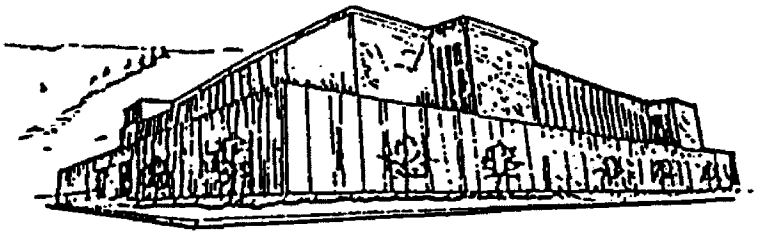
1994

Role of insulin resistance in cardiovascular abnormalities associated with non-insulin-dependent diabetes mellitus

Bernadine Heather Fraser
The University of Montana



Maureen and Mike MANSFIELD LIBRARY

The University of Montana

ROLE OF INSULIN RESISTANCE IN CARDIOVASCULAR ABNORMALITIES ASSOCIATED WITH NON-INSULIN-DEPENDENT DIABETES MELLITUS.

By

Bernadine Heather Fraser

B.Sc., University of British Columbia, Vancouver, B.C., Canada, 1990

Presented in partial fulfillment of the requirements for the

Degree of Master of Science

The University of Montana

1994

Approved by:

Vernon R. Grund

Chairman, Board of Examiners

Dean, Graduate School

September 13, 1994

Date

Bernadine Heather Fraser, M. S., September 1994
Department of Pharmaceutical Sciences

Role of Insulin Resistance in Cardiovascular Abnormalities Associated with Non-Insulin Dependent Diabetes Mellitus (108 pp).

Director: Vernon R. Grund, Ph. D. VRG

Non-insulin dependent diabetes mellitus (NIDDM) is often associated with hyperinsulinemia and insulin resistance. Since there is little information about cardiovascular abnormalities in NIDDM, this study was conducted to evaluate the potential role of insulin resistance in two new animal models of insulin resistance, the young Zucker Diabetic Fatty (ZDF) and the Hybrid Zucker Diabetic Fatty/Spontaneously Hypertensive Heart Model (ZDF/SHHF). To evaluate the potential role of insulin resistance in cardiovascular abnormalities, the thiazolidinedione, pioglitazone (10 mg/kg/day p.o.), was used. This drug significantly decreased plasma insulin, glucose, triglycerides and cholesterol in young and hybrid diabetic rats.

Pioglitazone improved the insulin resistance in the two NIDDM models. Concurrently, there was impaired cardiac performance in young ZDF diabetic rats relative to non-diabetic rats; however, there was no impaired cardiac performance in untreated diabetic rats relative to untreated non-diabetic rats as seen in IDDM models. Pioglitazone also significantly increased body weights and heart weights, a potentially detrimental occurrence in the NIDDM rats. The increased heart weights seen in the pioglitazone treated diabetic rats may be responsible for the impaired cardiac performance at the lower end of the preload scale. Pioglitazone was able to decrease blood pressure in young hybrid diabetic rats relative to non-diabetic rats even though this diabetic rat model did not show elevated diabetic blood pressures. Correcting hyperinsulinemia with pioglitazone may be responsible for this drug's effects observed in the young ZDF and young hybrid ZDF/SHHF rats.

Vanadyl sulfate (0.75 mg/ml) resulted in decreased plasma glucose and triglycerides in young hybrid ZDF/SHHF diabetic rats without lowering plasma insulin or modifying cardiovascular parameters. Vanadyl sulfate also lowered plasma glucose without affecting plasma triglycerides and cholesterol in the IDDM model (older ZDF).

Hydralazine (5 mg/kg b.i.d.) did not significantly affect hyperinsulinemic young hybrid ZDF/SHHF rats except to decrease body weights increase heart weight to body weight ratios, and decreased total cholesterol and HDL cholesterol ratios. Hydralazine improved the rate of relaxation in hybrid ZDF/SHHF diabetic rats.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr. V. R. Grund, for his patience, guidance and generosity (including financial support). I am particularly indebted to Dr. Grund because he had faith in my abilities as a graduate student when others did not, particularly myself. He has also seen me through many difficult times in my life and has helped me grow as a student and a person.

I would also like to thank Dr. J. R. Smith for sharing his immense knowledge, friendship and ideas with me. His help with learning new techniques was greatly appreciated. Thank you also goes to the other members of my committee, Dr. C. A. Johnston and Dr. G. L. Card. I have great respect for the knowledge these men possess and appreciate the help and advice they gave me.

Special thanks go to all those who helped me over the years to accomplish my goals. These people include Dean J.H. McNeill, V. Yuen, A. Scarim, E. Olson, M. Cunningham, M. Gattu, D. Murphy, R. Snijder, D. Bednarczyk and K. Harrison. These people have contributed in their own way, mostly through their dedication and expert assistance with my projects over the years. I also extend "kudos" to the entire faculty and staff of the School of Pharmacy. All have been kind, generous and a pleasure to work with. These wishes also extend to the faculty of the Department of Biological Sciences who worked closely with me during my stay at The University of Montana.

Finally, but most importantly, I thank my parents (Gail Mair and James Fraser) and my brother (Danny) for their support, both emotionally and financially. Their encouragement, guidance and love have made me who I am today. I love them dearly.

1.0 INTRODUCTION

Diabetes mellitus, hypertension, hyperlipidemia, and hyperinsulinemia separately are believed to have deleterious effects on the heart. Together, diabetes, hypertension and hyperlipidemia have been shown to have cumulative deleterious effects in insulin-dependent diabetes mellitus (IDDM) as measured in the working heart model. This effect was also seen in conscious deoxycorticosterone treated hypertensive diabetic rats (Schenk and McNeill, 1991). In non-insulin dependent diabetic (NIDDM) patients, many or all of these phenomena may be present and it is speculated that hyperinsulinemia or insulin resistance may be associated with one or more of these clinical aspects. The overall purpose of this study was to examine the potential role of insulin resistance (a key factor of NIDDM) in the development of hypertension or other cardiovascular complications of diabetes.

Several animal models of NIDDM involving insulin resistance have recently been developed. These animals have in common the fact that they are obese and have elevated lipids. The purpose of this study was to evaluate whether or not drugs capable of reducing insulin resistance have the potential to reduce cardiovascular complications of diabetes. Three drugs (pioglitazone, vanadyl and hydralazine) were evaluated in two animal models of NIDDM. The Zucker diabetic fatty (ZDF) rat was selected as the first NIDDM-insulin resistant model. In the early stages of these animals' lives, they are hyperinsulinemic, insulin resistant, hypertriglyceridemic and hyperglycemic, closely resembling human NIDDM. At a later stage in these rats' development, they become hypoinsulinemic and more accurately resemble IDDM. These animals remain hyperglycemic and hypertriglyceridemic whether in the hyperinsulinemic phase

or in the hypoinsulinemic phase. The second animal model used was a hybrid rat model. These ZDF-SHHF hybrid rats are a cross between the non-hypertensive Zucker diabetic fatty (ZDF/Drt-*fa*) rat and the hypertensive SHHF/Mcc-*cp* rat. The hybrid rats are obese, insulin-resistant, hypertriglyceridemic and a good rat model of NIDDM. Using their lean litter mates as controls, the three drugs were used to evaluate their ability to improve the diabetic and cardiovascular state.

1.1 Diabetes Mellitus

Diabetes is one of the world's most common diseases. There are several forms of diabetes mellitus, including: type I, which is insulin-dependent (IDDM); type II, which is non-insulin-dependent (NIDDM); and gestational diabetes. Gestational diabetes is observed as impaired glucose tolerance during pregnancy (Brisco, 1993).

Characteristically, IDDM patients are usually not overweight, are variably aged, have a rapid onset of the disease and require insulin therapy to stay alive. Characteristics shared among IDDM and NIDDM include hyperglycemia, polyuria, polydipsia and polyphagia. Most diabetics, if not properly controlled, also exhibit hyperlipidemia. Patients with IDDM release inadequate pancreatic insulin. Insulin is produced and secreted by β (beta) cells in the Islets of Langerhans. It is believed that IDDM occurs as a result of an autoimmune attack on these islet cells resulting in the destruction of β cells. In this scenario, autoreactive T lymphocytes initiate and oversee β cell specific immune destruction (Eisenberth, 1986).

The key to treating this type of diabetes mellitus is insulin therapy. Insulin comes in many formulations and preparations. Patients self-monitor their blood glucose and administer their insulin subcutaneously on a daily basis. IDDM accounts for approximately 5 to 10 percent of all diabetic patients in the United States (ADA, 1991). Recently, a strong association between IDDM and certain genetic markers (HLA DR3 and DR4) has been found; however, to date, most individuals with these genetic markers do not develop diabetes. This makes predicting those who might become diabetic difficult. Some studies are being conducted to look at treating potential diabetics, based on presence of these genetic markers, or insulin antibodies with immunosuppressants such as cyclosporine and azathioprine to prevent or delay the onset of the disease.

NIDDM, on the other hand, is characterized by obesity (usually), hyperglycemia and insulin resistance (decreased tissue sensitivity or responsiveness to insulin). This form of diabetes mellitus accounts for 90 to 95 percent of the diabetics in the United States, approximately 12 million cases. Patients with NIDDM have a functioning pancreas and produce some insulin, but it is either insufficient in quantity or ineffective at the tissue level. This form of the disease can be asymptomatic for many years, has a slow progression, and is usually inherited.

The 1991 Diabetes Vital Statistics handbook lists several major risk factors for NIDDM: a family history of diabetes; obesity (body weight, due to fat, above 20% ideal); race (Native American, Hispanic or African American); age older than 40; previous identification of impaired glucose tolerance; hypertension or significant hyperlipidemia; and a history of gestational diabetes mellitus or delivery of a baby over 9 pounds. Of these risk factors, obesity is the most

important. A 3-fold higher incidence of NIDDM occurs in patients 40 % above desirable weight compared to patients of normal body weight (ADA, 1991). There are many drug therapies for NIDDM. For details see section 1.5.

1.2 Complications

There are many complications of diabetes mellitus, both acute and chronic. Chronic complications include cardiovascular disease, retinopathy, nephropathy and neuropathy. Acute complications include diabetic ketoacidosis, hyperglycemic coma, and infections. Hypoglycemic episodes due to overdoses of insulin or oral hypoglycemics are another complication.

Cardiovascular disease (CVD) is a term used to describe a group of diseases that affect the heart and blood vessels. Diseases in this group include strokes, ischemic heart diseases such as angina and coronary artery disease (atherosclerosis), peripheral vascular disease, ventricular hypertrophy, cardiomyopathy and heart failure. CVD is tremendously more likely in diabetic patients than the general population. In the United States, diabetics are 2-4 times more prone to die from heart disease than the non-diabetic population. Evidence of coronary heart disease is present in 7.5 to 16.6 percent of diabetics older than 45 years of age (ADA, 1991). Within the diabetic population, cardiovascular disease accounts for two-thirds of the total deaths. Cardiovascular disease is more common in NIDDM than IDDM, mostly because of the age of the population. As IDDM patients age, the risk increases, but the primary cause of death in IDDM is kidney failure. IDDM patients also represent a large portion of the kidney transplant patients today. Risk factors for cardiovascular disease include hypertension, high blood-cholesterol (low

density lipoprotein, LDL, and very low density lipoprotein, VLDL) with low high density lipoprotein (HDL) levels and cigarette smoking. Diabetes is itself a risk factor for atherosclerotic cardiovascular disease, increasing the risk two- to threefold (ADA, 1991). Hypertension is reported in 50% of the adult diabetic population compared to 25% of non-diabetics of the same age.

Lipoproteins are a group of molecules that transport cholesterol throughout the blood stream. There are four main types of lipoproteins. Chylomicrons are the largest of the lipoproteins and are rich in triglycerides and transport dietary fat and cholesterol from the intestine to the liver. Manufactured in the liver, VLDLs are also rich in triglyceride and cholesterol, but transport the endogenous forms of these lipids. On capillary surfaces, VLDLs and chylomicrons interact with lipoprotein lipase causing their triglycerides to be hydrolyzed, producing remnants called intermediate density lipoproteins (IDL) which are believed to be atherogenic. When VLDLs are metabolized, they form another lipoprotein, the LDL. This lipoprotein is high in cholesterol and is referred to as bad cholesterol and has a high association with coronary heart disease (Howard and Howard, 1994). LDL transport cholesterol from the liver to the peripheral tissues. HDL, good cholesterol, acquires free cholesterol from peripheral tissues and is esterified by the enzyme lecithin:cholesterol acyltransferase (LCAT) to cholesteryl ester which may be transferred to LDL, IDL or VLDL by the cholesteryl ester transfer protein (CEPT). Chylomicrons can transform into HDL with the transfer of apolipoproteins. One of the consequences of elevated lipoproteins is atherosclerosis. In excess, LDLs begin to deposit in artery walls accumulate and eventually reduce and possibly totally occlude, blood flow. This is atherogenesis. LDL attaches to damaged endothelium and activated macrophages and begins to transform macrophages into foam cells with

cholesterol accumulation. After a period of time, these foam cells become calcified and form a plaque. Diabetics, who have excess plasma glucose, also run the risk of glycosylating and oxidizing their LDLs. This form of LDL is more likely to be found in atherosclerotic lesions. Oxidized LDL may also recruit smooth muscle cells from the media to the intima and injure proliferating cells, leading to accumulation of dead cells (Autio et al., 1990). Hypertriglyceridemia has also been shown to induce lipid peroxidation and ultimately aid in destruction of cells in areas of atherosclerotic lesions (Hiramatsu et al., 1988).

Secondary hyperlipidemia, not attributable to genetics, is often a result of metabolic disorders such as diabetes or hypothyroidism, although there are many other causes of this condition such as alcoholism. Hyperlipidemia is a condition where VLDL and LDL levels are elevated (greater than 160 mg/dl) and HDL levels are reduced below 35 mg/dl. Generally, untreated NIDDM patients have normal to slightly reduced lipoprotein lipase (LPL) levels, which some have shown to increase with insulin treatment (Taskinen, 1987). NIDDM patients typically have fasting hypertriglyceridemia and low HDL. The difficulty in treating these patients is that they are usually obese and insulin resistant. It has even been noted that with tight control of NIDDM, elevated lipids sometimes do not decrease. Thus, any treatment strategy which can control hyperglycemia, reduce the elevated triglycerides and LDL cholesterol, and increase HDL cholesterol and LPL levels would be a benefit to the NIDDM patient.

Myocardial infarction and congestive heart failure occur at a much higher rate in diabetic populations (Jaffe, 1989). Reduced diastolic filling rate along with prolonged isovolumic relaxation has been observed in diabetes (Pozzoli et al.,

1984). There are many theories explaining cardiovascular abnormalities in the diabetic population, including: deterioration of the microcirculation (Friedmann, 1989), abnormalities in the conduction system (Garber and Neely, 1983), abnormalities in the myocardium such as depressed myocardial metabolism (Fevury et al., 1979), impaired tissue antioxidant status (Wohaieb and Godin, 1987), and decreased sarcolemmal enzyme activity (Pierce et al., 1983). The exact mechanism causing or initiating diabetic cardiomyopathy is unknown; however, intracellular calcium is believed to be crucial (Pierce, 1988; Weir, 1990, Ganguly et al, 1983). It is known that the calcium pump of the sarcoplasmic reticulum, which controls calcium removal in normal tissue, is defective in diabetic tissue, as evidenced by decreased ATP-dependent calcium transport and calcium stimulated ATPase activity (Ganguly et al., 1983). Calcium regulation is impaired by other mechanisms as well, including: decreased Na^+-K^+ (Pierce et al, 1990), Na^+-H^+ (Pierce et al, 1990), and Na^+-Ca^{++} exchange processes (Makino et al., 1987), and inhibition of Ca ATPase (Heyliger et al., 1987).

Cardiac performance abnormalities have been shown in many drug induced and genetic animal models of type I diabetes. There is very little evidence suggesting cardiovascular problems with type II models of diabetes. A standard method of determining cardiac performance is the working heart model. This procedure allows one to test the ability of the heart to respond to changes in preload. Transducers measure maximum and minimum pressures in the heart and heart rate. With a computer program, left ventricular developed pressure (LVDP), rate of contraction (+dP/dT), and rate of relaxation (-dP/dT) can be measured directly in a heart that is perfused through the pulmonary vein and out the aorta.

Much recent evidence suggests that tight control of blood glucose and the elimination of as many risk factors as possible can delay, if not prevent, the development of these diabetic complications. Appropriate lifestyle changes are often sufficient to eliminate the signs and symptoms of NIDDM. In fact, NIDDM patients are first treated with education and lifestyle changes including weight loss and exercise. Only as a last resort are patients with NIDDM treated with drug therapy.

1.3 Insulin

Among the important historical discoveries with insulin are the clinical description of the disease in 20 A.D., the discovery of pancreatic islets by Langerhans in 1869, the discovery of insulin in 1921 by Banting and Best, the isolation and crystalization of insulin by Abel in 1927 and the synthesis of recombinant human insulin in E. coli in 1979.

Insulin is a peptide hormone manufactured and released from the pancreas. Insulin mRNA is transcribed in the nucleus of the β islet cell. After being modified, the mRNA leaves the nucleus and begins the translation process on the rough endoplasmic reticulum. Here, a single chain of pre-pro-insulin is made. This is also where the pre (24 amino acids) segment is cleaved. The pro insulin is released in microvesicles which are taken up into Golgi where packaging into granules occurs. In the granules three disulfide bonds form between cys residues and the C-peptide segment (86 amino acids) is cleaved from the pro insulin leaving equal amounts of C-peptide and insulin which are secreted into the portal blood. Insulin exists as an A chain (20 amino acids) and

a B chain (30 amino acids) linked by two disulfide bonds with an intra-A disulfide bond. It is released in response to as little as a 10 mg/dl increase in plasma glucose levels as well as by amino acids, free fatty acids, glucagon, and other less potent secretagogues and sympathetic nervous stimulation (β adrenergic, epinephrine).

Insulin is released from the β cells into the blood where it binds to membrane bound receptors at target tissues and facilitates glucose uptake into the tissues where it can be used. If glucose cannot be used, the body relies on free fatty acids and amino acids as precursors. This can lead to muscle wasting, weight loss and ketoacidosis. Insulin's actions are anabolic and anti-ketogenic and favor the storage of fat, protein and carbohydrate. Insulin decreases glycogenolysis, lipolysis, proteolysis and gluconeogenesis. Counter-regulatory hormones to insulin include glucagon, epinephrine, growth hormone and cortisol. Fifty percent of endogenous insulin is removed by the liver with most of the remaining insulin excreted by the kidneys.

The insulin receptor consists of 4 protein subunits, 2 α and 2 β. It is proposed that 2 insulins bind to the 2 α-subunits of the insulin receptor resulting in autophosphorylation of tyrosine sites on the 2 β-subunits. The tyrosine kinase activity catalyzes the phosphorylation of serine kinases or phosphoprotein phosphatases, which phosphorylate or dephosphorylate target enzymes resulting in the alteration of metabolic pathways typical of insulin action. In contrast, the glucose transporter is not phosphorylated in response to insulin and the transporter is translocated to the plasma membrane to take up glucose. Cellularly, insulin secretion is stimulated when intracellular calcium concentrations increase due to depolarization of a resting cell. Insulin clears

glucose from the blood by preventing the liver from releasing additional glucose and by enhancing glucose transport into muscle and fat cells.

There are 5 known glucose transporters. They are termed GLUT 1-5. These different transporters are located in different tissues and differ in terms of the glucose requirements of each tissue. Some transporters are found in the same tissue. GLUT 1 and GLUT 3 transporters are found in most tissues; whereas GLUT 4 is found primarily in muscle and fat; GLUT 2 is found in liver; and GLUT 5 is found in the small intestine and kidney. There is a 20 fold increase in glucose transporters when muscle and fat are exposed to insulin. This involves recruitment of transporters from the cytoplasm to the surface and increases in the maximum transport capacity of those already there. To enter the cell, glucose first occupies its binding site on the outside of the cell. Next, the glucose molecule with its transporter change conformation so that the glucose now occupies the binding site that faces into the cell. The transporter then releases the glucose into the cytoplasm and then changes its conformation to face outward again (Lienhard et al., 1992).

1.4 Insulin Resistance

Insulin resistance occurs when the pancreas makes insulin, but for some reason the insulin is not very effective at promoting the transport of glucose from the blood into the cells of the body. There are many who believe that insulin resistance is due to a specific insulin-receptor gene defect. Some believe that this insulin-receptor mutation contributes to insulin resistance in a sub-population of NIDDM patients (O'Rahilly et al., 1991; Cocozza et al., 1992; Kim et al., 1992). Insulin resistance has also been implicated in a number of other

disorders such as obesity, essential hypertension, lipid abnormalities and atherosclerotic cardiovascular disease (Reaven, 1988; DeFronzo and Ferranninni, 1991). Although genetic defects have been implicated in some cases of insulin resistance, it is believed that most insulin resistance occurs at the postreceptor level (Clauser et al., 1992).

There are three types of insulin resistance: prereceptor (involving insulin structure, degradation or function), receptor defects (defects of insulin receptors at the surface of cells) and postreceptor insulin resistance (Kahn, 1985). Mechanisms for prereceptor insulin resistance include mutation of the insulin gene, increased degradation of insulin and formation of anti-insulin antibodies which block insulin's action. Defects in insulin receptors include decreased numbers of receptors, decreased affinity of the receptor for insulin or defective tyrosine kinase.

There are currently several animal models used to study insulin resistance. The first animal used to study this phenomenon was the mouse. These mice are obese and diabetic. There are three strains of mice including C57BL/6J obese (*ob*), KK mice and their hybrids, C57BL/Ks (*db*) and the NZO (New Zealand obese) mouse. Rats with concurrent diabetes and obesity include the Zucker fatty (*fa*), Diabetic fatty (ZDF/Drt-*fa*), Wistar-Kyoto diabetic and Wistar-Kyoto fatty. A newer strain of rat used to study diabetes is the corpulent SHR/N-*cp* with nephropathy, the SHHF/Mcc-cp with congestive heart failure, and the Jcr:LA-*cp* with ischaemic cardiovascular disease. Corpulent strains tend to be hypertensive diabetic rat strains. There are other more obscure models to study insulin resistance and/or other NIDDM-like syndromes. However, the models mentioned offer symptoms believed to most closely simulate human NIDDM.

Animal models of NIDDM are reviewed in two recent papers (Shafrir, 1992 and Bailey and Flatt, 1993).

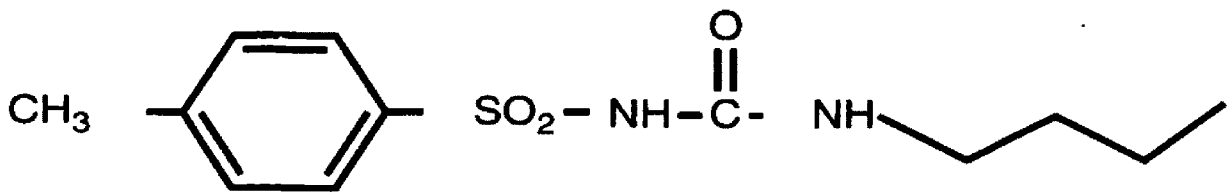
1.5 Drug Therapy for NIDDM

Most NIDDM patients can be treated successfully without drugs. Initial therapy is always diet, exercise and education. This is particularly true for the patient who is obese since insulin resistance can be caused by obesity. Weight loss and exercise are both associated with a decrease in insulin resistance. The primary aim in treating diabetes is to relieve symptoms, improve quality of life and prevent both acute and chronic complications (Rifkin, 1988). Hyperglycemia has been associated with chronic diabetic complications in both animal and human studies. However, this is not always the case. Some patients with poor control never develop complications (Pirart, 1978). Since insulin resistance and impaired insulin secretion are key factors in the pathogenesis of NIDDM, treatment should be directed at restoring intermediary metabolism towards normal by improving insulin secretion and reducing insulin resistance (Gerich, 1989).

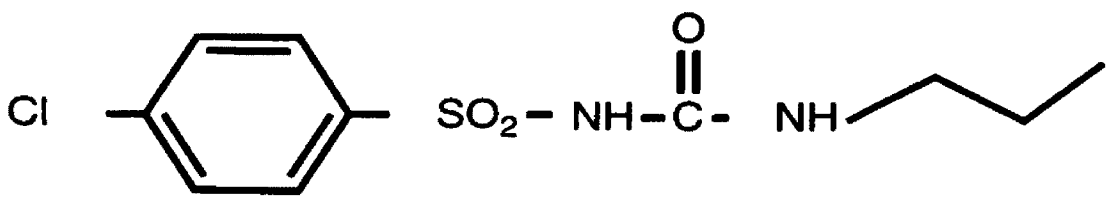
1.5.1 Sulfonylureas

There are five commercially available sulfonylureas in the United States. They all consist of a similar sulfonylurea core with different aromatic and alkyl substituents that produce pharmacokinetic and pharmacologic differences. The sulfonamide group is essential for hypoglycemic action (Loubatieres, 1944). This class of drugs is further subdivided. The first generation drugs, discovered in the 1960's, consist of tolbutamide, tolazamide and chlorpropamide. The

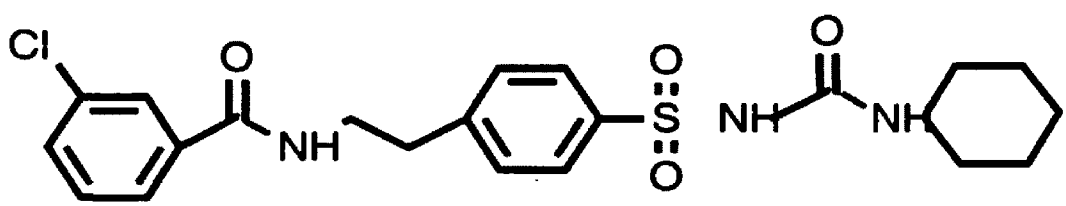
second generation drugs, discovered in the 1970's, consists of glipizide and glyburide. Three quarters of the hypoglycemic market from 1964 to 1986 was accounted for by three drugs: chlorpropamide, glyburide and glipizide (Kennedy et al., 1988). Tolbutamide causes the fewest serious side effects of all the sulfonylureas; however, it is the shortest-acting and least potent. Chlorpropamide is the longest acting and causes the most serious side effects, among which hypoglycemia and severe hyponatremia are the most significant. Glipizide and glyburide, the most potent of the available agents, are 100 to 150 times more potent than tolbutamide.



TOLBUTAMIDE



CHLORPROPAMIDE



GLYBURIDE

Sulfonylureas stimulate the release of insulin by increasing beta-cell sensitivity to glucose so that more insulin is released at every glucose level, but they do not increase the synthesis of insulin (Grodsky et al., 1977). It appears that all of the sulfonylureas have a similar mechanism of action. A sulfonylurea receptor has been found on the β cell and may be closely linked to or part of an ATP-sensitive potassium-ion channel. Inhibition of the efflux of potassium ions by sulfonylureas may lead to depolarization of the β-cell membrane and the entry of calcium which activates protein kinase C associated with exocrine secretory granules (Oberwetter et al., 1987; Zawalich et al., 1988) resulting in exocytosis of insulin-containing secretory granules. These drugs are ineffective in patients without functional β-cells (IDDM); however, some patients will experience decreases in glucose concentrations despite unchanging insulin concentrations, indicating an increase in insulin receptor binding or insulin tissue sensitivity.

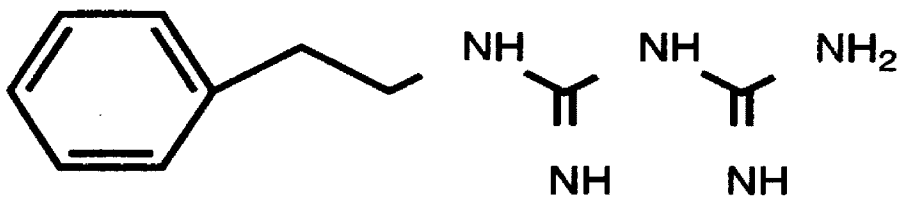
Along with decreased plasma glucose, sulfonylureas are known to decrease total plasma cholesterol, total triglyceride, and VLDL triglyceride and cholesterol with either no change (Taskinen et al., 1986) or a modest increase (Moorehouse, 1967) in HDL levels.

One of the problems with the use of sulfonylureas is the possibility of hypoglycemic episodes; another is that their use has not been associated with a decrease in cardiovascular complications of NIDDM. An additional problem is that approximately one third of NIDDM patients do not at first achieve satisfactory glycemic control with these drugs. The main reason for the failure is lack of dietary compliance (Melander, 1988) and severely impaired islet β-cell function (Rendell, 1983). Five to ten percent of those patients achieving

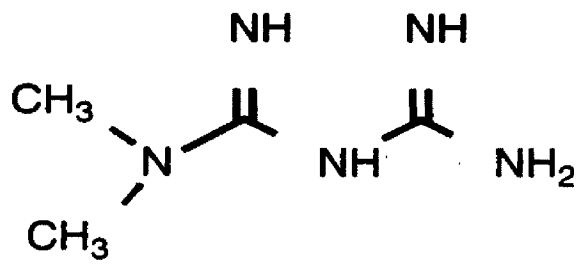
glycemic control have secondary failure after 10 years of treatment with these drugs. Other common side effects include gastrointestinal, hematologic, and dermatologic reactions in less than 2 percent of patients.

1.5.2 Biguanides

Buformin, phenformin and metformin comprise this chemical class of drugs and are not marketed in the United States. Metformin is widely used in Europe and Canada, where it accounts for 25 percent of the prescriptions for oral hypoglycemic agents (Bailey and Nattrass, 1988). These compounds are different from first generation sulfonylureas in that they do not undergo biotransformation, are eliminated solely by the kidney and are not bound to plasma proteins.



