini et al. (1988) demonstrated in obese adolescents that not only is obesity associated with the presence of structural changes in the forearm resistant vessels but also that these changes directly correlate with the degree of insulin resistance. In a group of obese adolescents they determined whether or not structural changes in the forearm resistance vessels were present by measuring forearm vascular resistance after 10 minutes of ischemic exercise. Obese individuals had significantly elevated minimum forearm vascular resistance and reduced maximal forearm blood flows.

The second method by which hyperinsulinemia/insulin resistance can lead to hypertension is with regard to insulin's ability to stimulate the sympathetic nervous system. This is an area which has long been emphasized by Landsberg and Young (Young et al., 1984; Landsberg and Young, 1978; Young et al., 1985; Young et al., 1982; Landsberg and Kriger, 1989; Rowe et al., 1981). They and others have documented that under conditions of euglycemic hyperinsulinemia, an activation of the sympathetic nervous system (SNS) can be observed in obese and normal humans and animals (Rocchini et al., 1990; Liang et al., 1982). The increase in the SNS activity is manifested as an increase in heart rate, blood pressure and plasma norepinephrine. More recently, Anderson et al. (1991) demonstrated that hyperinsulinemia in humans is not only associated with an increase in plasma catecholamines but also with an increase in sympathetic nerve activity. In addition, overfeeding with both carbohydrates and fat are associated with stimulation of the sympathetic nervous activity. Fisher rats fed a high fat diet develop obesity, hypertension and insulin resistance. When euglycemic insulin infusions are performed, increases in systolic blood pressure are seen in the fat-fed animals but not
in the control animals. As this blood pressure rise was reversible by combined alpha and beta-blockade, a role for increased sympathetic nervous activity was suggested (Assy et al., 1991). A recent cross-sectional epidemiological study demonstrated that abdominal obesity correlated with plasma insulin level and urinary epinephrine excretion (an index of sympathetic activity) (Landsberg et al., 1991).

The third proposed mechanism through which elevated insulin levels can lead to high blood pressure is via sodium and water retention. There is human and animal data which suggest that insulin resistance and/or hyperinsulinemia can result in chronic sodium retention (DeFronzo et al., 1975; Baum 1987). Insulin can enhance chronic renal sodium retention both directly, through its effects on renal tubules, and indirectly through stimulation of the sympathetic nervous system, augmenting angiotensin II mediated aldosterone production and by altering the secretion of atrial natriuretic peptide (Vierhapper et al., 1983). Rocchini et al. (1989) also showed in obese adolescents that insulin resistance and sodium sensitivity of blood pressure are directly related. They demonstrated that the blood pressure of obese adolescents is more dependent on dietary sodium intake than the blood pressure of non-obese individuals and that hyperinsulinemia and increased sympathetic nervous system activity appear to be responsible for the observed sodium sensitivity and hypertension. In addition, Antishin et al. (1990) demonstrated that the endogenous hyperinsulinemia that occurs in obese subjects following a glucose meal can result in urinary sodium retention. In that study, the investigators also showed that the obese adolescents who were the most sodium sensitive had significantly higher fasting insulin concentrations, higher glucose-stimulated insulin levels and greater urine sodium retention in response to an oral glucose load. There are animal data that suggest that insulin resistance may be in part responsible for the sodium retention associated with obesity induced hypertension. In a dog model of obesity-induced hypertension, Rocchini et al. (1987) demonstrated that during the first week of the high fat diet, the increase in sodium
retention appeared to best related to an increase in the plasma norepinephrine activity; whereas, during the latter weeks of the high fat diet, an increase in plasma insulin appeared to be the best predictor of sodium retention. Finally, Rocchini et al. (1990) recently demonstrated that the hypertension associated with weight gain in the dog occurs only if adequate salt is present in the diet. Thus, in both obese man and dog, insulin appears to play an important role in sodium retention.

Another suggested mechanism through which elevated insulin levels/insulin resistance can lead to hypertension is through alterations in cation transport. Insulin has been shown to affect both sodium and calcium transport, although controversy still exists regarding the molecular mechanism of this effect. A direct effect of insulin on sodium/hydrogen exchange has been demonstrated in vitro (Moore 1985; Lagadie-Grossman et al., 1988). Insulin has been reported to increase and decrease Na-K-ATPase activity (Tedde et al., 1988; Khadouri et al., 1987). Insulin has been linked with Na-Li countertransport and Na-K co-transport (Hunt et al., 1986).

The question that has been raised by several investigators is that if the above mentioned mechanisms are physiological actions of insulin, then in the face of insulin insensitivity/resistance shouldn't these actions be down-regulated? A view that is gaining acceptance is that of "selective insulin resistance". Selective insulin resistance implies that although an individual or animal may have an impaired ability of insulin to cause whole body glucose uptake, some of the other physiological actions of insulin may be preserved. With respect to hypertension, one of the potentially important actions of insulin is its ability to induce renal sodium retention (Rocchini et al., 1989). Rocchini et al. (Rocchini et al., 1989) recently demonstrated that obese adolescents have selective insulin resistance in that they are resistant with respect to glucose uptake yet are still sensitive to the renal sodium retaining effects of insulin.

