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CHANGES IN CARDIAC FUNCTION DUE TO THE INTERACTIONS OF DIABETES AND HYPERTENSION IN THE RAT

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CHANGES IN CARDIAC FUNCTION DUE TO THE
INTERACTIONS OF DIABETES AND HYPERTENSION
IN THE RAT

BY

AMY JANE DAVIDOFF

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
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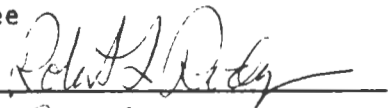




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DEAN OF THE GRADUATE SCHOOL

UNIVERSITY OF RHODE ISLAND

1989

ABSTRACT

Both clinical and experimental evidence suggest that diabetes mellitus impairs cardiac performance to a greater extent in the presence of hypertension than it does in the absence of hypertension. There is also indirect evidence to suggest that a low thyroid state, which often coincides with experimental and clinical diabetes, may contribute to this impairment. The following study was designed to characterize selected cardiovascular sequelae of diabetes in the hypertensive rat, and to determine whether involvement of attendant hypothyroidism is functionally significant. The hypotheses of this study can be summarized as follows: 1) The marked depression in cardiac performance caused by diabetes in hypertensive rats is associated with impairments in sarcoplasmic reticular (SR) calcium uptake and with prolongations in the duration of ventricular muscle action potentials; and 2) Demonstrated disturbances in cardiac performance, SR calcium uptake, and action potential duration caused by diabetes could then be altered by either thyroid hormone or insulin treatment in vivo. To test these hypotheses, spontaneously hypertensive rats (SHR) were made diabetic with a single injection of streptozotocin (STZ; 45 mg/kg). Subgroups of diabetic animals were treated with either thyroid hormone (T_3 ; 8-10 μ g/kg/day) or insulin (10-20 U/kg/day). After 8 weeks of untreated and treated diabetes, the following measurements were taken: 1) Indices of performance (contraction and relaxation) of perfused, working hearts ex vivo; 2) Rate and extent of calcium uptake by microsomal preparations of ventricular homogenates in vitro; and 3) Action potential profiles of papillary muscle in vitro. Control groups for various studies

included nonhypertensive Wistar Kyoto (WKY) rats, and renovascular hypertensive (RVH) rats, to rule out strain-dependent effects in the SHR model, food-restricted nondiabetic rats to control primarily for reductions in heart size, and rats pretreated with 3-O-methylglucose to protect against the diabetogenic effects of STZ (as a control for nonselective STZ-induced cardiotoxicity). The results showed, first, that indices of left ventricular (LV) performance ex vivo (e.g., LV pressure generation and rates of contraction and relaxation) of SHR, but not WKY rats, were depressed by diabetes. Diabetes-induced LV mechanical deficits of SHR were ameliorated by either insulin or T_3 treatment. The adverse effects of diabetes on mechanical function of SHR hearts were independent of coronary macrolesions, as evidenced by normal coronary flow rates, myocardial oxygen consumption and coronary vascular resistance in the diabetic SHR group. These data provide additional evidence that a "diabetic cardiomyopathy" at least partially contributes to depressed function in the SHR and that thyroid hormone treatment can prevent at least a portion of the mechanical deficits. Second, the results showed that diabetes impaired LV relaxation and depressed rates of SR calcium uptake in the SHR left ventricle without affecting either measurement in the WKY group. Thyroid hormone treatment completely prevented these mechanical and biochemical changes in the diabetic SHR heart. The third component of the results indicated that diabetes prolonged the ventricular action potential to the same extent in the SHR as it did in the WKY. Either insulin or T_3 treatment of diabetic SHR completely prevented the prolongation of the action potential. As it did in the SHR strain, diabetes also caused a marked depression in

the performance in hearts of RVH rats. Similarly, T_3 treatment of diabetic RVH, like T_3 treatment of diabetic SHR, prevented the impairment of cardiac performance. Collectively, these results suggest that left ventricular hypertrophy, as a common element of SHR and RVH models of hypertension, predisposes the myocardium to the adverse effects of diabetes. The thyroid hormone deficit associated with diabetes, in both the SHR and RVH models of hypertension, may contribute to this myocardial dysfunction. Furthermore, since thyroid hormone treatment of diabetic SHR prevents impaired SR calcium uptake and action potential prolongation, attendant hypothyroidism may play a significant role in the etiology of diabetic cardiomyopathy in hypertrophic left ventricle.

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PREFACE

This dissertation was prepared according to the manuscript thesis plan. The first section of the dissertation consists of a general introduction and provides background information, rationale, hypotheses and specific aims for the following study. The second section consists of three manuscripts. The first two manuscripts are prepared according to the format of the journal Hypertension. The third manuscript is prepared according to the format of the American Journal of Physiology. All three papers describe the cardiovascular effects of either insulin or thyroid hormone treatment in diabetic genetically hypertensive rats. The first paper provides detailed analyses of these effects on ventricular performance of isolated perfused working hearts. The second paper focuses specifically on myocardial relaxation of the working hearts and a biochemical correlate to this function, Ca^{2+} uptake by the sarcoplasmic reticulum. The third paper characterizes the effects of diabetes and thyroid hormone treatment on ventricular action potentials in the hypertensive rat.

Section three of the dissertation is a general discussion that encompasses all of the results obtained in each of the three studies listed above along with data presented in Appendix A. Appendix A details the effects of diabetes on cardiac performance in another experimental model of hypertension, the renovascular hypertensive rat. Appendix B provides the computer programs that were developed and used to acquire the data for most of the analyses. The last section is a bibliography for the entire dissertation.

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GENERAL INTRODUCTION

Cardiovascular disease contributes significantly to morbidity and mortality in the diabetic population (for review see Dillmann 1989). The incidence of coronary artery disease (such as atherosclerosis) and congestive heart failure (CHF) is 2 - 5 times greater in the presence of diabetes than it is in the general population (Kannel et al. 1974). Cardiac functional abnormalities that are commonly associated with diabetes include decreases in ejection fraction and stroke volume and increases in left ventricular (LV) end diastolic pressure and wall stiffness (Hamby et al. 1974; Regan et al. 1977). Impaired diastolic filling may be the first clinical sign of diabetes-induced cardiac dysfunction (Park et al. 1988; Ruddy et al. 1988). In addition to coronary artery disease, the etiology of CHF in diabetes may also involve either an autonomic neuropathy or a specific cardiomyopathy or both (Kannel et al. 1974; Regan et al. 1977; Fisher et al. 1986; Sunni et al. 1986). Even in the absence of coronary vascular disorder, the frequency of CHF is still higher than expected in diabetic patients (Kannel et al. 1974; Regan et al. 1977). The term diabetic cardiomyopathy is used to define depressed heart mechanical performance that results from myocardial dysfunction independent of the coronary vasculature (Dhalla et al. 1985; Fein and Sonnenblick 1985; Dillmann 1989).

Diabetic cardiomyopathy may be associated with specific cellular lesions. Studies using animals with chemically-induced diabetes (Penpargkul et al. 1980; Garber and Neely 1983) and animals which are genetically predisposed to diabetes (Lopaschuk and Tsang 1987; Brady and Brady 1989; Rodrigues and McNeill 1989) have been useful for

determining specific areas of cardiomyocyte disruption. For example, diabetes promotes an increase in phospholipid incorporation in the plasma, sarcoplasmic reticular (SR), and mitochondrial membranes (Gudbjarnason et al. 1987; Lopaschuk and Tsang 1987; Makino et al. 1987; Xiang et al. 1988; Black et al. 1989; Lopaschuk and Spafford 1989). Decreases in specific enzyme activities (e.g., Na^+/K^+ ATPase and Ca^{2+} ATPase) and prolonged duration of the action potential (AP) are often correlated with this altered membrane composition (Fozzard and Arnsdorf 1986). These and other cellular changes can interfere with the heart's normal pump function. Diabetes depresses indices of mechanical performance in situ (Regan et al. 1974) and ex vivo (Penpargkul et al. 1980; Fein et al. 1983; Rodgers 1986). The diabetic heart generally exhibits lower ventricular pressure generation, decreased contractility and prolonged relaxation when compared to a normal heart (Garber and Neely 1983; Kobayashi and Neely 1983; Tahiliani and McNeill 1984; Rodgers 1986).

The risk of cardiovascular complications appears to be greater when diabetes is combined with hypertension both clinically and experimentally (Kannel et al. 1974; Factor et al. 1980, 1981; Drury 1985; Fuller 1985; Rodgers 1986; Rodrigues and McNeill 1986; Sparafka et al. 1988; Bell 1989; Fein et al. 1989; Tzagournis 1989). Chemically-induced diabetes in hypertensive rats has a more profound effect on cardiac function than it does in normotensive (nonhypertensive) models (Fein et al. 1984; Rodgers 1986; Rodrigues and McNeill 1986). For instance, histological and ultrastructural changes are more evident in the diabetic renovascular hypertensive (RVH) rat heart than in control rat hearts (Factor et al. 1980, 1981;

Fein et al. 1989). Diabetes depresses LV performance to a greater extent in the spontaneously hypertensive rat (SHR) than in the normotensive Wistar Kyoto (WKY) or Sprague-Dawley rat (Rodgers 1986). It may be the presence of preexisting LV hypertrophy, a consequence of elevated arterial pressure, that exacerbates the functional defects in diabetic myocardium (Factor et al. 1983). Hypertrophic muscle progresses from an adaptive phase, during which an increase in muscle mass benefits the heart by redistributing the increase in workload, to a compromised phase, during which force of contraction is significantly reduced (Pfeffer et al. 1979; Scheuer and Buttrick 1987). Even when diabetes is induced in animals whose cardiac function is still well within the adaptive phase of hypertrophy, cardiac function subsequently is more severely impaired than it is in nonhypertrophic muscle (Rodgers 1986; Rodrigues and McNeill 1986). Since diabetes depresses systolic arterial pressure and can reverse LV hypertrophy in SHR (Rodgers 1986), neither sustained hypertension nor LV hypertrophy is required in order to manifest these cardiodepressive effects. Thus, LV hypertrophy may predispose the heart to the adverse effects of diabetes.

Hypothyroidism, or a "low thyroid state", is often reported as a secondary metabolic disorder of diabetes (Pittman et al. 1979; Bagchi 1982; Dillmann 1989), and insulin replacement therapy has been shown to restore normal thyroid status in diabetic rats (Garber et al. 1983). Hypothyroidism and diabetes have a number of similar effects on cardiac metabolism and function. For example, both pathological conditions impair myocardial contractility, depress myosin ATPase activity and SR Ca^{2+} uptake (Suko 1973; Takacs et al. 1982; Morkin et

al. 1983; Dhalla et al. 1985; Rodgers et al. 1986), prolong the cardiac AP (Freedberg et al. 1970; Nordin et al. 1985; Sauviat and Feuvray 1986; Capasso et al. 1986; Binah et al. 1987) and reduce sensitivity to adrenergic agonists (Bilezikian and Loeb 1983; Gotzsche 1986). Whether the cardiovascular effects of diabetes are at least in part due to the attendant hypothyroid state has been addressed in a number of studies (Tahiliani and McNeill 1986). Thyroid hormone replacement therapy in normotensive diabetic rats has been shown to be either ineffective (Tahiliani and McNeill 1984; Tahiliani and McNeill 1985; Barbee et al. 1988) or only partially effective (Garber et al. 1983; Goyal et al. 1987) in restoring cardiac function and atrial AP configuration (LeGaye et al. 1988). The prevailing view up to now is that the attendant hypothyroidism does not play a significant role in diabetic cardiomyopathy in the absence of hypertension (Tahiliani and McNeill 1986; Dillmann 1989).

Indirect evidence, however, suggests that the influence of an imbalance in thyroid status on the cardiovascular system may have a greater impact in the diabetic SHR than it does in diabetic normotensive strains. Thyroidectomy (Tx) of young SHR prevents the onset of elevated systolic arterial pressure (Rioux and Berkowitz 1977), and either Tx or methimazole treatment of SHR decreases contractility and pressure generation of the isolated working heart (Rodgers 1986; Rodgers and McNeill 1986). These effects of hypothyroidism in the SHR strain are reminiscent of, but less pronounced than, those of diabetes (Rodgers 1986).

A common manifestation of diabetes and hypothyroidism is reduced rate of Ca^{2+} uptake by the SR (Suko, 1973; Penpargkul, 1981). This

biochemical impairment is closely associated with depressed rates of myocardial relaxation in both diabetes (Penpargkul et al. 1980; Penpargkul et al. 1981) and hypothyroidism (Rodgers and McNeill 1986). Load-dependent relaxation of cardiac muscle is dependent upon the activity of SR in order to lower intracellular free Ca^{2+} concentrations (Brutsaert et al. 1980; Bers 1987). The adverse effects of diabetes on indices of relaxation do not appear to be dependent on thyroid state in nonhypertrophic muscle (Tahiliani and McNeill 1984). While the effects of LV hypertrophy on SR Ca^{2+} uptake and relaxation of heart muscle are not consistent (Limas and Cohn 1977; Capasso et al. 1986; Rodgers et al. 1986; Cuneo and Grassi de Gende 1988), the combined effects of diabetes and LV hypertrophy are more pronounced on isometric relaxation of RVH rat papillary muscle (Fein et al. 1984). The interaction of diabetes and LV hypertrophy myocardial relaxation and SR Ca^{2+} uptake have not been investigated.

The cardiac AP configuration is similarly altered by the effects of either diabetes or hypothyroidism (Aronson and Keung 1983; Fein et al. 1983; Nordin et al. 1985; Capasso et al. 1986; Capasso et al. 1986; Sauviat and Feuvray 1986). Prolongation of the cardiac AP is the most consistent consequence common to these abnormalities, and may be functionally related to associated mechanical alterations which are also characteristic of each condition. The presence of hypertrophy alone is not always associated with changes in AP configuration. The etiology and duration of the hypertrophy may influence the extent of the electrophysiological changes. Neither right ventricular hypertrophy in cat (Kaufmann et al. 1971) nor papillary muscle hypertrophy in SHR (Volkman et al. 1989)

significantly prolong the cardiac AP when compared to nonhypertrophic controls. However, the hypertrophy of RVH papillary muscle prolongs AP duration (Aronson 1980) and this increase is magnified in a time dependent manner (Capasso et al. 1986). A combination of any of these disease states (diabetes, hypothyroidism or hypertrophy) may have additive effects on ventricular transmembrane potentials (Fein et al. 1984; LeGaye et al. 1988), as they seem to have on mechanical function (Fein et al. 1984; Rodgers 1986; Rodrigues and McNeill 1986).

Ventricular muscle contraction begins with the depolarization of the sarcolemma. The influx of calcium ions during the AP appears to trigger the release of additional Ca^{2+} from the SR, raising intracellular free Ca^{2+} levels and promoting contraction (Fabiato and Fabiato 1979). Changes in membrane potential properties can therefore alter mechanical activities. Too much or too little intracellular free Ca^{2+} is detrimental to cell function (Dhalla et al 1985; Morad and Cleemann 1987). The accumulative effects of prolonged AP duration and lower rates of Ca^{2+} uptake may be to increase intracellular free Ca^{2+} concentrations, reduce Ca^{2+} dissociation from the contractile filaments, and lead to depressed rates of myocardial relaxation. The interaction of these and other cellular events provide the basis for the experimental design of the following studies.

The working hypotheses of this study, and their rationales, are listed below.

- 1) Thyroid hormone treatment of diabetic SHR will at least partially prevent the decline in cardiac performance affected by chronic (8 weeks) diabetes mellitus. Depressed cardiac function in thyroidectomized SHR is similar to, but not as severe as in diabetic SHR (Rodgers 1986; Rodgers and McNeill 1986). Furthermore, thyroidectomy as well as diabetes depresses systolic arterial pressure in SHR (Rioux and Berkowitz 1977; Rodgers 1986). The cardiodepressive effects of diabetes in SHR may in part be influenced by a low thyroid state.
- 2) Chronic diabetes should impose a more pronounced impairment in the rate and extent of LV relaxation and SR Ca^{2+} uptake in the SHR than it will in nonhypertrophic, nonhypertensive rats. A common physiological consequence of either hypertrophy or diabetes is impaired myocardial relaxation (Fein et al. 1980; Penpargkul et al. 1980; Capasso et al. 1986). The combination of diabetes and hypertrophy has a greater detrimental effect on myocardial relaxation than does either disease state alone (Fein et al. 1984; Rodrigues and McNeill 1986). Some studies have reported depressed rates of SR Ca^{2+} uptake in both hypertrophic and diabetic heart muscle (Limas and Cohn 1977; Penpargkul et al. 1981). The adverse effects of diabetes on LV relaxation and SR Ca^{2+} uptake may be greater in hypertrophic than in nonhypertrophic myocardium.
- 3) Thyroid hormone treatment of diabetic SHR will at least partially

prevent the decline in LV relaxation and SR Ca^{2+} uptake affected by chronic diabetes mellitus. Like diabetes, hypothyroidism is associated with impaired myocardial relaxation and depressed rates of Ca^{2+} sequestration by the SR (Suko 1973; Penpargkul et al. 1980; Penpargkul et al. 1981; Takacs et al. 1982; Rohrer and Dillmann 1988). The hypothyroidism that attends diabetes may influence indices of myocardial relaxation in SHR.

- 4) Chronic diabetes will prolong the ventricular AP to a greater extent in SHR than in nonhypertrophic, nonhypertensive rats. A common electrophysiological effect of diabetes and some models of hypertrophy is the prolongation of the cardiac AP (Gulch et al. 1979; Aronson and Keung 1983; Fein et al. 1983; Nordin et al. 1985; Thollon et al. 1985; Sauviat and Feuvray 1986; Volkmann et al. 1989). Diabetes exacerbates the prolonged AP in the hypertrophic RVH ventricular AP (Aronson 1980; Fein et al. 1984). Diabetes may have a more pronounced effect on AP duration in SHR papillary muscle when compared to WKY muscle.
- 5) Thyroid hormone treatment of diabetic SHR will at least partially prevent the diabetes-induced prolongation of the ventricular AP. Either diabetes or hypothyroidism alone, prolongs the ventricular AP in nonhypertensive rats (Capasso et al. 1986; Sauviat and Feuvray 1986). Thyroid hormone treatment prevents some of the AP configurational changes of atrial muscle in diabetic rats (LeGaye et al. 1988).
- 6) Chronic diabetes will depress LV performance in isolated RVH rat hearts to a greater extent than in hearts from normotensive rats. LV function is profoundly depressed in isolated working

hearts of diabetic SHR (Rodgers 1986; Rodrigues and McNeill 1986). Diabetes may impair isometric relaxation in RVH papillary muscles more so than in nonhypertensive, nonhypertrophic muscles (Fein et al. 1983; Fein et al. 1984). Hypertrophy, regardless of the etiology, may predispose the myocardium to the adverse mechanical effects of diabetes.

- 7) Thyroid hormone treatment of RVH rats will at least partially prevent the cardiodepressive effects of diabetes. This last study will be conducted primarily to ascertain whether the cardiac impairments seen in the diabetic SHR isolated heart and the effectiveness of T_3 treatment on heart performance, is independent of the etiology of hypertension in the rat.

The specific aims of the study are as follows:

- 1) LV performance of SHR will be evaluated using an isolated perfused working heart preparation (Rodgers 1986). Diabetes will be chemically induced and certain animals will be chronically treated with either insulin or thyroid (T_3) hormone. Various other control groups will be included in order to identify contributing factors that may be secondary to diabetes. For example, to assess the cardiovascular effects of malnutrition and non-specific effects of the diabetogenic agent streptozotocin, food restriction and pretreatment with 3-O-methylglucose (Ramanadham et al. 1987) will be employed, respectively. T_3 -treated nondiabetic SHR will also be included.
- 2) A detailed examination of ventricular relaxation and a biochemical correlate, SR Ca^{2+} uptake by isolated LV microsomes, will be analyzed in both SHR and normotensive WKY. These indices of relaxation will be evaluated in hearts from diabetic and nondiabetic animals with or without insulin or T_3 treatment.
- 3) Resting membrane potential and AP will be recorded from papillary muscle fibers of normal and diabetic SHR and WKY, and insulin- or T_3 - treated diabetic SHR.
- 4) The effects of diabetes and hormone treatment upon cardiac performance will be assessed in isolated working hearts of RVH rats. Control groups similar to those described for the SHR study (#1 above) will be included.

Insulin, Thyroid Hormone and Heart Function
of Diabetic Spontaneously Hypertensive Rat

SUMMARY

Diabetes impairs cardiac performance more extensively in hypertensive rats than it does in nonhypertensive strains. A "low thyroid state" may contribute to the adverse cardiovascular effects of diabetes in the spontaneously hypertensive rat (SHR). We tested this hypothesis by comparing the effects of thyroid hormone with those of insulin treatment on cardiac performance of diabetic SHR. Diabetes was induced with streptozotocin (45 mg/kg). Subsets of diabetic animals were treated with either insulin (10-20 U/kg/day) or triiodothyronine (8-10 μ g/kg/day). Heart rate and systolic arterial pressure were obtained at weekly intervals. After eight weeks, cardiac function was assessed using an isolated working heart preparation. Diabetes reduced arterial pressure and heart rate in vivo, and markedly depressed cardiac performance under volume and pressure loading conditions ex vivo, confirming previous observations. As expected, insulin treatment prevented the bradycardia and depressor effect in vivo, and the impairment of cardiac performance ex vivo caused by diabetes. The triiodothyronine treatment duplicated the effects of insulin on the hemodynamic measurements in vivo, and corrected nearly all depressed indices of performance of diabetic SHR hearts ex vivo. Both treatment regimes successfully reduced eight-week mortality when compared with the untreated diabetic group. The results support the hypothesis that a low thyroid state may contribute to the cardiovascular dysfunction in diabetic SHR. Left ventricular hypertrophy may be an important factor in this phenomenon.

INTRODUCTION

Diabetes mellitus is associated with cardiac impairments in humans¹ and in experimental animals^{2,3}. Both clinical and experimental "diabetic cardiomyopathy" may be exacerbated by hypertension⁴. Chemically induced diabetes in hypertensive rats has a more profound effect on cardiac function than it does in normotensive models⁵⁻⁷. Collectively, the data suggest that pressure load-induced left ventricular hypertrophy increases the susceptibility of the myocardium to diabetes related derangements¹⁻⁷.