PHENFORMIN



METFORMIN

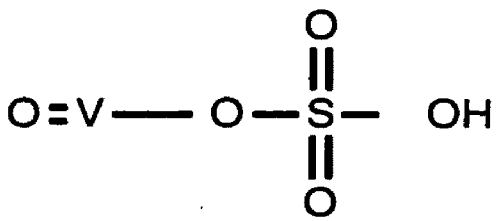
Biguanides contain two linked guanidine molecules. These compounds lower blood glucose concentrations by producing insulin-like effects on skeletal

muscle, liver, kidney and fat (Hermann, 1979), the exact mechanism of action being unknown. It is known that they suppress hepatic gluconeogenesis, stimulate glycolysis, and inhibit glucose absorption from the intestine (Jackson et al., 1987). These agents do not stimulate insulin secretion and are only effective in patients with some endogenous insulin. Biguanides are reported to not cause hypoglycemia. Interestingly, a recent report states that metformin improves cardiac function in isolated STZ-diabetic rat hearts (Verma and McNeill, 1994). Some reports suggest that these compounds cause lactic acidosis, but in the 20 years of Metformin use in Canada, there have been no cases of lactic acidosis reported (Vigneri and Goldfine, 1987). Phenformin, on the other hand, was discontinued in 1977 in the United States due to several deaths attributed to lactic acidosis.

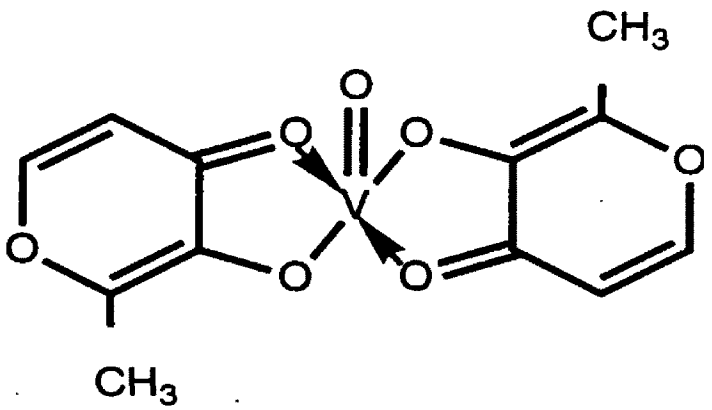
1.5.3 Insulin Sensitizers

It was previously indicated that one of the worst side effects of sulfonylurea use is severe hypoglycemia resulting from over-stimulation of insulin secretion. This suggests that the development of oral hypoglycemics which control hyperglycemia without stimulating insulin release would be an attractive alternative. A second pharmacotherapeutic option to treat NIDDM would be to restore tissue sensitivity to insulin. Two chemically distinct groups of agents have been synthesized which may accomplish this task, vanadyl sulfate (and its derivatives) and the thiazolidinediones. Insulin sensitizers are compounds that mimic the actions of insulin or increase tissue sensitivity to insulin. One such compound, vanadyl, has been shown to possess insulin-like properties in various cellular models such as isolated adipocytes, myocytes and hepatocytes (Tolman et al., 1979; Dubyak et al, 1980; Clark et al, 1985; Duckworth et al,

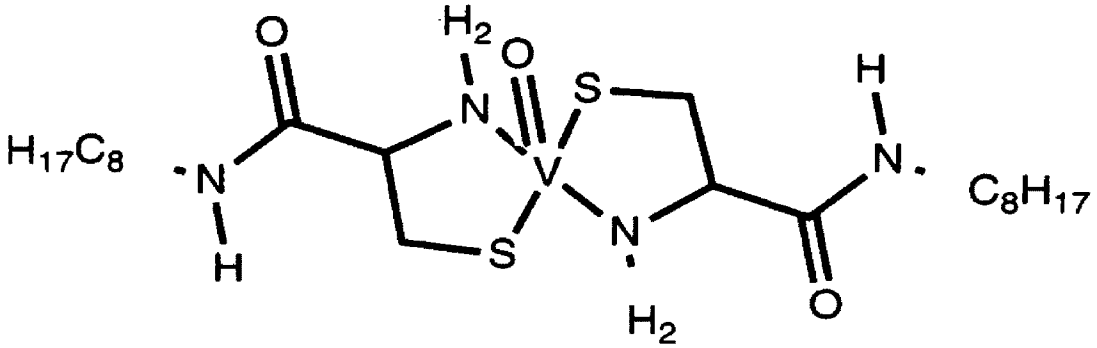
1988; Jackson et al., 1988; Gomez-Foix et al., 1988). This compound is able to stimulate glucose transport, inhibit glycogenolysis and inhibit lipolysis. Its mechanism of action is still unclear, but it has been suggested that it might work by inhibiting phosphotyrosyl-protein phosphatases (Swarup et al., 1982), inducting tyrosine phosphorylation (Tamura et al., 1984; Gherzi et al., 1988; Tracey et al., 1986), activating glucose transport through activation of the GLUT4 transporter gene (Strout et al., 1990), translocating the transporter from the cellular organelles to the plasma membrane (Paquet et al., 1990), or increaseing intracellular calcium secondary to Ca-Mg-ATPase inhibition (Delfert and McDonald, 1985).



VANADYL SULFATE



BIS(MALTOLATO)OXOVANADIUM (IV)



NAGLIVAN

Vanadyl's anti-diabetic activities were first demonstrated by Heyliger et al. in streptozotocin (STZ)-treated rats, a model of IDDM with some properties in common with NIDDM (1985). In these studies, 0.8 mg/ml sodium orthovanadate, administered in drinking water, was able to control hyperglycemia and prevent the impairment of cardiac function without any change in plasma insulin levels in diabetic rats, but decreased levels in control-treated rats. This suggests that insulin secretion is not related to the activity of vanadium, but that it replaces or potentiates endogenous insulin. It was interesting to discover that vanadate prevented insulin-resistance in STZ-induced diabetic rats (Blondel et al., 1989) and ob/ob genetically obese mice (Brichard et al., 1990) and corrected blood glucose in BB spontaneously diabetic (type I) rats (Ramanadham et al., 1990).

Vanadyl sulfate and other related compounds, bis(molatolato)oxovanadium (BMOV) and naglivan, work by a similar mechanism of action to reduce insulin resistance and restore euglycemia. BMOV reduces insulin resistance and normalizes plasma glucose levels in STZ-diabetic rats and improves the diabetic pathology observed in non-treated diabetic rats relative to non-diabetic rats (Dai et al., 1993). Similarly, vanadyl 's actions mimic insulin by increasing glucose transport in adipose and muscle tissues, increasing glucose

metabolism, activating glycogen synthesis and inhibiting the breakdown of triglycerides. In addition, it has been shown that vanadyl can reduce non-diabetic plasma insulin levels without affecting plasma glucose. This implies that vanadyl works by potentiating insulin's action (Ramanadham et al., 1989).

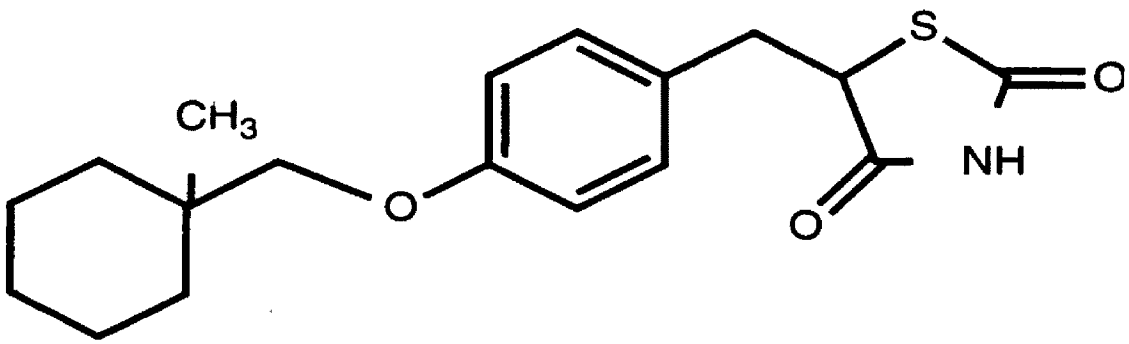
Vanadium has some reported toxicity, particularly digestive intolerance (diarrhea) leading to dehydration and death of animals. Vanadyl sulfate has fewer toxicity problems. This compound exhibits the same glucose lowering effects and after chronic treatment, the effects can still be seen 13 weeks after cessation of treatment (Ramanadham et al., 1989). It is doubtful that this is due to accumulation in tissues since the blood and kidney levels (main depot sites) were barely detectable (Ramanadham et al., 1989).

Given vanadyl's effectiveness at lowering plasma glucose and reversing insulin resistance, it has been proposed as a potential therapy in human diabetes in general, and in insulin-resistant states in particular (Cros et al., 1992). Vanadyl has the ability to prevent the myocardial and metabolic abnormalities seen in diabetic rats during treatment as well as 13 weeks after withdrawal from vanadyl (Ramanadhan et al., 1989). It has been suggested that this effect is due to normalization of plasma insulin levels and that insulin has a positive inotropic effect in the heart (Ramanadhan et al., 1989).

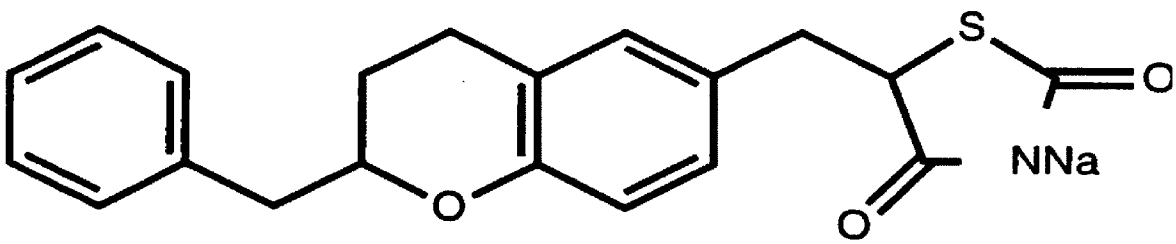
1.5.4 Thiazolidinediones

The most recent chemical class of oral hypoglycemics to be introduced is the thiazolidinediones (5-(4-alkoxybenzyl)-2,4-thiazolidinediones). These compounds appear to reduce insulin resistance or potentiate insulin action in

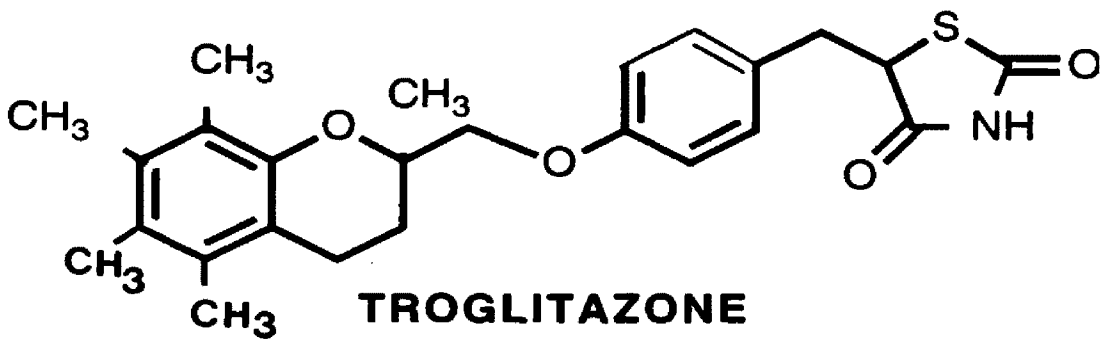
genetically diabetic and/or obese animals (Sohda et al., 1992), but do not induce hypoglycemia. The presence of a pyridyl ring on the p-alkoxy chain of the benzyl moiety is necessary for biological activity. Another important factor for activity is the location of the pyridine nitrogen α to the oxyethyl chain (Sohda et al., 1992). It has also been reported that the 4-oxybenzyl group and the 2,4-thiazolidinedione moiety are necessary for hypoglycemic and hypolipidemic activities (Momose et al., 1991), respectfully.



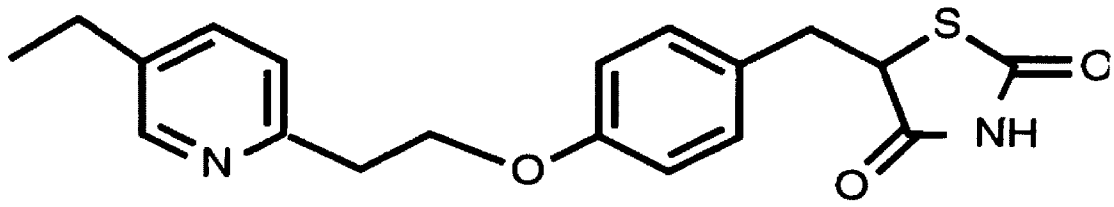
CIGLITAZONE



ENGLITAZONE



TROGLITAZONE



PIOGLITAZONE

Given the known effects of this class of drugs, it appears that the primary effect of these drugs is to improve peripheral target tissue responses to insulin. It has been suggested that these effects are due to enhanced post-binding events mediating insulin action (Hofmann and Colca, 1992). One example is the demonstration of increased GLUT 4 glucose transporter expression. This was observed in epididymal fat pads of insulin resistant KKAY mice after treatment with pioglitazone (Hofmann et al., 1991). This effect was seen only in animals where insulin was present (NIDDM) (Hofmann et al., 1991), supporting the hypothesis of an amplified insulin signal.

The first of these compounds to be synthesized in large quantities was **ciglitazone**, 5-[4-(1-methylcyclohexylmethoxy)benzyl]thiazolidine-2,4-dione, (AD-3878, U-63,287). It was discovered by scientists at Takeda Chemical Industries, Ltd., of Osaka, Japan, (Sohda et al., 1982; Fujita et al., 1983). This compound normalizes hyperglycemia, hyperinsulinemia and hypertriglyceridemia in various animal models of insulin resistance (Fugita et al., 1983; Chang et al., 1983) including KKAY mice (Ikeda et al., 1990; Fujiwara et al., 1988), Wistar Fatty rats (Ikeda et al., 1981) and ob/ob mice (Fujiwara et al., 1988; Stevenson et al., 1990). This compound requires a dose of 100 mg/kg/day to lower glucose in ob/ob and db/db mice (Chang et al., 1983).

Troglitazone, (CS-045), [(±)-5-[4-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl-methoxy)benzyl]-2,4-thiazolidinedione] is another compound in the thiazolidinedione group. In animal experiments using insulin resistant Zucker Fatty rats, it was determined that 150 mg/day p.o. of this compound improved glucose tolerance and decreased insulin levels after oral glucose loading due to increasing insulin responsiveness (Fujiwara et al., 1988). It was also demonstrated that troglitazone increased insulin binding, increased insulin receptor numbers in adipocytes and enhanced both insulin sensitivity and responsiveness (Fujiwara et al., 1988). Along with these effects, troglitazone decreased plasma lactate, free fatty acids and ketone body levels without decreasing food intake or body weight (Fujiwara et al., 1988). This is an important finding since it has been suggested that body weight loss improves glucose tolerance and that this is what causes the glycemic improvements. In all studies conducted with this compound, mean body weight did not change suggesting that improved glucose tolerance is not due to body weight loss in patients.

In the first clinical study with troglitazone, 204 patients were given either 200 or 400 mg per day either alone or in combination with sulfonylureas. Kuzuya and coworkers were able to find a dose-dependent glucose lowering effect with 25% efficacy with 200 mg/day and 46% efficacy with 400 mg/day (Kuzuya et al., 1991). They also found that the drug was as effective with or without the addition of sulfonylureas. This suggests that troglitazone works by a different mechanism than that of the sulfonylureas. These researchers found some adverse effects associated with the drug including gastrointestinal problems (6%), decreased red blood cell count (3.5%) and increased LDH levels (6%)

(Kuzuya et al., 1991). In other clinical studies using the same 400 mg/day dose, it was determined that hemoglobin A_{1c} was also decreased (Iwamoto et al., 1991) and that the use of this drug was associated with significantly increased HDL-cholesterol levels (Suter et al., 1992). Suter was able to determine that the increased HDL is comparable to that achieved with lipid-lowering drugs in hyperlipidemic patients (Frick et al., 1987). The inverse relationship between plasma HDL-cholesterol concentrations and cardiovascular risk is well established; this compound would be useful in those NIDDM patients at high risk of developing cardiovascular complications.

Englitazone (CP-68722), [(±)-5-[(3,4-dihydro-2-phenylmethyl-2H-1-benzopyran-6-yl)methyl}thiazolidine-2,4-dione, is another compound in this class. This compound, given at a dose of 5-50 mg/kg/day to ob/ob mice, showed a dose-dependent glucose and insulin lowering effect and decreased free fatty acids, glycerol, triglycerides and cholesterol after 11 days of treatment, but not after a single dose (Stevenson et al., 1990). It also reversed the defects in insulin-stimulated glycolysis and glycogenesis and basal glucose oxidation in isolated soleus muscle with no hypoglycemic effects seen in either the treated control or diabetic groups (Stevenson et al., 1990). This compound appears to be able to normalize plasma glucose at lower doses than either ciglitazone or troglitazone.

In studies using 3T3-L1 adipocytes, englitazone overcame insulin resistance primarily by increasing V_{max} of 2-deoxyglucose uptake. Like insulin, it appears to stimulate glucose uptake by a mechanism that involves translocation of intracellular glucose transporters to the plasma membrane and *de novo* protein synthesis since the uptake of glucose could be inhibited by protein synthesis

inhibitors and was temperature dependent (Kreutter et al., 1990). These effects are greater and require lower doses than those seen with sulfonylureas. Interestingly, sulfonylureas require insulin to exert their full effect. Sulfonylureas alone can induce the expression of glucose transporters, but require insulin for translocation to the cell membrane (Wang et al., 1989). Englitazone can do both independent of insulin. This suggests it might be an insulinomimetic rather than a true sensitizer. The other thiazolidinediones have not been tested for this.

Pioglitazone, (5-[4-[2-(5-etyl-2-pyridyl)ethoxy]benzyl]-2,4-thiazolidinedione (AD-4833), the final member of this class of drugs, has been studied extensively for its glucose and lipid lowering effects in animals. There have been no studies concerned with the potential beneficial or deleterious cardiovascular effects of the drug. These are concerns regarding clinical use since pioglitazone has been reported to cause cardiac enlargement at doses considered therapeutic (Colca, J., personal communications). As with other compounds of the thiazolidinedione drug class, pioglitazone corrected abnormalities in glucose, lipids and insulin in KKAY mice (2.4-24.5 mg/kg/d for 4 days) and Zucker fatty rats (0.1-10 mg/kg/d for 4 days) (Ikeda et al., 1990). As seen with the other thiazolidinediones, pioglitazone lowered plasma glucose and lipids in obese animals, but not in lean or STZ-diabetic rats, suggesting that the lipid and glucose lowering effects are associated with reduced insulin resistance and enhanced insulin action on peripheral tissues (Ikeda et al., 1990). When ED25 values were compared in KKAY mice, pioglitazone was much more potent (6 mg/kg/d) than was ciglitazone (31 mg/kg/d) (Ikeda et al., 1990). The ability of pioglitazone to decrease plasma glucose, insulin, triglycerides and fatty acids is dose dependent as is its promotion of body

weight gain (the higher the dose, the greater the absolute weight gain) (Ikeda et al., 1990).

Pioglitazone, when given at a dose of 3 mg/kg/day for 6 days, has been shown to decrease insulin resistance in peripheral tissues and livers of Wistar fatty rats, while having no effect on the lean litter mates (Sugiyama et al., 1990a). This was determined by the observation that pioglitazone restored hepatic glycogen production and peripheral glucose utilization in the presence of infused insulin in fatty rats. In addition, glucose-6-phosphatase decreased and glucokinase increased, suggesting an increased response of the liver to insulin and a resulting suppression of hepatic glucose production (glycolytic pathway becomes dominant against gluconeogenic pathway). These findings suggest that pioglitazone also reduces insulin resistance via hepatic enzyme regulation (Sugiyama et al., 1990a). These studies also indicate that pioglitazone can normalize plasma glucose levels at a much lower dose than the other thiazolidinediones.

In addition to the effects on liver insulin resistance, it was determined that pioglitazone increased insulin-stimulated glycogen synthesis and glycolysis in the isolated soleus muscle and insulin stimulated glucose oxidation and lipogenesis in adipocytes without any effect on insulin binding (Sugiyama et al., 1990b). Vanadate, the insulinomimetic, which is known to work at post receptor binding sites, was potentiated by pioglitazone, suggesting that the glucose and lipid lowering effects of the drug are caused by reducing insulin resistance at post-receptor binding sites (Sugiyama et al., 1990b).

Pioglitazone has been shown to inhibit cholesterol absorption and to lower plasma cholesterol concentrations in cholesterol-fed rats (Colca et al., 1991). These effects only occur when insulin is present, working synergistically to lower absorption of cholesterol and circulating cholesterol and triglycerides (Colca et al., 1991). It has been suggested, given the aforementioned results, that treatments that improve insulin sensitivity may also have a positive impact on coronary artery disease associated with diabetes. Although the plasma cholesterol levels in rats not fed a high cholesterol diet did not change, the lipoprotein profiles were different. In KKAY mice, the majority of plasma cholesterol is carried in HDL and HDL levels are very high (Castle et al., 1993). Pioglitazone is known to lower elevated lipids in other animal models such as insulin resistant obese Rhesus Monkeys and although the exact mechanism is unknown, it does enhance triglyceride clearance rate (Kemnitz et al., 1994) possibly through stimulation of lipoprotein lipase or triglyceride lipase.

As mentioned earlier, GLUT4 glucose transporters are membrane proteins expressed only in tissues in which glucose uptake is regulated by insulin (fat, skeletal muscle and heart). In fat and muscle insulin stimulates glucose entry by recruiting glucose transporters from intracellular pools to the plasma membrane (Cushman and Wardzala, 1980). GLUT4 expression is decreased in KKAY mice. Pioglitazone corrects the GLUT4 transporter defect (Hofmann et al., 1991). This seems to be more prominent in fat than muscle and requires the presence of insulin. GLUT2 transporters, the major liver glucose transporters, remained unchanged by pioglitazone treatment; however, phosphoenolpyruvate carboxykinase (a major rate-limiting enzyme for gluconeogenesis) activity, which is elevated in untreated diabetics, was

normalized, suggesting that pioglitazone restored sensitivity to insulin's normal inhibitory actions (Hofmann et al., 1992).

To further determine the mechanisms by which pioglitazone decreases insulin resistance, insulin receptor kinase levels were evaluated. Pioglitazone caused no change in receptor binding in Wistar fatty rats, but increased insulin-stimulated autophosphorylation of insulin receptors to 78% over the level in the control (Kobayashi et al., 1992). Kinase activity affecting the exogenous substrate poly Glu^4Tyr^1 also increased to 87% over control obese rats. Lean rats treated with pioglitazone, however, showed no change in autophosphorylation and kinase activity (Kobayashi et al., 1992). These results suggest that pioglitazone increased insulin sensitivity in part by activating the kinase of its receptors through indirect effects on insulin receptors. It was suggested that if the conformational change occurs when insulin binds to its receptor, pioglitazone may potentiate the change to activate kinase or signal transduction between the two subunits of the receptor (Kobayashi et al., 1992). This effect was seen in the skeletal muscle of rats fed a high fat diet (Iwanishi and Kobayashi, 1993). These cumulative results suggest that pioglitazone's ability to decrease insulin resistance may result from improving the sensitivity of target tissues to insulin rather than from a direct corrective action of the agent on glucose transporters (Hofmann et al, 1991). It could be acting through a pathway other than activating tyrosine kinase in the insulin-signaling mechanism and the increased kinase activity may reflect only a secondary effect of metabolic improvement (Iwanishi and Kobayashi, 1993).

1.6 General Hypothesis

A new chemical class of antidiabetic agents, the thiazolidinediones, by correcting or preventing insulin resistance in genetically diabetic animal models, can cause physiologic or metabolic changes that may reduce the risk of developing one or more cardiovascular complications of type II diabetes.

1.7 Objectives

1. To determine the ability of pioglitazone, a thiazolidinedione, to prevent or correct (reverse) diabetic hyperglycemia in 3 sets of genetically diabetic (NIDDM) rats known to exhibit a phase of hyperinsulinemia (insulin resistance). To fulfill this objective, plasma glucose (every two weeks), insulin (every two weeks) and glycosylated hemoglobin (at termination) were determined.

   a. Administered prior to onset of hyperglycemia (vs. no drug) -- In young ZDF rats (7 weeks of age at initiation of treatment)

   b. Administered after onset of hyperglycemia (vs. no drug, hydralazine and vanadyl) -- In older ZDF rats (13 weeks of age at initiation of treatment)

   c. Administered prior to onset of hyperglycemia diabetic rats which may be prone to develop hypertension or congestive heart failure (vs. no drug, hydralazine and vanadyl) -- In young ZDF/SHHF rats (6 weeks of age at initiation of treatment)

2. (a,b,c) To determine if the prevention or correction of hyperglycemia by pioglitazone is paralleled by corrections in plasma triglycerides, cholesterol, lipoproteins or free fatty acids, elevations of which may be associated with

enhanced rates of atherogenesis and whether pioglitazone causes favorable or unfavorable effects on HDL cholesterol.

3. (a,b,c) To determine whether cardiovascular abnormalities exist in the animal models of insulin resistance listed in objective 1, and whether correction of insulin resistance with pioglitazone is associated with beneficial changes in the following cardiovascular parameters:

i. heart size and heart weight vs. body weight ratios
ii. cardiac lipid profiles: cholesterol and triglycerides
iii. aortic (vascular) sensitivity
iv. cardiac performance

4. (b,c) To evaluate the usefulness of ZDF or ZDF-SHHF rats as models of hypertension associated with NIDDM and to assess the potential of pioglitazone or other thiazolidinediones to treat hypertension by altering the state of insulin resistance.

2.0 METHODS

2.1 Study Groups

Study 1a, 2a, and 3a were conducted using twenty-four young male Zucker Diabetic Fatty Rats (ZDF, Drt-*fa*), obtained from Genetic Models, Indianapolis, Indiana, and twenty-three male, non-diabetic, Zucker Lean Rats (ZL) littermates. These groups were each divided into two sub-groups: one received 10 mg/kg/day pioglitazone HCl suspended in sodium citrate, administered by oral gavage, while the other group received the vehicle (0.5 M sodium citrate). The subdivision of animals resulted in four groups which will be designated control (CU, n=11), control treated (CP, n=12), diabetic (DU, n=12), and diabetic treated (DP, n=12). Diabetes was not chemically induced, rather, these genetically derived non-insulin-dependent-diabetic rats became spontaneously hyperglycemic between 7 and 10 weeks of age. Treatment was initiated in all groups of rats at 7 weeks of age and continued for 6 weeks to a final age of 13 weeks at study termination. Diabetic rats were housed individually in hanging metal cages and lean rats were housed in shoebox cages. They were housed in a 12 hour light-dark cycle environment and received food (Purina 5008 rat chow) and water ad libitum. All animals were allowed a minimum of 5 days to acclimate before initiation of experiments.

Studies 1b, 2b, 3b and 4b used the same animal model (ZDF rats), however, the rats used were of an older age. Treatment was begun when they were 13 weeks of age (rather than 7) having fully established hyperglycemia and continued 8 weeks rather than 6, ending at 21 weeks of age. This study also involved additional treatment groups. Along with pioglitazone treated animals

(10 mg/kg/day) and vehicle treated control animals, one group of animals received vanadyl (incremental dosages from 0.05 mg/ml to 0.75 mg/ml administered in deionized drinking water ad libitum) and another group received hydralazine (5 mg/kg twice daily by oral gavage). The subdivision of animals resulted in 8 groups, which were designated control (CU, n=5), control treated (CP, n=5), diabetic (DU, n=5), diabetic treated (DP, n=5), control hydralazine (CH, n=4), diabetic hydralazine (DH, n=4), control vanadyl (CV, n=5) and diabetic vanadyl (DV, n=5). Initially DH and CH began with 5 rats, but one rat in each group died. These rats were housed identically to the first group of rats, but were fed Purina 5015 rat chow which is higher in fat.

Studies 1c, 2c, 3c and 4c consisted of a young hybrid animal model. These rats are a hybrid between the ZDF model and the SHHF (spontaneously hypertensive) animal models. Treatment was begun at 6 weeks of age and continued for 8 weeks to age 14 weeks at termination. The subdivision of animals resulted in 8 groups which will be designated control (CU, n=7), control treated (CP, n=7), diabetic (DU, n=7), diabetic treated (DP, n=7), control hydralazine (CH, n=4), diabetic hydralazine (DH, n=4), control vanadyl (CV, n=4) and diabetic vanadyl (DV, n=4). Lean rats were housed in metal hanging cages and diabetic rats in shoebox cages, as in the other studies, and received the same regular rat chow as in studies 1a, 2a, and 3a.

2.2 Drug Therapy

Pioglitazone hydrochloride was provided by Jerry Colca, Ph.D., Upjohn Co., (Kalamazoo, MI) and was suspended in 0.5 M sodium citrate (Sigma, St. Louis,

MO), pH 4.5, by homogenization with a Biohomogenizer (M133/1281-0, Fisher Scientific, Fair Lawn, New Jersey), before administration to rats by oral gavage at a dose of 10 mg/kg/day. In study 1a, 2a, and 3a, dosages were calculated and administered each day at 2:00 pm. In the other studies, drugs were administered at approximately 8:00 am daily. An equivalent volume (1 ml/kg) of sodium citrate vehicle was administered as a vehicle control to the non-treated animals via the same method for studies a and b.

Hydralazine, 5 mg/kg, (Sigma Chemical Co., St. Louis, MO) was dissolved in deionized water and administered twice daily (approximately 12 hours apart) by oral gavage.

Vanadyl Sulfate (Eastman Kodak Co., Rochester, NY) was dissolved in deionized drinking water to reduce oxidation. The concentration was changed every 3 ($\pm$) days from an initial concentration of 0.05 mg/ml to a final concentration of 0.75 mg/ml which is known to correct IDDM. This approach was used to minimize toxicity due to dehydration. If an animal failed to drink adequate amounts of fluid, the dosage was reduced for a day until the animal rehydrated.