It may be speculated that in the present study metformin caused a decrease in the plasma insulin level which via one or more of the above mechanisms led to a
decrease in blood pressure in both the SHR and fructose rat models. As this study aimed at establishing a role for insulin in the regulation of blood pressure, no mechanistic studies were performed to identify which pathway was affected by lowering insulin levels with metformin.

The association of hyperinsulinemia/insulin resistance and hypertension is well established but the causal relationship remains unclear. Basically there are three possibilities: firstly, hypertension leads to insulin resistance and hyperinsulinemia, secondly, insulin resistance and hyperinsulinemia lead to hypertension and lastly, both insulin resistance/hyperinsulinemia co-exist in the same individual but are not related to each other (Ulf 1991).

The first relationship seems unlikely since there is evidence that hypertension caused by renovascular disorders is not associated with insulin resistance (Marigliano et al., 1990) Furthermore, treating hypertension with most commonly used antihypertensive agents does not improve insulin sensitivity, but if anything leads to a further deterioration (Review Lithell, 1991).

In the context of the second relationship, many studies have shown a correlation between elevated insulin levels and blood pressure. In most studies, this remains even when concomitant obesity or other confounding factors are accounted for. However it also clear that not all individuals with hypertension are hyperinsulinemic or that all individuals with hyperinsulinemia are hypertensive. For instance there is evidence that patients with an insulinoma do not develop hypertension to an excessive extent (Tsutsu et al., 1990). A possibility is that only certain individuals are genetically susceptible to the effects of hyperinsulinemia. This could account for the increased prevalence of hypertension in other insulin-resistant states such as obesity and impaired glucose tolerance. Thus, any factor leading to insulin resistance and hyperinsulinemia would then lead to hypertension in susceptible
individuals. Such a concept could also account for the correlation seen between blood pressure and ambient insulin levels (Ulf, 1991).

The third possibility that insulin resistance and hypertension co-exist but are unrelated to each other cannot be excluded. However, the consistent relationship and the improvement of both factors by weight reduction, exercise and agents that improve insulin sensitivity (metformin) make it less likely (Landin et al., 1991).

Further studies addressing these three possibilities need to be carried out to establish a cause-effect relationship of insulin and hypertension and to define the mechanisms and pathways which are involved. It is likely that as our knowledge of the importance of insulin resistance/hyperinsulinemia in hypertension increases there will be new possibilities to treat both the blood pressure elevation and the many important risk factors linked to hypertension and coronary artery disease.
CONCLUSIONS

1. Induction of diabetes with STZ resulted in hyperglycemia, hypoinsulinemia, elevated triglycerides and depressed cardiac function when compared to age matched non-diabetic rats.

2. Treatment of the diabetic rats with metformin hydrochloride at a dose of 650 mg/kg/day improved glucose homeostasis without an increase in the plasma insulin level, decreased the elevated triglycerides and improved isolated cardiac function assessed under conditions of increasing preload in terms of +dP/dt, -dP/dt, LVDP and T₁/₂R. Metformin significantly decreased the plasma insulin level in the control animals. The treatment had no effect on the heart function of control animals.

3. SHR rats exhibited hyperinsulinemia when compared to their genetic controls, the WKY. The hyperinsulinemia was persisted throughout the experiment.

4. Paralleling the elevation in plasma insulin, the SHR exhibited full blown systolic hypertension by nine weeks of age when compared to the WKY. This effect was also sustained throughout the experiment.

5. Metformin treatment of the SHR prevented the development of hyperinsulinemia and alleviated hypertension when compared to untreated SHR.
6. Artificially raising plasma insulin by insulin-implants caused a marked elevation in plasma insulin level in the SHRM group. This was accompanied by an increase in systolic blood pressure.

7. Fructose feeding of Sprague Dawley rats caused hyperinsulinemia by week nine when compared to control rats. As with the previous experiment, the hyperinsulinemia was sustained and maintained over the experimental period.

8. This dietary manipulation also caused an increase in systolic blood pressure by approximately 20-25 mm Hg. This effect was seen throughout the experiment.

9. Pre-treatment of Sprague Dawley rats with metformin prior to the administration of fructose completely prevented the hyperinsulinemia and rise in blood pressure when compared to untreated fructose fed rats.


Bhanot S and McNeill JH (1993) Bis (maltolato) oxo vanadium (IV) lowers plasma insulin and blood pressure in spontaneously hypertensive rats (abstract). *Proceedings Endocrine Society Annual Meeting* p 96 (104)


Prager R and Schernthaner G (1983) Insulin receptor binding to monocytes,insulin secretion and glucose tolerance following metformin treatment. *Diabetes* **32**:1083-1086.


97