Hypothyroidism, or a "low thyroid state", is often reported as a secondary metabolic disorder of diabetes⁸, and insulin replacement therapy has been shown to restore normal thyroid status in diabetic rats⁹. Hypothyroidism and diabetes have a number of similar effects on cardiac metabolism and function. For example, both pathological conditions impair myocardial contractility, depress myosin ATPase activity and sarcoplasmic reticular Ca²⁺ uptake¹⁰⁻¹², and reduce sensitivity to adrenergic agonists¹³. Whether the cardiovascular effects of diabetes are at least in part due to the attendant low thyroid state has been addressed in a number of studies. Thyroid hormone treatment of normotensive diabetic rats has been shown to be either ineffective^{14,15} or only partially effective⁹ in restoring cardiac function.

Indirect evidence suggests that the influence of an imbalance in thyroid status on the cardiovascular system may have a greater impact in the diabetic spontaneously hypertensive rat (SHR) than it does in diabetic normotensive strains. Thyroidectomy (Tx) of SHR prevents the onset of elevated systolic arterial pressure (SAP)^{16,17}, and

either Tx or methimazole treatment decreases contractility and pressure generation of the isolated working heart^{6,17}. These effects of hypothyroidism in the SHR strain are reminiscent of, but less pronounced than, those of diabetes⁶.

The following investigation was designed to assess the impact of the attendant hypothyroidism on cardiac function in the diabetic SHR by comparing the effects of triiodothyronine (T_3) treatment with those of insulin therapy. The results add support to the hypothesis that attendant hypothyroidism contributes to the adverse cardiac effects of diabetes in the SHR.

METHODS

Animal Groups. Male SHR were obtained from Charles River Breeding Laboratories (Wilmington, MA), housed communally and fed ad libitum unless otherwise indicated. At 15 weeks of age animals were weight matched and earmarked for either nondiabetic or diabetic groups. Diabetes was induced with a single tail vein injection of streptozotocin (STZ), 45 mg/kg bw (Sigma Chemical Co., St. Louis, MO) after animals were lightly etherized. The STZ was dissolved in citrate buffer (0.1 M at pH 4.5) just prior to use. A subset of animals was first injected with 3-O-methylglucose (OMG; 1.2 mmol/150 g BW), followed by STZ within one minute¹⁸. Animals in the nondiabetic groups were also etherized and given similar volumes of the citrate buffer alone. Urine glucose levels were estimated using DIASTIX^(R) (Ames, Elkhart, IN). Diabetes was confirmed in all animals that received STZ. No urinary glucose was detected in the OMG group.

The following treatment regimes were begun three days after the tail vein injections. Diabetic animals were further divided into four subgroups and were treated with one of the following: Lente insulin (LI; Sigma), 20 U/kg/day s.c.; protamine zinc insulin (PZI; Eli Lilly Co., Indianapolis, IN), 10 U/kg/day s.c.; or T₃ (Sigma), 8-10 ug/kg/day s.c. The fourth group of diabetic animals remained untreated for the duration of the experiment. A preliminary study was conducted in order to determine an appropriate T₃ dose in diabetic SHR¹⁹. An initial T₃ dose of 30 µg/kg/day¹⁵ resulted in elevated systolic arterial pressure and heart rate compared to untreated nondiabetic animals. The T₃ dose was subsequently reduced

to 10 $\mu\text{g}/\text{kg}/\text{day}$ and readjusted to 8 $\mu\text{g}/\text{kg}/\text{day}$ after 6 weeks so that heart rates of the treated, diabetic animals matched those of the nondiabetic controls (see Figure 1B). The nondiabetic animals were also subdivided: untreated; food restricted (FR); or T_3 , 8-10 $\mu\text{g}/\text{kg}/\text{day}$ s.c. The FR animals were housed individually, and their food allowance was adjusted so that their weights were similar to those of the untreated diabetic group.

In Vivo Measurements. Weekly measurements of SAP, heart rate (HR) and body weight (BW) were taken just prior to the tail vein injections and then for eight subsequent weeks. SAP and HR were obtained in triplicate using standard tail-cuff sphygmomanometry, after the animals were placed in a temperature controlled chamber (34°C) for 10-15 minutes. These measurements were recorded before the daily injections of T_3 or insulin, and after the food restricted group was fed.

Serum Assays. The animals were killed by rapid decapitation eight weeks after the STZ or citrate buffer injections. The serum samples were collected 15-18 hours subsequent to the final insulin or T_3 injection, and stored at -20°C for future determinations. The degree of diabetes was assessed by serum glucose values using a glucose oxidase assay (Sigma). Animals were considered diabetic if serum glucose concentrations exceeded 300 mg/dL. Serum immunoreactive insulin activity, total T_3 and total thyroxine (T_4) were determined by radioimmunoassay (Cambridge Medical Diagnostics, Billerica, MA). Serum levels of insulin were quantified using rat insulin (generously provided by Dr. R. Chance of Eli Lilly, Inc.) as a standard.

Cardiac Performance. The hearts were quickly removed and perfused as described previously⁶. In brief, the perfusion apparatus was designed to allow manipulations in both volume loading (left atrial filling pressure, LAFP) and pressure loading (resistance to aortic ejection). Left ventricular pressure (LVP) and aortic pressure (AP) were monitored continually through indwelling cannulae coupled to pulse transducers (Stratham, Hato Rey, PR). Aortic flow rate (AFR) and coronary flow rate (CFR) were quantified intermittently. Myocardial oxygen consumption (VO_2) was assessed with an in-line O_2 electrode (Transidyne Corp., Ann Arbor, MI) confluent with the coronary perfusate (see calculations below).

Hearts were perfused with nonrecirculating Krebs-Henseleit buffer maintained at 37°C. The buffer contained 120 mM NaCl, 5.6 mM KCl, 0.65 mM $MgSO_4$, 1.21 mM NaH_2PO_4 , 25 mM $NaHCO_3$, 2.4 mM $CaCl_2$, 0.2 mM EDTA and 10 mM glucose (pH 7.4 when gassed with 95% O_2 and 5% CO_2). Bipolar electrodes (attached to a Grass SD9 Stimulator; Grass Instruments, Quincy, MA) were placed on the right atrium, and hearts were paced at 300 beats per minute. Occasionally, endogenous rhythmicity exceeded 300 bpm at which time external pacing was discontinued. The data were continually recorded on a chart recorder (Narco Trace Model 80; Narco BioSystems, Houston, TX) and a dedicated computer (Buxco Corp., Sharon, CT). LVP waves were also recorded and stored in an IBM PCII using ASYST^(R) software (Macmillan Software Co., New York, NY) and A/D conversion hardware (Data Translation Inc., Marlborough, MA). To ensure the stability of each preparation, LVP generation was monitored prior to data collection for 10-15 minutes while LAFP was set at 15 cm H_2O and resistance to

aortic ejection was set at $1.54 \text{ kPa/cm}^3 \cdot \text{min}^{-1}$. Measurements were first recorded during volume loading at LAFPs of 5, 10, 15, and 20 cm H_2O while holding resistance constant at $1.54 \text{ kPa/cm}^3 \cdot \text{min}^{-1}$. They were then recorded during pressure loading at aortic resistances of 0.19, 0.41, 1.54 and $3.13 \text{ kPa/cm}^3 \cdot \text{min}^{-1}$ while holding LAFP at 15 cm H_2O . Total perfusion time rarely exceeded 30 minutes.

Heart Weight Ratios. Once the data were collected, the hearts were removed from the perfusion apparatus and blotted dry. The aorta was removed, and the following wet weights were obtained; whole heart (HW), left ventricular (LV) including the septum, and right ventricular (RV). These weights were used to assess the degree of left ventricular hypertrophy⁶.

Calculations. Direct measurements included LVP, left ventricular end-diastolic pressure (EDP), AFR, CFR, aortic pressures (including mean aortic pressure (MAP)), and pO_2 of the coronary effluent. Indices of heart performance included left ventricular pulse pressure (LVPP as maximum LVP minus EDP), maximum rate of left ventricular pressure generation (LV + dP/dt), hydraulic power (power as the product of LVPP and cardiac output [CO=AFR plus CFR] per gram HW) and stroke work (SW as the ratio of power to HR). Myocardial oxygen consumption (VO_2) was calculated as the product of oxygen extracted from the buffer, CFR and oxygen solubility in buffer at 37°C ($31.25 \mu\text{L/cm}^3$) and normalized for HW. The efficiency of contraction (E) was then calculated as the ratio of power to VO_2 . Coronary vascular resistance (CVR) was estimated from the slope of the regression line when MAP was plotted against CFR at the four pressure loads⁶.

Statistical Analyses. The differential effects of diabetes and the hormone treatments were evaluated by analysis of variance (with repeated measures where appropriate), and the Student Newman-Keuls test for multiple comparisons (Statistical Analysis System, Cary, NC). Group means were considered statistically different from each other if the probability value was less than 0.05. Lower probabilities were not reported. All group comparisons were made statistically; however, in most cases it was only necessary to present comparisons of each experimental group to the untreated, nondiabetic SHR. Additional comparisons, between experimental groups, were noted only if they had obvious relevance to the discussion.

RESULTS

Serum Assays. Serum glucose levels were elevated in all STZ-injected groups except those which had been pretreated with OMG (Table 1). Treatment with PZI controlled the hyperglycemia more effectively than LI did, but neither treatment restored serum glucose levels to that of the nondiabetic animals. Glucose levels of the FR and T_3 -treated nondiabetic SHR were not different from those of the untreated, nondiabetic group.

Hypoinsulinemia was evident in both the untreated and the T_3 -treated diabetic rats. Both PZI and LI treatments markedly elevated serum immunoreactive insulin activity; PZI effected the most pronounced increase. The elevated levels may reflect a subsensitivity to exogenous insulin in the diabetic rat. The serum insulin levels of all the other groups were normal.

Diabetes also reduced serum T_3 and T_4 levels. Either insulin or T_3 treatment prevented the decline in T_3 concentration. Insulin treatment also reversed the drop in T_4 levels. However, T_3 treatment of either diabetic or nondiabetic SHR markedly suppressed serum T_4 concentrations. The OMG and FR groups had normal T_3 values but the FR group had slightly depressed T_4 levels.

Body and Heart Weights. Diabetes reduced both heart and body weights of SHR (Table 2). Treatment with PZI completely prevented these changes, whereas LI was only partially effective. In contrast, T_3 therapy did not affect the decline in HW, but increased the extent of the loss in BW, of diabetic SHR. Administration of T_3 to nondiabetic SHR had no effect on BW or HW. The effects of STZ-induced diabetes

on BW and HW were nearly duplicated by food restriction, and completely blocked by OMG pretreatment.

The ratios HW/BW and LV/BW, which are indices of relative cardiac hypertrophy, and LV/RV, which is an index of absolute left ventricular hypertrophy⁶, were not significantly affected by any of the experimental manipulations except one; the extent of relative hypertrophy was increased in the T₃-treated diabetic group.

Mortality. The mortality rate of the diabetic SHR was about 64%, which was much higher than that of any other group (Table 3). As expected, insulin treatment (either PZI or LI) prevented the eight week mortality of diabetic SHR. Unexpectedly, T₃ treatment was as effective as insulin was in preventing (or delaying) mortality of the diabetic animals. The mortality in the FR group occurred early in the study, as a result of attempts to exactly duplicate the mean BW of the diabetic group. Slight upward adjustments of the food allotment prevented further mortality, and resulted in mean BW which was slightly higher than that of the diabetic group by the end of the eight week period (see Table 2). The mortality of diabetic SHR was completely prevented by treatment with OMG prior to STZ injection.

Systolic Arterial Pressure and Heart Rate. Diabetes depressed SAP of SHR in vivo, beginning about four weeks after STZ injection (Figure 1A), confirming previous results⁶. It also caused a progressive decline in HR (Figure 1B). Either insulin (PZI or LI) or T₃ treatment prevented both the depressor and bradycardic effects of diabetes. Since both PZI and LI restored SAP and HR to control values, the two insulin-treated groups were pooled in Figure 1 and Table 4. A summary of the SAP and HR measurements in the remaining

control groups at the eight week time point is provided in Table 4. With one exception, none of these values is significantly different from that of the untreated, nondiabetic group. Only the HR of the FR group was significantly depressed.

Cardiac Performance Ex Vivo. Diabetes impaired the performance of SHR hearts ex vivo, under either volume- or pressure-loading conditions (Figures 2 and 3). The impairment was evident as a decline in LVPP and SW at higher volume loads (Figure 2), and in LVPP at higher pressure loads (Figure 3). Diabetes also depressed left ventricular contractility (LV + dP/dt; Table 5). These results generally confirm those of previous reports^{6,7}, which had also demonstrated that the magnitude of the cardiac impairment due to diabetes is greater in the SHR than it is in normotensive rats. Both insulin treatments of diabetic SHR (either PZI or LI) completely prevented the decline in cardiac performance; in the interest of clarity, therefore, the data for the two insulin-treated groups were pooled in Figures 2 and 3 and in Tables 5-7. Treatment of diabetic SHR with T₃ was nearly as effective as was insulin therapy (Figure 4); it completely prevented all effects of diabetes except that it only partially maintained LVPP at the highest volume load (Figure 2A and Table 5) and depressed LVPP at the lowest pressure load (Figure 3A). In addition, T₃ treatment of diabetic animals significantly increased SW at a LVFP of 10 cm H₂O (Figure 2B). Neither LVPP, SW, nor contractility of any of the other control groups was different from the corresponding value of the nondiabetic control under either volume-loading (Table 5) or pressure-loading conditions (data not shown). Thus, neither malnutrition nor direct cardiotoxic effects of

STZ appear to contribute significantly to the cardiac impairments of the diabetic SHR.

Diabetes had no effect on VO_2 , CO, hydraulic power, or efficiency of contraction (E; Table 6). None of the experimental groups differed significantly from the control with regard to any of these variables, except that the CO (per gram HW) of the T_3 -treated diabetic group was elevated. This was related more to the reduction in heart size than it was to any increase in total output²⁰.

Although diabetes reduced total coronary flow rate (CFR), it had no effect on either CFR per gram HW or on CVR (Table 7). Thus, the impaired LVPP, SW and contractility of diabetic SHR hearts were not associated with restricted coronary flow.

DISCUSSION

Recent results from this laboratory suggested that a low thyroid state might contribute significantly to changes in cardiac performance of diabetic SHR^{6,17}. Accordingly, we expected that T₃ treatment of diabetic SHR might reduce the magnitude of the subsequent impairment in mechanical function. We were somewhat surprised, though, that the dose of T₃ used in this study was almost as effective as insulin was in preventing the depressions in measurements of heart function, including pressure generation, rates of contraction, and stroke work.

Our results obtained from SHR differ from previous reports of the effects of T₃ treatment on cardiac function and hemodynamic measurements in diabetic normotensive rats^{9,14,15}. In the earlier studies, T₃ treatment - at doses ranging from approximately 8 to 30 µg/kg/day - was either ineffective or partially effective in preventing diabetes-induced myocardial functional impairments. For the present study, we selected the dose of T₃ according to its ability to maintain normal HR in vivo, an index which is a sensitive indirect indicator of thyroid status. This turned out to be about 2-3 fold greater than "physiologic" replacement doses in nonhypertensive rat strains²¹. The T₃ dose used herein did not appear, however, to impose a hyperthyroid condition in diabetic SHR for several reasons: 1) It did not elevate serum T₃ levels above normal, when measured at the same time point after injection, in either the diabetic or the nondiabetic SHR groups (Table 1); 2) The same dose given to nondiabetic SHR (Table 4) or to diabetic SD rats²² had no effect on SAP or HR in vivo; and 3) T₃ treatment of

nondiabetic SHR had no effect on any of the indices of cardiac performance ex vivo (Tables 5-7). Although T_3 treatment reduced body weights of the diabetic SHR, it had no effect on this measurement in the nondiabetic control group (Table 2). It may not be appropriate to assume that doses of T_3 which are adequate for therapy of simple hypothyroidism should be enough to correct the hypothyroid state induced by diabetes. Diabetic hypothyroidism in the rat is characterized not only by low serum hormone levels, but also by target tissue resistance. For example, diabetes impairs peripheral T_4 to T_3 conversion, tissue T_3 uptake, and both affinity and density of nuclear T_3 receptors^{23,24}.

The differences in the effects of T_3 treatment between the SHR and normotensive diabetic rats are probably related to the elevated arterial pressure of SHR, and not to differences related specifically to the genetic strain. We have preliminary results (unpublished observations) that T_3 is as effective in the diabetic renovascular hypertensive (RVH) rat as it is in the diabetic SHR. A common feature of the two models, left ventricular hypertrophy (LVH), may increase cardiac susceptibility to a number of stresses including those imposed by diabetes^{4,6,7}, hypothyroidism¹², or ischaemia²⁵. Our results suggest that the hypothyroidism accompanying diabetes has a more profound cardiovascular effect in animals with elevated arterial pressure, and that hypertrophied myocardium may be functionally more dependent upon thyroid status than is nonhypertrophic heart muscle. This hypothesis is supported by the observation that thyroidectomy reduces left ventricular contractility to a greater extent in the SHR than it does in the WKY¹⁷.

There are a number of mechanisms by which T_3 treatment might effectively reverse the cardiac functional impairments of diabetes, especially of hypertrophic heart muscle. A common manifestation of diabetes, hypertrophy and hypothyroidism on rat ventricular muscle is a shift from the predominant V_1 to the V_3 myosin isozyme^{9,11,21,26-28}. The effects of hypertension and diabetes (with or without hypothyroidism) on myosin isozyme profiles may thus be additive, but this hypothesis has not been tested. T_3 treatment of the diabetic SHR may change the ratio of myosin isozyme activity in favor of V_1 and this may in turn help to explain the increased left ventricular contractility in this group (Table 5). Another biochemical change frequently observed in hearts from either diabetic, hypertensive or hypothyroid animals is depressed sarcoplasmic reticular (SR) Ca^{2+} uptake, which correlates with impaired myocardial contraction and relaxation^{10,12,28}. Although thyroid hormone treatment did not restore SR Ca^{2+} uptake of diabetic normotensive rats^{15,29}, its effectiveness on diabetic hypertensive muscle has recently been demonstrated (see accompanying manuscript). Metabolic derangements in the diabetic heart include decreased glucose utilization and increased lipid accumulation³⁰. These in turn have been linked to changes in energy substrate availability and membrane integrity and function in the heart¹⁰. Hypertrophy may exacerbate these metabolic effects. Increased vulnerability of hypertrophic myocardium to metabolic stress may be related either to a lower energy state³¹ or to abnormal lipid metabolism in the SHR^{7,32-34}. Interestingly, the vasodilator hydralazine reduced serum lipids and improved cardiac function but also corrected the attendant

hypothyroid state in diabetic nonhypertensive rats³⁵. Thyroid hormone may relieve some of these stresses in diabetic SHR, perhaps by increasing the utilization of fatty acid by the myocardium³⁶, by stimulating glucose uptake and utilization³⁷, or by augmentating glucose-induced insulin secretion³⁸, although our data (Table 1) did not support the latter possibility. Thyroid hormone may also enhance the responsiveness of the myocardium to sympathoadrenal influences (see below).

Several alternative interpretations of our results could be eliminated by the control studies. Amelioration of the cardiodepression and mortality by either insulin or T₃ treatment, and by pretreatment with the glucose analog OMG¹⁸, ruled out the possibility that direct cardiotoxicity of STZ contributed to the morbidity and mortality of diabetes. Complete glycemc control was not necessary for preservation of cardiac functional activity, supporting previous observations^{34,39}, both PZI and LI maintained pump function without normalizing serum glucose values (Table 1). Malnutrition alone could not account for the observed cardiodepressive effects of diabetes in our study, since performance was not impaired in the food restricted group (Tables 5 and 6; see also references 3 and 6).

Hypothyroidism probably also contributes to the reduction in SAP in vivo of diabetic SHR⁶ (Table 4). Thyroidectomy prevents the development of hypertension in this model^{16,17}, whereas T₃ treatment of diabetic SHR prevents the decline in SAP (Table 4). The latter result also illustrates that the restorative effects of T₃ (and insulin) treatment are manifest in the face of sustained elevations

in arterial pressure load. The depressor effect of diabetes in SHR likely involves both vascular and cardiac components. Yamamoto and Nakai⁴⁰ seemed to show in their Table 3 that moderate diabetes reduced total peripheral resistance of SHR by about 10-20%, depending on the duration of diabetes. Administration of T_3 may increase TPR in the diabetic SHR. On the other hand, variations in SAP of either diabetic or hypothyroid SHR, whether treated or untreated, always seem to be associated with parallel changes in cardiac performance^{6,7,17} (Table 4 and 5). Tsujimoto and Hashimoto⁴¹ attributed most of the rise in SAP of T_3 -induced hyperthyroid rats to cardiac, rather than to vascular, effects of T_3 .

Treatment with T_3 , like insulin, also corrected the bradycardia of diabetic SHR (Figure 1B). The bradycardia associated with either diabetes or hypothyroidism has been attributed, at least in part, to depressed myocardial responsiveness to adrenergic stimulation^{13,42}. Both hypertrophic⁴³ and diabetic⁴⁴ myocardium have been shown to possess a lower density of functional β -adrenoceptors, and both insulin and T_3 may be involved in the regulation of cardiac β -adrenoceptor density. Insulin may act indirectly by restoring serum T_3 levels. Depressed myocardial β -adrenoceptor numbers of diabetic SD rats were restored to normal with either insulin or T_4 treatment, while those of thyroidectomized diabetic rats were not affected by insulin treatment alone⁴⁵. The chronotropic action of T_3 in vivo may also involve a direct effect of the hormone on pacemaker cells⁴⁶. The available evidence implies that at least a significant portion of the bradycardia of experimental diabetes, in the presence

or absence of hypertension, can be attributed to the attendant hypothyroidism.