As an overt measure of drug toxicity, animals were observed on a daily basis to ensure that no diarrhea was present and their body weights were recorded. The animals were given water ad libitum and were fed regular rat chow, Purina Chow 5008, Richmond, Indiana in a and c studies, but were fed Purina 5015 in the b studies.

All other chemicals for general lab use (buffers, etc.) were obtained from Fisher Scientific Co., Fair Lawn, NJ.

2.3 Measurement of Plasma Components

Blood was removed from pentobarbital anesthetized rats at study termination through the abdominal aorta using a 22 gauge needle attached to a 10 cc plastic syringe, containing a small amount of the anticoagulant EDTA. Blood was centrifuged at 4°C for 10 minutes in a Beckman Model TJ-6 Centrifuge at 2000 rpm and the plasma was separated and distributed into several microfuge tubes for plasma analyses. Plasma analyses included glucose, cholesterol, triglycerides, free fatty acids and insulin. All plasma samples were stored at -70°C for later analyses. Some termination plasma samples were analyzed on an automated chemical analyzer (Hitachi) while most were analyzed using common biochemical kits (see section 2.3.1).

Blood was also collected at week 0, 2, 4, 6 and 8 for time course evaluation of plasma triglycerides, cholesterol and insulin. These samples were obtained through the tail vein by nicking the tail with a sterile scalpel and stroking the tail to collect the blood in microfuge tubes containing EDTA. Blood was separated and plasma stored at -20 °C for later analyses.

2.3.1 Glucose, Triglyceride and Cholesterol Determinations

Blood glucose was measured weekly on a One Touch II Glucose Analyzer (Johnson and Johnson Co., Milpitas, California) using One Touch test strips.

For these samples, the blood was obtained from the tail. These values were determined weekly using this method on 5 hour fasted rats.

On weeks 2, 4, 6 and 8, plasma glucose and triglycerides were determined using Stanbio enzymatic colorimetric kits obtained from Fisher Scientific Co., Fair Lawn, NJ. Cholesterol was determined using a similar procedure with kits obtained from Sigma Chemical Co., St. Louis, MO. The standard procedure for all of these kits was to use 10 µl of plasma plus 1 ml of reagent and incubate for 10 minutes followed by reading the optical density on a Bausch and Lomb Spectrophotometer at 500 nm.

2.3.2 Free Fatty Acid Determinations

Free fatty acids from a and b studies were measured at study termination using a chloroform extraction technique followed by colorimetric determination of free fatty acid levels following the methods of Duncombe,WW (1963), as modified by Itaya, K, and Ui, M (1965). For study c, a micro-assay technique from Boehringer Mannheim, Indianapolis, Indiana, was used. Determinations were made at weeks 0, 4 and 8.

2.3.3 Plasma Insulin Determinations

Plasma insulin was determined at weeks 0, 2, 4, 6 and 8 weeks. Plasma insulin was measured using a double antibody radioimmunoassay obtained from Linco (Linco Research Inc., St. Louis, MO) according to the method of Morgan and Lazarow (1963). This kit uses polyclonal antibodies raised in guinea pigs against rat insulin (100% specific to rat insulin). The assay used ^{125}I labeled rat

insulin of specific activity 0.02 μCi per sample. The sensitivity of the assay as used was 0.1 ng/ml. Intra and inter assay coefficients of variation were 1.34% and 3.17%, respectively.

2.4 Measurements of Cardiac Function

Following six or eight weeks of oral drug treatment, the rats were overdosed with Pentobarbital Sodium (Fort Dodge Laboratories, Inc., Iowa) 100 mg/kg i.p. After loss of pain and righting reflexes, rats were exanguinated via the abdominal aorta with a 10 ml plastic syringe and a 22 g needle. The blood was centrifuged and separated for later plasma analyses. During the exanguination process, the heart was rapidly removed and placed into a dish of warm Chenoweth-Koelle (CK) buffer for transport to the working heart apparatus. The heart was first cannulated through the aorta, according to the Langendorff procedure, and perfused with warm, oxygenated (95% O_2 and 5% CO_2), CK buffer (millimolar concentrations of: NaCl, 120; KCl, 5.6; $CaCl_2$, 2.18; $MgCl_2$, 2.1; $NaHCO_3$, 19.2; and glucose, 10). Once the pulmonary vein was cannulated, the system was switched to an isolated working heart preparation. This allows perfusion first through the left atria into the left ventricle and finally out the aorta. The buffer was not recirculated. PE-10 tubing was used to provide resistance. The experiment involved subjecting the heart to changes in preload and observing changes in cardiac performance. The criteria measured were left ventricular developed pressure (LVDP), rate of force development (+dP/dT) and rate of relaxation (-dP/dT) as previously described (Rodrigues and McNeill, 1986). At the completion of the working heart analysis, the whole heart was weighed to determine the heart weight to body weight ratio then freeze

clamped in liquid nitrogen to preserve samples for subsequent determinations of myocardial triglyceride and cholesterol levels.

2.5 Blood Pressure

Blood pressure values were determined for each rat in study b and c. This method involves the use of a semi-automated, amplified tail cuff inflation device from IITC Inc., Woodland Hills, California. Rats were placed in appropriately sized holders and sensors and allowed to acclimate to the surroundings for 20-30 minutes. The tail inflation device containing a light sensor was subsequently inflated to 300 mmHg and allowed to deflate in a linear fashion. The amplifier transfers the signal from the sensor and translates it into a blood pressure trace which is seen on the chart recorder. From this tracing, systolic blood pressure can be measured from the initial pulses on the trace and the mean arterial pressure is measured from the first maximum pulse. The diastolic pressure is calculated from the systolic and mean pressures. The calculation is as follows:

$$D.P.= \frac{3(mean)-systolic}{2}$$

2.6 Vascular Reactivity

Endothelium dependent relaxation is believed to be impaired in IDDM, but is questionably affected in NIDDM; thus a series of experiments was designed to investigate this phenomenon in NIDDM by measuring aortic sensitivity. Aortic sensitivity was measured by determining the ability of 4 mm aortic rings to contract in the presence of norepinephrine. Aortic strips were taken from the

region just distal to the aortic arch of the heart. A 4 mm strip was suspended in a water jacketed tissue bath containing 25 ml of Krebs buffer (containing mM concentrations of: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $NaHCO_3$, 25; KH_2PO_4, 1.2; $MgSO_4$, 1.2; Glucose, 11) and oxygenated with 95% O_2 and 5% CO_2 at 37°C. Two grams of resting tension was placed on the strip to provide resistance against which to contract. The tissue was held in place by two small fish hooks which are attached to an F-60 Myograph Pressure Transducer (Narco Biosystems). The contractions were translated by the coupling device and produced a read out on the physiograph (Narco Biosystems).

The tissue was first allowed a 30 minute equilibration period. Then, to establish whether the tissue worked, the aortic strip was suspended in a high potassium bath (40 mM KCl; replacing NaCl). The tissue's ability to contract due to depolarization of the membrane indicated that the strip was working. A norepinephrine (NE) dose response curve was determined on each strip of tissue using concentrations of NE from 10^{-9} M to 10^{-5} M. Norepinephrine is an alpha-adrenergic agonist in blood vessels which causes contraction of the vascular tissue. Contractile force was expressed in grams.

2.7 Statistics

Comparison between group means was accomplished using a one way analysis of variance (ANOVA) followed by Scheffe's F-test, where applicable. A probability of $p<0.05$ was used to indicate statistical significance.

3.0 RESULTS

For the purpose of convenience, the legends in the figures have been condensed to two letter acronyms for most treatment groups as follows:

CU	Control/ untreated rats	DU	Diabetic untreated rats
CP	Control pioglitazone	DP	Diabetic pioglitazone
CV	Control vanadyl	DV	Diabetic vanadyl
CH	Control hydralazine	DH	Diabetic hydralazine

NOTE: Control refers to non-diabetic rats.

To review, the study groups are as follows:

a. Administered prior to onset of hyperglycemia (vs. no drug) -- In young ZDF rats (7 weeks of age at initiation of treatment)

b. Administered after onset of hyperglycemia (vs. no drug, hydralazine and vanadyl) -- In older ZDF rats (13 weeks of age at initiation of treatment)

c. Administered prior to onset of hyperglycemia diabetic rats which may be prone to develop hypertension or congestive heart failure (vs. no drug, hydralazine and vanadyl) -- In young ZDF/SHHF rats (6 weeks of age at initiation of treatment)

3.1 Body Weights

Body weights of young (7-13 weeks of age) ZDF diabetic rats were significantly greater than their non-diabetic littermates throughout the study. After one week of oral treatment with 10 mg/kg/day pioglitazone HCl, body weights were

significantly higher than the untreated diabetic rats. Six weeks of pioglitazone treatment caused a ~1.5 fold greater increase in body weight relative to untreated diabetics and ~1.8 fold increase over control rats. Non-diabetic rats had a steady body weight increase whether given pioglitazone or not (FIG. 1).

Body weights of older ZDF diabetic rats were significantly higher than non-diabetic littermates at 13 weeks of age. After 7 weeks of treatment (20 weeks of age), body weight was not significantly different except in the vanadyl treated group (FIG. 2B). Vanadyl lowered body weights in both diabetic and non-diabetic older ZDF rats. Pioglitazone and hydralazine had no effect on body weight gain in the older ZDF rats (FIG. 2A,C).

At 6 weeks of age (before treatment) young hybrid ZDF/SHHF diabetic rats (DU, DP) did not have elevated body weights compared to their non-diabetic littermates (CU). Unexpectedly, randomization of the control rats led to the CV and CH groups beginning treatment at a lower body weight than either the CU or DU groups. After 3 weeks of pioglitazone treatment (9 weeks of age) body weights of the DP group were significantly elevated compared to DU and by the end of the 8 weeks of treatment, DP was ~1.25 fold greater than DU (FIG. 3A). There was no effect on body weight observed in the non-diabetic group following pioglitazone treatment. Also at 9 weeks of age, the body weights of the DU group became significantly elevated compared to the CU group and remained elevated throughout the rest of the study. CU body weights exceeded those of the CV and CH rats throughout the study. Vanadyl significantly decreased body weight in the diabetic group after 8 weeks of treatment. Hydralazine had no effect on body weight in either diabetic or non-diabetic rats (FIG. 3B,C).

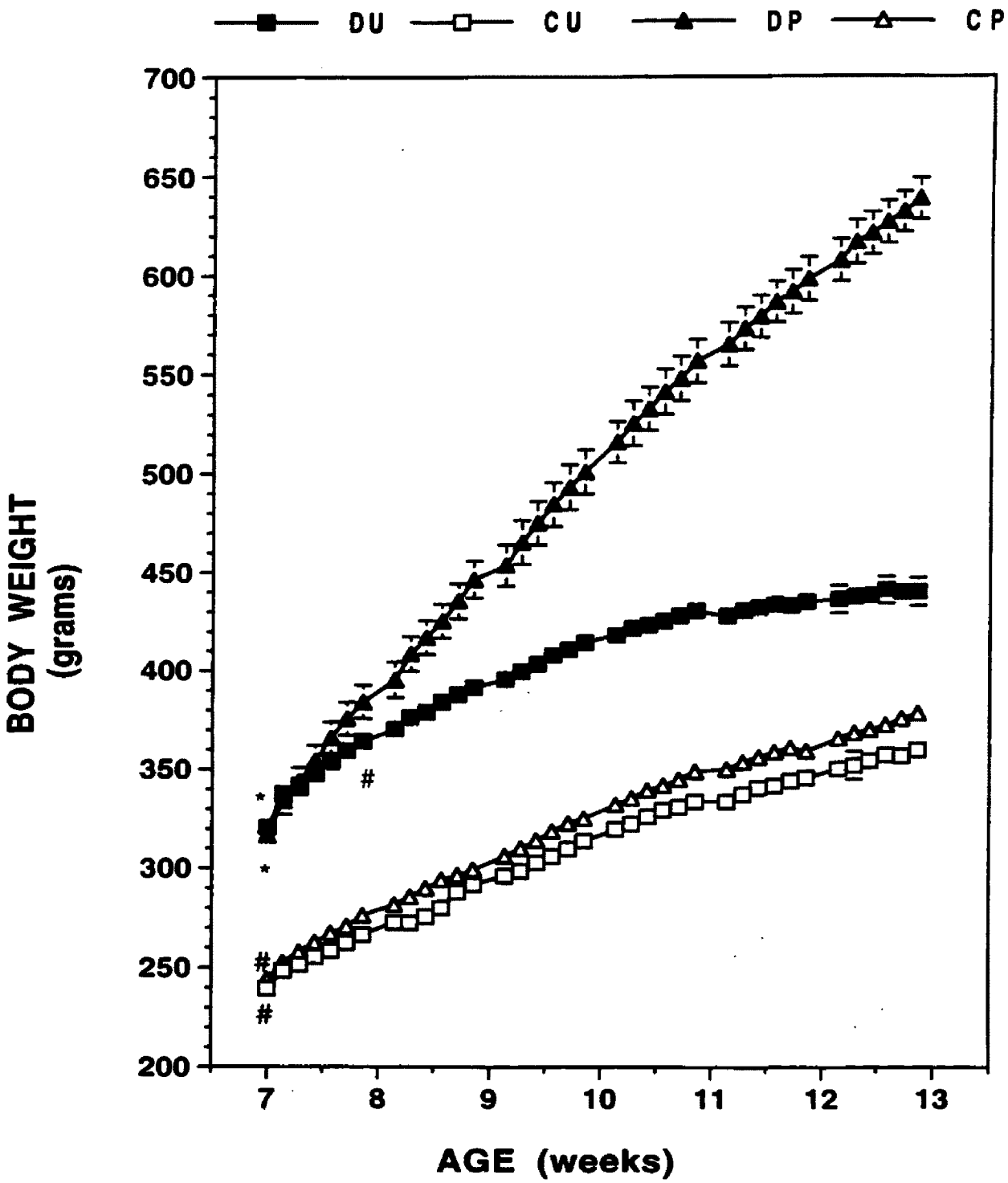


FIG. 1. Time course of oral pioglitazone effect on body weight in young ZDF diabetic rats and non-diabetic littermates. Data represent the means ± SEM. Error bars smaller than symbols are not shown.
#, significantly different from diabetic untreated from this point through week 13.
*, significantly different from control untreated from this point through week 13.

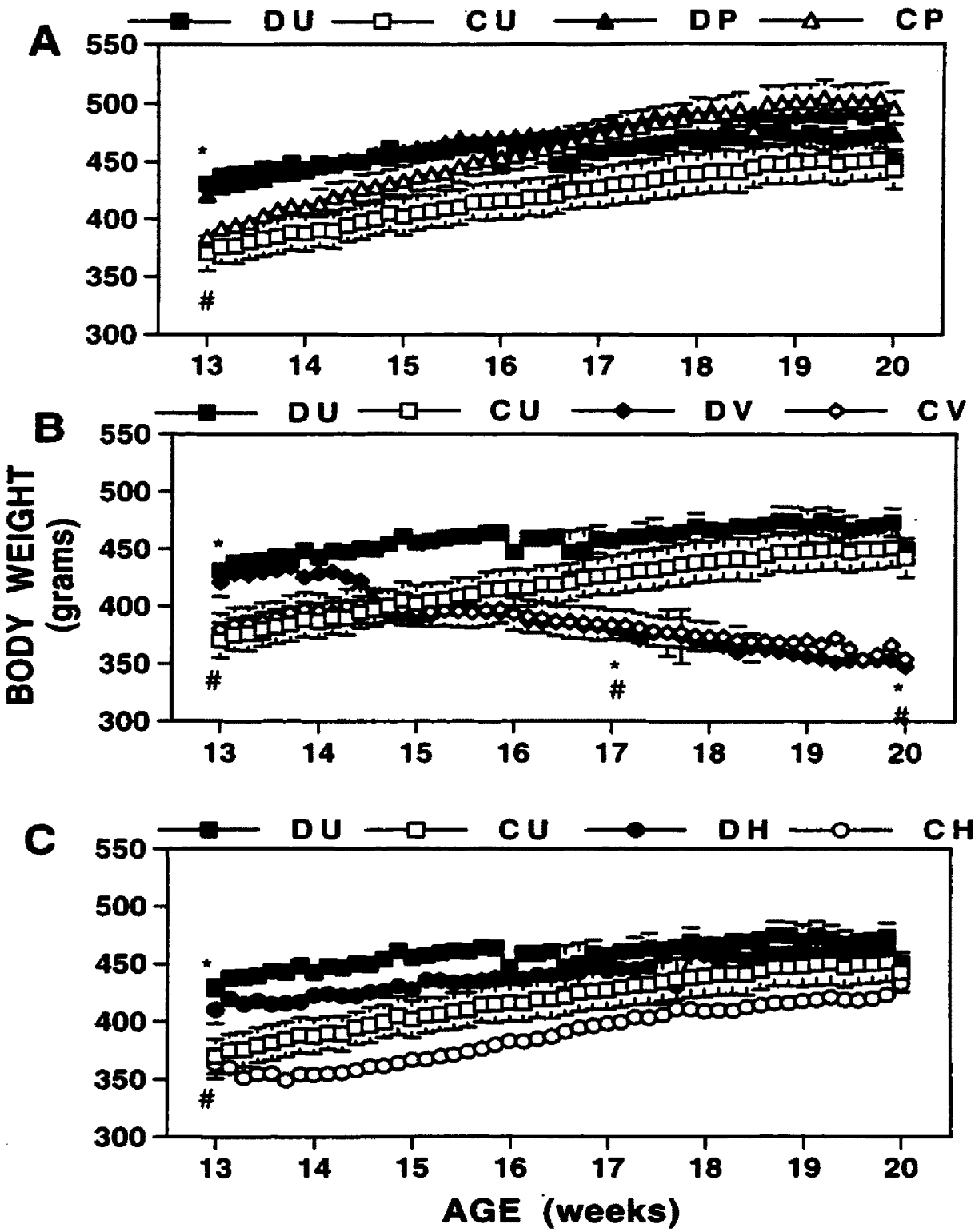


FIG. 2. Time course of oral (A) pioglitazone, (B) vanadyl and (C) hydralazine effects on body weight in older ZDF diabetic rats and non-diabetic littermates. Data represent the means ± SEM. Error bars smaller than symbols are not shown. #, significantly different from diabetic untreated. *, significantly different from control untreated.

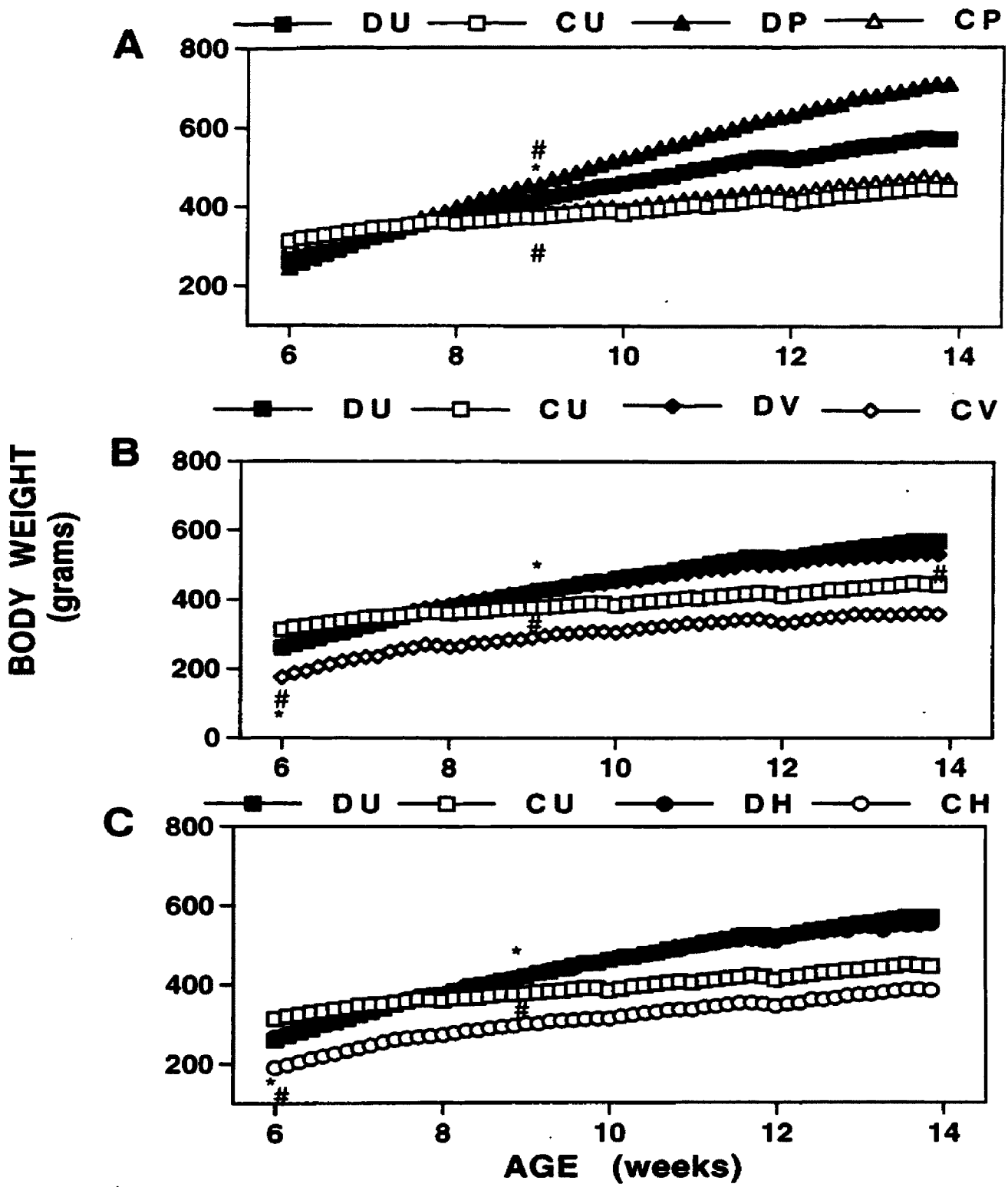


FIG. 3. Time course of oral (A) pioglitazone, (B) vanadyl and (C) hydralazine effects on body weight in young hybrid ZDF/SHHF diabetic rats and non-diabetic littermates. Data represent the means ± SEM. Error bars smaller than symbols are not shown. #, significantly different from diabetic untreated from this point through 14 weeks of age. *, significantly different from control untreated from this point through 14 weeks.

3.2 Plasma Glucose

Fasting plasma glucose levels of 7 week old young ZDF rats were not significantly different from levels in control rats. By 9 weeks of age, DU plasma glucose levels were significantly elevated relative to those of CU and CP rats. Fasted plasma glucose levels were significantly reduced by pioglitazone in young ZDF diabetic rats beginning near the time of developing hyperglycemia. After two weeks of treatment with pioglitazone, glucose levels of diabetic rats were completely normalized and remained so throughout the study. Pioglitazone had no effect on the blood glucose in non-diabetic sham treated rats (given 1ml/kg/day of 0.5 M sodium citrate by oral gavage) (FIG.4).

At 13 weeks of age, older ZDF diabetic rats had significantly elevated plasma glucoses compared to control rats. Older ZDF diabetic rats, that exhibited consistent hyperglycemia, showed no effect from pioglitazone treatment on plasma glucose at any point during the 7 weeks of treatment (FIG. 5A). However, vanadyl significantly decrease plasma glucose after two weeks of treatment with the drug (FIG. 5B). During this period of time, rats were subjected to steadily increasing concentrations of vanadyl from 0.05 mg/ml to 0.75 mg/ml to avoid toxicity. Hydralazine had no effect on plasma glucose (FIG. 5C). Pioglitazone, vanadyl or hydralazine had no effect on plasma glucose in non-diabetic rats (FIG. 5).

At the beginning of treatment in the young hybrid rats (6 weeks old), there was no significant difference in blood glucose levels between any of the rat groups. Diabetic rats did not develop significantly elevated plasma glucose levels until 12 weeks of age. As the rats developed hyperglycemia, it was evident that both

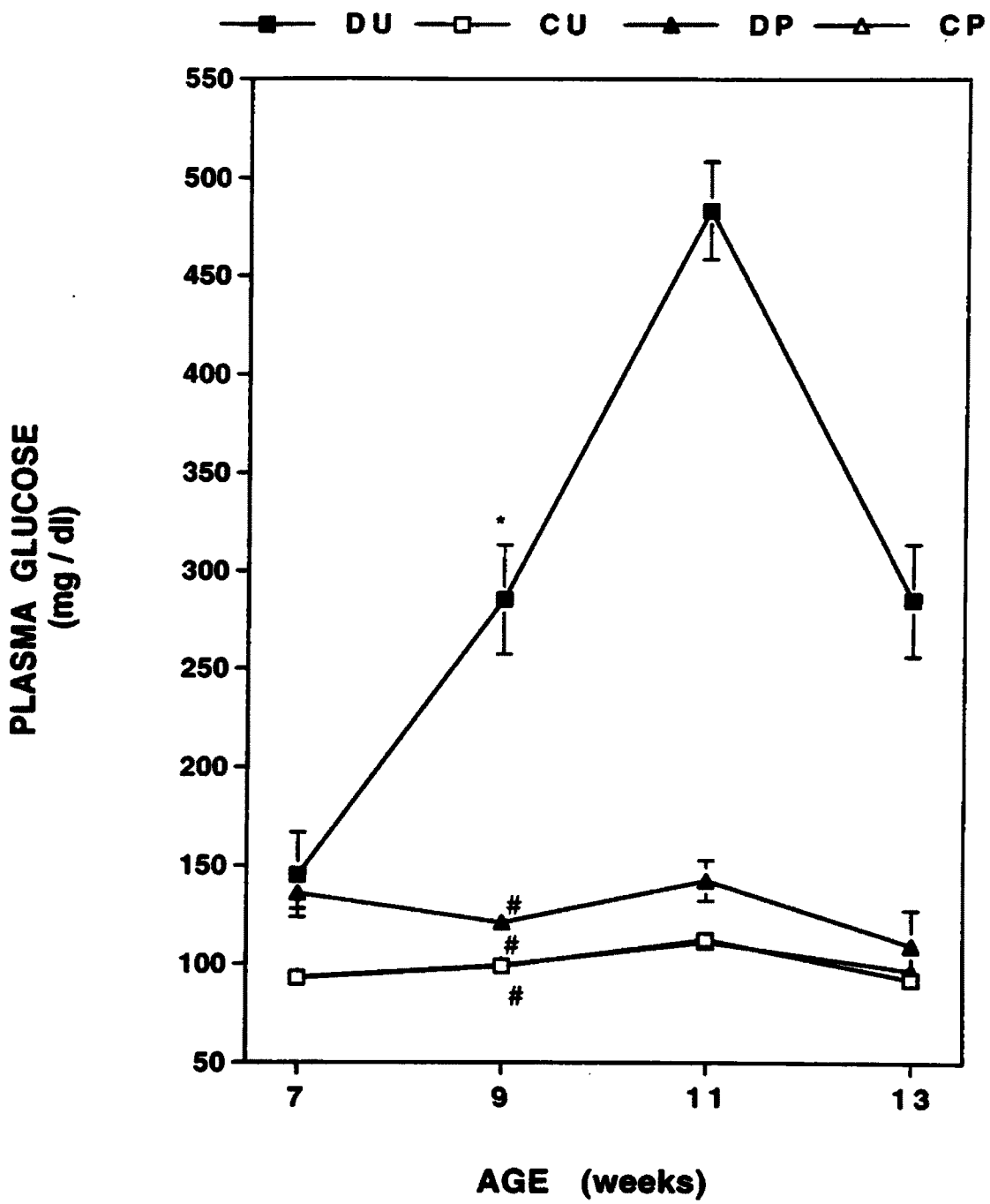


FIG. 4. Time course of oral pioglitazone effect on fasted plasma glucose levels in young ZDF diabetic rats and non-diabetic littermates. Data represent the means ± SEM. Error bars smaller than symbols are not shown. #, significantly different from diabetic untreated from this point through 13 weeks. *, significantly different from control untreated from this point through 13 weeks.

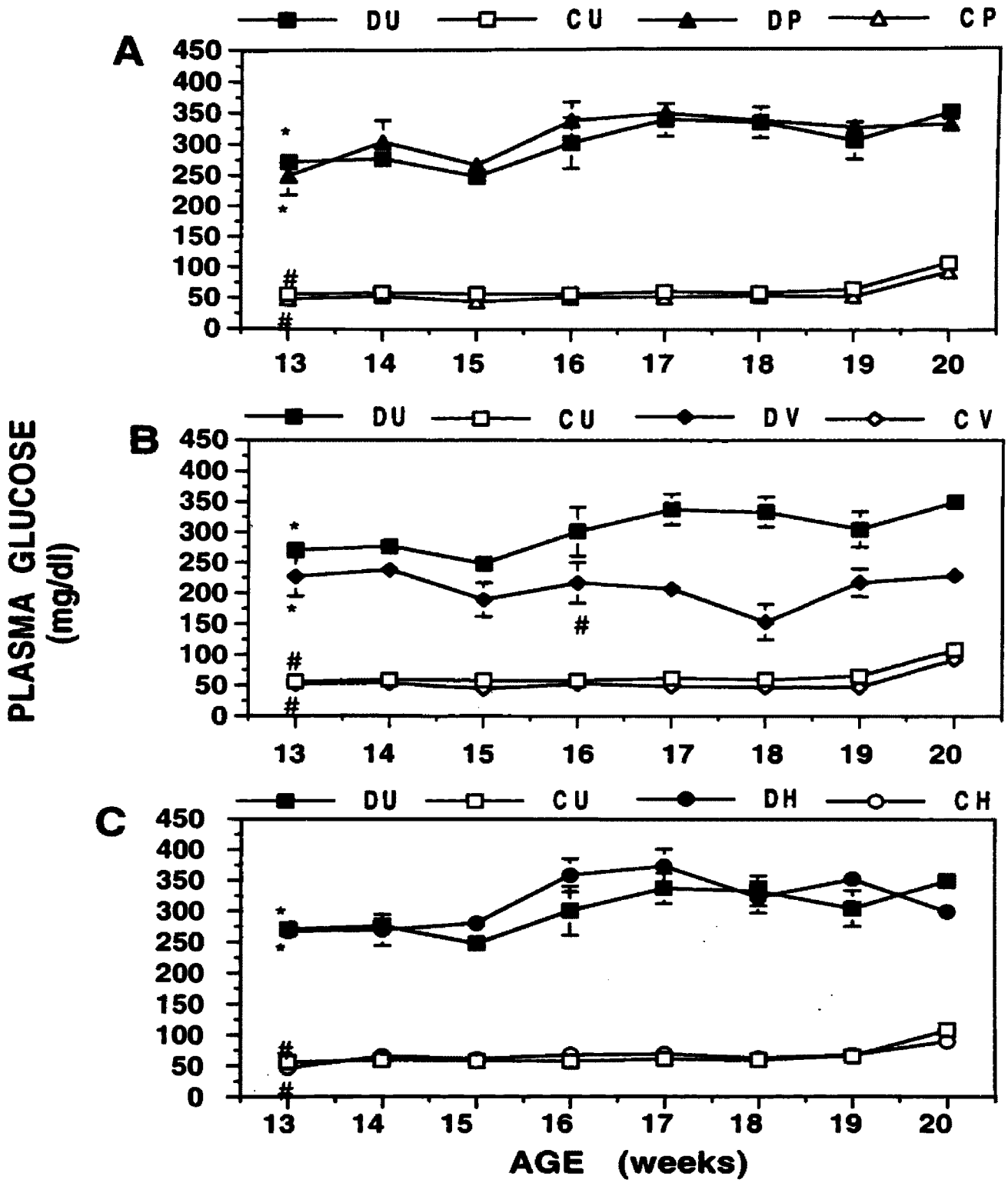


FIG. 5. Time course of oral (A) pioglitazone, (B) vanadyl and (C) hydralazine effects on fasting plasma glucose levels in older ZDF diabetic rats and non-diabetic littermates. Data represent the means ± SEM. Error bars smaller than symbols are not shown. #, significantly different from diabetic untreated from this point through week 20. *, significantly different from control untreated from this point through week 20.

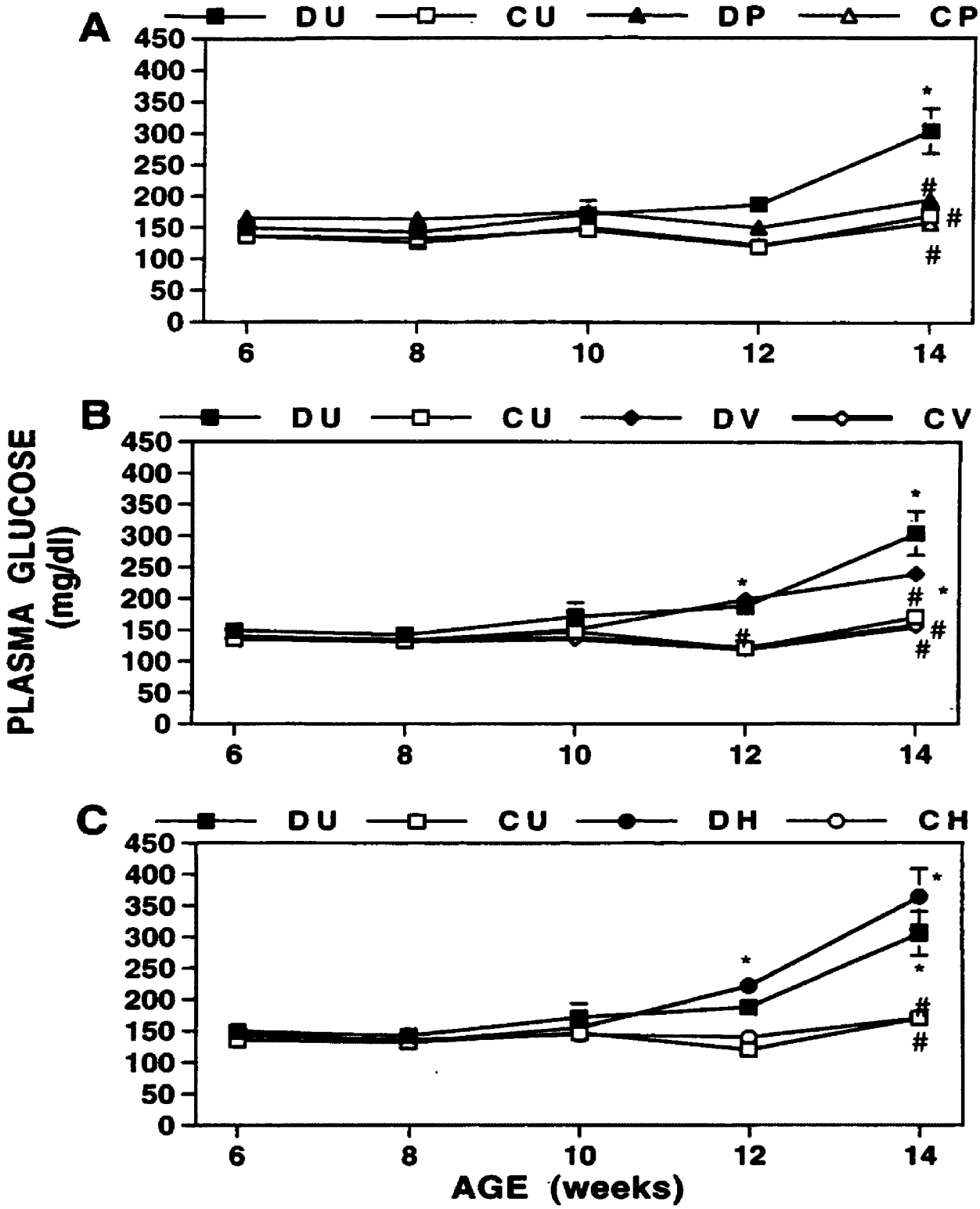


FIG 6. Time course of oral (A) pioglitazone, (B) vanadyl and (C) hydralazine effects on fasted plasma glucose levels in young hybrid ZDF/SHHF diabetic rats and non-diabetic littermates. Data represent the means ± SEM. Error bars smaller than symbols are not shown. #, significantly different from diabetic untreated. *, significantly different from control untreated.

the pioglitazone and vanadyl treatment groups had significantly lower plasma than the untreated diabetic rat group (FIG. 6A,B). Hydralazine treatment was without effect in the diabetic or non-diabetic groups (FIG. 6C). Pioglitazone, vanadyl or hydralazine had no effect on the non-diabetic rats.

3.3 Plasma Triglycerides

At seven weeks of age, young ZDF diabetic rats already had significantly higher plasma triglyceride levels (~ 7 times) than the non-diabetic littermates. Within 2 weeks, pioglitazone normalized the elevated triglyceride levels and maintained these levels throughout the study (FIG. 7).

Thirteen week old, older ZDF diabetic rats had significantly elevated plasma triglyceride levels (~5 times) compared to non-diabetic rats. Pioglitazone significantly decreased triglyceride levels from DU levels at weeks 3 and 7 of treatment (FIG. 8A). Vanadyl had no effect on plasma triglyceride levels in diabetic rats (FIG. 8B). Hydralazine treatment significantly decreased plasma triglycerides in the diabetic rats at weeks 1, 3 and 7 of treatment (FIG. 8C). Control rats, treated with either pioglitazone, vanadyl or hydralazine, had significantly lower triglyceride levels compared to CU after 3 weeks of treatment.

Six week old, young hybrid diabetic rats were not hypertriglyceridemic. By 8 weeks of age, diabetic rats had significantly elevated triglyceride levels which progressively increased through 12 weeks of age at which time they plateaued. Pioglitazone significantly reduced, but did not normalize, these elevated triglyceride levels and had no effect in the non-diabetic rats (FIG. 9A). Vanadyl

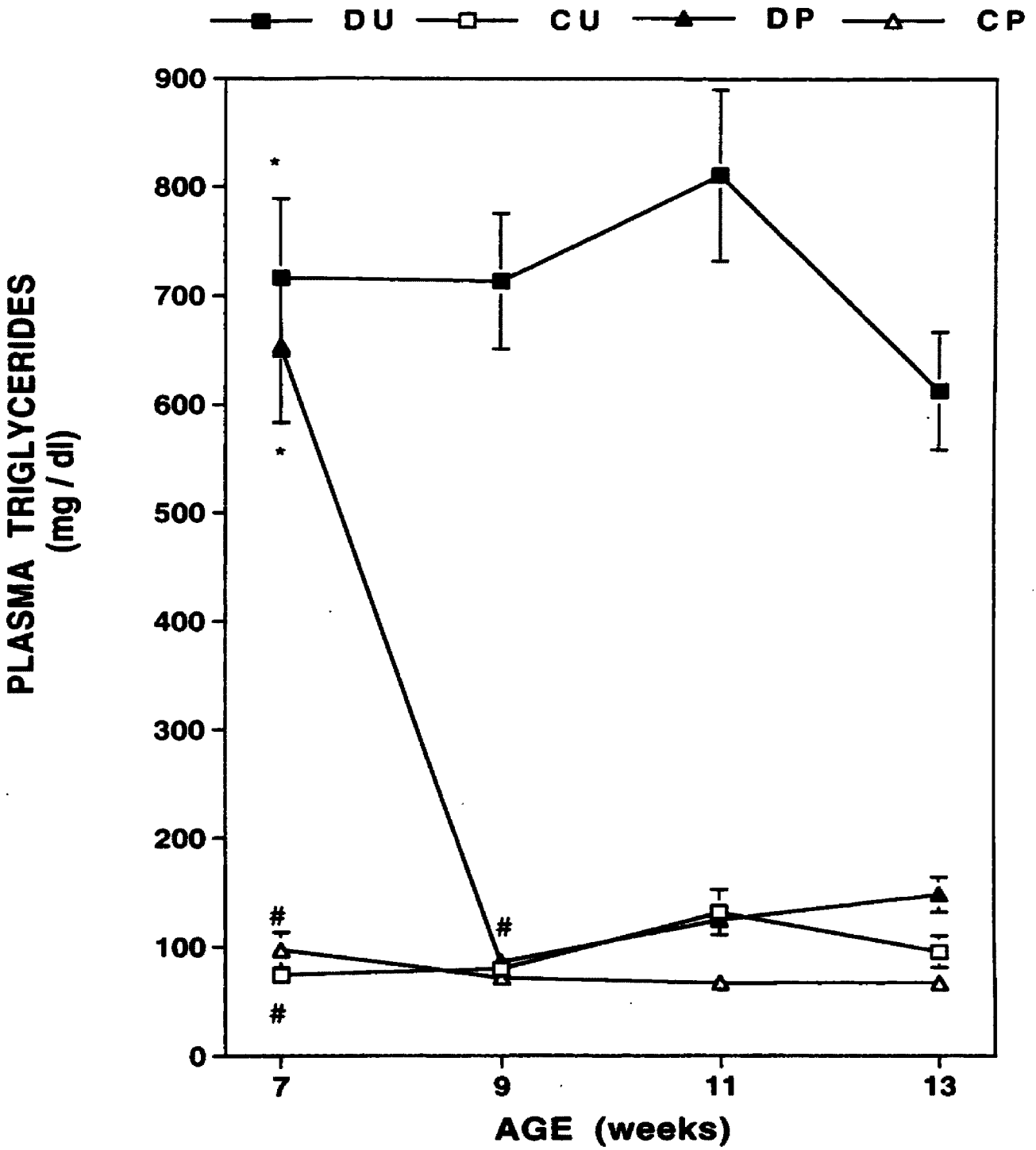


FIG. 7. Time course of oral pioglitazone effect on fasted plasma triglyceride levels in young ZDFdiabetic rats and non-diabetic littermates. Data represent means ± SEM. Error bars smaller than symbols are not shown. #, significantly different from diabetic untreated from this point through 13 weeks.
*, significantly different from control untreated from this point through 13 weeks.

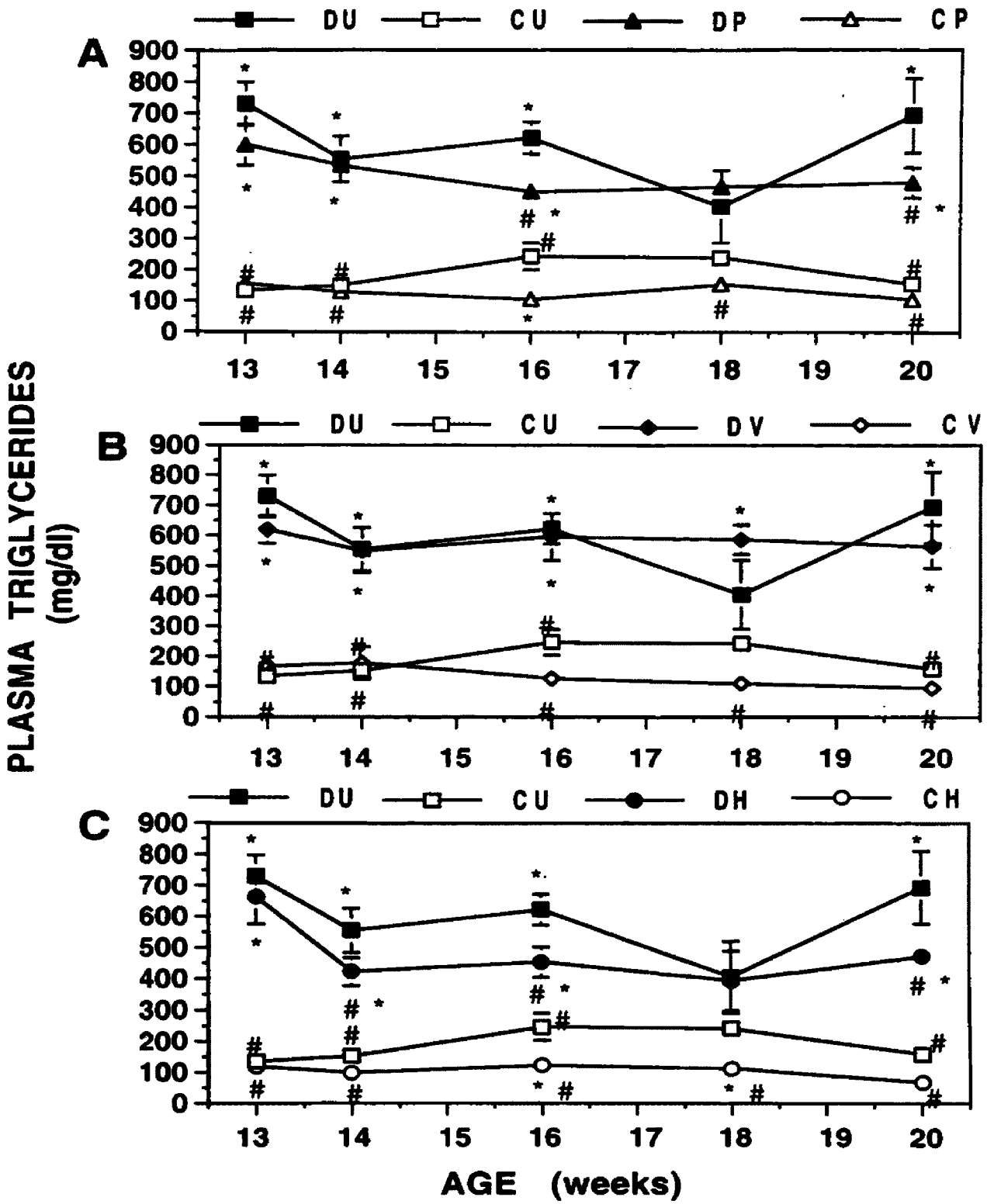


FIG. 8 . Time course of oral (A) pioglitazone, (B) vanadyl and (C) hydralazine effects on fasted plasma triglyceride levels in older ZDF diabetic rats and non-diabetic littermates. Data represent means ± SEM. Error bars smaller than symbols are not shown. #, significantly different from diabetic untreated. *, significantly different from control untreated.

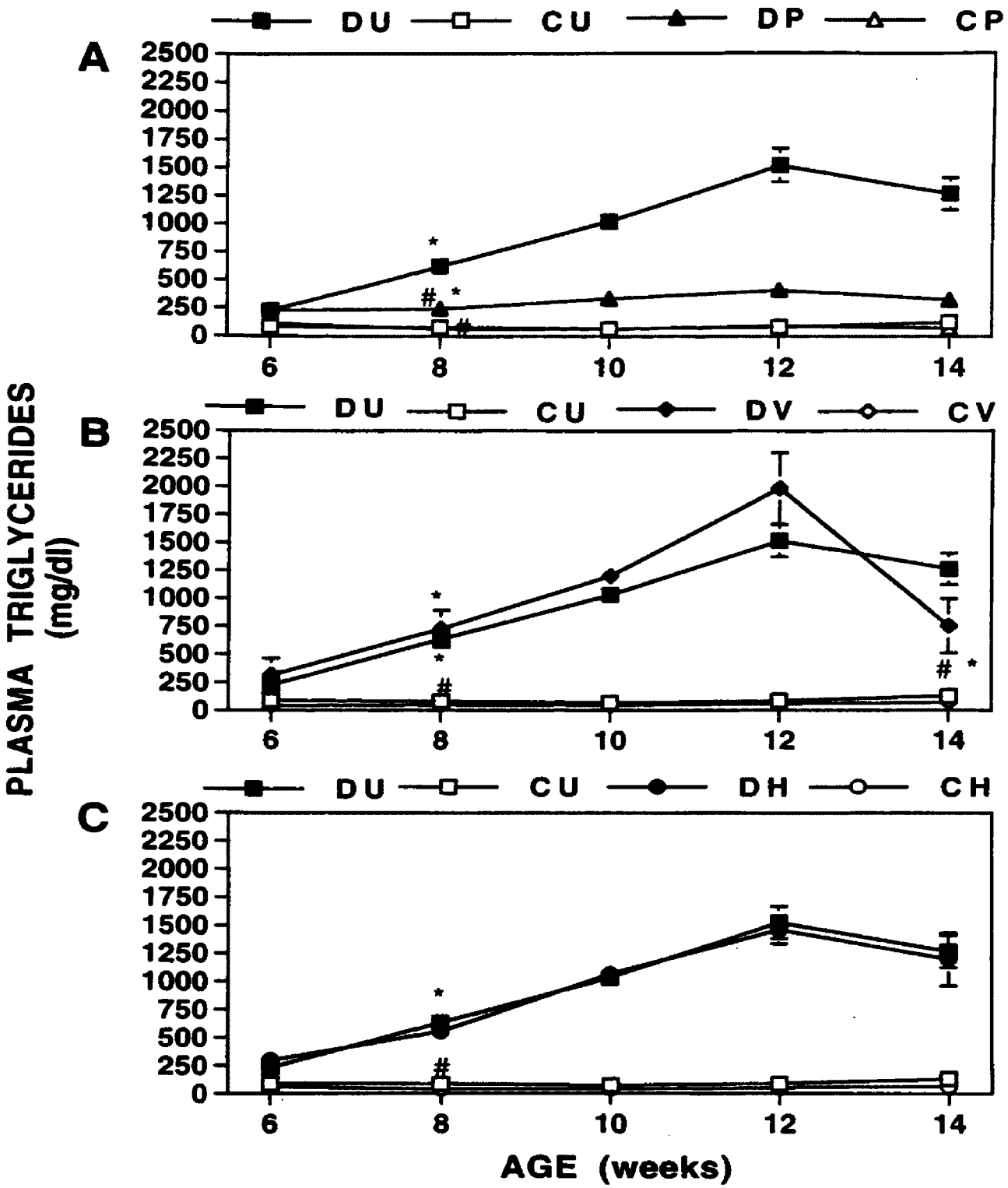


FIG. 9. Time course of oral (A) pioglitazone, (B) vanadyl and (C) hydralazine effects on fasted plasma triglyceride levels in young hybrid ZDF/SHHF diabetic rats and non-diabetic littermates. Data represent means ± SEM. Error bars smaller than symbols are not shown. #, significantly different than diabetic untreated from this point through week 14. *, significantly different from control untreated from this point through week 14.

caused a significant reduction in triglyceride levels compared to DU at week 14 when vanadyl dosing was at its highest (FIG. 9B). Hydralazine had no effect on triglycerides in diabetic or non-diabetic hybrid rats (FIG. 9C).

3.4 Plasma Cholesterol

Fasted plasma values following 6 weeks of vehicle treatment in young ZDF diabetic rats (7-13 weeks of age) indicated that DU rats had significantly higher glucose, triglyceride, total and HDL cholesterol and free fatty acids than did non-diabetic rats. Pioglitazone, administered for 6 weeks, significantly reduced the hyperglycemia, hypertriglyceridemia and hypercholesterolemia but had no effect on HDL or free fatty acid levels in the DU rats . Pioglitazone given to non-diabetic rats had no effect on any of the mentioned plasma parameters (Table 1).

Older ZDF diabetic rats (13-20 weeks of age) exhibited significantly elevated fasted glucose, triglycerides, total cholesterol, free fatty acids and hemoglobin A1c than did control rats after 7 weeks of treatment. There was no difference between CU and DU with respect to HDL cholesterol. Insulin values were significantly reduced in diabetic groups compared to control groups. Pioglitazone and hydralazine treatment groups had plasma triglyceride levels that were intermediate between those of DU and CU rats. Vanadyl significantly reduced plasma glucose in diabetic rats. Only pioglitazone significantly decreased free fatty acid levels relative to CU and DU rats (Table 2).

TABLE 1. Effect of oral pioglitazone treatment on plasma glucose, triglycerides, total and HDL cholesterol, free fatty acids and insulin after 6 weeks of treatment in young ZDF diabetic rats and non-diabetic littermates.

PLASMA PARAMETER	**DU** (n=12)	**DP** (n=12)	**CU** (n=11)	**CP** (n=12)
Glucose (mg/dl)	285.6 ± 30.0 *	109.8 ± 8.1 #	92.4 ± 4.0 #	96.8 ± 6.2 #
Triglycerides (mg/dl)	613.8 ± 54.1 *	148.7 ± 16.2 #	95.9 ± 14.6 #	67.0 ± 5.0 #
Total Cholesterol (mg/dl)	174.0 ± 14.0 *	135.0 ± 11.0 * #	82.0 ± 11.0 #	78.0 ± 9.0 #
HDL Cholesterol (mg/dl)	147.0 ± 29.0 *	121.0 ± 21.0 *	64.0 ± 7.0 #	64.0 ± 12.0 #
FFA (µmol/l)	127.0 ± 10.6 *	123.4 ± 15.2 *	39.0 ± 8.2 #	49.2 ± 5.8 #
Insulin (ng/ml)	16.6 ± 2.6	29.5 ± 3.0 *	12.4 ± 2.4	9.6 ± 2.4
HbA1c (percent)	--	--	--	--

NOTE: A dash indicates that this parameter was not measured. Results represent means ± SEM; n, number of animals. Numbers represent fasted values.

Significantly different from diabetic untreated.

* Significantly different from control untreated.

TABLE 2. Effect of oral pioglitazone, vanadyl and hydralazine treatment on plasma glucose, triglycerides, total and HDL cholesterol, free fatty acids and insulin after 7 weeks of treatment in older ZDF diabetic rats and non-diabetic littermates.

PLASMA PARAMETER	DU (n=5)	DP (n=5)	DV (n=5)	DH (n=4)	CU (n=5)	CP (n=5)	CV (n=5)	CH (n=4)
Glucose (mg/dl)	350.2 * ± 5.9	330.8 * ± 12.5	229.4 #* ± 17.0	299.5 * ± 9.3	108.6 # ± 7.1	94.2 # ± 6.2	92.6 # ± 1.1	90.0 # ± 6.6
Triglycerides (mg/dl)	694.2 * ± 117.3	481.6 #* ± 49.5	565.0 * ± 71.5	471.8 #* ± 34.8	158.2 # ± 21.9	106.8 # ± 7.2	95.8 # ±22.2	68.3 # ± 17.5
Total Cholesterol (mg/dl)	153.6 ±13.4*	147.4 ± 8.4*	132.4 ± 6.9*	142.8 ± 8.2*	56.6 ±2.65#	53.4 ± 1.2 #	68.2 ± 4.0 #	51.5 ± 3.3 #
HDL Cholesterol (mg/dl)	83.6 ± 37.4	55.1 ± 19.5	71.1 ±14.9	95.5 ± 14.7	39.2 ± 2.2	36.3 ± 1.4	49.0 ± 3.5	38.5 ± 1.6
FFA (µmol/l)	292.8 * ± 25.1	247.9 * ± 15.4	245.3 * ± 40.9	300.4 * ± 28.9	171.5 # ± 3.6	142.8 #* ± 9.7	173.68 # ± 6.4	195.4 # ± 40.8
Insulin (ng/ml)	5.9 ± 1.9 *	3.2 ± 0.4 *	6.8 ± 1.0 *	5.0 ± 0.6 *	22.6 ± 7.4 #	14.1 ± 2.8 #	6.3 ± 1.4 #	17.7 ± 4.5 #
HbA1c (percent)	8.7 ± 0.4 *	9.5 ± 0.4 *	6.2 ± 0.4 *	9.6 ± 0.6 *	5.7 ± 0.4 #	5.8 ± 0.4 #	6.1 ± 0.7 #	6.6 ± 1.3 #

NOTE: Results represent means ± SEM; n, number of animals. Numbers represent fasted values.
Significantly different from diabetic untreated.
* Significantly different from control untreated.

TABLE 3. Effect of oral pioglitazone, vanadyl and hydralazine treatment on plasma glucose, triglycerides, total and HDL cholesterol, free fatty acids and insulin after 8 weeks of treatment in young hybrid ZDF/SHHF diabetic rats and non-diabetic littermates.

PLASMA PARAMETER	DU (n=7)	DP (n=7)	DV (n=4)	DH (n=4)	CU (n=7)	CP (n=7)	CV (n=4)	CH (n=4)
Glucose (mg/dl)	304.8 ±34.9*	195.1 ±10.5#	239.4 ±15.5#*	365.3 ±45.0*	170.3 ± 8.0 #	158.6 ± 7.0#	156.2 ±17.6#	170.4 ± 5.4#
Triglycerides (mg/dl)	1267.2 ± 142.2*	328.9 ± 35.0#*	755.9 ± 243.2#*	1197.6 ± 235.8*	128.1 ± 10.8#	77.1 ± 4.5#	70.2 ± 13.3#	62.2 ± 8.3#
Total Cholesterol (mg/dl)	189.1 ± 11.4*	92.2 ± 6.5#	149.2 ± 24.4*	102.8 ± 17.0#*	79.6 ± 10.0#	54.7 ± 3.7#	59.0 ± 6.3#	54.7 ± 6.1#
HDL Cholesterol (mg/dl)	67.6 ± 1.6*	61.6 ± 3.3*	72.0 ± 4.2*	78.5 ± 1.4*#	48.1 ± 2.0#	39.7 ± 1.6#	44.7 ± 6.7#	41.2 ± 2.1#
FFA (µmol/l)	259 ± 20	253 ± 26	262 ± 31	276 ± 10	247± 11	186 ± 19	230 ± 62	243 ± 22
Insulin (ng/ml)	33.8 ± 5.2*	13.1 ± 1.6#	26.6 ± 3.5*	29.4 ± 8.7*	7.2 ± 1.1#	5.3 ± 0.4#	5.7 ± 2.1#	7.1 ± 1.5#
HbA1c (percent)	6.39 ± 0.4*	5.20 ± 0.38#	6.33 ± 0.47*	6.67 ± 0.59*	4.22 ± 0.34#	4.66 ± 0.40#	5.06 ± 0.39#	4.68 ± 0.82#

NOTE: Results represent means ± SEM; n, number of animals. Numbers represent fasted values.
Significantly different from diabetic untreated.
* Significantly different from control untreated.

Young hybrid ZDF/SHHF diabetic rats (6-14 weeks of age) showed significantly elevated fasted plasma glucose, triglycerides, total and HDL cholesterols, insulin and hemoglobin A1c compared to CU rats following 8 weeks of treatment (Table 3). Pioglitazone normalized plasma glucose, total cholesterol, insulin and hemoglobin A1c in the DP group and significantly decreased triglycerides relative to DU. Vanadyl significantly reduced plasma glucose and triglyceride levels whereas hydralazine significantly reduced total and elevated HDL cholesterol levels. There was no effect by any drug on control rats. There was no difference between any groups with respect to free fatty acid levels.

3.5 Cardiac Performance

Cardiac performance was measured by three parameters: left ventricular developed pressure (LVDP), rate of force development by the left ventricle (+dP/dt), and rate of relaxation by the left ventricle (-dP/dt). LVDP was not affected by diabetes alone (DU) at any of the preload pressures from 7.5 to 22.5 cm H_2O in young ZDF diabetic rats (FIG. 10), in older ZDF diabetic rats (FIG. 11) or in young hybrid ZDF/SHHF diabetic rats (FIG. 12). Pioglitazone treatment caused a significantly reduced function in young ZDF diabetic rats at the two lowest preloads (7.5 and 10 cm H_2O) compared to CU rats. In young hybrid ZDF/SHHF rats, pioglitazone caused control rats to have significantly improved function at the three highest preloads (17.5, 20 and 22.5 cm H_2O) compared to CU (FIG. 12A). Vanadyl and hydralazine did not influence LVDP in either diabetic or non-diabetic rats in either older ZDF (FIG. 11B,C) or young hybrid ZDF/SHHF rats (FIG. 12B,C).

Rate of force development (+dP/dt) was also not affected by diabetes in young, old or hybrid ZDF diabetic rats compared to non-diabetic rats (FIG. 13, 14, 15). As with LVDP, pioglitazone treatment significantly reduced diabetic heart function, at the two lowest preloads tested (7.5 and 10 cm H2O) in young ZDF diabetic rats (DP; FIG. 13), but not in the older ZDF diabetic or young hybrid ZDF/SHHF diabetic rats compared to CU (FIG. 13). Young ZDF diabetic rats have impaired function compared to CU rats, but this trend was not seen in older ZDF diabetic rats or young hybrid ZDF/SHHF diabetic rats. Vanadyl and hydralazine had no significant effect on rate of force development in either diabetic or control young, old or hybrid ZDF rats (FIG. 14B,C; 15B,C).