In conclusion, the cardiac impairments associated with diabetes in the SHR can be prevented by either insulin or T_3 treatment. To our knowledge this is the first account of a moderate dose of T_3 successfully restoring cardiac performance in experimental diabetes. In addition, T_3 treatment, like insulin, prevented the fall in arterial pressure and bradycardia induced by diabetes in the SHR in vivo, and either reduced or delayed mortality. The results suggest that attendant hypothyroidism may play a more significant role in the development of impaired cardiac function in diabetic SHR than it does in normotensive rats. The possible contribution of left ventricular hypertrophy to this phenomenon remains to be studied. These results may have some clinical applicability. Whether or not hypertension occurs more frequently among diabetic patients⁴⁷, it is generally regarded as an important risk factor for the higher cardiovascular morbidity and mortality in this group^{1,47}. The incidence of impaired thyroid function among diabetics may be as high as 30%, is most severe during poor metabolic control, and is most evident in IDDM²³. While diabetic patients with hypothyroidism may be at increased risk of cardiovascular morbidity and mortality⁴⁸, this risk may be further intensified by hypertension.

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TABLE 1. Serum glucose, insulin, triiodothyronine and thyroxine levels of nondiabetic, diabetic and treated SHR.

Group	n	Glucose (mg/dl)	Insulin (μ U/ml)	T ₃ (ng/dl)	T ₄ (μ g/dl)
Nondiabetic	12	122 \pm 22	56 \pm 25	94 \pm 17	6.96 \pm 1.53
Diabetic	9	540 \pm 61*	14 \pm 7*	53 \pm 13*	2.92 \pm 0.58*
T ₃ -treated diabetic	9	578 \pm 87*	18 \pm 7*	73 \pm 13	0.28 \pm 0.09**
LI-treated diabetic	7	546 \pm 116*	229 \pm 129**	77 \pm 15	5.46 \pm 0.94
PZI-treated diabetic	7	370 \pm 179**	355 \pm 183**	85 \pm 22	5.49 \pm 1.57
T ₃ -treated control	7	115 \pm 12	67 \pm 15	88 \pm 26	0.34 \pm 0.12**
FR control	9	120 \pm 36	42 \pm 29	88 \pm 14	4.36 \pm 1.31**
OMG control	9	136 \pm 20	50 \pm 13	87 \pm 23	5.87 \pm 1.01

Values are means \pm SD, obtained 8 weeks after initiation of diabetes or treatment. T₃, triiodothyronine; T₄, thyroxine; LI, lente insulin; PZI, protamine zinc insulin; FR, food restricted nondiabetic group; OMG 3-O-methylglucose pretreated, STZ-injected group. *Significantly different from the nondiabetic group. **Significantly different from the nondiabetic and diabetic groups.

TABLE 2. Body and heart weights of nondiabetic, diabetic and treated SHR.

Group	n	BW (g)	HW (mg)	HW/BW (mg/g)	LV/BW (mg/g)	LV/RV (mg/mg)
Nondiabetic	12	324±28	1370±132	4.24±0.46	3.41±0.38	5.56±0.81
Diabetic	9	208±20 [*]	907±85 [*]	4.38±0.30	3.48±0.24	5.25±0.61
T ₃ -treated diabetic	9	179±26 ^{**}	929±146 [*]	5.22±0.69 [*]	4.11±0.59 [*]	4.93±0.51
LI-treated diabetic	7	283±24 ^{**}	1213±112 ^{**}	4.29±0.25	3.41±0.21	6.16±0.56
PZI-treated diabetic	7	312±27	1371±83	4.41±0.36	3.60±0.32	5.40±0.56
T ₃ treated control	7	300±20	1406±75	4.72±0.55	3.79±0.49	5.44±0.68
FR control	9	241±11 ^{**}	1064±112 ^{**}	4.41±0.39	3.62±0.38	6.04±0.77
OMG control	9	328±24	1370±94	4.19±0.24	3.43±0.21	6.16±0.56

Values are means ± SD, obtained 8 weeks after initiation of diabetes or treatment. BW, body weight; HW, heart weight; LV, left ventricular weight; RV, right ventricular weight; T₃, triiodothyronine; LI, lente insulin; PZI, protamine zinc insulin; FR, food restricted nondiabetic group; OMG, 3-O-methylglucose pretreated, STZ-injected group. ^{*}Significantly different from the nondiabetic group. ^{**}Significantly different from the nondiabetic and diabetic groups.

TABLE 3. Mortality of nondiabetic, diabetic and treated SHR.

Group	Mortality ^s	
	Fraction	%
Nondiabetic	1/18	5.6
Diabetic	21/33	63.6
T ₃ -treated diabetic	1/14	7.1
LI-treated diabetic	1/10	10.0
PZI-treated diabetic	1/10	10.0
T ₃ -treated control	0/12	0
FR control	3/13	23.1
OMG control	0/10	0

^sThe values represent the proportion of animals which had died during the eight week study period. T₃, triiodothyronine; LI, lente insulin; PZI, protamine zinc insulin; FR, food restricted nondiabetic group; OMG, 3-O-methylglucose pretreated, STZ-injected group.

TABLE 4. Systolic arterial pressure and heart rate of nondiabetic, diabetic and treated SHR.

Group	n	SAP (kPa)		HR (beats/min)	
		Week 0	Week 8	Week 0	Week 8
Nondiabetic	12	24.0 ± 3.7	26.7 ± 2.9	385 ± 47	401 ± 34
Diabetic	9	23.7 ± 2.5	21.1 ± 1.9*	366 ± 28	273 ± 22*
T ₃ -treated diabetic	9	24.8 ± 1.9	24.4 ± 4.1	407 ± 46	413 ± 37
I-treated diabetic	14	23.7 ± 1.6	26.5 ± 3.3	397 ± 35	366 ± 42
T ₃ -treated control	7	24.0 ± 1.1	30.0 ± 3.5	405 ± 33	429 ± 60
FR control	9	25.3 ± 1.9	26.3 ± 2.0	372 ± 18	358 ± 40
OMG control	9	23.6 ± 2.4	29.7 ± 3.5	377 ± 19	384 ± 27

Values are means ± SD, obtained before (Week 0) and 8 weeks after initiation of diabetes or treatment. SAP, systolic arterial pressure; HR, heart rate; T₃, triiodothyronine; I, PZI and Lente insulin; FR, food restricted nondiabetic group; OMG, 3-O-methylglucose pretreated, STZ-injected group. *Significantly different from the nondiabetic group.

TABLE 5. Left ventricular pulse pressure (LVPP), stroke work and contractility (LV+dP/dt) of SHR perfused hearts under various experimental conditions.

Group	n	LVPP (kPa)	Stroke Work (mJ/g)	LV+dP/dt (kPa/sec)
Nondiabetic	12	21.6 ± 1.8	2.47 ± 0.46	913 ± 124
Diabetic	9	15.5 ± 1.8 [*]	1.76 ± 0.42 [*]	572 ± 132 [*]
T ₃ -treated diabetic	9	18.7 ± 0.8 ^{**}	2.57 ± 0.47	854 ± 120
I-treated diabetic	14	21.3 ± 2.5	2.49 ± 0.48	851 ± 177
T ₃ -treated control	7	23.3 ± 1.9	2.76 ± 0.31	1101 ± 147
FR control	9	20.0 ± 2.3	2.36 ± 0.66	802 ± 162
OMG control	9	23.5 ± 2.9	2.48 ± 0.56	917 ± 139

Values are means ± SD, obtained 8 weeks after initiation of diabetes or treatment. Left atrial filling pressure was fixed at 20 cm H₂O and resistance to aortic ejection was fixed at 1.54 kPa/cm³·min⁻¹. Values for LVPP and stroke work of the first four groups are also depicted in Figure 2. I-PZI or Lente insulin; T₃, triiodothyronine; FR, food restricted nondiabetic group; OMG, 3-O-methylglucose pretreated, STZ-injected group. ^{*} Significantly different from the nondiabetic group. ^{**} Significantly different from the nondiabetic and diabetic groups.

TABLE 6: Myocardial oxygen consumption (VO_2), cardiac output (CO), power and efficiency of contraction (E) of nondiabetic, diabetic and treated SHR.

Group	n	VO_2	CO	Power	E
		($\mu LO_2/\text{sec}\cdot\text{g}^{-1}$)	($\text{cm}^3/\text{sec}\cdot\text{g}^{-1}$)	(mW/g)	(mJ/ μLO_2)
Nondiabetic	12	4.78 \pm 0.81	0.49 \pm 0.08	9.30 \pm 1.84	1.94 \pm 0.21
Diabetic	9	4.26 \pm 0.39	0.51 \pm 0.07	7.53 \pm 1.50	1.76 \pm 0.24
T_3 -treated diabetic	9	6.24 \pm 1.68	0.64 \pm 0.12 [*]	11.49 \pm 2.14	1.92 \pm 0.40
I-treated diabetic	14	5.29 \pm 0.92	0.53 \pm 0.07	9.93 \pm 2.07	1.88 \pm 0.27
T_3 -treated control	7	5.98 \pm 1.15	0.53 \pm 0.06	11.07 \pm 1.62	1.89 \pm 0.24
FR control	9	6.16 \pm 2.12	0.52 \pm 0.13	9.58 \pm 2.98	1.58 \pm 0.31
OMG control	9	4.83 \pm 0.88	0.43 \pm 0.07	8.91 \pm 2.01	1.83 \pm 0.22

Values are mean \pm SD, obtained 8 weeks after initiation of diabetes or treatment. Left atrial filling pressure was fixed at 15 cm H_2O and resistance to aortic ejection was fixed at 1.54 kPa/ $\text{cm}^3\cdot\text{min}^{-1}$. I-PZI and Lente insulin; T_3 , triiodothyronine; FR, food restricted nondiabetic group; OMG, 3-O-methylglucose pretreated, STZ-injected group. *Significantly different from the nondiabetic group.

TABLE 7. Coronary flow rate (CFR) and coronary vascular resistance (CVR) of nondiabetic, diabetic and treated SHR.

Group	n	CFR (cm ³ /sec)	CFR (cm ³ /sec.g ⁻¹)	CVR (kPa/cm ³ .sec ⁻¹ .g ⁻¹)
Nondiabetic	12	0.40 ± 0.08	0.30 ± 0.06	50.20 ± 3.14
Diabetic	9	0.23 ± 0.04*	0.26 ± 0.05	49.22 ± 3.26
T ₃ -treated diabetic	9	0.31 ± 0.08	0.34 ± 0.09	40.64 ± 3.88
I-treated diabetic	14	0.41 ± 0.07	0.31 ± 0.05	45.26 ± 3.36
T ₃ -treated control	7	0.45 ± 0.08	0.32 ± 0.06	50.42 ± 4.70
FR control	9	0.33 ± 0.11	0.31 ± 0.10	48.86 ± 5.56
OMG control	9	0.38 ± 0.08	0.29 ± 0.06	50.39 ± 3.42

CFR values are means ± SD and CVR values are means ± SE, obtained 8 weeks after initiation of diabetes or treatment. CFR was measured when left atrial filling pressure was fixed at 15 cm H₂O and resistance to aortic ejection was fixed at 1.54 kPa/cm³.min⁻¹. CVR is determined by plotting mean aortic pressure against coronary flow at the four resistances to aortic ejection, and obtaining the slope of the regression (see Methods). I-PZI and Lente insulin; T₃, triiodothyronine; FR, food restricted nondiabetic group; OMG, 3-O-methylglucose pretreated, STZ-injected group. *Significantly different from the nondiabetic group.

Figure 1. (A) Systolic arterial pressure (SAP) and (B) heart rate (HR) in vivo, of nondiabetic (○), diabetic (●), insulin-treated diabetic (■) and triiodothyronine (T₃)-treated diabetic (▲) SHR. The number of animals in each group is shown in parentheses. Values are means ± SD, obtained 8 weeks after initiation of diabetes or treatment. *Significantly different from the nondiabetic group.

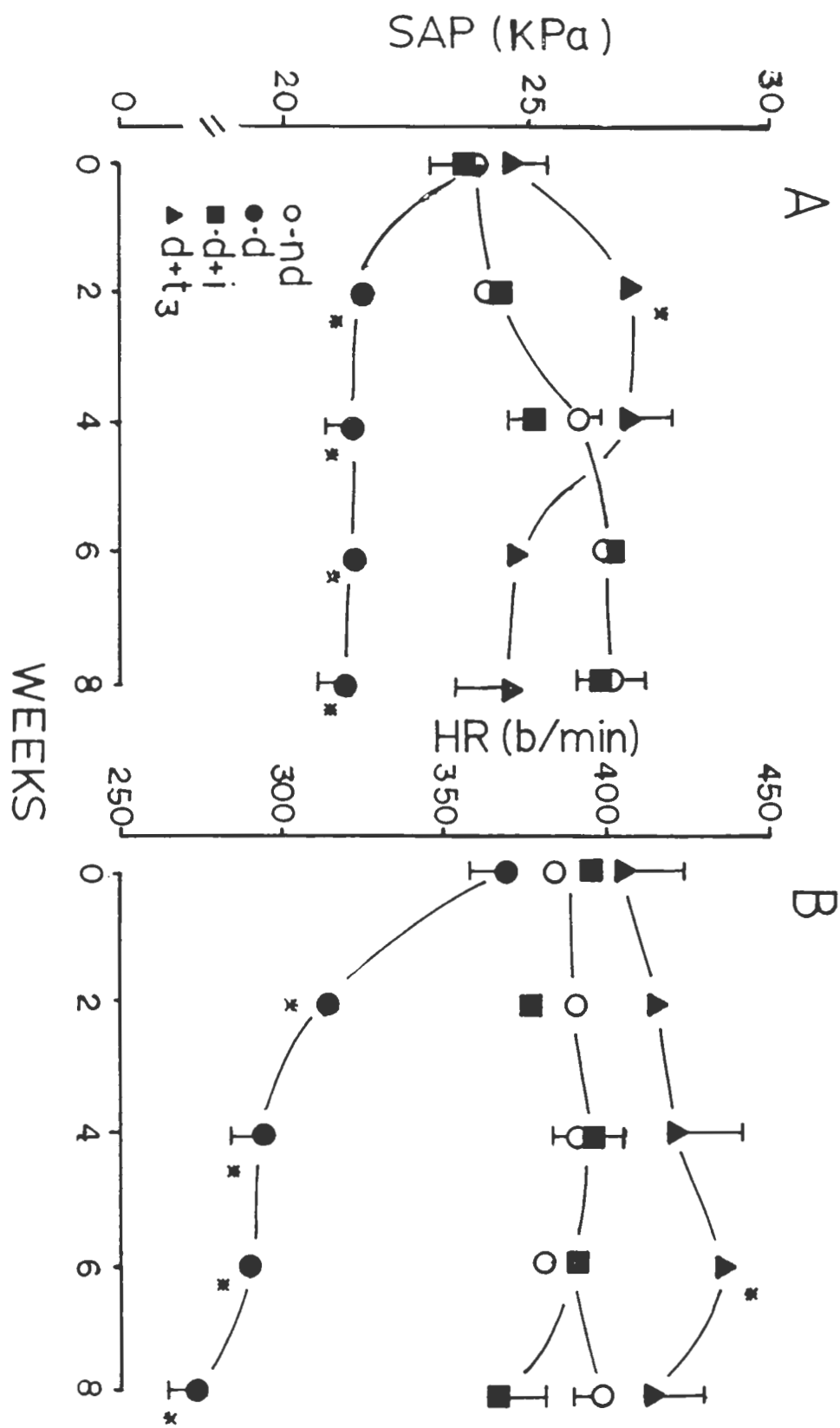


Figure 2. Cardiac performance ex vivo, as measured by (A) left ventricular pulse pressure (LVPP) or (B) stroke work, of nondiabetic (○), diabetic (●), insulin-treated diabetic (■), and triiodothyronine (T₃)-treated diabetic (▲) SHR. The hearts were volume loaded by varying left atrial filling pressure (LAFP) between 5 and 20 cm H₂O. Resistance to left ventricular ejection was fixed at 1.54 kPa/cm³.min⁻¹. Values are means ± SD, obtained 8 weeks after initiation of diabetes or treatment (n=7-12 per group). *Significantly different from the nondiabetic group. **Significantly different from the nondiabetic and diabetic groups.

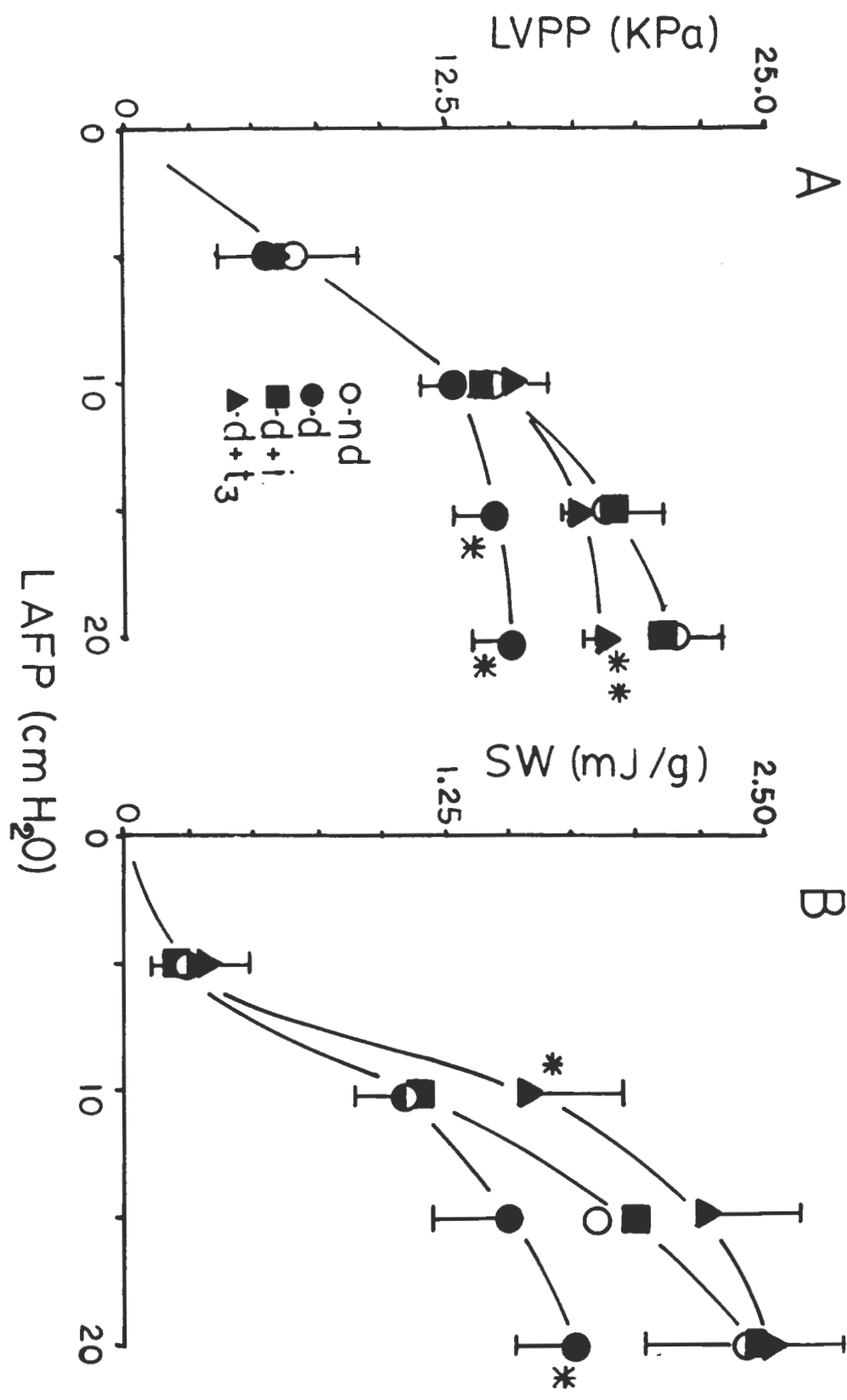


Figure 3. Cardiac performance ex vivo, as measured by (A) left ventricular pulse pressure (LVPP) or (B) stroke work, of nondiabetic (○), diabetic (●), insulin-treated diabetic (■), and triiodothyronine (T₃)-treated diabetic (▲) SHR. The hearts were pressure loaded by varying resistance to aortic ejection between 0.19 and 3.13 kPa/cm³.min⁻¹. Left atrial filling pressure was fixed at 15 cm H₂O. Values are means ± SD, obtained 8 weeks after initiation of diabetes or treatment (n=7-12 per group). *Significantly different from the nondiabetic group.