Rate of ventricular relaxation (-dP/dt) showed similar responses to +dP/dt and LVDP in that there was no significant effect of untreated diabetes on this cardiovascular parameter in old, young or hybrid ZDF diabetic rats relative to non-diabetic littermates. A trend seems apparent, suggesting that diabetic hearts have impaired function in young ZDF diabetic rats (FIG. 16). Pioglitazone significantly impaired diabetic heart function, but only at the two lowest preloads tested (7.5 and 10 cm H2O) in the young ZDF diabetic rats (FIG. 16). Hydralazine caused diabetic rat hearts to relaxed significantly slower (-dP/dt) than control hearts at the three highest pressures (17.5, 20 and 22.5 cm H2O) and caused hearts of the CH rats to have significantly increased function at the same three high pressures compared CU or DU (FIG. 17C). Pioglitazone and vanadyl had no effect on -dP/dt in diabetic or non-diabetic older ZDF and young hybrid ZDF/SHHF rats (FIG. 17A,B; 18A,B). Hydralazine also affected -dP/dt in young hybrid ZDF/SHHF diabetic hearts (FIG. 18C). Diabetic hearts exposed to hydralazine had significantly elevated rates of relaxation compared to CU at the 4 highest preloads (15, 17.5, 20 and 22.5 cm H2O).

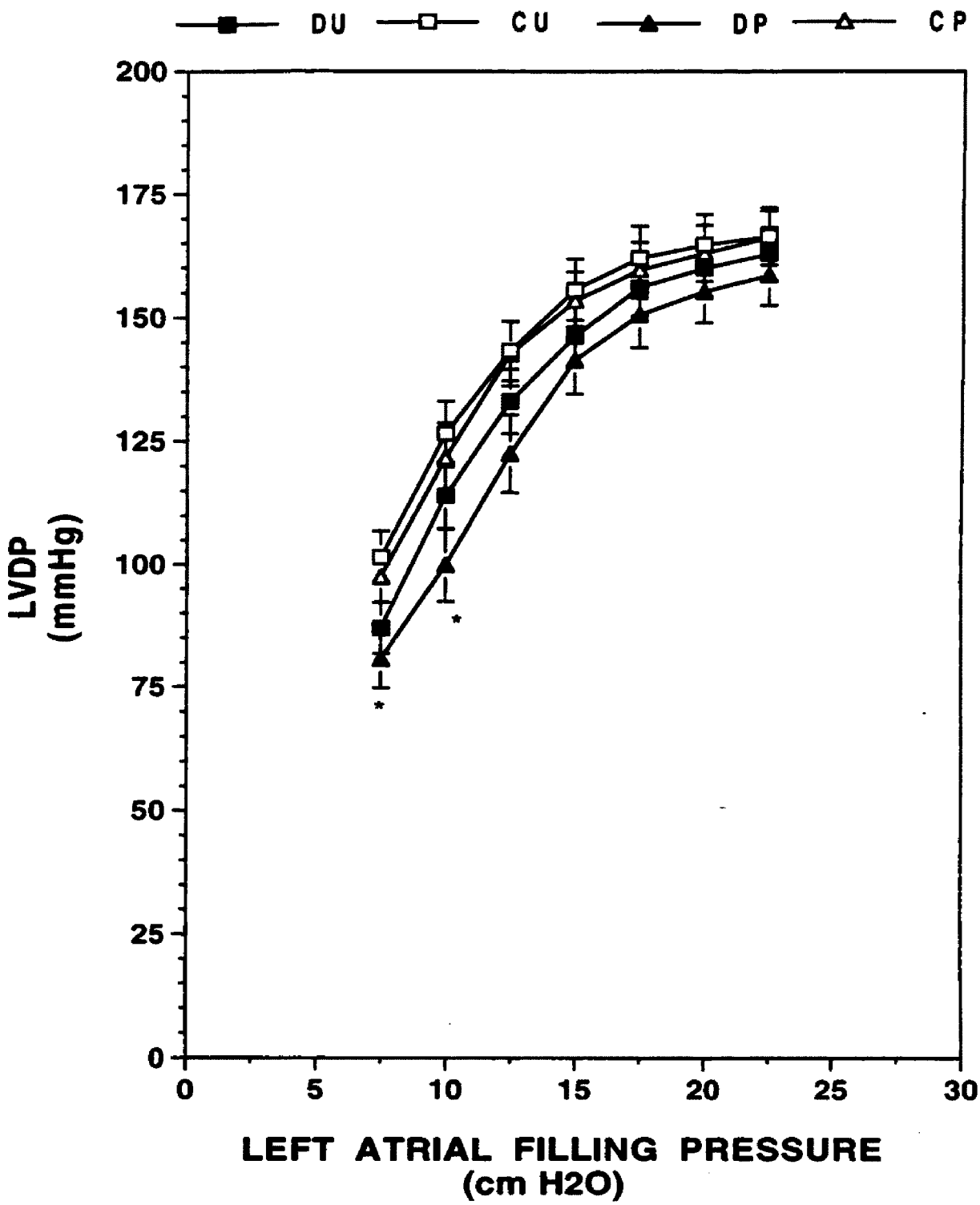


FIG.10. Influence of oral pioglitazone on left ventricular developed pressure (LVDP) in isolated working hearts of young ZDF diabetic rats and non-diabetic littermates. Results illustrate the LVDP as left atrial filling pressures changed at 2.5 cm H2O intervals from 7.5 to 22.5 cm. Data represent mean ± SEM. *, significantly different than control untreated.

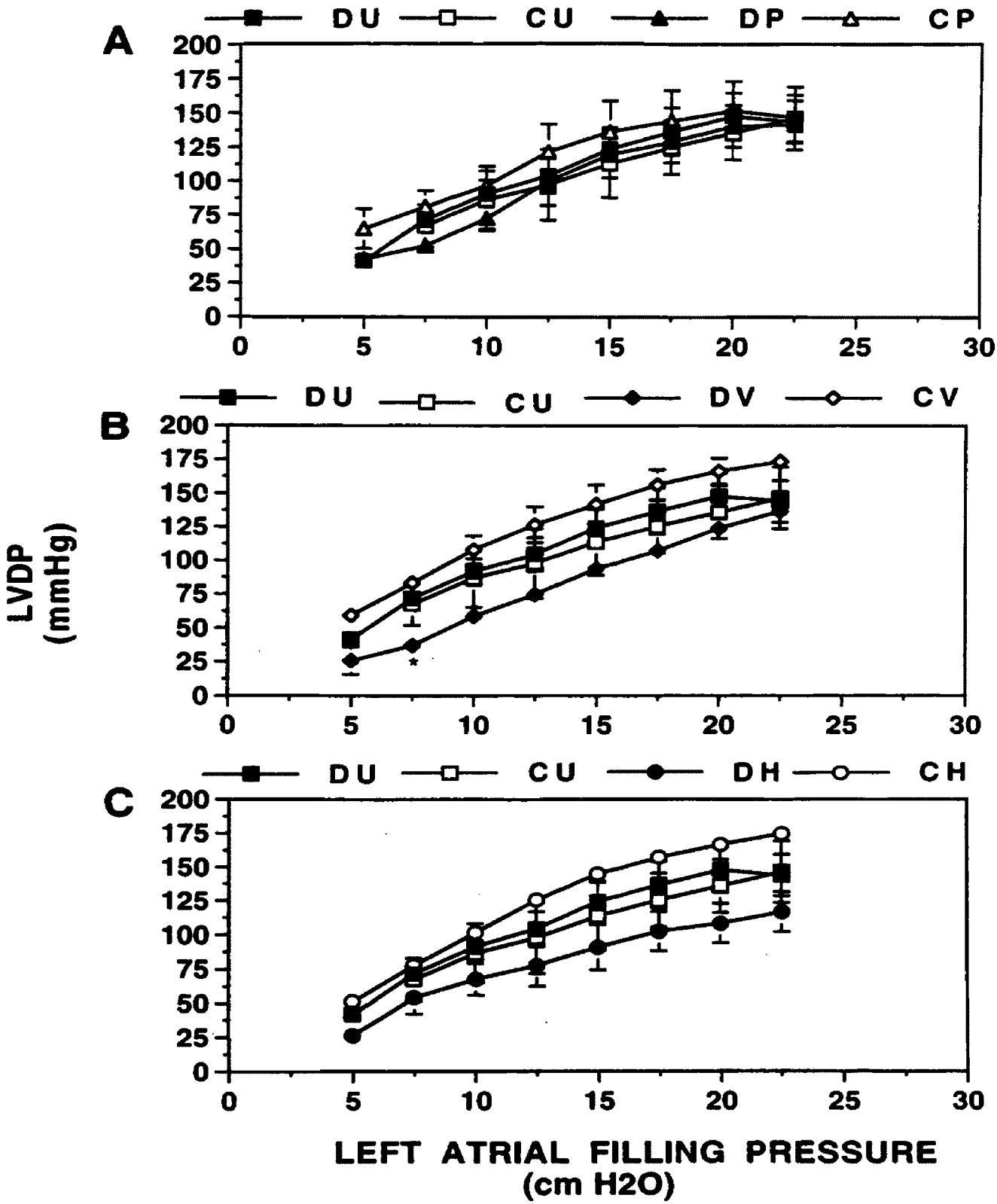


FIG. 11. Influence of oral (A) pioglitazone, (B) vanadyl and (C) hydralazine on left ventricular developed pressure (LVDP) in isolated working hearts of older ZDF diabetic rats and non-diabetic littermates. Results illustrate the LVDP as left atrial filling pressures changed at 2.5 cm H2O intervals from 7.5 to 22.5 cm. Data represent means ± SEM. *, significantly different from control untreated.

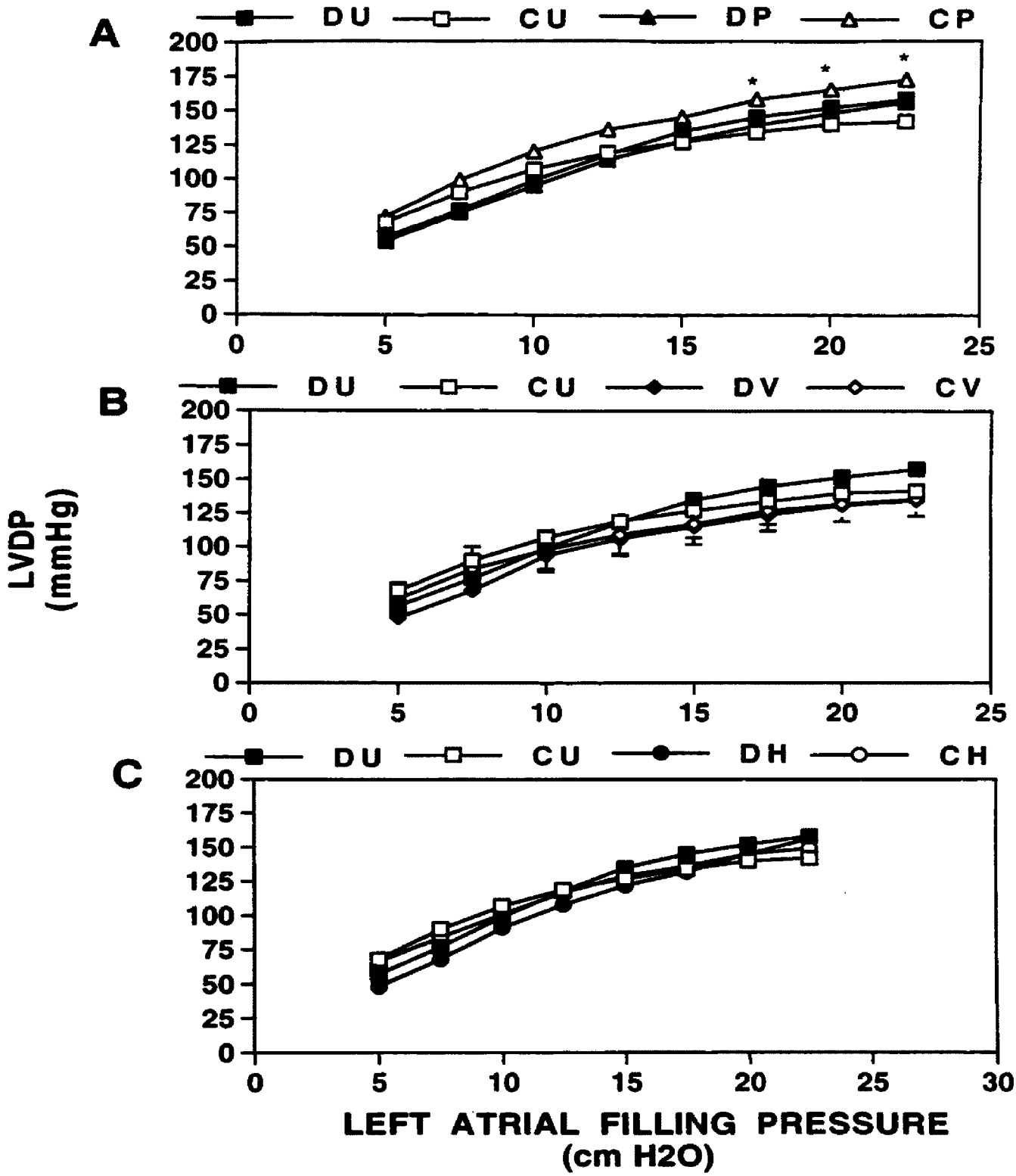


FIG. 12. Influence of oral (A) pioglitazone, (B) vanadyl and (C) hydralazine on left ventricular developed pressure (LVDP) in isolated working hearts of young hybrid ZDF/SHHF diabetic rats and non-diabetic littermates. Results illustrate LVDP as left atrial filling pressures changed at 2.5 cm H2O intervals from 7.5 to 22.5 cm. Data represent mean ± SEM. *, significantly different than control untreated.

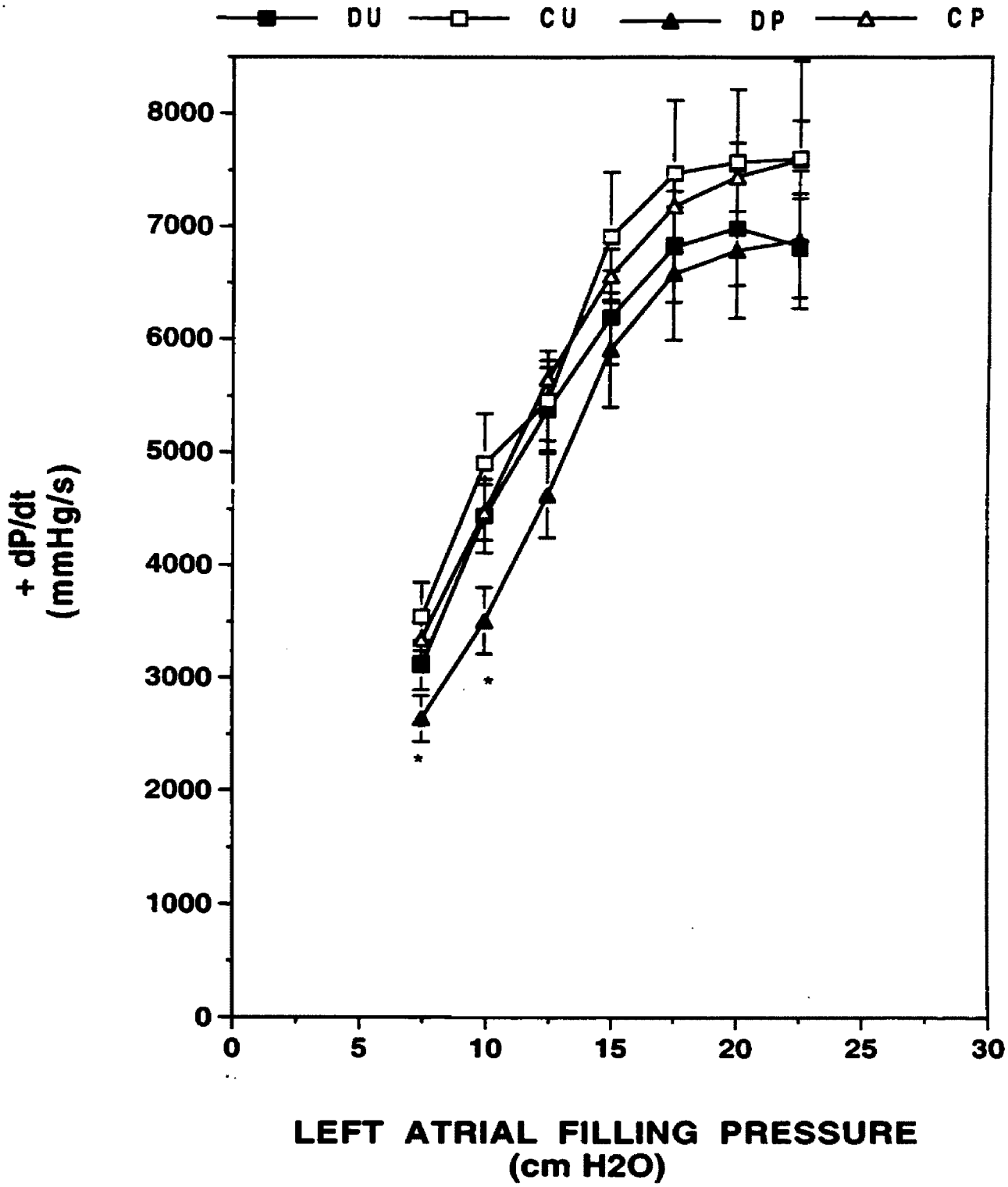


FIG. 13. Influence of oral pioglitazone on rate of force development (+dP/dt) in isolated working hearts of young ZDF diabetic rats and non-diabetic littermates. Results illustrate +dP/dt in the left ventricle as left atrial filling pressures changed at 2.5 cm H2O intervals from 7.5 to 22.5 cm. Data represent mean ± SEM. *, significantly different from control untreated.

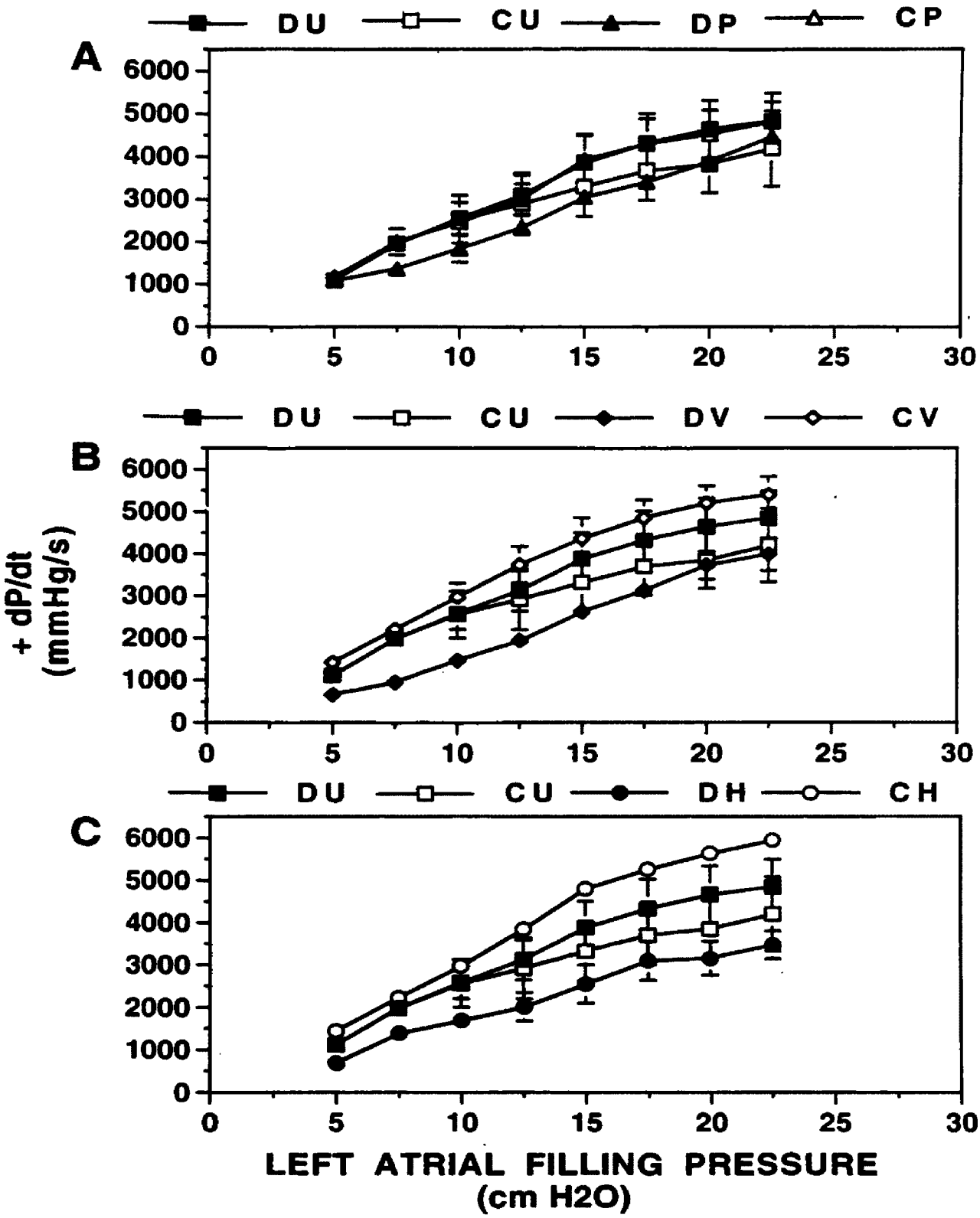


FIG. 14. Influence of oral (A) pioglitazone, (B) vanadyl and (C) hydralazine on rate of force development (+dP/dt) in isolated working hearts of older ZDF diabetic rats and non-diabetic littermates. Results illustrate the +dP/dt in the left ventricle as left atrial filling pressures changed at 2.5 cm H2O intervals from 7.5 to 22.5 cm. Data represent mean ± SEM.

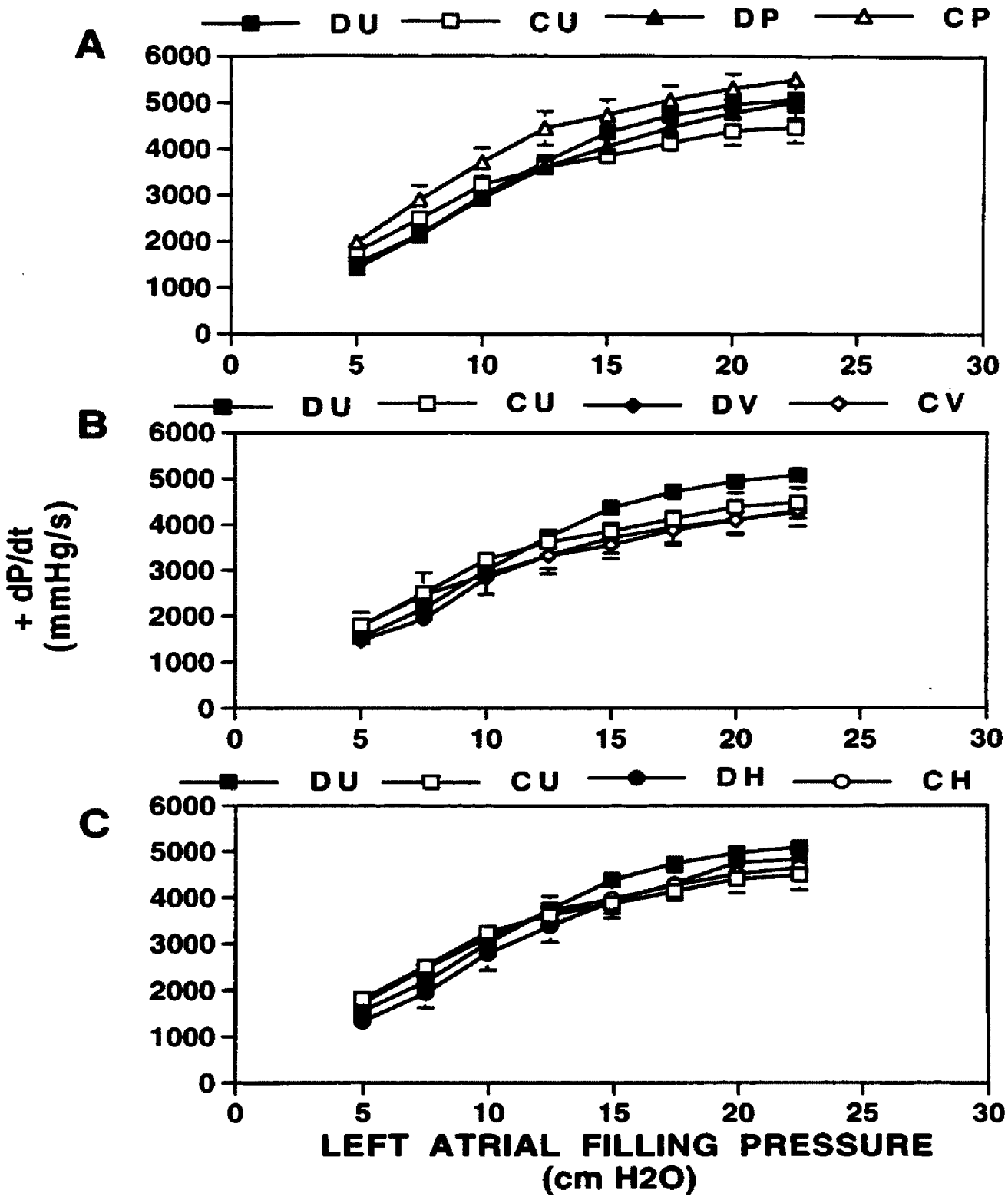


FIG. 15. Influence of oral (A) pioglitazone, (B) vanadyl and (C) hydralazine on rate of force development (+dP/dt) in isolated working hearts in young hybrid ZDF/SHHF diabetic rats and non-diabetic littermates. Results illustrate the +dP/dt in the left ventricle as left atrial filling pressures changed at 2.5 cm H2O intervals from 7.5 to 22.5 cm. Data represent mean ± SEM.

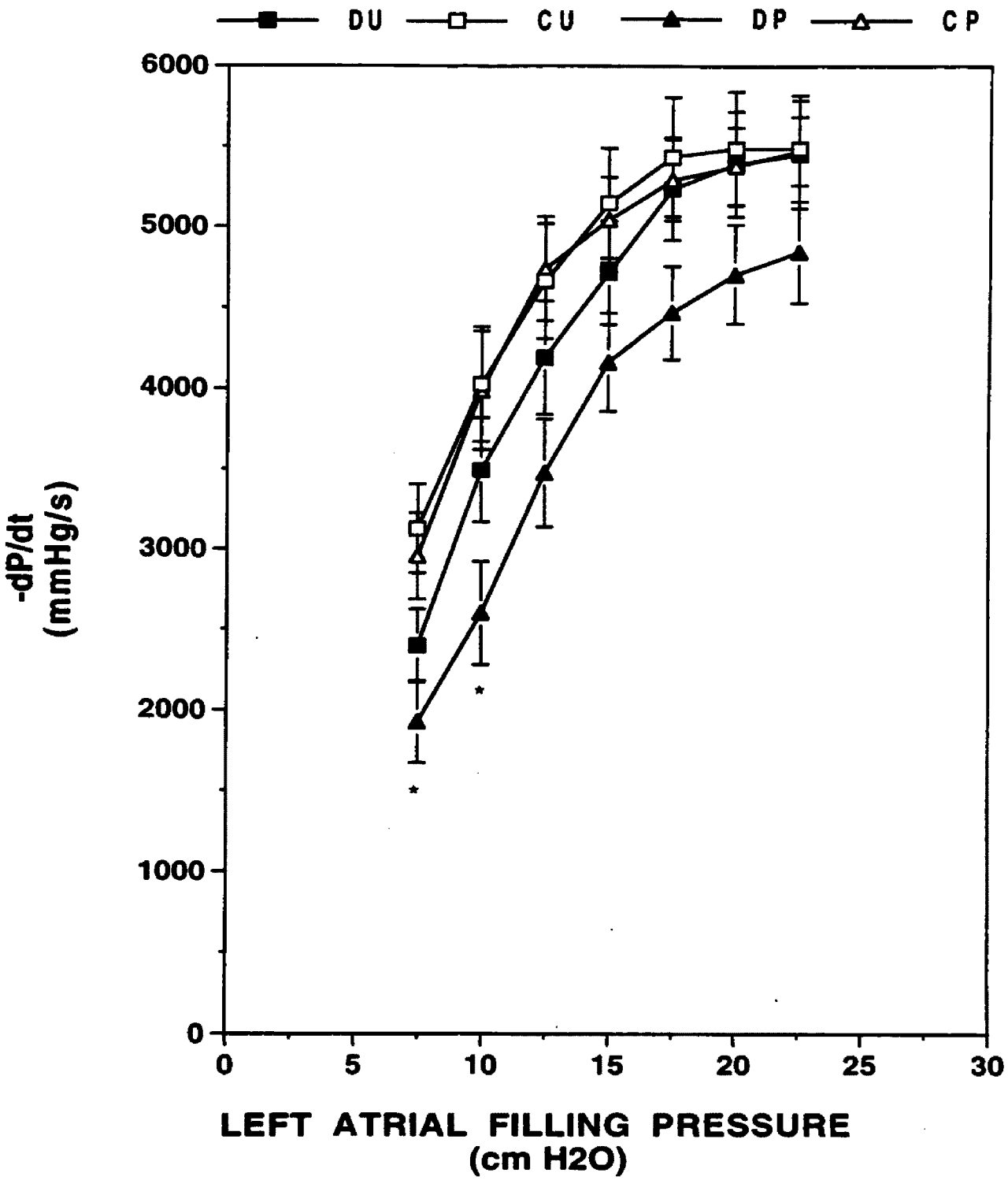


FIG. 16. Influence of pioglitazone on rate of relaxation (-dP/dt) in isolated working hearts of young ZDF diabetic rats and non-diabetic littermates. Results illustrate the -dP/dt of the left ventricle as left atrial filling pressures changed at 2.5 cm H2O intervals from 7.5 to 22.5 cm. Data represent mean ± SEM. *, significantly different than control untreated.

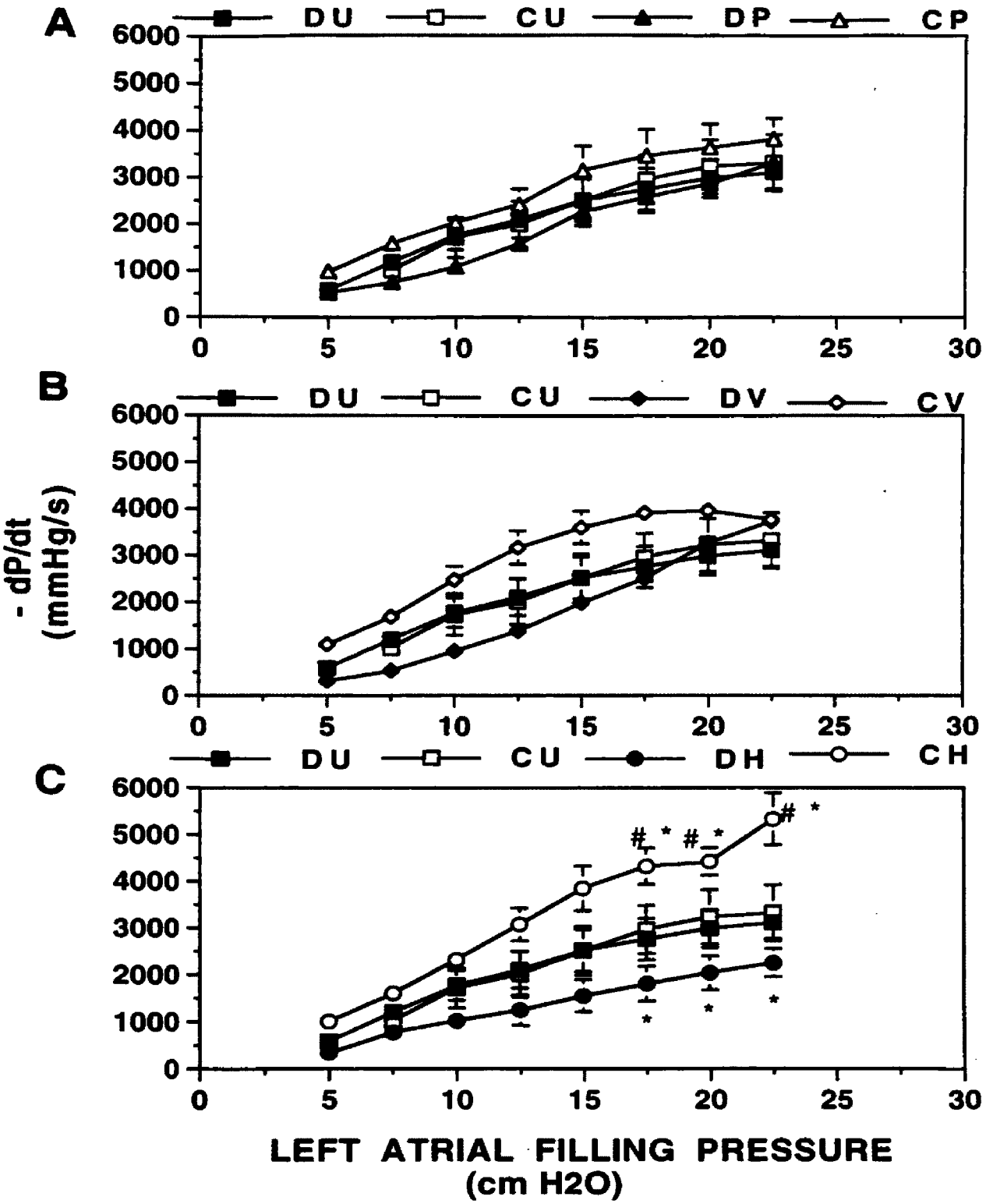


FIG. 17. Influence of oral (A) pioglitazone, (B) vanadyl and (C) hydralazine on rate of relaxation (-dP/dt) in isolated working hearts in older ZDF diabetic rats and non-diabetic littermates. Results illustrate the -dP/dt of the left ventricle as left atrial pressures changed at 2.5 cm H2O intervals from 7.5 to 22.5 cm. Data represent mean ± SEM. #, significantly different from diabetic untreated. *, significantly different from control untreated.

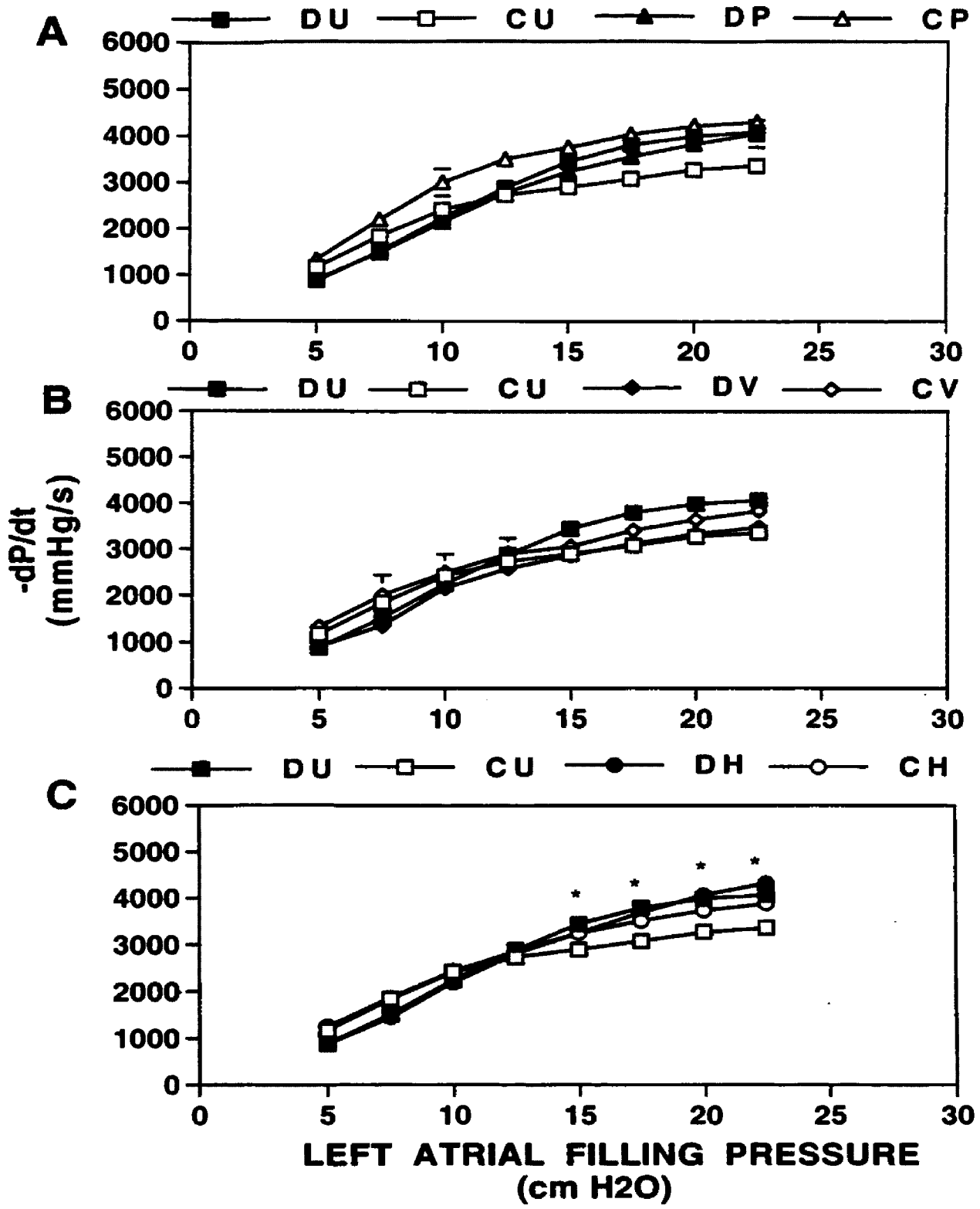


FIG. 18. Influence of oral (A) pioglitazone, (B) vanadyl and (C) hydralazine on rate of relaxation (-dP/dt) in isolated working hearts of young hybrid ZDF/SHHF diabetic rats and non-diabetic littermates. Results illustrate the -dP/dt of the left ventricle as left atrial filling pressures changed at 2.5 cm H2O intervals from 7.5 to 22.5 cm. Data represent mean ± SEM.
*, significantly different from control untreated.

3.6 Vascular Reactivity

Vascular reactivity was measured by determining the ability of aortic rings to contract after incremental doses of norepinephrine (NE). Data were compiled as dose response curves. In older ZDF diabetic rats, aortic sensitivity to NE was significantly increased compared to untreated non-diabetic littermates. Pioglitazone significantly attenuated the aortic sensitivity of DU rats, but does not normalize it to CU levels. Pioglitazone had no effect on responsiveness in non-diabetic rats (FIG. 19A). Vanadyl lowered NE responsiveness similarly, but more potently and completely than pioglitazone relative to aortas of DU rats. Vanadyl was without effect on non-diabetic rats (FIG. 19B). Hydralazine not only failed to reduce aortic sensitivity to NE, but actually increased sensitivity above that seen in tissues from untreated diabetic rats. Regardless of diabetic status or drug treatment, vascular reactivity was not changed in young hybrid ZDF/SHHF rats (FIG. 20A,B,C).

The maximal response to NE was significantly elevated in older ZDF diabetic rats compared to CU rats. Pioglitazone and vanadyl decreased NE sensitivity in diabetic rat aortas. Hydralazine, on the other hand, significantly increased sensitivity in non-diabetic rats with no effect relative to DU (Table 4). The maximal response to NE in young hybrid ZDF/SHHF diabetic rats was not different than that of non-diabetic rats, nor did piogitazone, vanadyl or hydralazine have any sigificant effect on this parameter (Table 5).

The maximal response to 40 mM KCl was also significantly higher in older ZDF diabetic rats compared to CU rats. Pioglitazone and vanadyl significantly reduced these levels in diabetic rats while vanadyl and hydralazine significantly

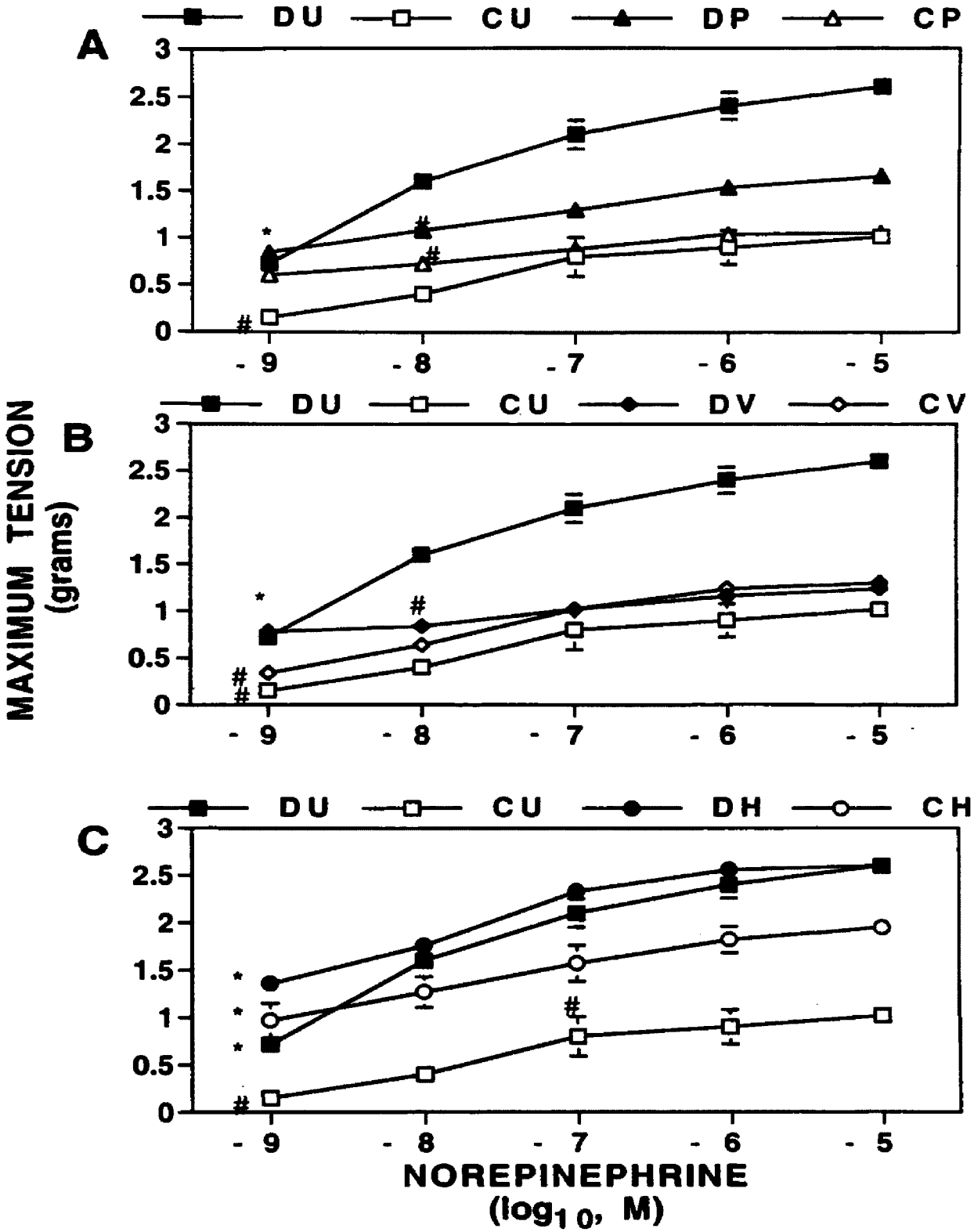


FIG. 19. Effect of oral (A) pioglitazone, (B) vanadyl and (C) hydralazine on norepinephrine dose response curves in isolated aorta of older ZDF diabetic rats and non-diabetic littermates. Data represent mean ± SEM. #, significantly different from diabetic untreated from that point throug the rest of the curve. *, significantly different from control untreated from that point through the rest of the curve.

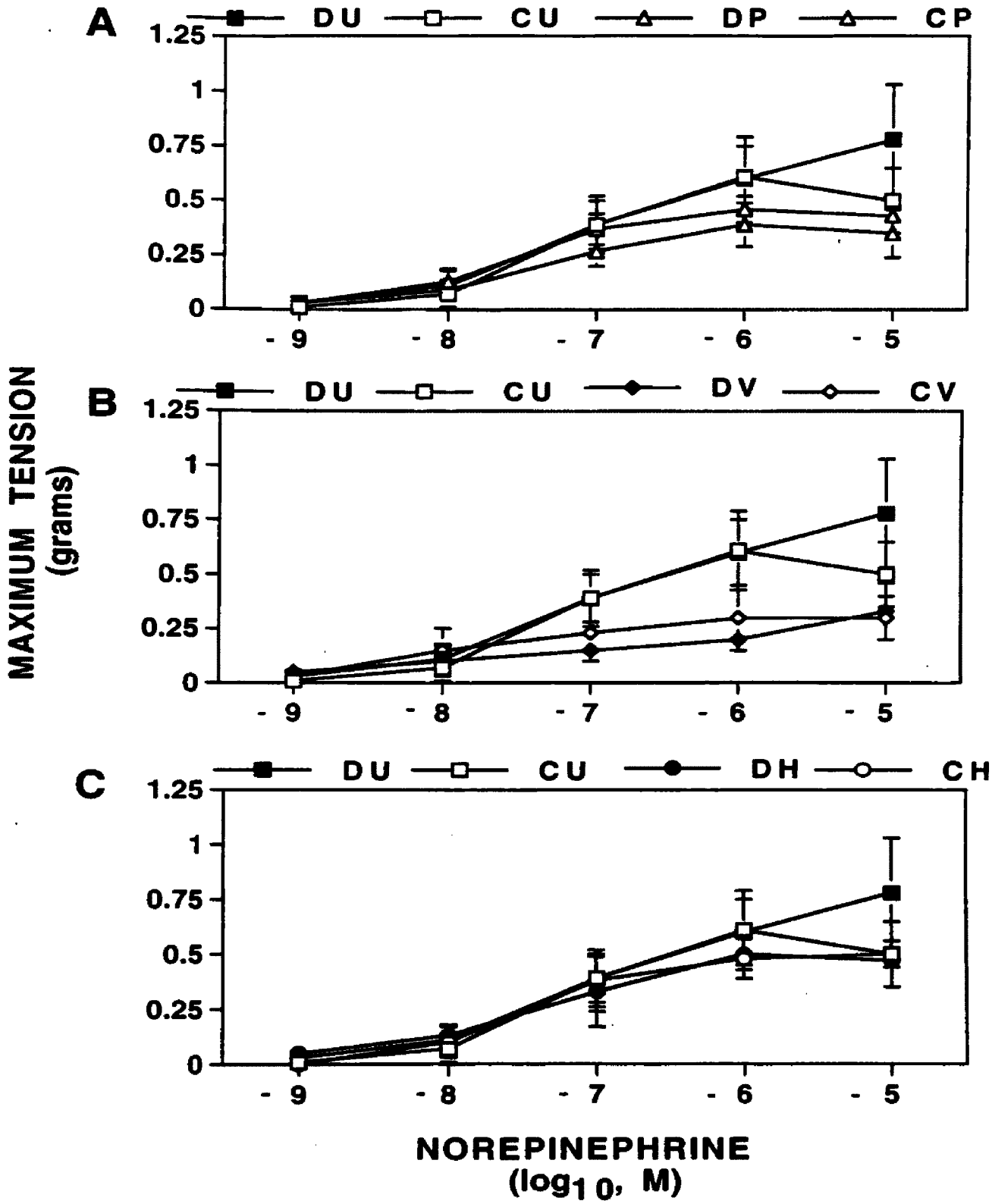


FIG. 20. Effect of oral (A) pioglitazone, (B) vanadyl and (C) hydralazine on norepinephrine dose response curves in isolated aorta of young hybrid ZDF/SHHF diabetic rats and non-diabetic littermates. Data represent mean ± SEM.

TABLE 4. Effect of oral pioglitazone, vanadyl and hydralazine on the maximal response to 40 mM KCl and norepinephrine (NE) after 7 weeks of treatment in older ZDF diabetic rats and non-diabetic littermates.

	DU (n=5)	DP (n=5)	DV (n=5)	DH (n=4)	CU (n=5)	CP (n=5)	CV (n=5)	CH (n=4)
40 mM KCl (grams of tension)	1.60 ± 0.12 *	1.06 ±0.05*#	1.08 ±0.12*#	1.30 ± 0.06 *	0.63 ± 0.09 #	0.68 ± 0.10 #	1.10 ±0.12*#	1.08 ± 0.25 #
MAX. NE RESPONSE (grams of tension)	2.68 ± 0.05 *	1.66 ±0.04*#	1.24 ± 0.10 #	2.60 ± 0.12 *	1.03 ± 0.13 #	1.06 ± 0.07 #	1.30 ± 0.09 #	1.95 ±0.06*#

NOTE: Data represent means ± SEM; n, number of animals. Numbers represent fasted values.
#Significantly different from diabetic untreated.
*Significantly different from control untreated.

TABLE 5. Effect of oral pioglitazone, vanadyl and hydralazine on the maximal response to 40 mM KCl and norepinephrine (NE) after 8 weeks of treatment in young hybrid ZDF/SHHF diabetic rats and non-diabetic littermates.

	DU (n=7)	DP (n=7)	DV (n=4)	DH (n=4)	CU (n=7)	CP (n=7)	CV (n=4)	CH (n=4)
40 mM KCl (grams of tension)	0.44 ± 0.08	0.40 ± 0.08	0.38 ± 0.05	0.53 ± 0.10	0.44 ± 0.09	0.44 ± 0.07	0.40 ± 0.06	0.43 ± 0.06
MAX. NE RESPONSE (grams of tension)	0.78 ± 0.25	0.39 ± 0.10	0.33 ± 0.13	0.50 ± 0.11	0.61 ± 0.18	0.46 ± 0.06	0.30 ± 0.10	0.50 ± 0.06

NOTE: Data represent means ± SEM; n, number of animals. Numbers represent fasted values.
#Significantly different from diabetic untreated.
*Significantly different from control untreated.

increased the response in non-diabetic littermates (Table 4). In young hybrid ZDF/SHHF rats there was no significant difference with respect to the response to 40 mM KCl in any treatment group (Table5).

3.7 Cardiovascular Parameters

Additional cardiovascular parameters measured included systolic and diastolic blood pressure, mean arterial blood pressure, cardiac cholesterol and triglycerides, heart weight and heart weight to body weight ratios. Systolic, diastolic and mean arterial blood pressures were not measured in young ZDF diabetic and non-diabetic rats due to lack of necessary equipment. Cardiac cholesterol did not differ between diabetic and non-diabetic rats and no effect was observed with pioglitazone treatment. There was a significant difference between diabetic and non-diabetic levels of cardiac triglycerides. Pioglitazone treatment lowered cardiac triglycerides in both diabetic and non-diabetic rats. Pioglitazone significantly increased heart sizes in diabetic rats and in conjunction with significantly elevated body weights resulted in significantly lower heart weight to body weight ratios compared to untreated and non-diabetic rats (Table 6).

Hydralazine, but not pioglitazone or vanadyl, significantly decreased systolic, diastolic and mean arterial blood pressures in both diabetic and non-diabetic older ZDF rats. Cardiac cholesterol in diabetic rats was significantly decreased compared to non-diabetic rats. Hydralazine, but not pioglitazone and vanadyl, significantly decreased cardiac cholesterol in control rats with no effect seen in diabetic rats. Cardiac triglycerides are significantly higher in diabetic compared

to non-diabetic rats. Vanadyl therapy significantly reduced elevated cardiac triglyceride levels in DU rats. Diabetic heart weight and body weights were not significantly different from heart weights in non-diabetic rats. Vanadyl significantly lowered body weights in both diabetic and non-diabetic rats and significantly lowered heart weights in diabetic rats. None of the drug treatments modified heart weight to body weight ratios (Table 7).

Although a relative trend appears for hydralazine to decrease systolic, diastolic and mean arterial blood pressures in young hybrid ZDF/SHHF rats compared to untreated diabetic and non-diabetic young hybrid ZDF/SHHF rats, significant changes were not observed. Pioglitazone did decrease systolic, diastolic and mean arterial blood pressures in diabetic rats compared to CU rats. Cardiac cholesterol and triglyceride levels were not altered by pioglitazone, vanadyl or hydralazine in either diabetic or non-diabetic rats. Pioglitazone treatment caused significantly increased heart weights in diabetic rats compared to CU and DU. Diabetic rats had significantly elevated body weights compared to control rats. Pioglitazone treatment significantly increased the body weights of diabetic rats whereas vanadyl significantly decreased body weights compared to DU rats. Vanadyl and hydralazine treatment also significantly reduced body weights in non-diabetic rats compared to CU and DU rats; however, this phenomenon occurred from the initiation of the study and was probably not reflective of drug effect. Heart weight to body weight ratios were significantly increased by hydralazine in control rats but not diabetic rats.

TABLE 6. Effect of oral pioglitazone treatment on systolic, diastolic and mean arterial pressures, cardiac cholesterol and triglycerides, heart weight, body weight and heart weight to body weight ratio after 6 weeks of treatment in young ZDF diabetic rats and non-diabetic littermates.

CARDIAC PARAMETER	**DU** (n=12)	**DP** (n=12)	**CU** (n=11)	**CP** (n=12)
Systolic/Diastolic (mmHg)	--	--	--	--
Mean Arterial Pressure (mmHg)	--	--	--	--
Cardiac Cholesterol (nmol/mg protein)	237.5 ± 26.4	217.6 ± 26.1	226.0 ± 32.1	183.0 ± 22.3
Cardiac Triglycerides (nmol/mg protein)	280.0 ± 35.5 *	168.0 ± 25.9 #	142.0 ± 30.2 #	81.0 ± 12.8 #*
Heart Weight (grams)	1.912 ± 0.078	2.223 ± 0.087 #*	1.755 ± 0.053	1.931 ± 0.068
Body Weight (grams)	439.9 ± 7.4 *	633.4 ± 10.7 * #	363.4 ± 6.8#	379.8 ± 6.3#
Heart:Body Weight Ratio ($x10^{-3}$)	4.34 ± 0.15	3.51 ± 0.11 * #	4.85 ± 0.20	5.09 ± 0.18

NOTE: A dash indicates that this parameter was not measured. Results represent means ± SEM; n, number of animals. Numbers represent fasted values.

Significantly different from diabetic untreated.

* Significantly different from control untreated.

TABLE 7. Effect of oral pioglitazone, vanadyl and hydralazine treatment on systolic, diastolic and mean arterial blood pressures, cardiac cholesterol and triglycerides, heart weight, body weight and heart weight to body weight ratio after 7 weeks of treatment in older ZDF diabetic rats and non-diabetic littermates.

CARDIAC PARAMETER	DU (n=5)	DP (n=5)	DV (n=5)	DH (n=4)	CU (n=5)	CP (n=5)	CV (n=5)	CH (n=4)
Systolic/ Diastolic (mmHg)	143/105	140/116	140/102	104#*/78 #*	136/111	145/111	142/105	101#*/80 #*
Mean Arterial Blood Pressure (mmHg)	118 ± 8	124 ± 8	116 ± 11	91 ± 7 #*	120 ± 10	126 ± 6	117 ± 7	87 ± 8 #*
Cardiac Cholesterol (nmol/mg protein)	13.80 ±1.9*	18.07 ± 1.5	15.26 ± 2.8	16.08 ± 3.6	20.78 ±1.0#	16.20 ± 1.6	18.68 ± 1.9	7.55 ± 0.6#*
Cardiac Triglycerides (nmol/mg protein)	16.08 ±1.6 *	18.96 ±2.0*	9.10 ± 1.1 #	24.1±3.0#*	8.17 ± 0.4#	5.81 ± 0.7#	4.70 ± 0.6#	7.54 ± 0.3#
Heart Weight (grams)	1.7 ± 0.1	1.8 ± 0.2	1.3 ± 0.1#	1.6 ± 0.1	1.7 ± 0.2	1.7 ± 0.1	1.4 ± 0.1	1.6 ± 0.2
Body Weight (grams)	450.0 ± 10.8	472.8 ± 9.8	347.6 ± 14.7#*	432.8 ± 8.0	442.6 ± 17.1	495.6 ± 13.8	354.0 ± 9.2#*	432.8 ± 20.5
Heart:Body Weight ratio (x 10^{-3})	3.83 ± 0.04	3.88 ± 0.19	3.84 ± 0.08	3.73 ± 0.12	3.83 ± 0.11	3.51 ± 0.13	4.00 ± 0.11	3.74 ± 0.22

NOTE: Results represent means ± SEM; n, number of animals. Numbers represent fasted plasma values.
#Significantly different from diabetic untreated.
*Significantly different from control untreated.

TABLE 8. Effect of oral pioglitazone, vanadyl and hydralazine treatment on systolic, diastolic and mean arterial blood pressure, cardiac cholesterol and triglycerides, heart weight, body weight and heart weight to body weight ratio after 8 weeks of treatment in young hybrid ZDF/SHHF diabetic rats and non-diabetic littermates.

CARDIAC PARAMETER	DU (n=7)	DP (n=7)	DV (n=4)	DH (n=4)	CU (n=7)	CP (n=7)	CV (n=4)	CH (n=4)
Systolic/ Diastolic (mmHg)	130 / 88	117* / 78*	140 / 94	118 / 79	154 / 106	140 / 93	149 / 104	125 / 77
Mean Arterial Blood Pressure (mmHg)	103 ± 6	91 ± 4*	109 ± 8	92 ± 6	123 ± 4	109 ± 7	119 ± 12	93 ± 17
Cardiac Cholesterol (nmol/mg protein)	10.81 ± 1.3	12.71 ± 1.5	12.30 ± 2.1	13.75 ± 2.7	9.72 ± 1.2	12.03 ± 2.0	13.24 ± 3.7	16.72 ± 2.0
Cardiac Triglycerides (nmol/mg protein)	8.49 ± 1.4	8.68 ± 1.6	8.53 ± 1.3	9.05 ± 1.6	4.67 ± 0.3	5.70 ± 0.7	4.92 ± 1.4	9.33 ± 1.75
Heart Weight (grams)	1.818 ± 0.034	2.027 ± 0.049 #*	1.738 ± 0.058	1.737 ± 0.013	1.760 ± 0.027	1.883 ± 0.039	1.534 ± 0.13	1.741 ± 0.063
Body Weight (grams)	571 ± 6 *	708 ± 8 #*	541 ± 7 #*	548 ± 16*	446 ± 6 #	469 ± 10 #	360 ± 14 #*	385 ± 14 #*
Heart:Body Weight ratio ($x10^{-3}$)	3.186 ± 0.067 *	2.866 ± 0.074 *	3.209 ± 0.074 *	3.174 ± 0.068 *	3.952 ± 0.082 #	4.027 ± 0.099 #	4.248 ± 0.240 #	4.542 ± 0.204 #*

NOTE: Results represent means ± SEM; n, number of animals. Numbers represent fasted values.
#Significantly different from diabetic untreated.
*Significantly different from control untreated.

4.0 DISCUSSION

Increased body weight is apparently an important factor in insulin resistance and hyperlipidemia in NIDDM. This is particularly true in obese NIDDM patients, in whom it has been shown that significant weight loss through diet and exercise can alleviate insulin resistance and possibly eliminate the need to take oral hypoglycemic agents. If adequate control can not be attained with diet and exercise, drug therapy is necessary.