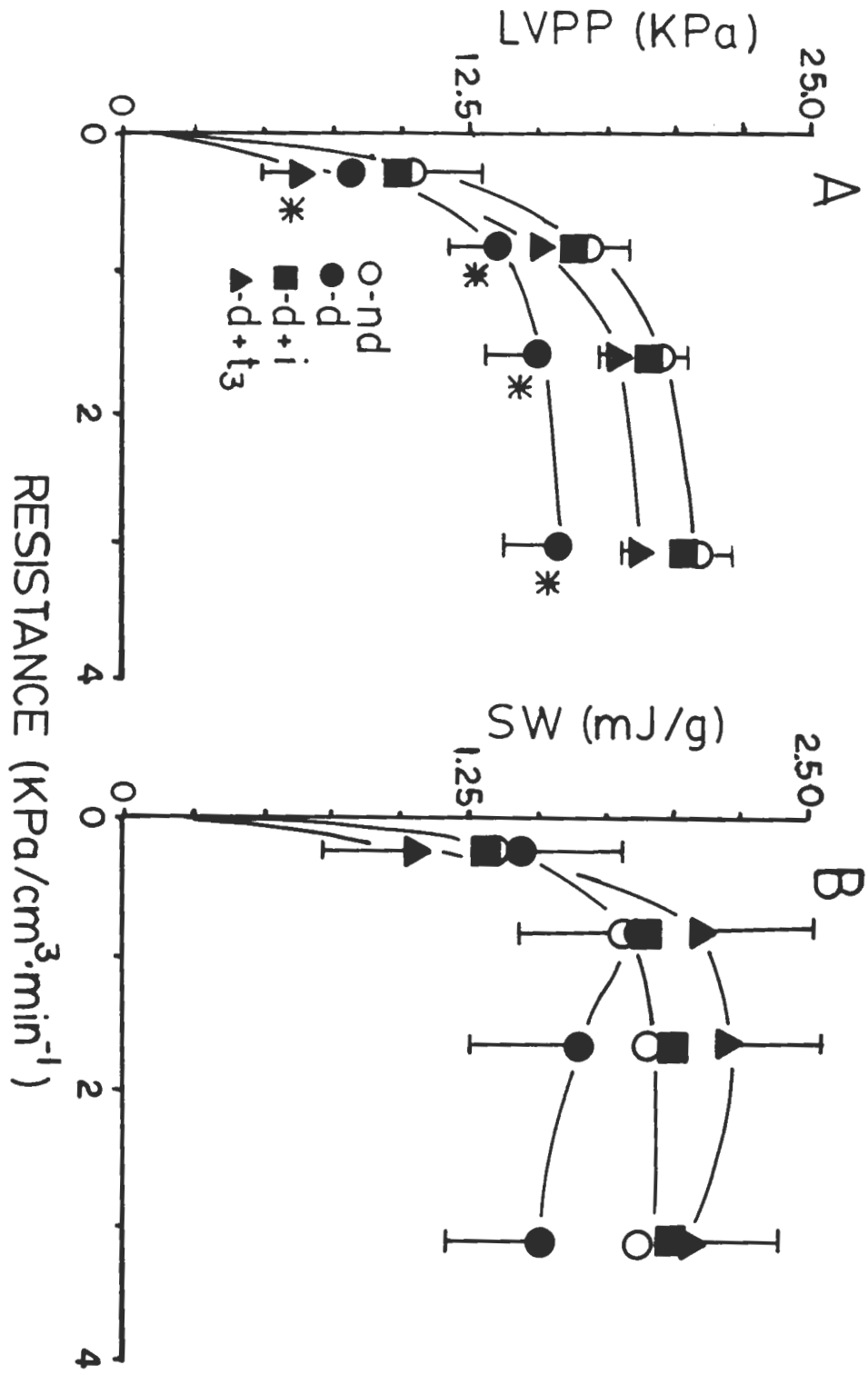
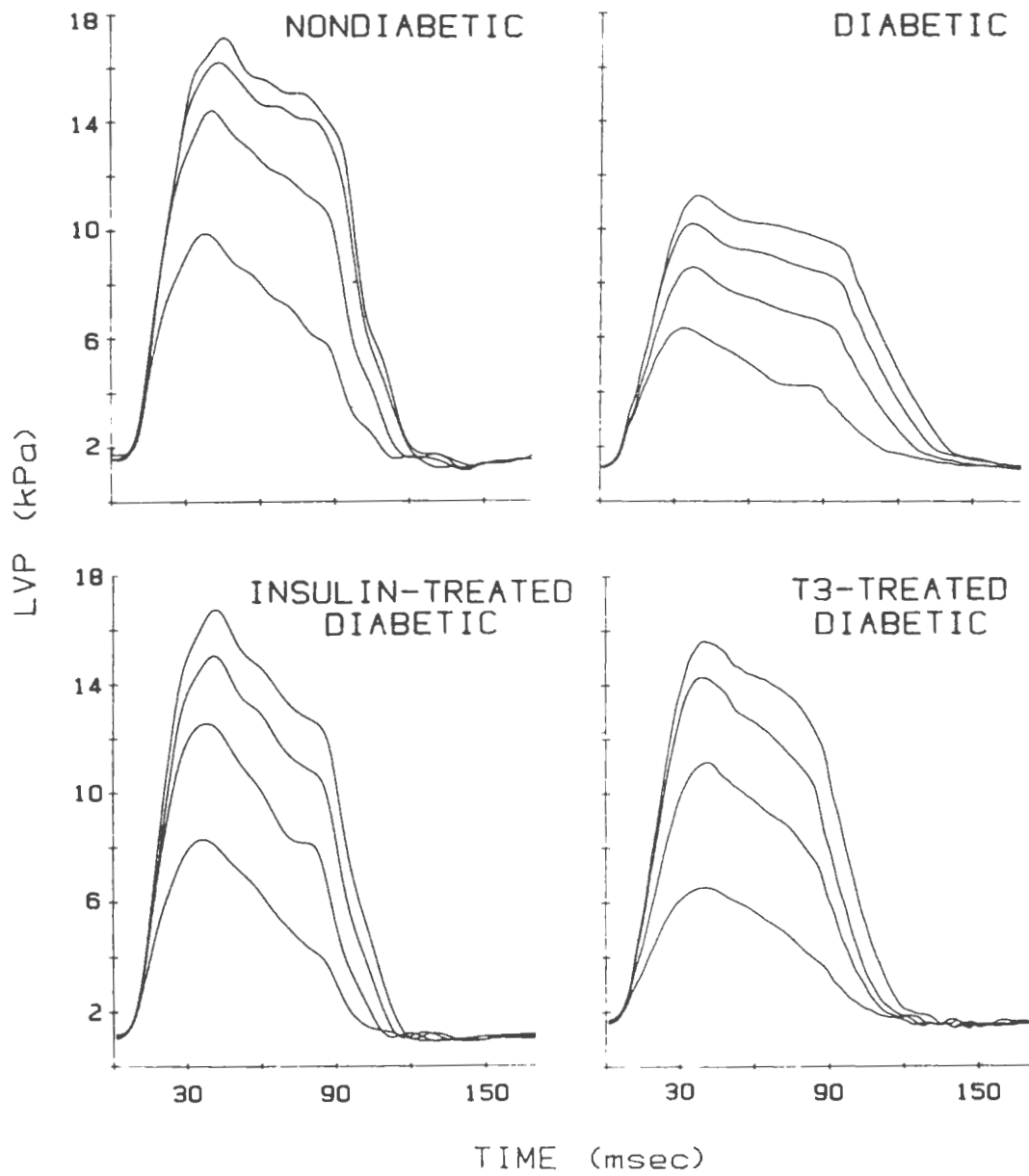


Figure 4. Left ventricular pressure (LVP) waves from perfused working hearts of nondiabetic, diabetic, insulin-treated diabetic and triiodothyronine (T_3)-treated diabetic SHR, under increasing pressure-loading conditions. Each of the four superimposed LVP waves was obtained at one of four resistances to aortic ejection: 0.19, 0.41, 1.54 and 3.13 kPa/cm³.min⁻¹. Left atrial filling pressure was fixed at 15 cm H₂O. LVP waves were recorded from a single heart in each group and were obtained eight weeks after initiation of untreated or treated diabetes. Analog signals were digitized and stored on a diskette and regenerated using an IBM PC II with ASYST^(R) software and an HP7470 plotter. Individual records were selected with the aim of approximating, as closely as possible, the mean maximum LVP values for each group as shown in Figure 3A.

SHR



Ventricular Relaxation of Diabetic Spontaneously
Hypertensive Rat

SUMMARY

Diabetes - and possibly the hypothyroidism which attends diabetes - impairs mechanical relaxation of ventricular muscle, in part by depressing the rate of Ca^{2+} uptake by sarcoplasmic reticulum. Left ventricular hypertrophy exacerbates the adverse effects of diabetes on cardiac performance but its effects on relaxation variables have not been well characterized. We examined the impact of streptozotocin-induced diabetes (8 weeks) on ventricular pressure load-dependent relaxation and sarcoplasmic reticular calcium uptake of hearts from spontaneously hypertensive rats and Wistar Kyoto rats. Subsets of diabetic hypertensive rats were treated with either insulin (10 U/kg/day) or triiodothyronine (8-10 $\mu\text{g}/\text{kg}/\text{day}$). Diabetes impaired load-dependent relaxation and depressed sarcoplasmic reticular calcium uptake only in spontaneously hypertensive rat hearts. Either insulin or triiodothyronine treatment prevented the diabetes-induced depressions of both mechanical and biochemical indices of relaxation. The results suggest that: 1) Hypertrophic ventricles of spontaneously hypertensive rats are more susceptible to the detrimental effects of diabetes on relaxation indices than are the nonhypertrophic Wistar Kyoto rat ventricles; and 2) The hypothyroidism which attends diabetes may contribute to the impaired relaxation of diabetic spontaneously hypertensive rat left ventricle.

INTRODUCTION

Impaired mechanical relaxation is an important component of the adverse effects of diabetes on cardiac performance¹⁻³. Diastolic dysfunction involving prolonged relaxation is an early indication of clinical diabetic cardiomyopathy^{4,5}. Characteristics of abnormal ventricular relaxation in diabetes include increased durations and reduced rates of decline in either the tension of isolated muscle¹ or the pressure of the intact ventricle^{2,3}.

One mechanism by which diabetes produces an impairment of ventricular muscle relaxation may involve the rate of calcium sequestration by the sarcoplasmic reticulum (SR). This biochemical measurement is assumed to be a determinant of mechanical relaxation⁶. Diabetes often depresses SR calcium uptake, and the activity of the SR Ca^{2+} - Mg^{2+} -ATPase, in rat ventricle⁷⁻¹⁰.

Recent evidence suggests that left ventricular hypertrophy (LVH) exacerbates functional defects of diabetic myocardium^{1,11-13}, including those which may have an impact on ventricular relaxation. The presence of LVH alone may or may not be associated with impaired relaxation¹⁴⁻¹⁷. However, when diabetes coexists with LVH in the spontaneously hypertensive rat (SHR), the rate of left ventricular pressure decline of perfused hearts is more profoundly depressed when compared to the effects of diabetes in normotensive rat strains^{11,13}. Isometric relaxation is also prolonged in hypertrophic papillary muscles from the diabetic renovascular hypertensive rat¹². These results would suggest that combined effects of diabetes and LVH on SR Ca^{2+} uptake might be more pronounced than the influence of either condition individually. However, the interaction between diabetes

and LVH on the relationship between myocardial relaxation and SR calcium uptake has not been characterized.

Reduced serum levels of thyroid hormones are often associated with diabetes^{1,2,12}, and this hypothyroidism (or "low thyroid state") may contribute to diabetic cardiomyopathy. Thyroid hormone deficiencies, like diabetes, can lead to prolonged relaxation and depressed rates of SR calcium uptake in the myocardium^{18,19}. The effects of hypothyroidism on these measurements are well correlated, both in nonhypertrophic and hypertrophic ventricles²⁰. Treatment of the diabetic SHR with triiodothyronine (T_3) effectively prevents the resultant cardiac dysfunction²¹, but the influence of T_3 treatment in nonhypertensive diabetic rat models is inconsistent²²⁻²⁴.

Circumstantial evidence therefore provides a rationale for the following hypotheses: 1) The impairment in mechanical relaxation caused by diabetes can be correlated with the depression of SR Ca^{2+} uptake activity; 2) Both of these effects of diabetes are more pronounced on hypertrophic ventricles than they are on nonhypertrophic ventricles; and 3) Attendant hypothyroidism contributes to the effects of diabetes on mechanical and biochemical indices of ventricular relaxation in the SHR. The results of the study provide evidence in support of each hypothesis, demonstrating that T_3 treatment can prevent both mechanical and biochemical defects in relaxation of the diabetic hypertrophic ventricle in the SHR model.

METHODS

Animal Groups. Male SHR and Wistar Kyoto (WKY) rats were purchased from Charles River Breeding Labs (Wilmington, MA), then housed communally by strain and fed ad libitum. At 15 weeks of age, animals were weight matched and divided into either diabetic or nondiabetic groups. Diabetes was induced in lightly etherized animals by a single tail vein injection of streptozotocin (STZ; Sigma Chemical Co., St. Louis, MO). The dose of STZ for each strain was adjusted in order to induce similar degrees of diabetes as assessed by serum glucose concentrations. Based on preliminary results, equieffective doses of STZ in the WKY and SHR strains, as indexed by serum glucose elevations, were determined to be 50 mg STZ/kg BW and 45 mg STZ/kg BW respectively. The STZ was dissolved in citrate buffer (0.1 M at pH 4.5) just prior to use. The nondiabetic animals were also etherized and were injected with comparable volumes of the citrate buffer alone. Urinary glucose was detected in all animals of the diabetic groups (DIASTIX^(R)). All animals were sacrificed eight weeks later.

The diabetic SHR were divided into three subgroups corresponding to the treatment regimes initiated three days after STZ injection: 1) protamine zinc insulin (I; Eli Lilly Co., Indianapolis, IN, 10 U/kg/day sc); 2) T₃ (Sigma, 8-10 µg/kg/day sc); and 3) untreated. We observed initially that treatment of diabetic SHR with 10 µg/kg/day of T₃ was sufficient to just reverse the bradycardia in vivo. After 6 weeks of T₃ treatment, the dose was reduced to 8 µg/kg/day in order to avoid a progressive T₃-induced tachycardia (see accompanying manuscript). The nondiabetic SHR were subdivided into two groups of untreated and T₃-treated (8-10 µg/kg/day sc) animals. Treatment of

diabetic WKY with either insulin or T_3 was unnecessary, because initial studies had shown that diabetes had little or no effect on mechanical indices of ventricular relaxation in this strain (see Results).

In Vivo Measurements. Systolic arterial pressure (SAP), heart rate (HR) and body weight (BW) were taken just prior to the tail vein injections, and then four and eight weeks later. A standard tail-cuff sphygmomanometer within a temperature controlled chamber (34°C) was used to measure SAP and HR, after the animals were prewarmed for 10-15 minutes. All measurements were recorded prior to the daily injections of T_3 or insulin.

Serum Assays. Eight weeks after they were injected with STZ or citrate buffer, the animals were rapidly decapitated, and their serum was collected and stored at -20°C . The rats received the final injection on the day prior to sacrifice. The degree of diabetes was evaluated by serum glucose concentration using a glucose oxidase assay (Sigma). Animals were classified as diabetic if serum glucose values exceeded 300 mg/dL. Radioimmunoassays were used to determine serum insulin (Micromedic Systems Inc., Horsham, PA) and total T_3 and thyroxine (T_4) concentrations (Cambridge Medical Diagnostics, Billerica, MA). Rat insulin was generously supplied by Dr. R. Chance (Eli Lilly) and was used as the standard for the insulin assays.

Heart Perfusion. A subset of each animal group was used to assay left ventricular (LV) relaxation of isolated perfused working hearts. The hearts were quickly excised and perfused as described previously¹¹. In brief, the perfusion apparatus was designed to manipulate LV pressure loads by altering resistance to aortic

ejection (see below). Left ventricular pressure (LVP) was continually monitored through an intraventricular cannula coupled to a pulse transducer (Statham, Hato Rey, PR). The aorta was tied to a 14 gauge cannula and the heart was first perfused in a retrograde fashion for 3-5 min, then in the working mode for the remainder of the experiment.

The hearts were perfused (nonrecirculating) with Krebs-Henseleit buffer maintained at 37°C. The buffer composition was 120 mM NaCl, 5.6 mM KCl, 0.65 mM MgSO₄, 1.21 mM NaH₂PO₄, 25 mM NaHCO₃, 2.4 mM CaCl₂, 0.2 mM EDTA and 10 mM glucose (pH 7.4 when warmed and gassed with 95% O₂ and 5% CO₂). Hearts were paced at 300 beats per minute with a bipolar stimulating electrode placed on the right atrium. Prior to data collection, LVP was monitored in the working heart continually for 10-15 minutes on a Narcotrace Model 80 chart recorder (Narco Biosystems, Houston, TX) interfaced with a dedicated digital computer (Buxco Corp., Sharon, CT) to ensure stability to each preparation. During this time, left atrial filling pressure (LAFFP) was fixed at 15 cm H₂O and resistance to aortic ejection was set at 1.54 kPa/cm³•min⁻¹. Aortic flow could be directed through one of four segments of PE tubing, the length and diameter of which was varied in order to impose a final resistance of either 0.19, 0.41, 1.54 or 3.13 kPa/cm³•min⁻¹ (calibrated at constant flow).

Quantification of Left Ventricular Relaxation. Samples of the LVP waves were taken during pressure loading at the four aortic resistances and at constant LAFFP (15 cm H₂O). LVP waves were recorded and stored in an IBM PC II. Data acquisition was accomplished, in part, with the aid of A/D conversion hardware (Data

Translation, Marlborough, MA) and ASYST^(R) software (Macmillan Software Co., New York, NY). At each pressure load, a series of five sequential LVP waves was recorded using an external trigger signal to initiate the data acquisition. Measurements obtained from the digitized left ventricular waveforms (see Figure 1) included maximum left ventricular pulse pressure (P_{\max}), the time from the onset of LVP rise at end-diastole to P_{\max} (T_p), the time from P_{\max} to minimum diastolic LVP (T_r), and the maximum rate of LVP decline (LV-dP/dt). Maxima and minima of the LVP wave were obtained at the time points corresponding to zero value of the first derivative. The area under the LVP wave over T_r (A_r) was used to quantify pressure load-dependent relaxation¹⁶. For each heart and at each pressure load, P_{\max} was plotted against A_r ; the slope of the regression line through the four points was used as the relaxation index (P_{\max}/A_r). Preliminary studies showed that sampling and averaging of successive pressure waves was unnecessary because the variability between waves never exceeded 0.1%. The resolution of the digitized and stored waveform was 1 msec, each point representing the average of 400 2.5 μ sec samples.

The hearts were removed from the perfusion apparatus and blotted dry after the data collection. The aorta and excess connective tissue were trimmed away and the following wet weights were obtained: whole heart (HW): left ventricular (LV) including the septum, and right ventricular (RV). These heart weights were used to assess the degree of ventricular hypertrophy.

SR Calcium Uptake. Microsomes were prepared from left ventricular tissue of subgroups within each experimental group. Membrane

fractions enriched with SR were isolated either from single left ventricles or from pools of two left ventricles, as determined by preliminary studies of SR protein yield, by the method of Penpargkul et al.⁹ with minor modifications. The need for pooling explains the differences in total sample size between groups in, for example, Tables 1 and 5. The hearts were removed, quickly trimmed, weighed and homogenized in 12 mL of a medium containing 0.3 M sucrose and 10 mM imidazole (pH 7.4). The homogenate was centrifuged for 15 minutes at 2,000 x g. The supernatant was then centrifuged at 8,700 x g for another 15 minutes. The second supernatant was centrifuged a third time at 50,000 x g for 30 min. The pellet was resuspended in 10 mL of 0.6 M KCl and 10 mM imidazole (pH 6.8), and centrifuged at 50,000 x g for 30 minutes. The final pellet was resuspended in 2-3 mL of 50 mM KCl and 10 mM imidazole to yield a final concentration of approximately 100 µg/100 µL⁹.

Calcium uptake was determined by using the Millipore filtration technique. Cardiac microsomes were characterized in the presence and absence of sodium azide, KCl, ATP and oxalate. Microsomes were pre-incubated for 3 min in 1 mL final volume, containing 5 mM ATP, 120 mM KCl, 40 mM histidine HCl, 5 mM oxalate, 5 mM sodium azide, and 5 mM MgCl₂ (pH 6.8 at 37°C). The reaction was initiated by adding a solution containing EGTA, ⁴⁵Ca and nonradioactive CaCl₂. The final concentration of EGTA was constant at 100 µM. Free calcium concentrations were calculated to range between 0.18 and 5.62 µM²⁵. The reaction was terminated by filtering over 0.45 micron Millipore filters and washed with 2 mL Tris-HCl (20 mM). Filters were dried, counted in a liquid scintillation counter in 5 mL scintillation fluid

(BetaBlend^(R), ICN Radiochemicals, Irvine, CA). Protein concentrations were determined by the method of Lowry et al.²⁶. Rates of SR calcium uptake were expressed as $\mu\text{moles/mg protein} \cdot \text{min}^{-1}$. Blanks consisted of microsomal preparations in the absence of added ATP.

Statistical Analyses. The effects of diabetes and the hormone treatments of SHR were evaluated by one- or two-factor analysis of variance (ANOVA) with repeated measures when appropriate (Statistical Analysis Systems, Cary, NC). Comparisons between rat strains were made using two- or three-factor designs, with repeated measures when appropriate. Significance was set at the 0.05 level. Simple effects and Student Newman-Keuls multiple comparisons tests were used to evaluate significance. The mean square error term(s) from the parent ANOVA were used to calculate all follow-up statistics.

RESULTS

Serum Assays. The injections of STZ induced a degree of diabetes, as quantified by serum glucose and insulin levels, which was equivalent in the SHR and WKY rat strains (Table 1). The diabetic animals were characterized by depressed serum T_3 and T_4 levels. The magnitudes of the depressions in thyroid hormone concentrations were about the same in the two strains, although both T_3 and T_4 levels of nondiabetic SHR were slightly higher than those of the nondiabetic WKY.

Treatment of diabetic SHR with T_3 prevented the decline in serum T_3 levels, but had no effect on the elevated serum glucose or depressed insulin values (Table 1). It also exacerbated the decline in serum T_4 , probably by inhibiting TSH production and glandular T_4 secretion. T_3 treatment of nondiabetic SHR had the same effect on T_4 levels, but otherwise did not alter glucose, insulin or T_3 values.

Treatment of diabetic SHR with insulin did not correct the hyperglycemia, at least at the 15-20 hour time point, even though it caused a pronounced increase in immunoreactive insulin activity (Table 1). This pattern may be evidence for subsensitivity to exogenous insulin in diabetic SHR. Insulin treatment was only partially effective in restoring serum T_3 and T_4 to normal.

Body and Heart Weights. As expected, nondiabetic SHR had lower BW, but higher ratios of LV weight to either BW or (RV weight), than did nondiabetic WKY (Table 2). Thus, the left ventricles of SHR were both relatively (LV/BW) and absolutely (LV/RV) hypertrophic¹¹. Diabetes reduced BW and HW of SHR and WKY rats, and reduced LV/RV of SHR without affecting LV/BW. This indicates that diabetes had no

effect on relative hypertrophy, but reversed absolute hypertrophy of SHR left ventricles¹¹.

Treatment of diabetic SHR with T_3 caused a further decline in BW, but had no effect on HW, thus it increased LV/BW (Table 2). However, T_3 had no effect on LV/RV, and therefore did not affect the reversal of hypertrophy induced by diabetes. Treatment of nondiabetic SHR with T_3 had no effect on any of the body or heart weight measurements.

Insulin treatment (Table 2) nearly restored BW to normal, and prevented the declines in HW and LV/BW in diabetic SHR, in spite of its failure to correct the hyperglycemia¹.

Arterial Pressure and Heart Rate In Vivo. As expected, nondiabetic SHR were hypertensive and tachycardic compared to the WKY animals (Table 3). The diabetic SHR were characterized by depressed SAP and HR after 8 weeks, confirming previous observations¹¹. However, diabetes had no effect on these measurements in the WKY strain.

Treatment of diabetic SHR with T_3 , at a dose which was adjusted to prevent the bradycardia, only partially reversed the depressor effect of diabetes (Table 3). In contrast, insulin treatment prevented the decline in SAP but did not fully restore HR to normal. Treatment of nondiabetic SHR with T_3 had no significant effect on either SAP or HR.

Left Ventricular Pressure Wave Characteristics. Perfused hearts from nondiabetic SHR had a shorter T_r but otherwise did not differ from those of the WKY strain with regard to P_{max} , T_p , and LV -dP/dt (Table 4). Diabetes increased T_p of both SHR and WKY hearts, but increased T_r and reduced P_{max} and LV-dP/dt only in the SHR. None of these

changes was associated with differences in coronary flow or resistance, either within or between strains, confirming previous observations^{11,20} (data not shown).

All of the effects of diabetes on left ventricular pressure wave characteristics in the SHR strain were prevented by either T_3 or insulin treatment (Table 4). However, none of these measurements was altered by T_3 treatment of nondiabetic SHR. The effects of diabetes and treatment with either insulin or T_3 on LVP waveforms are illustrated in Figure 1.

Pressure Load-Dependent Relaxation and SR Ca^{2+} Uptake. Pressure load-dependent relaxation of SHR ventricles was impaired by diabetes, but that of the WKY hearts was not affected (Table 5, Figure 2). The rate of left ventricular SR calcium uptake was also depressed by diabetes only in the SHR (Table 5, Figure 3), so that the effects of diabetes on the two variables were well correlated between experimental groups. Both the mechanical and biochemical defects of diabetic SHR ventricle were reversed by either insulin or T_3 treatment (Table 5, Figures 1-3). Therefore, T_3 treatment was as effective as insulin therapy in preventing the impairment in mechanical relaxation, and the depressed SR calcium uptake activity, of diabetic SHR ventricle. Treatment of nondiabetic SHR with T_3 had no significant effect on either the relaxation index (Table 5, Figure 2) or the rate of SR calcium uptake (Figure 3).

DISCUSSION

A common biochemical defect of diabetic myocardium is a depressed rate of calcium sequestration by SR microsomes⁷. Proposed mechanisms include reductions in either the density or the activity of $\text{Ca}^{2+}\text{-Mg}^{2+}$ ATPase^{8,9,18}, alterations in membrane lipid profiles^{8,27,28}, and changes in the degree of membrane phosphorylation²⁹. Until now, studies of this kind have been restricted to nonhypertensive, nonhypertrophic models. Our data suggest, however, that the adverse effect of diabetes on left ventricular SR calcium uptake activity is exacerbated by preexisting LVH (Figure 3, Table 5). Thus, diabetes of equal duration and severity - as measured by serum glucose and insulin levels - depressed SR Ca^{2+} uptake of SHR left ventricles, but had no effect in the nonhypertensive, nonhypertrophic WKY strain. These results extend those of earlier studies which had demonstrated that the detrimental effects of chronic diabetes on various indices of cardiac mechanical activity are more pronounced on hypertrophic than on nonhypertrophic ventricles of both SHR and renovascular hypertensive rats¹¹⁻¹³. (See accompanying manuscript.)

The mechanism for the apparent interaction between diabetes and LVH on SR Ca^{2+} uptake is a matter of speculation. In nondiabetic hypertrophic ventricle, SR Ca^{2+} uptake might be impaired^{20,30} or normal³¹, depending perhaps on either the experimental model or the duration of hypertension³². Like diabetes, LVH is associated with alterations in lipid metabolism^{33,34} and restricted fuel source availability^{35,36}. Elevated workloads, reduced coronary reserve³⁷, and possibly increased metabolic demand for carnitine³³ (see below)

may combine to predispose the hypertrophic ventricle to membrane-disrupting effects of diabetes which might otherwise be less apparent in the absence of preexisting LVH.

Our results also show that the activity of SR Ca^{2+} uptake was well correlated with indices of mechanical relaxation. Both measurements were depressed in the diabetic SHR, but neither one was significantly affected in any other experimental group (Table 5, Figure 3). Cardiac muscle relaxation can be categorized as consisting of inactivation-dependent and afterload-dependent mechanisms; the latter is considered to be more closely linked to intracellular calcium sequestering mechanisms³⁸. Load-dependent relaxation is most often demonstrated in isolated ventricular muscle preparations. A recent report from this laboratory suggested that the ratio P_{max}/A_r can serve as an index of load-dependent relaxation in the ejecting ventricle¹⁶. As expected, the value of P_{max}/A_r in the present study correlated with the rate of SR Ca^{2+} uptake between experimental groups (Table 5, Figures 2 and 3). The T_r variable did not correlate as well, because it differed between nondiabetic SHR and WKY groups, whereas the SR Ca^{2+} uptake activity did not (Tables 4 and 5). In contrast to the results of the previous report¹⁶, in this study LV $-dP/dt$ varied with P_{max}/A_r . However, the former variable is inconsistent and difficult to interpret, largely because of its dependence on the configuration of the pressure wave. Overall, our results strongly suggest that diabetes impairs load-dependent relaxation of hypertrophic ventricle, although diabetes likely compromises inactivation-dependent relaxation mechanisms as well^{1,12,38}. Whether or not diabetes can specifically depress

load-dependent relaxation of nonhypertrophic myocardium remains to be determined.

The effectiveness of T_3 treatment to completely prevent the diabetes-induced impairments in relaxation and SR Ca^{2+} uptake of SHR ventricle (Tables 4 and 5, Figures 2 and 3) was somewhat surprising. In two previous reports, hypothyroidism induced in SHR by either methimazole¹¹ or thyroidectomy²⁰ only partially duplicated the effects of diabetes on mechanical function. In normotensive diabetic rats, insulin therapy can ameliorate impaired mechanical function without completely correcting the depressed serum thyroid hormone levels¹. The dose of T_3 used in the current study, 8-10 $\mu\text{g}/\text{kg}/\text{day}$, was selected on the basis of its ability to restore the depressed HR in vivo of diabetic SHR to normal (see Table 3). This dose probably did not impose a hyperthyroid state in the diabetic SHR for several reasons: 1) It did not significantly increase serum T_3 levels above those of the nondiabetic controls, although it apparently did inhibit T_4 production by the thyroid gland (Table 1); 2) The same dose given to the nondiabetic SHR had no effect on heart or body weights, or on SAP or HR in vivo (Tables 2 and 3); 3) T_3 treatment of nondiabetic SHR also had no significant effect on either serum T_3 levels (Table 1), indices of mechanical relaxation, or the rate of SR Ca^{2+} uptake in vitro (Tables 4 and 5). Earlier studies had indicated that "physiological doses" of thyroid hormone - i.e., those which had been shown to be sufficient for replacement therapy of simple hypothyroidism - were only partially effective in restoring cardiac function of diabetic normotensive rats^{8,22,23,24}. Interestingly,

however, T_3 (10 $\mu\text{g}/\text{kg}$, twice/day) given for only the final 5 days of a 2-week period of diabetes, almost completely reversed the prolonged action potential duration in diabetic Wistar rat atria³⁹.

Our results suggest that hypertension, and probably left ventricular hypertrophy, increase the responsiveness of diabetic myocardium to the ameliorative effects of T_3 therapy, at least with regard to mechanical and biochemical indices of relaxation. The mechanism may involve, in part, differential effects of diabetes on membrane lipid profiles, and other aspects of lipid metabolism, of hypertrophic vs. nonhypertrophic ventricle. The acute effects of T_3 on Ca^{2+} - Mg^{2+} -ATPases are profoundly influenced by the lipid environment⁴⁰. Because hypertrophy⁴¹, hypothyroidism⁴⁰ and diabetes⁴² all affect membrane lipid composition, it seems reasonable to hypothesize that different combinations of these pathophysiologic conditions would have variable influences on the subsequent effects of T_3 administration. In normotensive rats, myoinositol coadministration increases the therapeutic efficacy of T_3 in diabetic cardiomyopathy⁴³. Fatty acyl esters of carnitine may be especially important. Enhanced incorporation of long chain acyl carnitines (LCAC) into the SR membrane is correlated with depressed rates of calcium uptake in diabetes²⁷ and T_3 treatment decreases LCAC levels in SR membranes⁴⁴. Another potential mechanism undoubtedly involves the regulation of SR calcium pump activity by thyroid hormone. Chronically, T_3 probably increases the rate of SR Ca^{2+} uptake by accelerating the synthesis of Ca^{2+} - Mg^{2+} -ATPase enzyme molecules, and hypothyroidism has opposite effects on these measurements⁴⁵. The

data from our study (Figure 3) and those of a previous report¹⁶ are consistent with such an effect of thyroid state in diabetic SHR.

In summary, the results of this study show that the interaction between diabetes and LV hypertrophy is manifested by defects in biochemical and mechanical measurements of ventricular muscle relaxation. They also demonstrate that this condition is completely preventable by treatment of diabetic SHR with T₃. The possibility that these two consequences of hypertensive hypertrophy in the SHR model - exacerbation of diabetic cardiomyopathy and predisposition to the ameliorative effects of T₃ treatment - might be mechanistically interrelated remains to be investigated.

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TABLE 1. Serum glucose, insulin, T₃ and T₄ values of nondiabetic and diabetic SHR and WKY rats.

Group	n	Glucose (mg/dL)	Insulin (μU/mL)	T ₃ (ng/dL)	T ₄ (μg/dL)
SHR					
Nondiabetic	20	124 ± 20	68 ± 26	99 ± 19 ^Y	5.52 ± 1.93 ^Y
Diabetic	18	580 ± 65 [*]	16 ± 6 [*]	47 ± 13 [*]	2.12 ± 0.76 [*]
Diabetic + T ₃	18	615 ± 77 [*]	25 ± 14 [*]	118 ± 64	0.21 ± 0.12 ^{**}
Diabetic + I	16	542 ± 196 [*]	287 ± 165 ^{**}	71 ± 23 ^{**}	4.12 ± 1.60 ^{**}
Nondiabetic + T ₃	15	132 ± 27	69 ± 15	109 ± 50	0.27 ± 0.15 ^{**}
WKY					
Nondiabetic	21	149 ± 34	77 ± 21	86 ± 13	4.62 ± 1.12
Diabetic	16	567 ± 118 [*]	22 ± 9 [*]	41 ± 11 [*]	2.60 ± 0.68 [*]

Values represent means ± SD, obtained 8 weeks after initiation of diabetes or treatment. T₃, triiodothyronine; I, protamine zinc insulin.

* Significantly different from the nondiabetic group (within strain).

** Significantly different from the nondiabetic and diabetic groups (within strain).

^Y Significantly different from the nondiabetic WKY.

TABLE 2. Body and heart weights of nondiabetic and diabetic SHR and WKY rats.

Group	n	BW (g)	HW (mg)	LV/BW (mg/g)	LV/RV (mg/mg)
<u>SHR</u>					
Nondiabetic	20	328 ± 25 ^Y	1370 ± 132	3.20 ± 0.40 ^Y	5.37 ± 0.72 ^Y
Diabetic	18	196 ± 21 [*]	907 ± 85 [*]	3.04 ± 0.51	4.42 ± 1.04 [*]
Diabetic + T ₃	18	174 ± 21 ^{**}	929 ± 146 [*]	3.68 ± 0.63 [*]	4.41 ± 0.73 [*]
Diabetic + I	16	305 ± 25 ^{**}	1371 ± 83	3.00 ± 0.59	4.84 ± 1.11
Nondiabetic + T ₃	15	322 ± 35	1406 ± 75	3.36 ± 0.58	4.86 ± 0.90
<u>WKY</u>					
Nondiabetic	21	386 ± 31	1428 ± 182	2.66 ± 0.37	4.31 ± 0.80
Diabetic	16	306 ± 37 [*]	1070 ± 75 [*]	2.75 ± 0.50	4.13 ± 0.66

Values represent means ± SD, obtained 8 weeks after initiation of diabetes or treatment. BW, body weight; HW, heart weight; LV, left ventricular weight; RV, right ventricular weight; T₃, triiodothyronine; I, protamine zinc insulin.

* Significantly different from the nondiabetic group (within strain).

** Significantly different from the nondiabetic and diabetic groups (within strain).

^Y Significantly different from the nondiabetic WKY.

TABLE 3. Systolic arterial pressure and heart rates of nondiabetic and diabetic SHR and WKY rats.

Group	n	SAP (kPa)		HR (beats/min)	
		Week 0	Week 8	Week 0	Week 8
<u>SHR</u>					
Nondiabetic	20	23.6 ± 3.2 ^Y	26.4 ± 2.4 ^Y	386 ± 40 ^Y	422 ± 46 ^Y
Diabetic	18	23.7 ± 2.8	20.0 ± 1.9 [*]	377 ± 33	287 ± 29 [*]
Diabetic + T ₃	18	24.4 ± 2.0	23.6 ± 3.2 ^{**}	390 ± 40	439 ± 50
Diabetic + I	16	23.5 ± 2.0	26.9 ± 2.7	384 ± 28	389 ± 36 ^{**}
Nondiabetic + T ₃	15	23.7 ± 1.6	28.2 ± 3.1	400 ± 29	456 ± 57
<u>WKY</u>					
Nondiabetic	21	17.1 ± 1.7	18.8 ± 1.5	315 ± 49	302 ± 29
Diabetic	16	17.3 ± 1.7	17.9 ± 2.3	311 ± 19	275 ± 22

Values represent means ± SD, obtained 8 weeks after initiation of diabetes or treatment. SAP, systolic arterial pressure; HR, heart rate; T₃, triiodothyronine; I, protamine zinc insulin.

^{*} Significantly different from the nondiabetic group (within strain).

^{**} Significantly different from the nondiabetic and diabetic groups (within strain).

^Y Significantly different from the nondiabetic WKY.

TABLE 4. Left ventricular pressure wave characteristics of nondiabetic and diabetic SHR and WKY rat hearts *ex vivo*.

Group	n	P _{max} (kPa)	T _p (msec)	T _r (msec)	LV-dP/dt (kPa/sec)
<u>SHR</u>					
Nondiabetic	12	19.1 ± 0.9	38 ± 3	83 ± 5 ^Y	600 ± 92
Diabetic	9	14.7 ± 1.9 [*]	42 ± 6 [*]	104 ± 8 [*]	416 ± 113 [*]
Diabetic + T ₃	9	17.9 ± 0.7	34 ± 5	82 ± 8	549 ± 76
Diabetic + I	7	20.0 ± 1.5	41 ± 3	80 ± 6	601 ± 70
Nondiabetic + T ₃	7	20.9 ± 1.6	34 ± 6	78 ± 5	645 ± 105
<u>WKY</u>					
Nondiabetic	13	18.9 ± 2.7	36 ± 4	99 ± 9	541 ± 134
Diabetic	9	17.1 ± 2.0	45 ± 5 [*]	98 ± 12	517 ± 108

Values represent means ± SD, obtained 8 weeks after initiation of diabetes or treatment. P_{max}, maximum left ventricular developed pressure (LVDP); T_p, time to P_{max}; T_r, time from P_{max} to minimum LVDP; LV-dP/dt, maximum rate of left ventricular pressure decline; T₃, triiodothyronine; I, protamine zinc insulin. Left ventricular filling pressure was fixed at 15 cm H₂O and resistance to aortic ejection was fixed at 1.54 kPa/cm³.min⁻¹.

^{*}Significantly different from the nondiabetic group (within strain).

^YSignificantly different from the nondiabetic WKY.

TABLE 5. Ventricular relaxation index and SR Ca²⁺ uptake of nondiabetic and diabetic SHR and WKY rats.

Group	n [§]	Relaxation Index (sec ⁻¹)	n [†]	SR Ca ⁺⁺ Uptake (nmoles/mg.min ⁻¹)
<u>SHR</u>				
Nondiabetic	12	16.7 ± 0.6	6	42.3 ± 7.4
Diabetic	9	12.6 ± 1.1*	4	14.2 ± 1.4*
Diabetic + T ₃	9	18.8 ± 0.7	4	45.7 ± 6.0
Diabetic + Insulin	7	18.1 ± 0.8	4	49.0 ± 3.8
Nondiabetic + T ₃	7	17.2 ± 0.6	4	59.6 ± 4.5
<u>WKY</u>				
Nondiabetic	13	15.4 ± 0.9	6	53.4 ± 3.0
Diabetic	9	13.3 ± 1.0	4	47.4 ± 8.3

Values represent means ± SE, obtained 8 weeks after initiation of diabetes or treatment. Microsomal Ca²⁺ uptake was stimulated by 10^{-5.5} M free calcium (see Figure 2). T₃, triiodothyronine; I, protamine zinc insulin.

§ Number of individual perfused hearts.

† Number of groups of pooled left ventricles.

* Significantly different from the nondiabetic SHR group.

Figure 1. Computer print-out of left ventricular pressure (LVP) waves from nondiabetic (nd), diabetic (d), insulin-treated diabetic (d+i) and T_3 treated diabetic (d+t₃) SHR hearts ex vivo. Each LVP wave was recorded from a single heart at a fixed pressure load (resistance to aortic ejection) of $3.13 \text{ kPa/cm}^3 \cdot \text{min}^{-1}$, and volume load of $15 \text{ cm H}_2\text{O}$. The LVP waves were chosen in order to closely represent the mean values of contraction and relaxation indices for each experimental group. The indices used to assess mechanical function are depicted on the nondiabetic SHR LVP wave (left) and include: maximum LVP (P_{max}); time to P_{max} (T_p); total time of pressure decline (Tr); and area under the LVP wave during Tr (Ar). For further details, see Methods.

SHR

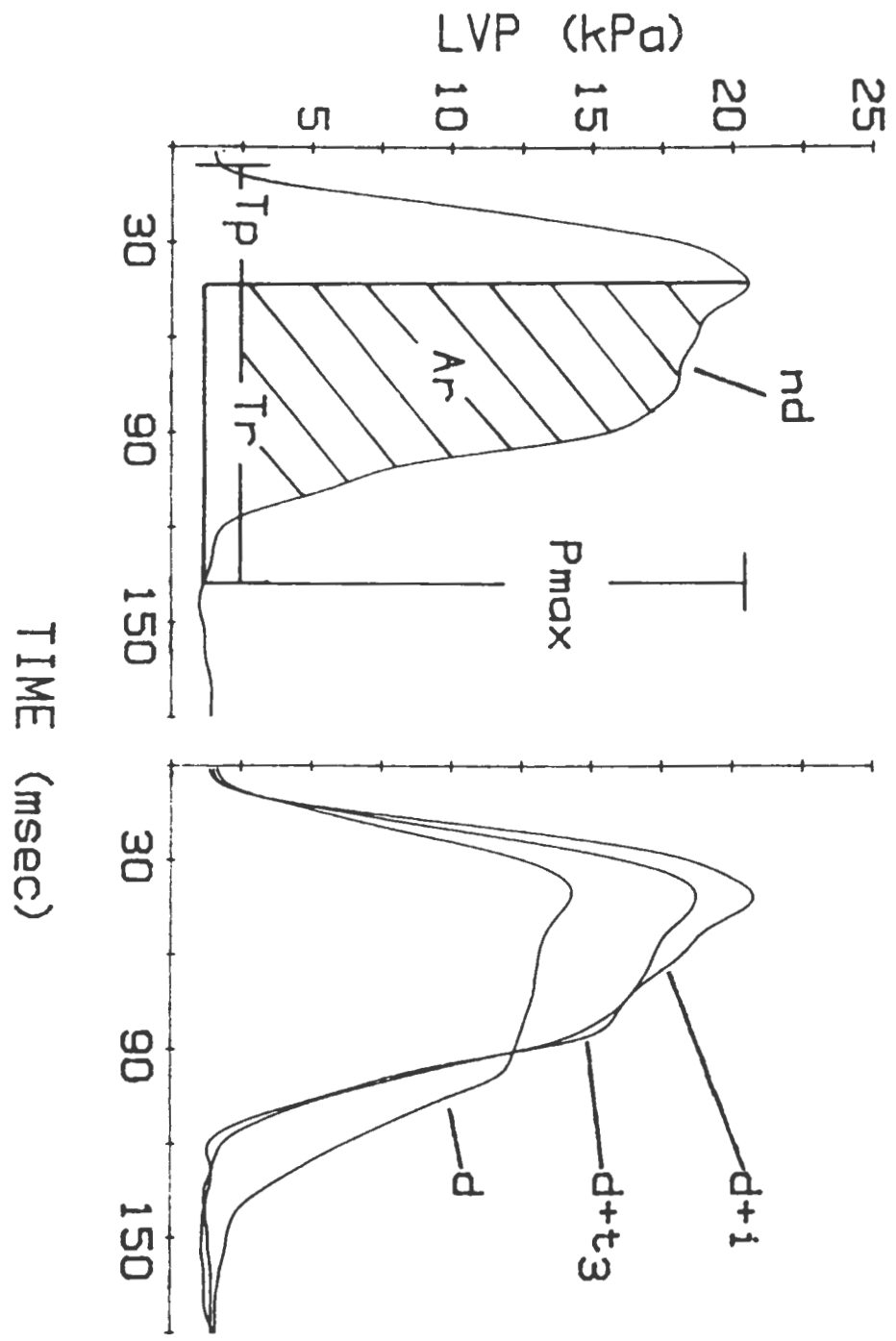


Figure 2. Pressure load-dependent relaxation of nondiabetic (○), diabetic (●), insulin-treated diabetic (■), T₃-treated diabetic (▲), and T₃-treated nondiabetic (△) SHR hearts ex vivo. The four points within each group represent maximum left ventricular pulse pressure (P_{max}) vs. the area under the falling phase of the pressure wave (A_r) at each of four pressure loads (see Figure 1). Loading was altered stepwise by fixing the resistance to aortic ejection at 0.19, 0.41, 1.54, or 3.13 kPa/cm³.min⁻¹ (see Methods). The slope of the regression line through the four points within each group is the value of the relaxation index (see Table 5). Only the untreated diabetic group was characterized by a significantly depressed relaxation index. Therefore, for simplicity, a single line representing the collective mean slope is depicted for the remaining experimental groups. Values represent means (n = 7-12), obtained 8 weeks after initiation of untreated and treated diabetes.