Zucker Diabetic Fatty (ZDF) rats are hyperinsulinemic, hyperglycemic, hypertriglyceridemic and hypercholesterolemic and simulate human NIDDM. At an older age, these ZDF rats develop IDDM, become hypoinsulinemic and remain hyperglycemic, hypertriglyceridemic and hyper-cholesterolemic. Hybrid ZDF/SHHF rats maintain the same characteristics of the young ZDF rats, but have the added potential of developing hypertension and/or congestive heart failure. In all 3 groups of rats, diabetic fatty rats had elevated body weight compared to control rats at some point. In the young and older ZDF, this increased body weight occurred from the initiation of the study (7 weeks and 13 weeks, respectively); however, the significantly increased body weight of young hybrid ZDF/SHHF diabetic rats occurred only after the rats reached 9 weeks of age.

Pioglitazone, a thiazolidinedione, caused a significant increase in body weights in the young ZDF diabetic rats and young hybrid ZDF-SHHF diabetic rats. There was not a significant body weight increase in young, old or hybrid non-diabetic rats treated with pioglitazone. In the older non-diabetic ZDF rats, pioglitazone appeared to cause an increase in body weight, but this trend was

not significant. By the end of the study in older ZDF rats (20 weeks of age) all diabetic and non-diabetic groups, except the vanadyl treated animals, had body weights that were not significantly different from each other. This body weight gain is consistent with results seen in KKA^{y} mice (Hofmann et al., 1991) and Wistar diabetic fatty rats (Sugiyama et al., 1990), even though their pioglitazone treatment lasted for a much shorter period of time. Sugiyama (1990) suggests that the increased body weight is attributed to increased food consumption in Wistar fatty rats (a rat model of NIDDM) treated with pioglitazone. It has also been shown that the effects of pioglitazone occur only in hyperinsulinemic rats and not normal or hypoinsulinemic states (Ikeda, 1990; Hofmann et al., 1991). This agrees with our study where body weight gains only existed in hyperinsulinemic young ZDF and young hybrid ZDF/SHHF rats, but not in hypoinsulinemic older ZDF rats. Food consumption was not directly measured in our experiments, but increased consumption could be observed on a daily basis by visual examination. Increased food consumption has also been reported as a result of insulin injection. It would seem that an increase in body weight would not be beneficial to NIDDM diabetic patients, the majority of whom are already obese; however, these and other studies conclude that there appears to be no detrimental effect on plasma components caused by pioglitazone.

Vanadyl sulfate treatment in older ZDF diabetic and non-diabetic littermates caused a significant reduction in body weights coincidental with a decrease in plasma glucose. This effect was also seen in the young hybrid ZDF/SHHF rats, but only after 8 weeks of treatment. Perhaps this is because the rats had not been at an adequate concentration for a sufficinet period of time since the rats were slowly brought up to a concentration known to decrease plasma glucose

study and at that point there was a significant decrease in plasma glucose in the diabetic rats treated with vanadyl. It has been suggested that the antidiabetic effects of vanadyl are mediated entirely through its appetite suppression caused weight loss (Malabu, U.H. et al., 1994). There are many reports, using pair fed animals, which contradict Malabu's theory. In these experiments, obese fa/fa rats were treated with food restrictions similar to the reduced consumption observed in vanadyl treated rats. These rats were compared to vanadyl treated obese fa/fa rats. It was determined that oral vanadate but not food restrictions decreased muscle insulin resistance in these obese rats (Brichard, S.M., 1992).

With optimal control of diabetes in humans and rats, plasma glucose levels decrease and approach 100 mg/dl. Clinically, fasting plasma glucose greater than 150 mg/dl is considered hyperglycemic. In old, young and hybrid diabetic rat groups (DU), plasma glucose levels were significantly elevated, albeit at different ages. The difference in ages of the animal models at the onset of treatments (7 weeks, young ZDF; 13 weeks, older ZDF; 6 weeks, hybrid ZDF/SHHF) resulted in two groups that were not hyperglycemic (young, a; hybrid, c) and one group that was hyperglycemic (older, b). In the young ZDF diabetic rats, hyperglycemia (286 ± 30 mg/dl) was established at 9 weeks of age; in young hybrid ZDF/SHHF rats, hyperglycemia (305 ± 35 mg/dl) became evident at 12 weeks of age. Regardless of treatment, non-diabetic rats did not develop hyperglycemia spontaneously nor did they become hypoglycemic after pioglitazone, vanadyl or hydralazine treatment.

Pioglitazone administration significantly reduced plasma glucose levels in the young ZDF diabetic and young hybrid ZDF/SHHF diabetic rats, but not in the older rat group. This is probably attributable to the fact that the

thiazolidinediones require some level of insulin to be effective; by the end of the study the older rats had apparently converted from type II (hyperinsulinemic) to type I (hypoinsulinemic) diabetics. This was indicated by the significant decrease in insulin levels in diabetic rats with respect to non-diabetic rats. This indicated a possible destruction of pancreatic β cells that originally tried to overcompensate for the elevated glucose levels in the diabetic rats. One suggested mechanism for the glucose lowering effect of pioglitazone is to stimulate expression of GLUT 4 glucose transporter mRNA. These new transporters subsequently translocate to membranes where they stimulate the rapid removal of glucose from the bloodstream into fat and muscle. This phenomenon has been observed in several models of insulin resistance in adipose tissue and muscle (Hofmann et al, 1991). By increasing transporter number and stabilizing message expression, stimulation of storage and metabolism of glucose can be expected as well as increased insulin sensitivity leading to decreased insulin resistance (Sandouk et al., 1993).

Also of interest was the observation that although rats were given a pioglitazone dose 3 fold greater than those reported necessary to control plasma glucose, no hypoglycemia was observed even when the rats were dosed and fasted concurrently. This is a significant advantage over the sulfonylureas in treating NIDDM. These compounds are known to cause hypoglycemia, which can be severe, as a side effect of treatment.

Hydralazine treatment had no effect on blood glucose levels in any of the rats. Vanadyl sulfate, however, did significantly lower plasma glucose in older ZDF diabetic rats, but did not normalize plasma glucose, possibly because the dose was not given in adequate amounts for a sufficient period of time. Vanadyl at

maximum concentrations lowered the plasma glucose levels of young hybrid ZDF diabetic rats once hyperglycemia was established. Vanadyl has been known to reduce plasma glucose levels in ob/ob mice, a model of NIDDM (Brichard et al., 1990) and in STZ-diabetic rats, a model of IDDM with low levels of circulating insulin (Heyliger et al., 1985).

Vanadyl is known to improve insulin sensitivity and reduce insulin resistance in diabetic rats, consistent with our results from old and hybrid diabetic rats. Vanadyl's reported ability to increase the diminished GLUT4 transporters in diabetic rats (Strout et al., 1990; Paquet et al., 1990) may be responsible for these effects.

Since elevated plasma insulin levels (hyperinsulinemia) are a good indicator of NIDDM and insulin resistance and given that the overall mechanism of action of pioglitazone is to reduce insulin resistance, plasma insulin levels were determined. If not corrected, hyperinsulinemia in diabetic and/or obese states can cause hepatic glucose production to increase and glucose utilization to decrease despite the presence of insulin (Defronzo et al., 1982). These metabolic abnormalities worsen the diabetes and make control very difficult.

Young ZDF rats maintained moderately elevated insulin values regardless of diabetic status. These levels were somewhat greater than those seen in the non-diabetic rats of hybrid origin (the other NIDDM group). Older ZDF diabetic rats had significantly lowered plasma insulin levels (similar to IDDM) compared to non-diabetic rats; whereas, young hybrid ZDF/SHHF diabetic rats had significantly elevated insulin levels compared to non-diabetic littermates (similar to NIDDM). Pioglitazone did not appear to reduce the hyperinsulinemia seen in

young ZDF diabetic rats. It also did not have an effect on the already low plasma insulin levels of the older ZDF diabetic rats. Pioglitazone did, however, significantly reduce plasma insulin levels in young hybrid ZDF/SHHF diabetic rats. These results are consistent with NIDDM models where pioglitazone works only when insulin is present. This explains why the drug was ineffective in the older rats that converted to IDDM with low insulin levels. By the end of the treatment period, the control rats had also begun to make the conversion to type II diabetes as evidenced by their significantly higher plasma insulin levels compared to the diabetic rats. A possible explanation for the failure of pioglitazone to eliminate hyperinsulinemia in the young ZDF diabetic rats (DU, 17 ± 3 ng/ml) might be that their insulin levels never reached the extremely elevated levels seen in the young hybrid ZDF/SHHF diabetic rats (DU, 35 ± 5 ng/ml).

Unlike pioglitazone, which improves insulin sensitivity, vanadyl tends to mimic insulin's actions. Vanadyl sulfate mimics the actions of insulin and thus is effective in improving glucose tolerance in NIDDM and IDDM models. No change in insulin levels were noted with vanadyl or hydralazine in diabetic or non-diabetic rats of young, old or hybrid origin.

It has been suggested that the insulin resistance in Wistar Fatty NIDDM rats may be ameliorated by the activation of tyrosine kinase activity in insulin receptors (Kobayashi et al., 1992). However, in that study, it was never established whether this was a secondary effect of metabolic improvement or a direct effect. Tyrosine kinase is necessary for properly functioning insulin receptors. After insulin binds to the α subunit, the receptor's β subunit undergoes autophosphorylation at its tyrosine residues. This results in activation of

tyrosine kinase which phosphorylates substrates eventually resulting in insulin's effects.

Insulin resistance has also been associated with elevated triglycerides and fatty acids since fatty acids are preferentially mobilized from adipose tissues and glucose disposal is inhibited (Kobayashi et al., 1992). Elevated triglycerides and cholesterol and decreased HDL cholesterol levels have been linked to increased incidence of coronary artery disease. Thus it is very important for diabetics and others with elevated lipids to normalize their lipids. This is especially true for diabetics who have a higher incidence of vascular abnormalities such as hypertension as a consequence of their disease.

Pioglitazone significantly lowers the elevated triglycerides observed in young, older and hybrid diabetic rats after 6, 7 and 8 weeks of treatment respectively. In the young ZDF diabetic rats, pioglitazone caused a complete normalization of plasma triglyceride levels by two weeks of treatment. In older ZDF diabetic rats, the effect was much more modest and plasma triglycerides were not completely normalized to control levels. In fact, the effect was only seen after 3 and 7 weeks of treatment, but not at 5 weeks of treatment. This could be a result of the conversion from type II to type I diabetes with its relative deficiency of insulin, a situation known to interdict pioglitazone activity. Young hybrid diabetic rats did not exhibit elevated triglycerides from the start of the experiments as did the young and older ZDF rats. However, when the hybrid ZDF diabetic rats developed hypertriglyceridemia, the degree of hypertriglyceridemia was ~2.4 fold greater than that seen in the young and older ZDF rats. This could relate to the additional genetic potential of developing cardiovascular abnormalities. Since this is the first study using the hybrid ZDF/SHHF rat model, it is difficult to

speculate on a contribution by genetic defects. Pioglitazone, given to these hybrid diabetic rats, dramatically improved, but did not totally normalize, plasma triglyceride levels compared to untreated hybrid ZDF rats. There was no effect seen in control rats from this drug. Vanadyl caused a significant decrease in plasma triglycerides, but only after 8 weeks of treatment and at the highest concentration, which coincides with a significant decrease in plasma glucose.

Decreased plasma triglyceride levels are probably due to an increase in clearance into tissues. This has been reported in both normal and obese insulin resistant rats (Sugiyama et al, 1990). The increased clearance of triglycerides can be explained by induction of the enzyme lipoprotein lipase that removes triglycerides from the blood stream. Insulin resistance may decrease triglyceride uptake by decreasing insulin's ability to induce lipoprotein lipase (Ikeda et al., 1990). Another potentially important reason for decreased triglyceride levels is that of enhanced insulin ability to inhibit adipose tissue triglyceride lipase.

Free fatty acid levels were significantly elevated in the young and older ZDF diabetic rat groups, but not in the young hybrid diabetic rats, compared to their non-diabetic littermates. Drug treatment did not significantly affect these elevated lipid levels. This suggests that correction of hypertriglyceridemia is not linked to enhanced insulin inhibition of triglyceride lipase since free fatty acids and glycerol are the major catabolic byproducts of triglyceride metabolism. Interestingly, the young ZDF rats, had much lower plasma free fatty acid levels compared to the older ZDF and young hybrid rats. The increased free fatty acid levels in the older ZDF rats could be due to their hypoinsulinemic state. Insulin is known to stimulate lipoprotein lipase and facilitate removal of free fatty acids

from the blood stream into tissues. In the presence of little insulin, free fatty acids rise, potentially explaining why free fatty acid levels were higher in the older than in the younger rats.

Lipoprotein profiles in ZDF rats were also affected by diabetic state. Young, older and hybrid ZDF diabetic rats showed elevated total cholesterol levels compared to non-diabetic littermates. Pioglitazone treatment significantly reduced, but did not normalize, plasma total cholesterol levels in the young ZDF diabetic group while it normalized total cholesterol in the young hybrid ZDF/SHHF diabetic rats. Pioglitazone did not change total cholesterol levels in the older ZDF diabetic rats. This failure to lower total cholesterol is probably due to the conversion from NIDDM to IDDM during the study. HDL cholesterol levels were elevated in young and hybrid ZDF diabetic rats compared to non-diabetic littermates. HDL levels in older ZDF diabetic rats appeared to be elevated, however a 45% standard error preclude significance. Although pioglitazone decreased plasma triglycerides in the young and hybrid diabetic rats, it did not decrease HDL levels implying that the triglycerides are derived from LDL and VLDL lipoproteins. There was no significant effect of pioglitazone in the young, old or hybrid non-diabetic rats with respect to total or HDL cholesterol. In KKAY mice, pioglitazone caused a 58% reduction in LDL and VLDL and a 25% increase in HDL cholesterol (Castle et al., 1993). It has been suggested that reduction in cholesterol levels may be due to a reduction in triglyceride levels that could produce an increase in lipoprotein lipase activity secondary to augmented insulin action (Sugiyama et al., 1990). Hydralazine had no significant effect on plasma lipoprotein profiles in older ZDF diabetic rats, but did significantly lower total cholesterol levels in young hybrid

ZDF/SHHF diabetic rats and significantly elevated HDL cholesterol levels in young hybid ZDF/SHHF diabetic rats.

Hemoglobin A1C (HbA1c) levels are a measure of glycosylated hemoglobin as a result of consistently elevated plasma glucose levels. It is indicative of relative plasma glucose control over the prolonged periods of 3 weeks or more. HbA1c was not measured in young ZDF rats, but was in the older and hybrid rats. In the older and hybrid rats a small, but significant, increase in HbA1c levels was observed in diabetic untreated rats compared to non-diabetic untreated rats. None of the drug treatments (pioglitazone, vanadyl or hydralazine) affected these levels in the older ZDF diabetic rats. Pioglitazone significantly decreased HbA1c levels in young hybrid ZDF/SHHF diabetic rats to levels seen in non-diabetic untreated rats. This indicates that pioglitazone provided excellent glucose control in young NIDDM (hybrid) diabetic rats. Vanadyl had no effect on HbA1c levels in either older or hybrid rats, possibly because the concentration of the drug was only at optimal levels for a week and would not significantly affect HbA1c levels during that short period of time.

Sustained obesity is associated with cardiac hypertrophy and compromised ventricular function. In order to assess cardiovascular performance and overall cardiovascular health, we measured left ventricular developed pressure, maximum rate of force development (+dP/dt) and maximum rate of relaxation (-dP/dt) in the isolated perfused working heart apparatus using glucose as the sole source of energy. We also determined ventricular levels of cholesterol and triglycerides and observed heart weights and heart weight to body weight ratios. In addition, systolic, diastolic and mean arterial blood pressures were

determined in conscious restrained rats using a semi-automated tail cuff device. As an added component, vascular reactivity was investigated in aortic strips.

Obesity results in increased metabolic demands, one of which is for oxygen. To compensate for this, cardiac output increases, more specifically either stroke volume increases induced due to increased filling pressures (preload) (Paulson and Tahiliani, 1992) or heart rate increases to increase cardiac output. This increased preload, over a period of time, results in left ventricular hypertrophy. In some cases, both preload and afterloads increase resulting in cardiomegaly. This is opposite to hypertension induced hypertrophy which results from increased arterial blood pressure (afterload). In obese patients, autopsy results indicate increased heart weights, primarily due to increased left ventricular mass, an increase which correlates well with increased body weight.

As a heart tries to compensate for increased filling pressures and thus becomes larger, there exists the possibility for ischemic episodes, myocardial infarction or heart failure. STZ-diabetic rats consistently show elevated heart weight to body weight ratios (McNeill, 1985); however, in STZ-diabetic animals, body weight is lower than in non-diabetic controls. To date there is no published data concerning heart weights in ZDF rats or hybrid ZDF/SHHF rats when exposed to oral hypoglycemic agents.

In our study, none of the diabetic untreated rats (young, old or hybrid) possessed altered heart weights compared to untreated non-diabetic littermates. Pioglitazone, however, caused a significant increase in heart weights in young ZDF and young hybrid ZDF/SHHF rats with no effect on the older ZDF rats (IDDM). Vanadyl and hydralazine had no effect on heart weights

in either diabetic or non-diabetic rats of young, old or hybrid ZDF origin. Hypertrophy can be beneficial initially, but if the condition progresses, it can lead to ventricular wall distention and weakening with the potential for decreased cardiac output and heart failure.

Pioglitazone did significantly increase heart weights and body weights; however, the degree of increase was not proportional, resulting in a significantly lowered heart weight to body weight ratio in the DP group compared to untreated young ZDF diabetic rats. There was no effect on heart weights or body weights caused by pioglitazone in the young non-diabetic rats or in either diabetic or non-diabetic older ZDF rats. Young hybrid ZDF/SHHF diabetic rats treated with pioglitazone also had decreased heart weight to body weight ratios which however, were not significant. Interestingly, hydralazine caused the non-diabetic group to have significantly elevated heart weight to body weight ratios compared to untreated control rats. Normal hearts weigh approximately 4 percent of the body weight. If the heart weight decreases compared to the body weight, the heart must work harder to properly perfuse the body. This can lead to ischemia and other cardiovascular abnormalities.

The differences seen in heart weight are interesting since the young ZDF diabetic rats evidenced significantly impaired cardiac performance as reflected by LVDP, +dP/dt and -dP/dt. In this group of rats, LVDP, +dP/dt and -dP/dt had reduced function at the lowest preload pressures tested. Since no blood pressure determinations were made in the young ZDF rats, no association between hypertension, hypertrophy and impaired overall cardiac performance at low preloads can be made. All that can be noted is that obesity can also cause these same effects and the DP rats were obese. This may be related to

ischemia in the tissues, since at low perfusion pressures, the heart is hypoperfused and may result in ischemia (personal communications, V. Yuen and S. Dai, UBC). Potentially, these DP rats may be more prone to ischemic episodes. To test this hypothesis, ischemia reperfusion studies would have to be conducted.

This phenomenon was not present in the young hybrid ZDF/SHHF diabetic pioglitazone treated rats which also exhibited increased heart weights. Pioglitazone caused a significant enhancement in LVDP of hybrid rats relative to diabetic untreated rats. None of the rat groups exhibited a significant impairment of cardiovascular performance which is a well documented phenomenon in animal models of IDDM. There may in fact be no good model for cardiovascular impairment in NIDDM rats. There appeared to be a trend for impaired function in the young ZDF diabetic rats, but this trend was not observed in the older ZDF model or the hybrid model.

Older ZDF rats were specifically chosen for the second study because we hypothesized that cardiomyopathy may not occur until later in the rat's life at a time when diabetes has significantly progressed. As it turned out, we probably did not use old enough rats. To compound this issue, we determined that the NIDDM rats slowly convert to IDDM rats, thus masking any cardiomyopathy that might have evolved during the phase of NIDDM. It has, however, recently been reported that cardiomyopathy does not occur in Zucker fatty rats until several months of age (Rosen et al., 1986). The greatest effect that occurred in this group of rats was due to hydralazine. Hydralazine significantly impaired diabetic -dP/dt and improved non-diabetic -dP/dt. Hydralazine lowered blood pressure, but had no effect on cardiac size. It impaired rates of relaxation in

diabetic rats, but improved rates of relaxation in non-diabetic rats. This can not be related to hyperinsulinemia since it did not exist in these older ZDF rats. Possibly, hydralazine interferes with electrolyte balance, ultimately affecting calcium levels and contractility in the heart.

Hybrid ZDF/SHHF rats not only are obese, insulin resistant and hyperlipidemic, but they also have the potential to develop hypertension and/or congestive heart failure due to an abnormal gene expressed in the ZDF strain. It is known that cardiovascular complications can be potentiated by hypertension and/or other congenital heart defects. The possibility exists, that since this new hybrid strain can develop other cardiovascular abnormalities, it possibly could develop the "diabetic cardiomyopathy" observed in STZ-diabetic rats (IDDM). Since impaired cardiovascular performance was not observed in either the young or older ZDF diabetic rats tested, this hybrid strain of rats was chosen to look for potential cardiomyopathy. The only effect seen in the young hybrid ZDF/SHHF diabetic rats was with hydralazine treatment, which significantly improved diabetic -dP/dt compared to non-diabetic rats.

Diabetic cardiomyopathy by definition is cardiomyopathy which occurs in the absence of any significant coronary artery disease. Rats typically do not develop coronary artery disease and thus are a good model for diabetic cardiomyopathy. Vanadyl, which corrects the diabetic cardiomyopathy in STZ-diabetic rats (Heyliger et al., 1986), had no effect in the older ZDF or hybrid ZDF/SHHF rats. Since significantly impaired cardiac performance was not observed in the diabetic rats, little improvement could probably be expected.

Cardiac tissue cholesterol levels, corrected for protein content were not increased in young or older ZDF diabetic rat hearts compared to non-diabetic rat hearts, but were significantly elevated in the young hybrid ZDF/SHHFdiabetic rats. This increase in cardiac cholesterol in young hybrid ZDF/SHHF diabetic rats is consistent with elevated plasma cholesterol levels seen in this group. Pioglitazone had no effect on young, older or hybrid ZDF rat heart cholesterol content despite an overall increase in heart weight and a decrease in plasma cholesterol in diabetic groups. Hydralazine also had no effect on cardiac cholesterol in young or older ZDF diabetic and non-diabetic rat hearts, but did significantly decrease cardiac cholesterol in non-diabetic rats relative to both diabetic and non-diabetic untreated rats.

Elevated cardiac triglycerides have been documented in the IDDM rat model, the BB rat. Elevated plasma lipid concentrations can accumulate intracellularly where lipids might contribute to cardiac stiffness and interfere with various enzyme systems. Cardiac tissue triglyceride levels were elevated in young and older ZDF diabetic rats compared to non-diabetic littermates. Diabetic and non-diabetic cardiac triglyceride levels in the young ZDF rats were significantly decreased by pioglitazone treatment. This could be due to a secondary effect of pioglitazone to decrease circulating plasma triglyceride levels. It could, however, be a direct effect of the drug to stimulate lipoprotein lipase in the cardiac tissue. Vanadyl normalized elevated cardiac triglyceride levels in the older diabetic rats, but had no effect in non-diabetic rats. Pioglitazone had no effect on this cardiac parameter in older ZDF diabetic rats. Once again this is probably due to the IDDM state. Although hydralazine reduced plasma triglycerides significantly after 7 weeks of treatment, it significantly increased the cardiac triglyceride levels of older ZDF diabetic rats. Perhaps this is because

plasma triglycerides are being removed and subsequently deposited in tissues such as the heart. Another possibility is that there is abnormal fatty acid metabolism, such as reduced amounts of carnitine involved in mitochondrial uptake of free fatty acids. Levels of cardiac triglycerides in the young hybrid ZDF/SHHF diabetic rats are not significantly different from the levels in non-diabetic rats. Pioglitazone, hydralazine and vanadyl had no effect in either diabetic or non-diabetic rats of hybrid origin. The amount of cardiac triglycerides is lower in the young hybrid rats compared to the older ZDF rats and the higher levels in older rats may be reflective of their IDDM where elevated triglyceride levels have been previously noted.

Decreasing cardiac lipids is important for diabetics. In contrast to non-diabetics, diabetic individuals tend to use more free fatty acids than glucose to function normally. If triglyceride levels are high, free fatty acid levels increase as triglyceride lipase breaks down triglycerides. Another potential problem with elevated cardiac lipids is coronary artery disease or hypertension, two important problems associated with diabetic populations. This suggests that any drug capable of decreasing both plasma and cardiac tissue lipids would be an added benefit to NIDDM patients. This is the case with pioglitazone in young ZDF diabetic rats and hydralazine in older ZDF diabetic rats.

Hypertension has been associated with hyperinsulinemia and insulin resistance in human subjects with essential hypertension and in various animal models of hypertension including spontaneously hypertensive (SH) rats. Several recent studies investigating the relationship between hyperinsulinemia and hypertension have indicated that hyperinsulinemia may contribute to the development of hypertension in SH rats. In separate studies, vanadyl sulfate

and bis(maltolato)oxovanadium(IV) (an organic vanadium complex) concomitantly decreased plasma insulin and blood pressure (Bhanot, S., et al, Hypertension, in press; Bhanot, S., et al, 1994). Vanadyl sulfate has also been shown to prevent fructose-induced hyperinsulinemia and hypertension in rats (Bhanot, S., et al., 1993).

CS-045, another thiazolidinedione, has also been observed to have concurrent insulin and blood pressure lowering effects in 7 month old obese Zucker rats (Yoshioka et al., 1993). Since these rats were not hyperglycemic, it rules out the possibility that hyperglycemia caused the hypertension. In a study using pioglitazone in insulin resistant rhesus monkeys, the drug decreased insulin, glucose, triglycerides and blood pressure, indicating an association between insulin resistance and hypertension in these animals (Kemnitz, J.W., et al., 1994). It has recently been suggested that the hypotensive activity of pioglitazone is not always associated with normal insulin sensitivity (Zhang, H.Y., et al., 1994). These authors suggest that pioglitazone's ability to decrease peripheral resistance in Dhal salt rats (insulin resistant) may be due to inhibited growth of vascular smooth muscle cells as a result of alterations in intracellular calcium. A study, investigating the relationship between intracellular calcium and hypertension in obese rats, determined that decreased insulin and blood pressure correlated with a significantly decreased capacity of platelet-derived growth factor BB homodimer to induce sustained increases in intracellular free calcium (Pershadsingh, H.A., et al, 1993). This study suggests that a novel pharmacological approach to the treatment of hypertension might be to modify cell calcium responses to pressor agents, growth factors, or both.

Systolic, diastolic and mean arterial blood pressures were not determined in the young ZDF rats. Older ZDF diabetic rats did not exhibit any hypertension compared to non-diabetic rats. The absolute value of systolic and diastolic blood pressures in both diabetic and non-diabetic rats is mildly elevated compared to what has been reported for non-obese rats. This agrees with studies in Zucker rats which indicate that significant cardiovascular abnormalities do not occur until several months of age. Although we named these ZDF rats older, they are only 5 months old at the end of the experiment.

In the young hybrid ZDF/SHHF rats, hydralazine significantly decreased systolic, diastolic and mean arterial blood pressures in both diabetic and non-diabetic rat groups compared with untreated littermates. Hydralazine is known to be a direct arterial vasodilator; moreover, hydralazine caused a concomitant decrease in plasma triglyceride levels in the diabetic rats. This triglyceride lowering effect was also seen by Rodrigues et al., (1986) in STZ-diabetic rats and occurred concurrently with decreased systolic blood pressure and improved cardiac performance. Our results do not indicate improved cardiac performance in the diabetic rats, but do suggest improved cardiac performance in non-diabetic rats. Hydralazine increased the rate of relaxation without changing the rate of force development or left ventricular developed pressure. Young hybrid ZDF/SHHF diabetic rats had similar systolic and mean arterial blood pressures to the older ZDF rats, but had lower diastolic blood pressures. As in the older ZDF rats, there was no difference in blood pressure between diabetic and non-diabetic rats. In this study, hydralazine tended to cause a decrease in blood pressure (~9% decrease in systolic; ~10% decrease in diastolic; ~12% decrease in mean arterial blood pressure in diabetic rats) which, however, did not attain significance. In this study, pioglitazone

significantly reduced the systolic (10%), diastolic (11%) and mean arterial (12%) blood pressures of diabetic rats compared to control untreated rats. This effect occurred concomitantly with decreased plasma glucose, triglycerides, cholesterol and insulin. Several of these parameters could have contributed to the hypotensive effects of pioglitazone. However, correcting the hyperinsulinemia present in these rats may play a significant role since no effect was seen in our older, insulin deficient, ZDF rats (IDDM).

Some members of the research community have expressed concern about the reliability of non-invasive, tail cuff blood pressure determinations. There are several reasons to believe the validity of our readings. First, the rats were acclimated to the surroundings on several occasions to establish baseline responses before their determinations. Second, the rats were housed in temperature controlled conditions. Third, there are several papers which have determined that there is no significant difference between invasive and non-invasive blood pressure readings (Bhanot, S., et al., 1994). Fourth, we observed an expected decrease in blood pressure upon treatment with a direct vasodilator, hydralazine.

Vascular reactivity is a measure of the sensitivity of vascular tissue, such as aortas, to certain pharmacological agents, such as norepinephrine. Many studies have been conducted to evaluate vascular sensitivity and reactivity. Results of these studies show increased, decreased or no change in vascular reactivity, depending on the experimental conditions and animal model used. To date, there is very little evidence that there is any change in NIDDM rats. It is believed that increased sensitivity may be associated with hypertension.