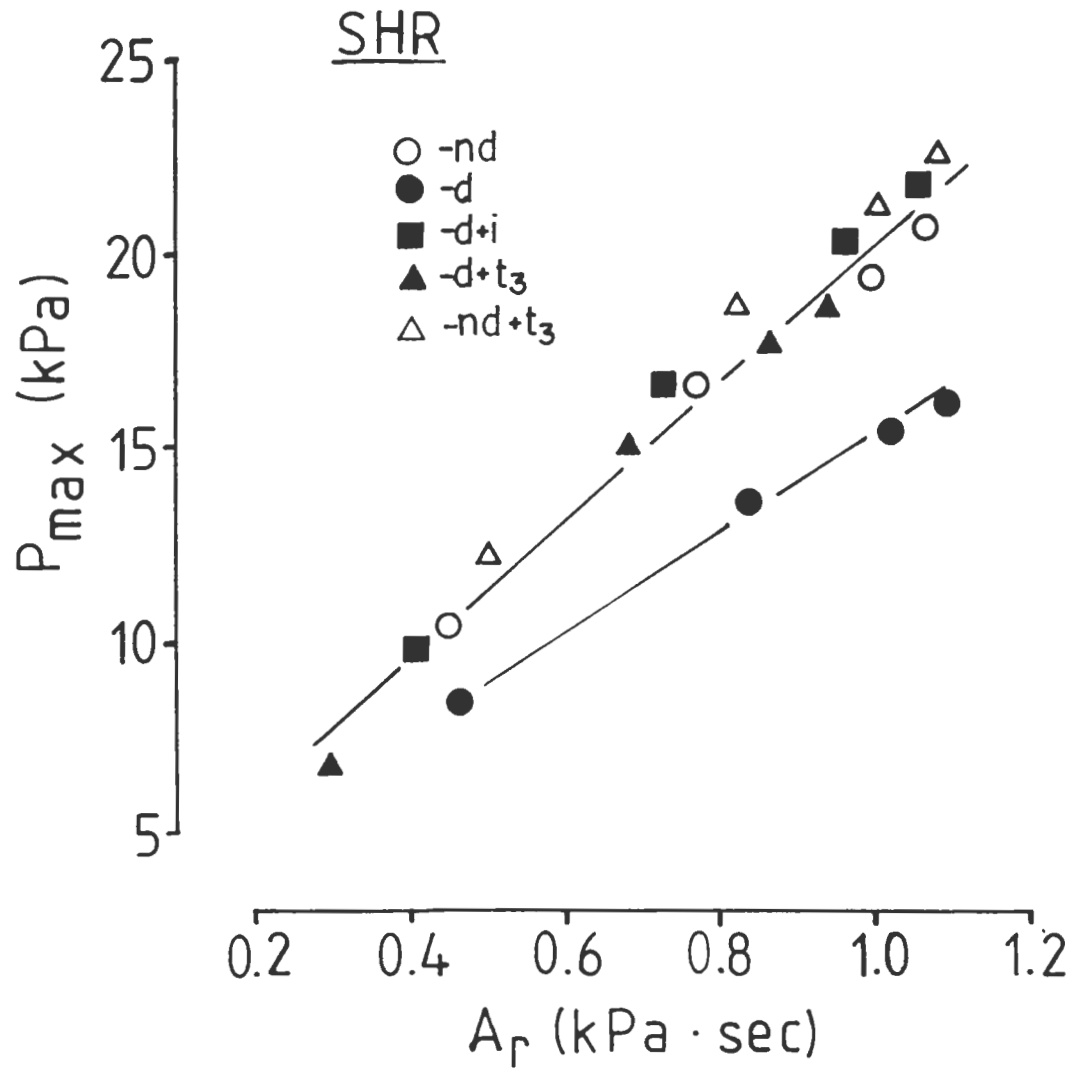
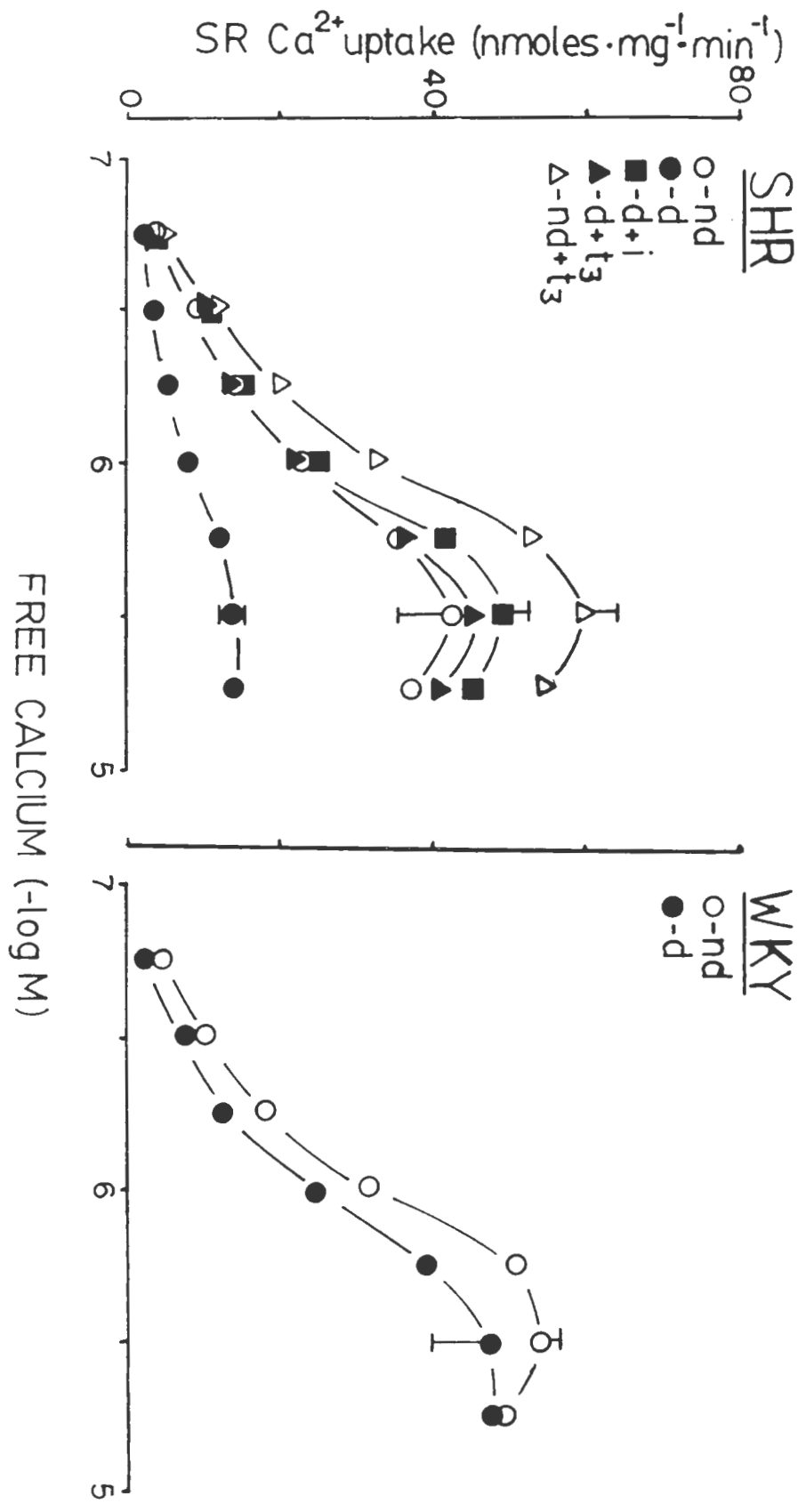


Figure 3. Rate of sarcoplasmic reticular (SR) calcium uptake of left ventricular microsomes from nondiabetic (○), diabetic (●), insulin-treated diabetic (■), T₃-treated diabetic (▲) and T₃-treated nondiabetic (△) SHR (left), and nondiabetic (○) and diabetic (●) WKY (right). Values represent means \pm SE, obtained 8 weeks after initiation of diabetes or treatment (n = 4-6 pooled samples).



Ventricular Action Potentials in Diabetic SHR:

Alterations prevented by Insulin or T_3

ABSTRACT

Diabetes imposes a pronounced cardiodepression in the spontaneously hypertensive rat (SHR), which may be attributable, at least in part, to attendant hypothyroidism. A prolongation of the ventricular action potential duration (APD) characteristic of either diabetes or hypothyroidism, may be associated with this impairment. Thyroid hormone treatment (in vivo) prevents mechanical and biochemical deficits in hearts from diabetic SHR. Chronic diabetes (8 weeks) was chemically-induced in SHR and normotensive Wistar Kyoto (WKY) rats by a single tail vein injection of streptozotocin (STZ; 45 mg/kg and 50 mg/kg, respectively). A subgroup of diabetic SHR were treated with daily injections of either insulin (10 U/kg/day) or triiodothyronine (T_3 ; 8-10 μ g/kg/day). Systolic arterial pressure (SAP) and heart rate (HR) were recorded in vivo, before and 8 weeks after STZ injections. As expected, diabetes reduced SAP in SHR and lowered HR in both the SHR and WKY rats. Either insulin or T_3 treatment of diabetic SHR prevented the drop in SAP and HR. Absolute left ventricular hypertrophy as assessed by the ratio of left to right ventricular weights, was reversed by diabetes in SHR. The reversal was partially prevented by insulin, but not by T_3 treatment. Diabetes prolonged APD to the same extent in SHR ventricular muscle as it did in WKY muscle, and these effects were independent of changes in resting membrane potential, action potential amplitude or rate of rise of the upstroke (V_{max}). Hypertrophy alone had no effect on APD, since this measurement was the same in both nondiabetic SHR and nondiabetic WKY rats. Thyroid hormone treatment was as effective as insulin was in preventing the

diabetes-induced changes of APD in SHR papillary muscle. These data suggest that prolongation of APD of ventricular muscle caused by chronic, untreated diabetes is not affected by left ventricular hypertrophy. Furthermore, the data provide additional support that the attending hypothyroidism is functionally significant in the diabetic cardiomyopathy of SHR.

INTRODUCTION

Hypertrophy may predispose the myocardium to the adverse effects of diabetes. Both clinical and experimental evidence indicate that cardiomyopathies are more severe and perhaps more frequent when these pathological conditions coexist (7, 15, 30). Experimental studies further show that mechanical, biochemical and morphological indices of cardiac function are more severely impaired in diabetic hypertensive rats than in animals with either disease alone (11, 12, 16, 18, 42, 57).

A hypothyroid state is often associated with poorly controlled diabetes (40). In addition to low serum thyroid hormone levels, diabetes impairs peripheral T_4 to T_3 conversion, tissue T_3 uptake, and both affinity and density of nuclear T_3 receptors (5, 8). The influences of attendant hypothyroidism on the cardiovascular system may be difficult to distinguish from those of diabetes. Indeed, certain cardiovascular manifestations of experimental diabetes, such as the bradycardia, may be entirely attributable to the impaired thyroid state (54). The effectiveness of thyroid hormone treatment on the progression of diabetic cardiomyopathies are not consistent (22, 53) but may be more apparent in the presence of left ventricular hypertrophy (11, 12). Recently we have shown that the functional impairments in diabetic spontaneously hypertensive rat (SHR) hearts can be prevented by treating animals with either insulin or triiodothyronine (T_3 ; 11). Treatment of SHR with T_3 also prevented the diabetes-induced depressions of sarcoplasmic reticular (SR) calcium uptake and its mechanical correlate, ventricular relaxation (12).

The relationship between the electrical properties of the sarcolemma and contractile events of cardiac muscle is well characterized (for reviews see 19 and 36). The cardiac action potential (AP) configuration is similarly altered by the effects of either hypertrophy, diabetes, or hypothyroidism (4, 9, 10, 17, 37, 47). Prolongation of the cardiac AP is the most consistent consequence common to all three abnormalities, and may be functionally related to associated mechanical alterations which are also characteristic of each condition. A combination of these disease states may have additive effects on ventricular transmembrane potentials (18), as they seem to have on mechanical function (11, 12).

The central purpose of this study was to characterize the influence of chronic, untreated diabetes on the configuration of the papillary muscle AP in SHR and normotensive WKY rats. Additional aims included the assessment of the effectiveness of T_3 treatment, compared to that of insulin therapy, on ventricular electrophysiology in diabetic SHR. The results show that diabetes prolongs the cardiac AP to the same extent in hypertrophic and nonhypertrophic papillary muscle. Furthermore, T_3 treatment is as effective as insulin in preventing the diabetes-induced AP prolongation.

METHODS

Animal Groups. Male spontaneously hypertensive rats (SHR) and Wistar Kyoto (WKY) rats were purchased from Charles River Breeding Laboratories (Wilmington, MA), then housed communally according to strain, and fed ad libitum. When the animals were 15 weeks of age, they were weight matched and assigned to either nondiabetic or diabetic groups. Diabetes was induced by a single tail vein injection of streptozotocin (STZ; Sigma Chemical Co., St. Louis, MO) in lightly etherized animals. The dose of STZ was adjusted for each strain in order to induce comparable degrees of diabetes as indexed by serum glucose levels (12): SHR, received 45 mg/kg, and WKY received 50 mg/kg. The STZ was dissolved in citrate buffer (0.1 M at pH 4.5) just prior to use. The nondiabetic animals were also etherized and given comparable volumes of citrate buffer alone. Glucose appeared in the urine (DIASTIX) of all STZ treated animals. All animals were sacrificed eight weeks after the tail vein injections.

The diabetic SHR were divided into three subgroups corresponding to specific treatment regimes which were initiated three days after the STZ injection. One subgroup of diabetic SHR was treated with protamine zinc insulin (I; Eli Lilly Co., Indianapolis, IN), 10 U/kg/day sc, administered once each day at 1600 hrs. The second subgroup of diabetic SHR was given a single daily injection of triiodothyronine (T_3 ; Sigma) 8-10 ug/kg/day sc (also at 1600 hrs). After 6 weeks of treatment, the T_3 dose was reduced from 10 ug/kg/day to 8 ug/kg/day in order to avoid the development of T_3 -induced tachycardia (11). This dose of T_3 was selected according to its

ability to just prevent the progression of bradycardia during the 8 week period of diabetes (11, 42). The third subgroup of diabetic SHR remained untreated for the entire 8 week period.

In Vivo Measurements. Systolic arterial pressure (SAP), heart rate (HR) and body weight (BW) were measured prior to the STZ injections, and then eight weeks subsequently. SAP was monitored continually until three or more consistent measurements were observed, using a standard tail-cuff sphygmomanometer contained in a temperature controlled chamber (34°C). HR was also monitored with the tail-cuff apparatus, before and after the SAP recordings. These measurements were obtained prior to the daily T_3 or insulin injections.

Intracellular Recordings. Eight weeks following the tail vein injections, all animals were rapidly decapitated, the hearts were quickly excised and a papillary muscle was removed and prepared for intracellular recordings. A papillary muscle with an origin on the left ventricular outer wall was isolated and superfused with nonrecirculating Krebs-Henseleit buffer maintained at 34°C . The buffer composition was (mM); 120 NaCl, 5.6 KCl, 0.65 MgSO_4 , 1.21 NaH_2PO_4 , 25 NaHCO_3 , 2.4 CaCl_2 , 0.2 EDTA and 10 glucose (pH 7.4 when warmed and gassed with 95% O_2 and 5% CO_2). In order to minimize the degree of contraction during electrical stimulation, muscles were loosely anchored to the base of the preparation chamber. The buffer capacity of the chamber was 8 ml and the flow rate through the chamber was maintained at approximately 3 ml/min. Each muscle preparation was allowed to equilibrate in buffer for 30 min prior to recording membrane potentials. A bipolar, silver wire suction electrode was used with a Grass SD9 stimulator (Grass Instruments,

Quincy, MA) to evoke cardiac action potentials at a frequency of 1 Hz. Stimulus intensities were set just above threshold voltages with square wave pulses of 0.5 ms duration. The stimulating electrode was placed at the apical end of the muscle. Standard intracellular electrodes were filled with 3 M KCl and connected to a DC preamplifier (Model SVC 2000, WPI, New Haven, CT). Transmembrane potentials were monitored continuously on a storage oscilloscope (Model 5103N, Tektronix, Beaverton, OR), and simultaneously monitored and stored, for off-line analysis, in an IBM PCII, using A/D conversion hardware (Data Translation, Marlborough, MA) and ASYST software (Macmillan Software Co, New York, NY). The sampling rate of the A/D conversion was 1.25 μ s, of which the digitized data were averaged, sampled and stored every 1 ms. Data acquisition was synchronized by externally triggering A/D conversion through the stimulator. For each muscle fiber sampled, 4-5 action potentials were recorded, and an average of 7 muscle fibers were sampled from each animal.

AP Configuration Analysis. The resting membrane potential (RP) was recorded and the action potential (AP) configuration was described using the following indices; AP amplitude (AMP), time course to complete repolarization (AP duration at 10% (APD10), 25% (APD25), 50% (APD50) and 75% (APD75) repolarization). The maximum rate of rise of the depolarization (V_{\max}) was determined by calculating the maximum first derivative of the stored AP.

Heart Weight Ratios. Prior to the removal of the papillary muscle, the hearts were quickly blotted dry and the following wet weights were obtained; whole heart (HW), left ventricle (LV) including the

septum, and right ventricle (RV). The degree of ventricular hypertrophy was assessed using these heart weights.

Statistical Analyses. The effects of diabetes on SAP and HR within and between each strain were assessed using a three-way analysis of variance (ANOVA) with repeated measures. These in vivo measurements were also assessed in diabetic SHR treated with either insulin or T₃ by means of a two-way ANOVA with repeated measures. To evaluate the treatment effects for all ex vivo measurements, similar statistical designs were employed, but without a repeated measures factor. The membrane potential data presented for each experimental group actually represent the average values from multiple samples within a given muscle fiber, and multiple fibers within a given papillary muscle. Thus, sample sizes presented in Table 3 reflect the number of animals within each experimental group, rather than the number of individual fibers sampled. Probability values of 0.05 or less were considered sufficient to indicate significant differences between group means. Simple effects and Student Newman-Keuls multiple comparisons tests were used to further evaluate significant F values. The mean square error term(s) from the parent ANOVA were used for the follow-up tests.

RESULTS

Systolic Arterial Pressure and Heart Rate. At 15 weeks of age (week 0 in Table 1), SHR were already hypertensive and tachycardic when compared to WKY. As previously reported, diabetes depressed systolic arterial pressure (SAP) in SHR but not in WKY (42). Either insulin or T_3 treatment prevented the depressor effects of diabetes in the SHR. Similarly, heart rate (HR) was also reduced in diabetic SHR, and maintained at nondiabetic levels by either insulin or T_3 treatment. Diabetes did not produced a bradycardia in the WKY.

Body and Heart Weights. Diabetes reduced body weight (BW) and whole heart weight (HW) in both the SHR and WKY strains (Table 2). Insulin treatment prevented this BW loss, but only partially prevented diabetes-induced HW loss. Treatment with T_3 did not influence the BW or HW in diabetic SHR. Absolute left ventricular hypertrophy (LV/RV), but not relative ventricular hypertrophy (LV/BW) was apparent in the hypertensive SHR when compared to the normotensive WKY. Diabetes decreased LV/RV in SHR, without altering LV/BW. Insulin treatment, unlike T_3 treatment, partially prevented the diabetes-induced reversal of LV/RV ratio in SHR. Diabetes had no effect on ventricular weight ratios in WKY.

Cardiac Action Potentials. The membrane potentials of papillary muscles from nondiabetic SHR were quantitatively similar to those of nondiabetic WKY muscles (Figure 1, Table 3 and Table 4). There were no significant strain-dependent differences with regard to resting membrane potential (RP), action potential (AP) amplitude (AMP), and AP duration to 10% repolarization (APD10), 25% repolarization

(APD25), 50% repolarization (APD50) and 75% repolarization (APD75), or V_{\max} .

Diabetes prolonged the APD to the same extent in both SHR and WKY papillary muscles (Figure 1, Table 3 and Table 4). This prolongation of the AP was observed at all points sampled after peak depolarization. Diabetes-induced changes in AP configuration were independent of changes in RP, AMP or V_{\max} .

Treating diabetic SHR with either insulin or T_3 in vivo completely prevented the changes in AP configuration in vitro (Figure 1 and Table 3). The RP, AMP and V_{\max} of muscles from insulin-treated and T_3 -treated diabetic SHR were the same as those from nondiabetic SHR. T_3 treatment as well as insulin treatment completely prevented the diabetes-induced increases in APD at all stages of repolarization.

DISCUSSION

The results of this study show that diabetes prolongs the cardiac action potential (AP) in SHR and in WKY papillary muscles, and that the AP duration (APD) of nondiabetic SHR and WKY rat muscle were not different (Table 3). The results provide additional evidence that the 23 week-old SHR is in the adaptive stage of hypertrophy (39), in which the ventricular muscle is electrically, mechanically (42) and, in part, biochemically (12) normal. Volkmann et al. (56) described similar results when comparing membrane potentials from 15 week-old SHR and WKY. Our data also show that the adverse effect of untreated diabetes on AP configuration in both hypertrophic and nonhypertrophic muscle are quantitatively similar.

A prolonged APD is characteristic of diabetic, hypothyroid and compromised hypertrophic ventricular muscle (9, 10, 17, 27, 37). The cellular basis for the increased APD is not yet known. It has been suggested that the net inward current during the plateau phase is increased with hypothyroidism (20) or hypertrophy (33), perhaps due to a decrease in outward K^+ current. An increased density of voltage-dependent calcium channels in hypothyroid rat hearts (26) could also explain these findings. Furthermore, the time course of the AP of hypertrophic muscle appears to be extended by a depressed rate of inactivation of the slow inward current, when compared to normal muscle cells (4, 33). It is still not clear whether the underlying mechanisms which prolong the AP in cardiac muscle of diabetic, hypertensive and hypothyroid animals have a common ionic basis.

The influx of calcium during the cardiac AP probably increases with extended depolarization times, regardless of the mechanism of APD prolongation. A number of investigators have linked depressed mechanical activity with increased intracellular calcium concentrations (1, 4, 37), since an excess of free intracellular calcium can be toxic to the cell (13). Whether calcium overload exists with diabetes is still a source of debate (13, 29, 48); nonetheless, abnormal intracellular calcium handling is a common phenomenon among diabetic, hypertrophic and hypothyroid cardiac tissue (21, 38, 43).

Increased duration of the cardiac AP is often associated with disrupted mechanical activity in diabetic (17, 37) and hypertrophic myocardium (4, 10, 25, 28, 55). The results of this study are consistent with the pronounced mechanical deficits imposed by diabetes on the intact ventricle of SHR (11, 12, 42, 45, 57). Diabetes reduces maximum left ventricular (LV) pressure and rates of contraction and relaxation in the isolated working SHR heart (11, 42). It also depresses a biochemical correlate of relaxation, the rate and extent of calcium uptake by the sarcoplasmic reticulum (SR) in SHR (12). Prolongation of the AP is also evident of renovascular hypertensive (RVH) rat in papillary muscle (3) and is exacerbated by superimposed diabetes (18). Diabetes also impairs isometric relaxation of these papillary muscles to a greater extent than in nonhypertrophic muscles (17, 18).

Configurational changes in the AP alone caused by diabetes may not be sufficient to influence contractile events in the heart. Our data also show that diabetes prolongs the AP to the same extent in

SHR and WKY papillary muscles (Table 3). While diabetes depresses cardiac function in the SHR (e.g., LV pressure and SR Ca^{2+} uptake), it has no effect on the function of the WKY heart (11, 12, 57). Conversely, other authors have reported mechanical changes in heart muscle imposed by different pathological conditions (including hypertrophy and hypothyroidism) without concomitant alterations in the AP (32, 41, 51, 56). Collectively, these data must call into question the direct association of disrupted membrane potentials and mechanical function. The evidence obtained from the WKY strain suggests that intracellular events subsequent to the AP (e.g., SR Ca^{2+} uptake and perhaps myosin ATPase activity) are the primary determinants of alterations in myocardial performance affected by diabetes in the SHR strain.

Changes in APD due to either diabetes, hypothyroidism, or hypertrophy are generally independent of resting membrane potential (RP), AP amplitude (AMP) or rate of depolarization (V_{max} ; 3, 9, 10, 17, 20, 47). Neither diabetes nor hypertrophy affected RP, AMP or V_{max} (Table 4); however, the values for these measurements differ somewhat from previously published data. A papillary muscle RP typically ranges from -70 to -78 mV (17, 47, 56) yet the average RP for all groups in our study is approximately -65 mV (Table 4). The RP is primarily dependent on the activity of sarcolemma (SL) Na^+/K^+ ATPase, which is highly sensitive to temperature and metabolic disturbances (e.g., hypoxia). Sauviat and Feuvray (47) and Fein et al. (17) reported RP values of -60 mV from papillary muscle superfused with buffer below 30°C. Exposure to either hypoxic conditions or ouabain inhibits Na^+/K^+ ATPase activity and reduces

papillary muscle RP (2, 17). A reduced RP may partially inactivate the voltage-dependent Na^+ channels which are primarily responsible for the initial depolarization. Often V_{max} is used as an indirect index of Na^+ channel conductance (50). Accordingly, low temperatures and metabolic inhibitors reduce V_{max} , and this might help explain a nearly 5-fold decrease in our V_{max} values when compared to published data. Decreased Na^+/K^+ ATPase activity may not, however, explain all of our discrepant results. Normal SHR (22 week-old) have significantly lower SL Na^+/K^+ ATPase and reduced ouabain sensitivity than do SL membranes from WKY (35), but do not differ with respect to either RP or V_{max} (Table 4; 56). The APD of hypoxic muscle is characteristically shorter than the APD of normal muscle (24). Despite the deviations between our data and those of others, the APD of nondiabetic SHR and WKY are quantitatively similar to other published values (56).

Treatment with either insulin or thyroid hormone prevents the diabetes-induced changes in AP configuration of SHR papillary muscle. The effectiveness of insulin treatment on the electrophysiological properties of diabetic SHR hearts is not surprising, since most of the in vivo metabolic changes are also preventable (Tables 1 and 2). However, until recently the hypothyroidism attending diabetes was thought to have only a minimal impact on cardiac mechanical function in the diabetic rat (6, 14, 21, 23, 53, 54). However LeGaye et al. (34) reported that treatment of diabetic normotensive rats with T_3 partially prevents the prolongation of atrial muscle AP. Our findings indicate that T_3 completely prevents the delayed repolarization of papillary muscle in diabetic SHR. These data,

coupled with similar findings on mechanical and biochemical indices of function (11, 12, 42), strongly suggest that the thyroid hormone deficit is responsible, at least in part, for the diabetes-induced effects on SHR heart function. Preliminary results also show that T_3 treatment of diabetic RVH can prevent cardiac functional impairments in this model of hypertensive diabetic cardiomyopathy as well (unpublished observations). Thus, the presence of preexisting hypertrophy may influence myocardial dependence on thyroid hormone during the diabetic state.

The cellular mechanisms by which insulin and T_3 affect cardiac excitation contraction coupling are not understood. Insulin may influence cardiac function by improving metabolic activities; for example by increasing glucose availability, reducing fatty acid (FA) accumulation and protein wasting, or by changing sympathetic nerve activity (13). Even though a single daily injection of insulin does not always adequately control the serum hyperglycemia or BW loss in diabetic SHR, the hormone treatment is sufficient to maintain normal heart function (11). Rubinstein et al. (46), and more recently Rodrigues et al. (44), have also demonstrated that incomplete insulin therapy restores cardiac mechanical activity without fully correcting all metabolic changes associated with diabetes in the rat. Similarly, T_3 treatment of diabetic SHR does not control the hyperglycemia or BW loss, yet effectively maintains normal cardiac performance ex vivo (Table 2, Table 3 and Figure 1; 11, 12). It is possible that a portion of insulin's actions is secondary to that of thyroid hormone (52), since insulin treatment also prevents the attendant hypothyroidism of diabetes (11).

The AP configuration of diabetic SHR papillary muscle may be influenced directly or indirectly by T_3 treatment. For example, thyroid hormone can decrease the density of Ca^{2+} channels in the rat ventricle (26), which would reduce the net inward current and enhance rates of repolarization. By preventing depressed rates of Ca^{2+} uptake by the SR, T_3 treatment may reduce a transient inward current activated by intracellular Ca^{2+} (12). Diabetes-induced increase of intracellular and membrane-bound FA (13) may disrupt the electrical activity of the heart, particularly when glucose is not an available fuel source. Katz and Messineo (31) demonstrated that the accumulation of FA can influence the AP configuration when glucose availability is restricted. T_3 treatment, known to increase glucose uptake and utilization by cardiac muscle (49), may prevent this impairment.

To our knowledge, this is the first study showing that diabetes induces a prolongation of the AP in papillary muscle of the SHR, and that in vivo T_3 treatment is as effective as insulin treatment in preventing this prolongation. T_3 treatment in diabetic SHR not only prevents the impairments in cardiac electrophysiology, but also those relating to cardiac performance and a biochemical index of function (11, 12). Thus, thyroid hormone deficits may be an important component in the etiology of diabetic cardiomyopathy in SHR.

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Table 1. Systolic arterial pressure and heart rates of nondiabetic and diabetic SHR and WKY rats.

Group	(n)	SAP (mmHg)		HR (beats/min)	
		Week 0	Week 8	Week 0	Week 8
SHR					
Nondiabetic	(7)	198 ± 20 ^Y	196 ± 17 ^Y	399 ± 19 ^Y	418 ± 27 ^Y
Diabetic	(7)	198 ± 10	140 ± 12*	401 ± 25	298 ± 27*
Diabetic + I	(8)	194 ± 24	185 ± 22	381 ± 27	378 ± 31
Diabetic + T ₃	(7)	189 ± 16	201 ± 12	383 ± 26	438 ± 48
WKY					
Nondiabetic	(6)	144 ± 16	142 ± 19	333 ± 26	307 ± 32
Diabetic	(7)	141 ± 16	134 ± 18	328 ± 20	283 ± 24

Values represent means ± SD, obtained 8 weeks after initiation of diabetes or treatment. SAP, systolic arterial pressure; HR, heart rate; I, protamine zinc insulin; T₃, triiodothyronine.

*Significantly different from the nondiabetic group (within strain).

^YSignificantly different from the nondiabetic WKY.

Table 2. Body and heart weights of nondiabetic and diabetic SHR and WKY rats.

Group	(n)	BW (g)	HW (mg)	LV/BW (mg/g)	LV/RV (mg/mg)
<u>SHR</u>					
Nondiabetic	(7)	338 ± 21 ^Y	1.27 ± 0.07	2.92 ± 0.12	5.43 ± 0.46 ^Y
Diabetic	(7)	195 ± 25*	0.70 ± 0.11*	2.60 ± 0.48	4.22 ± 0.32*
Diabetic + I	(8)	327 ± 19	1.15 ± 0.08**	2.67 ± 0.09	4.90 ± 0.25**
Diabetic + T ₃	(7)	187 ± 12*	0.83 ± 0.12**	3.12 ± 0.30	4.13 ± 0.29*
<u>WKY</u>					
Nondiabetic	(6)	385 ± 21	1.38 ± 0.25	2.57 ± 4.25	3.84 ± 0.52
Diabetic	(7)	302 ± 31*	1.09 ± 0.27*	2.56 ± 0.64	3.93 ± 0.32

Values represent means ± SD, obtained 8 weeks after initiation of diabetes or treatment. BW, body weight; HW, heart weight; LV, left ventricular weight; RV, right ventricular weight; I, protamine zinc insulin; T₃, triiodothyronine.

*Significantly different from the nondiabetic group (within strain).

**Significantly different from the nondiabetic and diabetic groups (within SHR).

^YSignificantly different from the nondiabetic WKY.

Table 3. Papillary muscle action potential duration of nondiabetic and diabetic SHR and WKY rats.

Group	(n)	APD ₁₀ (ms)	APD ₂₅ (ms)	APD ₅₀ (ms)	APD ₇₅ (ms)
<u>SHR</u>					
Nondiabetic	(7)	12.2 ± 1.5	16.5 ± 1.8	24.8 ± 2.2	39.5 ± 2.6
Diabetic	(7)	27.6 ± 3.5*	39.0 ± 3.7*	57.1 ± 5.0*	91.9 ± 7.8*
Diabetic + I	(8)	11.4 ± 1.2	15.1 ± 2.0	22.7 ± 2.3	36.2 ± 3.6
Diabetic + T ₃	(7)	14.0 ± 2.0	18.6 ± 2.3	26.5 ± 2.8	40.3 ± 4.1
<u>WKY</u>					
Nondiabetic	(6)	8.2 ± 2.0	11.4 ± 2.1	17.2 ± 2.7	28.5 ± 3.4
Diabetic	(7)	28.9 ± 5.9*	37.9 ± 7.1*	54.7 ± 9.8*	84.0 ± 13.8*

Values represent means ± SE, obtained 8 weeks after initiation of diabetes or treatment. Sample sizes reflect number of animals from which multiple fibers (4-10) were sampled and averaged. APD₁₀-APD₇₅, action potential duration at 10%-75% repolarization; I, protamine zinc insulin; T₃, triiodothyronine.

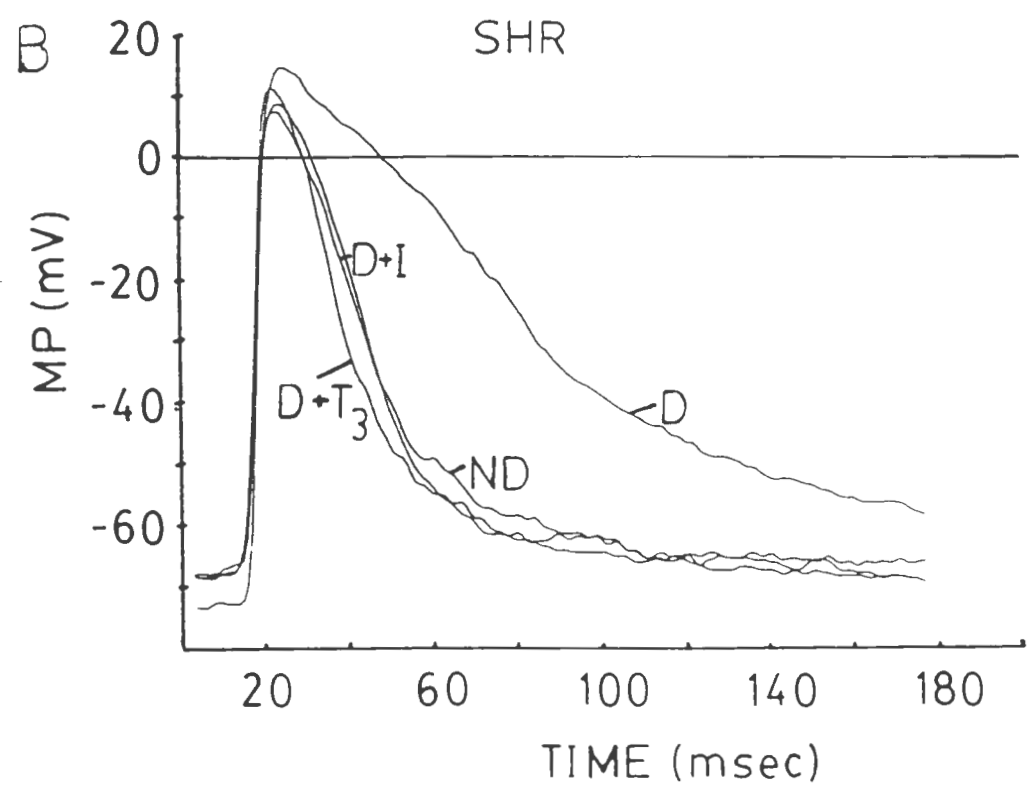
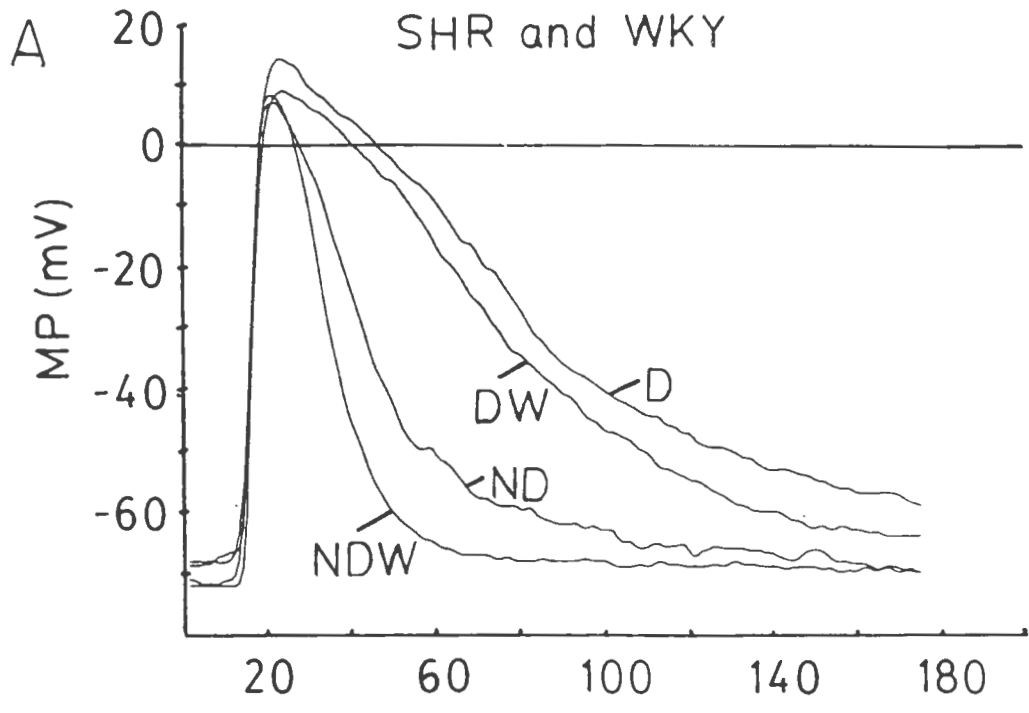
*Significantly different from the nondiabetic group (within strain).

Table 4. Papillary muscle membrane potential characteristics of nondiabetic and diabetic SHR and WKY rats.

Group	(n)	RP (-mV)	AMP (mV)	Vmax (V/S)
<u>SHR</u>				
Nondiabetic	(7)	65.4 ± 1.4	79.0 ± 2.5	28 ± 2
Diabetic	(7)	61.8 ± 1.4	82.9 ± 3.8	22 ± 3
Diabetic + I	(8)	64.6 ± 1.0	80.4 ± 1.5	27 ± 2
Diabetic + T ₃	(7)	64.9 ± 0.8	80.7 ± 1.9	26 ± 1
<u>WKY</u>				
Nondiabetic	(6)	64.9 ± 2.4	81.6 ± 2.8	27 ± 3
Diabetic	(7)	65.2 ± 2.0	84.1 ± 1.3	28 ± 2

Values represent means ± SE, obtained 8 weeks after initiation of diabetes or treatment. Sample sizes reflect number of animals from which multiple fibers (4-10) were sampled and averaged. RP, resting membrane potential; AMP, action potential amplitude; Vmax, maximum rate of rise; I, protamine zinc insulin; T₃, triiodothyronine.

Figure 1: Representative papillary muscle action potentials (AP). (A) The AP were recorded from nondiabetic SHR (ND), diabetic SHR (D), nondiabetic WKY (NDW) and diabetic WKY (DW), and (B) from T_3 -treated diabetic SHR (D+ T_3) and insulin-treated diabetic SHR (D+I). The individual AP were recorded from a single animal within each experimental group and were chosen because they best represented the average AP configuration as described in Table 3.



GENERAL DISCUSSION

The results of this study can be summarized according to the original aims and hypotheses as follows:

- 1) Untreated diabetes mellitus of 8 weeks duration caused a pronounced deficit in mechanical performance of spontaneously hypertensive rat (SHR). The depressed function in diabetic SHR hearts was not attributable to either malnutrition or a nonspecific effect of the diabetogen streptozotocin. Thyroid hormone treatment (in vivo) was nearly as effective as insulin treatment was in preventing the diabetes-induced cardiodepression in SHR.
- 2) Diabetes impaired left ventricular (LV) relaxation and Ca^{2+} uptake by the sarcoplasmic reticulum (SR) in the SHR but not in the nonhypertensive Wistar Kyoto (WKY) rat.
- 3) Thyroid hormone treatment, like insulin, completely prevented depressed rates of load-dependent relaxation and SR Ca^{2+} uptake in diabetic SHR hearts.
- 4) Diabetes prolonged ventricular action potential (AP) to the same extent in SHR as it did in WKY rats, and the AP duration (APD) was similar in both nondiabetic strains.
- 5) Either thyroid hormone or insulin treatment prevented the APD changes associated with diabetes in SHR papillary muscle.
- 6) Chronic diabetes had the same cardiodepressive effects on mechanical performance in the renovascular hypertensive (RVH) rat as it had in the SHR (Appendix A).
- 7) Either thyroid hormone or insulin treatment prevented

diabetes-induced mechanical impairments in RVH heart performance.

Thus, these results support the central hypotheses of the study, with the following exception: The effects of chronic diabetes on APD of SHR papillary muscle were not more pronounced than they were on the APD of nonhypertrophic WKY muscle.

The preexisting hypertrophy, rather than the etiology of hypertension, may be a primary factor influencing the exaggerated effects of diabetes on cardiac performance of hypertensive rats. The SHR and RVH are two experimental models which have been used to investigate the combined effects of diabetes and hypertrophy on cardiac function. Hypertension is induced in the RVH by surgically imposing elevated plasma renin activity (Goldblatt 1934). The SHR strain is genetically predisposed to elevated arterial pressures, which is believed to stem, in part, from enhanced central sympathetic activity (Yamori 1975) and is characterized by normal or below normal plasma renin activity (Sonkodi and Abraham 1988). One obvious common denominator of the RVH and SHR models of hypertension is the presence of LV hypertrophy. The presence of LV hypertrophy alone is not always associated with impaired cardiac mechanical performance (Pfeffer et al. 1979; Capasso et al. 1986; Rodgers et al. 1986). The results of this study provide additional evidence that cardiac function in 23 week-old SHR is still within the adaptive phase of hypertrophy. Cardiac mechanical performance is also normal after 12 weeks of imposed hypertension in 23 week-old RVH (Appendix A). However, when diabetes coexists with LV hypertrophy (in either SHR or RVH), LV contraction and relaxation of perfused hearts are depressed

more than when diabetes is imposed on nonhypertrophic hearts (Rodgers 1986; Rodrigues and McNeill 1986; Davidoff and Rodgers; Davidoff et al.; Appendix A). Isometric relaxation is also prolonged in papillary muscles from diabetic RVH (Fein et al. 1984). The effects of diabetes on cardiac function in hypertensive rats is independent of either sustained hypertension or LV hypertrophy. Diabetes depresses SAP in SHR without significantly changing SAP in RVH (Rodgers 1986; Davidoff and Rodgers: Appendix A). The depressor effects of diabetes in SHR is not always correlated with reversal of LV hypertrophy (Rodgers 1986; Davidoff and Rodgers; Davidoff et al.; Davidoff and Rodgers). Diabetes, however, consistently impairs mechanical performance in both SHR and RVH but it has no effect on cardiac function in nonhypertensive, nonhypertrophic WKY rats. Diabetes depresses LV pressure generation in nonhypertensive Sprague-Dawley rat hearts, but the magnitude of diabetes-induced effects are markedly greater in SHR (Rodgers 1986). Therefore, the key characteristic, which accounts for the exacerbation of the cardiodepressant effects of diabetes, seems to be preexisting LV hypertrophy.