Older ZDF diabetic rats had significantly higher maximum responses to norepinephrine than did non-diabetic littermates. As this group of rats closely resembles IDDM, this is consistent with previously published results (Gattu, M., 1993). Pioglitazone decreased aortic sensitivity in the older ZDF diabetic rats, but had no effect on non-diabetic littermates. The effects of pioglitazone in this instance are probably not a direct effect of the drug since these rats were hypoinsulinemic and insulin is necessary for pioglitazone to exert its effects. In these rats, there was no effect of the drug on blood pressure, so this improved vascular characteristic can not be entirely responsible for changes in blood pressure. Likewise, vanadyl sulfate improved vascular sensitivity in older ZDF diabetic rats with no effect on non-diabetic rats and no corresponding effects on blood pressure. Hydralazine, on the other hand, had no effect on diabetic rats, but significantly increased sensitivity in non-diabetic rats. Hydralazine also had a concomitant blood pressure lowering effect in both diabetic and non-diabetic rats. This does not agree with the theory that increased vascular sensitivity results in hypertension. Maximum vasoconstriction to the depolarizing agent, KCl (40 mM), in these older ZDF diabetic rats was also significantly greater than non-diabetic littermates. This is opposite to the decreased effect in IDDM reported by Gattu (1993) and Fulton (1991). Possibly this is because these rats had not been insulin dependent for a significant period of time as they belatedly converted from NIDDM to IDDM. Pioglitazone and vanadyl decreased vasoreactivity in diabetic rats while hydralazine increased non-diabetic responsiveness.

In the young hybrid ZDF/SHHF diabetic rats, a significant difference was not observed in vascular sensitivity compared with non-diabetic rats. Pioglitazone, vanadyl and hydralazine also had no effect on vascular sensitivity in this model

of NIDDM. This agrees with previous results in young ZDF diabetic rats (Gattu, M., 1993). Since there was a significant blood pressure lowering effect seen with pioglitazone treatment in these hyperinsulinemic rats, but no effect on vascular sensitivity, hypertension could be produced from some means other than vascular impairment. It is probably more likely that hyperinsulinemia or insulin resistance is responsible for, and certainly associated with, hypertension in young hybrid ZDF/SHHF rats. Maximum response to 40 mM KCl was also not affected by either disease or drug therapy. This is in agreement with the results of Gattu (1993), who observed similar results in young ZDF diabetic rats.

5.0 CONCLUSIONS

Pioglitazone reduced hyperglycemia, hyperlipidemia and hyperinsulinemia effectively at a dose an oral dose of 10 mg/kg/day when administered chronically to young ZDF and young hybrid ZDF/SHHF diabetic hyperinsulinemic rats. The reduction of plasma glucose in hyperinsulinemic but not hypoinsulinemic NIDDM rats indicates that pioglitazone's mechanism of action involves reversal of insulin resistance. The reduction of plasma cholesterol levels by pioglitazone appears to be associated with decreased LDL since HDL levels were not affected, and plasma triglyceridses were dramatically reduced. Pioglitazone promotes a potentially detrimental gain in body weight in ZDF and hybrid ZDF/SHHF NIDDM rats. It was evident that pioglitazone impaired maximum left ventricular pressure and rates of force development and relaxation in young ZDF diabetic rats relative to non-diabetic untreated rats at the lowest filling pressures and improved maximum left ventricular developed pressure in non-diabetic rats relative to untreated non-diabetic rats. Pioglitazone caused increased heart weights and body weights in both young and hybrid ZDF diabetic rats and potentially decreased heart weight to body weight ratios (seen in young ZDF diabetic rats, but not hybrid ZDF/SHHF diabetic rats). Pioglitazone, however, significantly lowered blood pressure in the young diabetic hybrid ZDF/SHHF rats relative to their non-diabetic littermates at a time when the untreated diabetic animals had blood pressures that approximated those of control littermates. Since pioglitazone lowered blood pressure in diabetic hybrid ZDF/SHHF rats, but not in their control littermates, the implication is that pioglitazone lowers blood pressure by its ability to correct hyperinsulinemia by re-establishing normal insulin sensitivity.

Vanadyl sulfate (0.75 mg/ml), administered orally in drinking water, improved hyperglycemia in older ZDF diabetic rats as a concomitant body weight loss occurred. It improved but did not normalize the hyperglycemia and hypertriglyceridemia of hybrid ZDF/SHHF diabetic rats without changing body weights. This suggests that vanadyl's mechanism of action is distinctly different from the mechanism of pioglitazone to reduce plasma glucose and lipids.

Hydralazine (5 mg/kg b.i.d.), administered orally, effectively decreased plasma cholesterol in hybrid ZDF/SHHF diabetic rats and plasma triglycerides in older ZDF diabetic rats. Hydralazine improved the rates of relaxation in non-diabetic older ZDF rats by improving rates of relaxation, in contrast, hydralazine impaired rates of relaxation in older ZDF diabetic rats at the higher preloads. However, in the hybrid ZDF/SHHF rats indicated that diabetic rats treated with hydralazine improved relative to non-diabetic rats with respect to rates of relaxation. There were no changes in cardiac cholesterol levels in the two NIDDM rat groups (young ZDF and hybrid ZDF/SHHF) while cardiac triglycerides decreased in young hybrid ZDF/SHHF diabetic rats treated with hydralazine compared to non-diabetic rats. Hydralazine significantly reduced blood pressure in the older ZDF rats and tended to lower blood pressures in the young hybrid ZDF/SHHF rats. The mechanism of action of hydralazine to reduce blood pressure is through direct vasodilation, which is obviously distinctly different from the mechanism by which of pioglitazone reduces blood pressure.

Evaluation of the cardiovascular parameters measured in these studies indicated that neither young, old or hybrid ZDF diabetic rats are a good model to

study diabetic cardiovascular abnormalities that manifest themselves as impaired cardiac performance or increased vascular reactivity. Since no impaired cardiovascular impairment occurred with respect to these two parameters in diabetic rats, it is premature to speculate on the potential beneficial or detrimental effects of pioglitazone, vanadyl or hydralazine. Thus, the NIDDM rat models used in the present study were not useful for determining cardiovascular abnormalities associated with diabetes in the isolated perfused working heart apparatus during the time frame tested.

In addition to the aforementioned results, systolic, diastolic and mean arterial blood pressures were not elevated in the insulin resistant NIDDM diabetic rats relative to non-diabetic littermates in the hybrid ZDF/SHHF model. Data suggest that the blood pressures in this genetic rat model are higher than normal regardless of diabetic status. Thus, none of these NIDDM rat models appear to be good models in which to study hypertension related to insulin resistance in NIDDM, and it can be speculated that hyperinsulinemia especially if short term is not predictive of an ensuing elevation of blood pressure.

6.0 REFERENCES

American Diabetes Association. 1991. Diabetes 1991 Vital statistics.

Autio, I., Jackal, O., Solakivi, T. and Nikkari, T. 1990. Oxidized low-density lipoprotein is chemotactic for arterial smooth muscle cells in culture. FEBS Lett 277:247-49.

Bailey, C.J. and Nattrass, M. 1988. Treatment--metformin. Baillieres Clin Endocrinol Metab 34:567-8.

Bailey, C.J. and Flatt, P.R. 1993. Islet defects and insulin resistance in models of obese non-insulin-dependent diabetes. Diabetes/Metab. Rev. 9(suppl1):43s-50s.

Bhanot, S., Bryer-Ash, M., Cheung, A. and McNeill, J.H. 1994. Bis(maltolato)oxovanadium(IV) attenuates hyperinsulinemia and hypertension in spontaneously hypertensive rats. Diabetes 43(7):857-861.

Bhanot, S. and McNeill, J.H. 1994. Vanadyl sulfate lowers plasma insulin and blood pressure in spontaneously hypertensive rats. Hypertension, In press.

Bhanot, S., McNeill, J.H. and Bryer-Ash, M. 1994. Vanadyl sulfate prevents fructose-induced hyperinsulineia and hypertension in rats. Hypertension 23:308-312.

Blondel, O., Bailbe, D. and Portha, B. 1989. In vivo insulin resistance in streptozotocin-diabetic rats: evidence for reversal following oral vanadate treatment. Diabetologia 32:185-190.

Brichard, S.M., Bailey, C.J. and Henquin, J.C. 1990. Vanadate improves glucose homeostasis in insulin-resistant diabetic ob/ob mice. (Abstr.) Fund Clin Pharmacol 4(Suppl1):54s.

Brichard, S.M., Ongemba, L.N. and Henquin, J.C. 1992. Oral vanadate decreases muscle insulin resistance in obese fa/fa rats. Diabetologia 35(6):522-527.

Brisco, P. 1993. Diabetes: Questions you have...Answers you need. People's Medical Society. Allentown, Pennsylvania.

Castle, C.K., Colca, J.R., and Melchoir, G.W. 1993. Lipoprotein profile characterization of the KKAy mouse, a rodent model of type II diabetes, before and after treatment with the insulin-sensitizing agent pioglitazone. Arterio. Thromb. 13:302-309.

Chang, A.Y., Wyse, B.M., Gilchrist, B.J., Peterson, T. and Diani, A.R. 1983. Ciglitazone, a new hypoglycemic agent. I. Studies on ob/ob and db/db mice,

diabetic chinese hamsters and normal and streptozotocin-diabetic rats. Diabetes 32:830-838.

Clark, A.S., Fagan, J.M.and Mitch, W.E. 1985. Selectivity of the insulin-like action of vanadate on glucose and protein metabolism in skeletal muiscle. Biochem J 232:273-276.

Clauser, E., Leconte, I. and Auzan, C. 1992. Molecular basis of insulin resistance. Horm Res 38:5-12.

Cocozza, S., Porcellini, A., Riccardi, G., Monticelli, A., Condorelli, G., Ferrara, A., Pianese, L., Miele, C., Capaldo, B., Beguinot, F. and Varrone,S. 1992. NIDDM associated with mutation in tyrosine kinase domain of insulin receptor gene. Diabetes 41:521-526.

Colca, J.R., Dailey, C.F., Palazuk, B.J., Hillman, R.M., Dinh, D.M., Melchior, G.W. and Spilman, C.H. 1991. Pioglitazone hydrochloride inhibits cholesterol absorption and lowers plasma cholesterol concentrations in cholesterol-fed rats. Diabetes 40:1669-1674.

Cros, G., Mongold, J.J., Serrano, J.J., Ramanadham, S. and McNeill, J.H. 1992. Effects of vanadyl derivatives on animal models of diabetes. Mol Cell Biochem 109:163-166.

Cushman, S.W. and Wardzala, L.J. 1980. Potential mechanisms of insulin action on glucose transport in the isolated rat adipose cell: apparent translocation of intracellular transport systems to the plasma membrane. J Biol Chem 255(10):4758-4762.

Dai, S., Yuen, V.G., Orvig, C. and McNeill, J.H. 1993. Prevention of diabetes-induced pathology in STZ-diabetic rats by bis(maltolato)oxovanadium (IV). Pharmacol. Commun. 3(4):311-321.

DeFronzo, R.A., Ferranninni, E. 1991. Insulin resistance: a multi-faceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 14:173-194.

Delfert, D.M. and McDonald, J.M. 1985. Vanadyl and vanadate inhibit Ca2+ transport systems of the adipocyte plasma membrane and endoplasmic reticulum. Arch Biochem Biophys 241:665-672.

Dubyak, G.R. and Kleinzeller, A. 1980. The insulin-mimetic effects of vanadate in isolated rat adipocytes. Dissociation of effects of vanadate as a (Na+/K+) ATPase inhibitor. J Biol Chem 255:5306-5312.

Duckworth, W.C., Solomon, S.S., Loiepnieks, J., Hamel, F.G., Hand, S. and Peavy, D.E. 1988. Insulin-like effects of vanadate in isolated rat adipocytes. Endocrinology 122:2285-2289.

Duncombe, W.W. 1963. The colorimetric micro-determination of long-chain fatty acids. Biochem J 88:7-10.

Eisenberth, G.S. 1986. Type I diabetes mellitus. A chronic autoimmune disease. N Engl J Med 314:1360-1368.

Fevury, D., Idell-wegner, J.A. and Neely, J.R. 1979. Effects of ischemia on rat myocardial function and metabolism in diabetes. Circ Res 44:322-329.

Friedmann, J.J. 1989. Vascular sensitivity and reactivity to norepinephrine in diabetes mellitus. Am J Physiol 256:H1134-H1138.

Frick, M.H., Elo, O., Haapa, K., Heinonen, O.P., Heinsalmi, P., Pekka, H., Huttenen, J.K., Kaitaniemi, P., Koskinen, P., Manninen, V., Maenpaa, H., Malkonen, M., Manttari, M., Norola, S., Pasternack, A., Pikkarainen, J., Romo, M., Sjoblom, T. and Nikkila, E.A. 1987. Helsinki heart study: primary-prevention trial with gemfibrozil in middle-aged men with dyslipidemia: safety of treatment, changes in risk factors, and incidence of coronary heart disease. N Engl J Med 31:1237-45.

Fujita, T., Sugiyama, Y., Taketomi, S., Sohda, T., Kawamatsu, Y., Iwatsuka, H. and Suzuoki, Z. 1983. Reduction of insulin resistance in obese and/or diabetic animals by 5-[4-(1-Methylcyclohexylmethoxy)benzyl]thiazolidine-2,4-dione (ADD-3878, U-63,287, Ciglitazone), a new antidiabetic agent. Diabetes 32:804-810.

Fujiwara, T., Yoshioka, S., Yoshioka, T., Ushiyama, I. and Horikoshi, H. 1988. Characterization of new oral antidiabetic agent CS-045. Diabetes 37(11):1549-1558.

Ganguly, P.K., Pierce, G.N., Dhalla, K.S. and Dhalla, N. 1983. Defective sarcoplasmic reticular calcium transport in diabetic cardiomyopathy. Am J Physiol 244:E528-E535.

Garber, D.W. and Neely, J.R. 1983. Decreased myocardial function and myosin ATPase in hearts from diabetic rats. Am J Physiol 244:H586-H591.

Gattu, M. 1993. Pioglitazone and cardiovascular function in diabetes mellitus. Masters Thesis. The University of Montana.

Gerich, J.E. 1989. Oral hypoglycemic agents. N Engl J Med 321(18):1231-1245.

Gherzi, R., Caratti, C., Andraghetti, G., Bertolini, S., Montemurro, A., Sesti, G. and Cordera, R. 1988. Direct modulation of insulin receptor protein kinase by vanadate and anti-receptor monoclonal antibodies. Biochem Biophys Res Commun 152:1474-1480.

Gomez-Foix, A.M., Rodriguez-Gil, J.E., Fillat, C., Guinovart, J.J. and Bosch, F. 1988. Vanadate raises fructose 2,6-biphosphate concentrations and activates glycolysis in rat hepatocytes. Biochem J 255:507-512.

Grodsky, G.M., Epstein, G.H. Fanska, R. and Karam, J.H. 1977. Pancreatic action of the sulfonyl-ureas. Fed Proc 36:2714-9.

Hermann, L.S. 1979. Metformin: a review of its pharmacological properties and therapeutic use. Diabetes Metab 5:233-45.

Heyliger, C.E., Prakash, A. and McNeill, J.H. 1987. Alterations in cardiac sarcolemmal Ca pump activity during diabetes mellitus. Am J Physiol 252:H540-H544.

Heyliger, C.E., Tahiliani, A.G. and McNeill, J.H. 1985. Effect of vanadate on elevated blood glucose and depressed cardiac performance of diabetic rats. Science 227:1474-1477.

Hiramatsu, K., and Arimori S. 1988. Increased superoxide production by monomuclear cells of patients with hypertriglyceridemia and diabetes. Diabetes 37:832-37.

Hofmann, C.A. and Colca, J.R. 1992. New oral thiazolidinedione antidiabetic agents act as insulin sensitizers. Diabetes Care 15(8):1075-1078.

Hofmann, C.A., EdwardsIII, C.W., Hillman, R.A. and Colca, R.J. 1992. Treatment of insulin-resistant mice with the oral antidiabetic agent pioglitazone: Evaluation of liver GLUT2 and phosphoenolpyruvate carboxykinase expression. Endocrinology 130(2):735-740.

Hofmann, C., Lorenz, K. and Colca, J.R. 1991. Glucose transport deficiency in diabetic animals is corrected by treatment with the oral antihyperglycemic agent pioglitazone. Endocrinology 129(4):1915-1925.

Howard, B.V. and Howard, W.J. 1994. Dyslipidemia in non-insulin-dependent diabetes mellitus. Endocrine Rev. 15(3):263-274.

Ikeda, H., Shino, A., Matsuo, T., Iwatsuka, H. and Suzuoki, Z.A. 1981. A new genetically obese-hyperglycemic rat (Wistar Fatty). Diabetes 30:1045-1050.

Ikeda, H., Taketomi, S., Sugiyama, Y., Shimura, Y., Sohda, T., Meguro, K. and Fujita, T. 1990. Effects of pioglitazone on glucose and lipid metabolism in normal and insulin resistant animals. Arzneim-Forsch/Drug Res 40(1):156-162.

Itaya, K and Ui, M. 1965. The colorimetric determination of fatty acids in biological fluids. J Lipid Res 6L:1360-1368.

Iwamoto, Y., Kazuya, T., Matsuda, A., Awata, T., Kumakura, S., Inooka, G. and Shiraishi, I. 1991. Effect of new oral antidiabetic agent CS-045 on glucose

tolerance and insulin secretion in patients with NIDDM. Diabetes Care 14(11):1083-1086.

Iwanishi, M. and Kobayashi, M. 1993. Effect of pioglitazone on insulin receptors of skeletal muscles from high-fat-fed rats. Metabolism 42(8):1017-1021.

Jackson, R.A., Hawa, M.I., Jaspan, J.B., Sim, B.M., Silvio, D., Featherbe, L. and Kurtz, D. 1987. Mechanism of metformin action in non-insulin-dependent diabetes. Diabetes 36:632-40.

Jackson, T.K., Salhanick, A.I., Sparks, J.D., Sparks, C.E., Bolognino, M. and Amatruda, J.M. 1988. Insulin-mimetic effects of vanadate in primary cultures of rat hepatocytes. Diabetes 37:1234-1240.

Jaffe, A.S. 1989. Cardiovascular effects of diabetes. Curr Op Cardiol 4:711-720.

Kahn, C.R. 1985. The molecular mechanism of insulin action. Ann Rev Med 36:429-451.

Kemnitz, J.W., Elson, D.F., Roecker, E.B., Baum, S.T., Bergman, R.N. and Meglasson, M.D. 1994. Pioglitazone increases insulin sensitivity, reduces blood glucose, insulin, and lipid levels, and lowers blood pressure in obese, insulin-resistant Rhesus monkeys. Diabetes 43:204-11.

Kennedy, D.L., Piper, J.M. and Baum, C. 1988. Trends in use of oral hypoglycemic agents, 1964-1986. Diabetes Care 11:558-62.

Kim, H., Kadowaki, H., Sakura, H., Odawara, M., Momomura, K., Takahashi, Y., Miyazaki, Y., Ohtani, T., Akanuma, Y., Yazaki, Y., Kasuga, M., Taylor, S.I. and Kadowaki, T. 1992. Detection of mutations in the insulin receptor gene in patients with insulin resistance by analysis of single-stranded conformational polymorphisms. Diabetologia 35:261-266.

Kobayashi, M., Iwanishi, M., Egawa, K. and Shigeta, Y. 1992. Pioglitazone increases insulin sensitivity by activating insulin receptor kinase. Diabetes 41:476-483.

Kreutter, D.K., Andrews, K.M., Gibbs, E.M., Hutson, N.J. and Stevenson, R.W. 1990. Insulin-like activity of new antidiabetic agent CP 68722 in 3T3-L1 adipocytes. Diabetes 39(11):1414-1419.

Kuzuya, T., Iwamoto, Y., Kosaka, K., Takebe, K., Yamanouchi, T., Kasuga, M., Kajinuma, H., Akanuma, Y., Yoshida, S., Shigeta, Y. and Baba, S. 1991. A pilot clinical trial of a new oral hypoglycemic agent, CS-045, in patients with non-insulin dependent diabetes mellitus. Diabetes Res and Clin Prac 111:147-154.

Lienhard, G.E., Slot, J.W., James, D.E. and Mueckler, M.M. 1992. How cells absorb glucose. Scientific Am 1:86-91.

Loubatieres, A. 1944. Relations entre la structure moleculaire et l-activate hypoglycemiante des aminosulfamides hypoglycemiantes. Arch Int Physiol 1:74-7.

Makino, N., Dhalla, K.S., Elimban, V. and Dhalla, N.S. 1987. Sarcolemmal Ca transport in streptozotocin induced diabetic cardiomyopathy in rats. Am J Physiol 253:E202-E207.

Malabu, U.H., Dryden, S., McCarthy, H.D., Kilpatrick, A. and Williams, G. 1994. Effects of chronic vanadate administration in the STZ-induced diabetic rat: the antihyperglycemic action of vanadate is attributable entirely to its suppression of feeding. Diabetes 43(1):9-15.

Melander, A. 1988. Sulphonylureas in the treatment of non-insulin-dependent diabetes. Baillieres Clin Endocrinol Metab 2:443-53.

Momose, Y., Meguro, K., Ikeda, H., Hatanaka, C., Ot, S. and Sohda, T. 1991. Studies on antidiabetic agents. X. Synthesis and biological activities of pioglitazone and related compounds. Chem Pharm Bull 39(6):1440-1445.

Moorehouse, J.A. 1967. A comparison of the effects of tolazamide and tolbutamide upon blood glucose and serum insulin and lipid levels in diabetic subjects. Can Med Assoc J 96:536-9.

Morgan, C.R. and Lazarow, A. 1963. Immunoassay of insulin: Two antibody system, plasma insulin levels of normal, subdiabetic and diabetic rats. Diabetes 12:115-126.

Oberwetter, J.M. and Boyd, A.E. III. 1987. High K+ rapidly stimulates Ca2+-dependent phosphorylation of three proteins concomitant with insulin secretion from HIT cells. Diabetes 36:864-71.

O'Rahilly, S., Choi, W.H., Patel, P., Turner, R.C., Flier, J.S. and Moller, D.E. 1991. Detection of mutations in insulin-receptor gene in NIDDM patients by analysis of single-stranded conformation polymorphisms. Diabetes 40:777-782.

Paquet, M.R., Romanek, R.J. and Sargent, R.J. 1990. Vanadate stimulates the recruitment of the glucose transporter (GLUT-4) to the plasma membrane in rat adipocytes. (Abst) Fund Clin Pharmacol 4(Suppl.1):44s.

Pershadsingh, H.A., Szollosi, J., Benson, S., Hyun, W.C., Feuerstein, B.G. and Kurtz, T.W. 1993. Effects of ciglitazone on blood pressure and intracellular calcium metabolism. Hypertension 21:1020-1023.

Pierce, G.N. and Dhalla, N.S. 1983. Sarcolemmal Na-K-ATPase activity in diabetic rat heart. Am J Physiol 245 (Cell Physiol. 14):C241-C247.

Pierce, G.N., Beamish, R.E. and Dhalla, S.N. 1988. Heart dysfunction in diabetes. Boca Raton, FL: CRC.

Pierce, G.N., Ramjiawan, B., Dhalla, S.N. and Ferrari, R. 1990. Na-H exchange in cardiac sarcolemmal vesicles isolated from diabetic rats. Am J Physiol 258:H255-H261.

Pirart, J. 1978. Diabetes mellitus and its degenerative complications: a prospective study of 4,400 patients observed between 1947 and 1973. Diabetes Care 1:168-88.

Pozzoli, G., Vitralo, E., Collini, P., DeMaria, R., Castelli, M.R. and Colombo, F. 1984. Assessment of left ventricular function with M-mode echocardiography in a selected group of diabetic patients. Acta Diabet Lat 21:71-84.

Ramanadham, S., Brownsey, R.W., Cros, G.H., Mongold, J.J. and McNeill, J.H. 1989. Sustained prevention of myocardial and metabolic abnormalities in diabetic rats following withdrawal from oral vanadyl treatment. Metabolism 38:1390-1395.

Ramanadham, S., Cros, G.H., Mongold, J.J., Serrano, J.J. and McNeill, J.H. 1990. Enhanced in vivo sensitivity of vanadyl-treated diabetic rats to insulin. Can J Physiol Pharmacol 68:486-491.

Reaven, G.M. 1988. Role of insulin resistance in human disease. Diabetes 37:1595-607.

Rendell, M. 1983. C-peptide as a criterion in treatment of maturity-onset diabetes. J Clin Endocrinol Metab 57:1198-206.

Rifkin, H., ed. 1988. Physician's guide to non-insulin-dependent (type II) diabetes: diagnosis and treatment. 2nd ed. Alexandria, Va.: American Diabetes Association.

Rodbell, M. 1964. Metabolism of isolated fat cells. J Biol Chem 239:375-380.

Rodrigues, B. and McNeill, J.H. 1986. Cardiac function in spontaneously hypertensive diabetic rats. Am J Physiol 251:H571-H580.

Rodrigues, B. and McNeill, J.H. 1986. Effects of hydralazine on streptozotocin-induced diabetic rats: prevention of hyperlipidemia and improvement in cardiac function. J PharmacolExp Ther 237(1):292-296.

Schenk, J. 1991. Examination of cardiovascular function in conscious hypertensive diabetic rats. Masters Thesis, University of British Columbia.

Shafrir, E. 1992. Animal models of non-insulin-dependent diabetes. Diabetes/Metabolism Reviews 8(3):179-208.

Sohda, T., Mizuno, K., Momose, Y., Ikeda, H., Fujita, T. and Meguro, K. 1992. Studies on antidiabetic agents. 1.1 Novel thiazolidinedione derivatives as potent hypoglycemic and hypolipidemic agents. J Med Chem 35(14):2617-2630.

Sohda, T., Mizuno, K., Imamiya, E., Sugiyama, Y., Fujita, T. and Kawamatsu, Y. 1982. Studies on antidiabetic agents. II. Sunthesis of 5-[4-(1-Methylcyclohexyl methoxy)benzyl]thiazolidine-2,4-dione (ADD-3878) and its derivatives. Chem Pharm Bull 30:3580-3600.

Stevenson, R.W., Hutson, N.J., Krupp, M.N., Volkmann, R.A., Holland, G.F., Eggler, J.F., Clark, D.A., McPherson, R.K., Hall, K.L., Danbury, B.H., Gibbs, E.M. and Kreutter, D.K. 1990. Actions of a novel antidiabetic agent englitazone in hyperglycemic hyperinsulinemic ob/ob mice. Diabetes 39(10):1218-1227.

Strout, H.V., Vicario, P.P., Biswas, C., Saperstein, R., Brady, E.J., Pilch, P.F. and Berger, J. 1990. Vanadate treatment of streptozotocin diabetic rats restores expression of the insulin-responsive glucose transporter in skeletal muscle. Endocrinology 126:2728-2732.

Sugiyama, Y., Shimura, Y. and Ikeda, H. 1990a. Effects of pioglitazone on hepatic and peripheral insulin resistance in wistar fatty rats. Arzneim-Forsch. Drug Res 40(1):436-440.

Sugiyama, Y., Taketomi, S., Shimura, Y., Ikeda, H. and Fujita, T. 1990b. Effects of pioglitazone on glucose and lipid metabolism in wistar fatty rats. Arzneim-Forsch/ Drug Res 40(1):263-267.

Suter, S.L., Nolan, J.J., Wallace, P., Gumbiner, B. and Olefsky, J.M. 1992. Metabolic effects of new oral hypoglycemic agent CS-405 in NIDDM subjects. Diabetes Care 15(2):193-203.

Swarup, G., Cohen, S. and Garbers, D.L. 1982. Inhibition of membrane phosphotyrosyl-protein phosphatase activity by vanadate. Biochem Biophys Res Commun 107:1104-1109.

Tamura, S., Brown, T.A., Whipple, J.H., Fujita-Yamaguchi, Y., Dubler, R.E., Cheng, K. and Lamer, J. 1984. A novel mechanism for the insulin-like effect of vanadate on glycogen synthase in rat adipocytes. J Biol Chem 259:6650-6658.

Taskinen, M. 1987. Lipoprotein lipase in diabetes. Diabetes Metab Rev 3:551-70.

Taskinen, M., Beltz, W.F. and Harper, I. 1986. Effects of NIDDM on very-low-density lipoprotein triglyceride and apolipoprotein metabolism: studies before and after sulfonylurea therapy. Diabetes 35:1268-77.

Tolman, E.L., Barris, E., Bums, M., Pansini, A. and Partridge, R. 1979. Effects of vanadium on glucose metabolism in vitro. Life Sciences 25:1159-164.

Tracey, A.S. and Gresser, M.J. 1986. Interaction of vanadate with phenol and tyrosine: implications for the effects on vanadate on systems regulated by tyrosine phosphorylation. Proc Natl Acad Sci USA 83:609-613.

Verma, S. and McNeill, J.H. 1994. Metformin improves cardiac function in isolated streptozotocin-diabetic rat hearts. Am J Physiol 266(35):H714-H719.

Vigneri, R. and Goldfine, I.D. 1987. Role of metformin in treatment of diabetes mellitus. Diabetes Care 10:118-20.

Wang, P.H., Moller, D. Flier, J., Nayak, R.C. and Smith, J.R. 1989. Coordinate regulation of glucose transport function, number, and gene expression by insulin and sulfonylureas in L6 rat skeletal muscle cells. J Clin Invest 84:62-67.

Weir, G.C. 1990. Cytoplasmic calcium in mammalian ventricle: dynamic control by cellular processes. Ann Rev Physiol 52:467-485.

Wohaieb, S.A. and Godin, D.V. 1987. Alterations in free radical tissue-defense mechanisms in streptozotocin-induced diabetes in rat: effects of insulin treatment. Diabetes 36:1014-1018.

Yoshioka, S., Nishino, H., Shiraki, T., Ikeda, KI, Koike, H., Okuno, A., Wada, M., Fujiwara, T. and Horikoshi, H. 1993. Antihypertensive effects of CS-045 treatment in obese Zucker rats. Metabolism 42(1):75-80.

Zhang, H.Y., Reddy, S.R. and Kotchen, T.A. 1994. Antihypertensive effects of pioglitazone is not invariably associated with increased insulin sensitivity. Hypertension 24:106-110.