The predisposition of LV hypertrophy on cardiodepressive effects of diabetes involves the reticular elements of the myocyte. A biochemical defect often found to be characteristic of diabetic myocardium is a depressed rate of Ca^{2+} sequestration by SR microsomes (Dhalla et al. 1985). A reduced rate of SR Ca^{2+} uptake is generally associated with impaired load-dependent relaxation (Brutsaert et al. 1980; Bers 1985; see also discussion in Davidoff et al.). Proposed mechanisms include reductions in either the density or the activity

of Ca^{2+} - Mg^{2+} ATPase (Penpargkul et al. 1981; Ganguly et al. 1983; Schaffer et al. 1989), alterations in membrane lipid profiles (Lopaschuk et al. 1983; Ganguly et al. 1983; Kim and LaBella 1988), or changes in the degree of membrane phosphorylation (Kranias et al. 1988). Until now, studies of this kind have been restricted to nonhypertensive, nonhypertrophic models. The data presented in this study suggest, however, that the adverse effects of diabetes on left ventricular SR Ca^{2+} uptake activity are exacerbated by preexisting hypertrophy. Thus, diabetes of equal duration and severity - as measured by serum glucose and insulin levels - depresses SR Ca^{2+} uptake of SHR left ventricles, but has no effect in the nonhypertensive WKY strain. These results extend those of earlier studies which had demonstrated that the detrimental effects of chronic diabetes on various indices of cardiac mechanical activity are more pronounced in hypertrophic than in nonhypertrophic ventricles of both the SHR and RVH models (Fein et al 1984; Rodrigues and McNeill 1986; Rodgers 1986; Davidoff and Rodgers; Appendix A).

Increased duration of the cardiac AP may be linked to disrupted mechanical activity in both diabetic (Fein et al. 1983; Nordin et al. 1985) and hypertrophic myocardium (Gulch et al. 1979; Heller and Stauffer 1981; Aronson and Keung 1983; Thollon et al. 1985; Capasso et al. 1986). There is a considerable amount of evidence to suggest that calcium metabolism is abnormal in either diabetic or hypertrophic myocardium. Heart muscle from both disease states may exhibit increased frequency of afterdepolarizations and triggered activity (Fein et al. 1983; Nordin et al. 1985), altered SR or sarcolemmal calcium fluxes (Penpargkul et al. 1980; Limas and Cohen

1979; Rodgers et al. 1986; Andrawis et al. 1988; Nakanishi et al. 1989; Davidoff et al.), and beneficial responses to calcium channel blockers (Afzal et al. 1988, 1989). In many of these studies, depressed mechanical activity of muscle have been linked to elevated intracellular calcium concentrations (Aronson and Keung 1983; Nordin et al. 1985; Afzal et al. 1988; Afzal et al. 1989), which in turn is assumed to be toxic to the cell (Dhalla et al. 1985; Andrawis et al. 1988). The influx of calcium during the cardiac AP probably increases with delayed repolarization and may contribute to changes in metabolism and mechanical functions. It has been suggested that the net inward current during the plateau phase is increased in some models of hypertrophy (Kleiman and Houser 1988), is perhaps due to a decrease in outward K^+ current. The time course of the AP of hypertrophic muscle appears to be prolonged by a depressed rate of inactivation of the slow inward (Ca^{2+}) current, when compared to normal muscle cells (Aronson and Keung 1983; Kleiman and Houser 1988). It is still not clear, however, whether the underlying mechanisms which prolong the AP in cardiac muscle of both diabetic and hypertensive animals have a common ionic basis.

The cardiac APD in several hypertensive animal models is significantly prolonged relative to that in normotensive animals. Aortic stenosis, renal artery constriction and chemically-induced hypertension promote ventricular hypertrophy and changes in AP configuration to varying degrees (Aronson 1980; Heller and Stauffer 1981; Thollon et al. 1985; Capasso et al. 1986; Nordin et al. 1989). The hypertrophic papillary muscle of SHR (Kawamura et al. 1976) does not, however, exhibit longer repolarization times when compared to

the nonhypertrophic WKY (Davidoff and Rodgers). While there were no statistical differences between APD of nondiabetic SHR and WKY papillary muscle in this study, there was an apparent trend toward higher APD values in SHR. Larger samples sizes may support statistical differences. Hayashi and Shibata (1974) recorded ventricular AP from Langendorff perfused hearts of SHR and WKY. It is not clear from their data whether the average APD₁₀₀ is significantly greater for SHR than for WKY (mean (ms) \pm SE (n=5), 104.0 \pm 3.1 and 93.9 \pm 3.2, respectively). Volkmann et al. (1989) recently reported APD₈₀ values for papillary muscle in SHR and WKY of 37.7 \pm 3.0 and 33.9 \pm 1.8, respectively (mean \pm SE, n= 9 and 10). The differences in APD between SHR and WKY are not as apparent as they are in other models of hypertrophy, but whether the discrepancies are related to the genetic strain, etiology or duration of hypertrophy, or other factors, remain to be tested.

These results suggest that alterations in electrophysiological characteristics of ventricular muscle may contribute to the etiology of diabetic cardiomyopathy in SHR. The data in this study also show, however, that diabetes prolongs the AP to the same extent in SHR and WKY papillary muscles, but depresses cardiac function only in the SHR (e.g., LV pressure and SR Ca²⁺ uptake; Yamamoto and Nakai 1988; Davidoff and Rodgers; Davidoff et al.). In other normotensive rat strains, however, diabetes prolongs APD and impairs mechanical function (Fein et al. 1980; Fein et al. 1983; Sauviat and Feuvray 1985). While the dissociation of electrical and mechanical events is not common, it is not without precedent. Indices of isometric contraction but not AP configuration of rat papillary muscles were

affected by chemically-induced thyroidectomy (6-7 weeks) and thyrotoxic doses of T_3 (2 weeks; Poggesi et al. 1987). An increase in isometric tension in SHR papillary muscle did not correspond to a change in AP configuration when compared to these measurements in WKY muscle (Volkman et al. 1989). Collectively, these data must call into question the direct association of disrupted membrane potentials and mechanical function. The evidence obtained from the WKY strain suggests that intracellular events subsequent to the AP (e.g., SR Ca^{2+} uptake and perhaps myosin ATPase activity) are the primary determinants of alterations in myocardial performance affected by diabetes in both hypertensive and normotensive rat strains.

The mechanism responsible for the apparent interaction between diabetes and LV hypertrophy with respect to cardiac function remains a matter of speculation. Metabolic derangements in the diabetic heart include decreases in lipolytic activity, fatty acid (FA) oxidation and glucose utilization (Paulson and Crass 1982; Vary and Neely 1982; Kobayashi and Neely 1983; Dhalla et al. 1985). These in turn are linked to changes in energy substrate availability, membrane integrity and function in the heart (Dhalla et al. 1985). Hypertrophy may exacerbate these metabolic effects. Like diabetes, LV hypertrophy is associated with alterations in lipid metabolism (Reibel et al. 1983; Reibel et al. 1987; Fujii et al. 1988; Rodrigues et al. 1988), restricted fuel source availability (Savabi 1988; Wexler et al. 1988), and changes in sarcolemmal (SL) ion transport (Lee et al. 1983; Nakanishi et al. 1989). Hypertrophic muscle may undergo compensatory intracellular changes to accommodate elevated

workloads and reduced coronary reserves (Shimamoto et al. 1982; Tubau et al. 1989). For example, an increase demand for FA oxidation, as the primary fuel source, may be met by increased myocardial carnitine content (Foster et al. 1985; Reibel et al. 1987). Changes in SL ion pump activities may enhance intracellular Ca^{2+} concentration and augment myocardial contractility. These and other cellular mechanisms may combine to predispose the hypertrophic ventricle to membrane-disrupting effects of diabetes.

A low thyroid state might contribute significantly to changes in cardiac performance of diabetic SHR. Methimazole treatment of SHR causes a depression in heart performance ex vivo that is qualitatively similar to the effects of diabetes, though lesser in magnitude (Rodgers 1986). These results were confirmed in a later study of the effects of surgical thyroidectomy on cardiac function in SHR (Rodgers and McNeill 1986). Based on these observations, it was expected that triiodothyronine (T_3) treatment of diabetic SHR might reduce the magnitude of the subsequent impairment in mechanical function. It was somewhat surprising that T_3 was almost as effective as insulin was in preventing the depressions in measurements of heart function, including pressure generation, rates of contraction and relaxation and stroke work.

The present results differ to some extent from previous reports of the effects of T_3 treatment on cardiac function and hemodynamic measurements in normotensive rat strains. Higher doses of T_3 (25-30 $\mu\text{g}/\text{kg}/\text{day}$), administered to diabetic Wistar rats for 6 weeks, failed to prevent the cardiac functional impairments (Tahiliani and McNeill 1984), but when given to diabetic Sprague-Dawley (SD) rats for only 5

days, restored mechanical indices of cardiac function to normal (Garber et al 1983). In the latter study, lower doses of T_3 (considered to be more appropriate for replacement therapy of hypothyroidism) were not completely effective. In a recent report, Barbee et al. (1988) found that approximately 4 μ g T_3 per rat per day for 3 weeks (by subcutaneous pellet implant) did not improve performance of unpaced hearts from diabetic SD rats. However, these investigators also failed to demonstrate any effect of diabetes on paced heart function. For the present studies, the dose of T_3 was selected according to its ability to restore to normal the depressed heart rate (HR) in vivo, an index which is a sensitive indirect indicator of thyroid status. This turned out to be about 2-3 fold greater than "physiologic" replacement doses in nonhypertensive rat strains (Dillmann 1982). The T_3 dose used herein did not appear, however, to impose a hyperthyroid condition in diabetic SHR. This conclusion is based on the following: 1) The dose used did not elevate serum T_3 levels above normal, when measured at the same time point after injection, in either the diabetic or nondiabetic SHR groups (Davidoff and Rodgers); 2) The same dose given to nondiabetic SHR had no effect on heart and body weights, or on SAP or HR in vivo; and 3) T_3 treatment of nondiabetic SHR had no effect on any of the indices of cardiac performance ex vivo. Although T_3 treatment reduced body weights of the diabetic SHR, it had no effect on this measurement in the nondiabetic control group.

The difference in the effectiveness of T_3 in diabetic SHR compared to normotensive rats are probably related to the elevated arterial pressure of SHR, and not to differences related specifically

to the genetic strain. Treatment with T_3 prevents the cardiodepression in diabetic RVH as well as in diabetic SHR (Appendix A). It is possible that hypertrophy, stimulated by elevated arterial pressure in both models, may increase cardiac susceptibility to a number of stresses including those imposed by diabetes (Factor et al. 1981; Rodgers 1986; Rodrigues and McNeill 1986), hypothyroidism (Rodgers et al. 1986), or ischemia (Mochizuki et al. 1986). This suggests that the hypothyroidism accompanying diabetes has a more profound cardiovascular effect in animals with elevated arterial pressure, and that hypertrophied myocardium may be functionally more dependent upon thyroid status than is nonhypertrophic heart muscle.

There are a number of mechanisms by which T_3 therapy might effectively prevent the cardiac functional impairments of diabetes, especially in hypertrophic heart muscle. Thyroid hormone may relieve some of the metabolic stresses by increasing myocardial utilization of FA and decreasing FA accumulation (Morkin et al. 1983; Muller and Seitz 1984a; Tanaka et al. 1985). Enhancing plasma lipid clearance with methionine treatment, for instances, improves cardiac performance in diabetic Wistar rats (Heyliger et al 1986). The vasodilator hydralazine reduces serum lipids in diabetic Wistars and improves cardiac function of isolated working hearts (Rodrigues et al. 1986). Interestingly, hydralazine treatment also corrects the hypothyroid state in these diabetic rats. Other actions of thyroid hormone include stimulation of glucose uptake and cellular metabolism (Muller and Seitz 1984b; Tanaka et al. 1985; Gordon et al. 1986; Segal 1989). T_3 directly stimulates glucose uptake in several rat tissues including the heart, by enhancing the activity of the plasma

membrane transport mechanism (Segal 1989). Treatment of diabetic SHR and RVH with T_3 may increase the availability of glucose and lipids as energy substrates and reduce FA accumulation, which would help to account for the improved cardiac function. This and other related metabolic actions may be more crucial, and more apparent, against a background of high workloads and associated metabolic demand.

A common biochemical change observed in hearts from either diabetic, hypertensive or hypothyroid animals is depressed SR Ca^{2+} uptake, which correlates with impaired myocardial relaxation (Suko 1973; Dhalla et al. 1985; Rodgers et al. 1986). Thyroid hormone treatment does not restore SR Ca^{2+} uptake or LV function in diabetic normotensive rats (Ganguly et al. 1983; Tahiliani and McNeill 1984), yet is completely effective on diabetic hypertensive muscle (Davidoff et al.). The presence of hypertrophy may account for the differential effects of diabetes and T_3 treatment on cardiac function, in part because of alterations in lipid metabolism (see above). The acute effects of T_3 on Ca^{2+} - Mg^{2+} ATPases are profoundly influenced by the lipid environment (Galo et al. 1981). Because hypertrophy (Petkov et al. 1988), hypothyroidism (Galo et al. 1981) and diabetes (Gudbjarason et al. 1987) all affect membrane lipid composition, it seems reasonable to hypothesize that different combinations of these pathophysiologic conditions will have variable influences on the subsequent effects of T_3 administration. In normotensive rats, myoinositol coadministration increases the therapeutic efficacy of T_3 in diabetic cardiomyopathy (Xiang et al. 1988). Fatty acyl esters of carnitine may be especially important. Enhanced incorporation of long chain acyl carnitines (LCAC) into the

SR membrane is correlated with depressed rates of Ca^{2+} uptake in diabetes (Lopaschuk et al. 1983) and T_3 treatment decreases LCAC levels in SR membranes (Black et al. 1988). Another potential mechanism undoubtedly involves the regulation of SR Ca^{2+} pump activity by thyroid hormone. Chronically, T_3 probably increases the rate of SR Ca^{2+} uptake by accelerating the synthesis of Ca^{2+} - Mg^{2+} ATPase enzyme molecules. Hypothyroidism has opposite effects on these measurements (Rohrer and Dillmann 1988).

Disruptions in myocardial excitation-contraction coupling characteristic of diabetes, hypertension and hypothyroidism (Fein et al 1983; Nordin et al. 1985; Capasso et al 1986) include prolongation of the cardiac AP. T_3 treatment prevents diabetes-induced alterations in SHR cardiac AP, and thus normalizes the first step leading to mechanical activity. The AP configuration of diabetic SHR papillary muscle may be influenced directly or indirectly by T_3 treatment. For example, thyroid hormone can decrease the density of Ca^{2+} channels in the rat ventricle (Hawthorn et al. 1988), which would reduce the net inward current and enhance rates of repolarization. Similarly, T_3 treatment may inhibit a transient inward current activated by intracellular Ca^{2+} , by restoring normal rates of SR Ca^{2+} uptake (T_3 prevents depression of Ca^{2+} uptake by the SR in diabetic SHR; Davidoff et al.). Diabetes-induced increase of FA (Dhalla et al. 1985) may disrupt the electrical activity of the heart, particularly when glucose is not an available fuel source. Katz and Messineo (1981) demonstrated that the accumulation of FA can influence the AP configuration when glucose availability is restricted. T_3 treatment, shown to increase glucose uptake and

utilization by cardiac muscle (Segal 1989), may prevent this impairment.

Another common manifestation of diabetes, hypertrophy and hypothyroidism in rat ventricular muscle is a shift from the predominant V_1 to the V_3 myosin isozyme (Malhotra et al. 1981; Mercardier et al. 1981; Dillmann 1982; Garber et al. 1983; Morkin et al 1983; Rupp and Jacob 1986; Schaffer et al. 1989). By extension, the effects of hypertension and diabetes (with or without hypothyroidism) on myosin isozyme profiles may be additive, but this hypothesis has not been tested. Thyroid hormone treatment in animals with a normally higher distribution of V_3 than V_1 promotes a shift to the more active V_1 form (Morkin et al. 1983; Williams and Ianuzzo 1988). Garber et al. (1983) were able to prevent the diabetes-induced V_3 shift with T_3 treatment in SD rats. T_3 administration to diabetic SHR and RVH may also change the ratio of myosin isozyme activity in favor of V_1 and this may in turn help to explain the increased rate of contractility in these groups (Davidoff and Rodgers; Appendix A). The effects of diabetes and T_3 treatment on ventricular myosin isozyme distribution in SHR and RVH are presently being investigated in this laboratory.

Several alternative interpretations of the results of this study could be eliminated by the control studies. Amelioration of the cardiodepression and mortality by either insulin or T_3 treatment and by pretreatment with the glucose analog 3-O-methylglucose (Ramanadham et al. 1987), ruled out the possibility that direct cardiotoxicity of streptozotocin contributed to the morbidity and mortality of diabetes in either SHR or RVH. Neither malnutrition nor reduced heart mass

alone could account for the observed cardiodepressive effects of diabetes, since performance was not impaired in either of the food restricted groups (Davidoff and Rodgers; Appendix A).

Complete glycemc control was not necessary to preserve cardiac function with diabetes. Treatment with either lente insulin or protamine zinc insulin (PZI) effectively maintained cardiac pump function and SR Ca^{2+} uptake, without normalizing serum glucose values of diabetic SHR (Davidoff and Rodgers; Davidoff et al.). Rubinstein et al. (1984) and Rodrigues et al. (1989) reported similar cardiovascular benefits from graded insulin treatment of normotensive diabetic rats, without complete normalization of blood glucose. Lopaschuk et al. (1983) restored SR Ca^{2+} uptake in diabetic Wistars with PZI treatment but did not completely restore blood glucose concentrations to normal levels. Strict glycemc control in humans also does not appear to be necessary for the prevention of diabetic nephropathies (Dodson and Horton 1988). T_3 replacement therapy in the diabetic SHR and RVH did not adequately control the hyperglycemia or hypoinsulinemia, yet effectively restored cardiac function (Davidoff and Rodgers; Davidoff et al.; Appendix A). T_3 treatment failed to reverse the weight loss incurred by diabetes while in general insulin treatment only partially restored body and heart weights.

The ability of T_3 to restore SAP of diabetic SHR as effectively as insulin did suggests strongly that hypothyroidism contributes to the depressor effect of diabetes in this model. The results of earlier studies also indicated that thyroid hormone is an important regulator of arterial pressure in SHR. Thyroidectomy of young SHR

prevents the development of hypertension in these animals (Rioux and Berkowitz 1977; Rodgers and McNeill 1986). Increased total peripheral resistance (TPR) is the primary factor contributing to SHR hypertension (Pfeffer and Frohlich 1973). Thus, the decline in SAP of diabetic SHR may be associated with a fall in TPR. Results reported by Yamamoto and Nakai (1988) seem to show that moderate diabetes reduces TPR (estimated by their cardiac index and mean arterial pressure values) of SHR by about 10-20%, depending on the duration of diabetes. Alternatively, the depressor effect of diabetes on SHR may be primarily cardiogenic. As mentioned earlier, T_3 treatment restored cardiac function ex vivo to near normal in diabetic SHR. Tsujimoto and Hashimoto (1986) attributed most of the rise in SAP of T_3 -induced hyperthyroid rats to the cardiac, rather than the vascular, effects of T_3 . Some of the in vivo hemodynamic effects of diabetes in SHR do not occur in RVH (Appendix A). Diabetes does not produce a reduction in SAP in RVH, but it does depress cardiac function. These observations lend additional support to suggest that there are direct myocardial effects of diabetes and the attendant hypothyroidism that are independent of hemodynamic changes in vivo.

Treatment with T_3 , like insulin, corrects the bradycardia of diabetic SHR. The bradycardia associated with either diabetes or hypothyroidism has been attributed, at least in part, to depressed myocardial responsiveness to adrenergic stimulation (Bilezikian and Loeb 1983; Gotzsche 1986). Both hypertrophic (Limas and Limas 1987) and diabetic (Heyliger et al. 1982; Kashiwagi et al. 1989) myocardium have been shown to possess a lower density of functional

β -adrenoceptors. Both insulin and T_3 may be involved in the regulation of cardiac β -adrenoceptor density (Hawthorn et al. 1988). Insulin may act indirectly, and possibly completely, by restoring serum T_3 levels (Davidoff and Rodgers; Davidoff et al.; Appendix A). Depressed myocardial β -adrenoceptor numbers of diabetic SD rats are restored to normal with either insulin or T_4 treatment, while those of thyroidectomized diabetic rats are not affected by insulin treatment alone (Sundaresan et al. 1984). The chronotropic action of T_3 in vivo may also involve a direct effect of the hormone on pacemaker cells (Kessler-Icekson 1988). The available evidence implies that perhaps all of the bradycardia associated with experimental diabetes, in the presence or absence of hypertension, can be attributed to the attendant hypothyroidism.

In conclusion, the results presented herein show that diabetes impairs cardiac function to a greater extent in the SHR and the RVH rat than in the normotensive WKY rat. Diabetes depresses LV function (contraction and relaxation) in these hypertensive rats without dramatically affecting function in normotensive strains (Rodgers 1986; Rodrigues and McNeill 1986; Davidoff and Rodgers; Davidoff et al.). Reduced rates of Ca^{2+} uptake by the SR (Davidoff et al.) from diabetic SHR hearts probably contribute to these mechanical deficits. The effects of diabetes on the LV AP are not consistent with those on performance, since AP configuration is altered comparably in both SHR and WKY papillary muscles. It appears that in hypertensive rats intracellular events subsequent to the cardiac AP are more susceptible to the adverse effects of diabetes than in normotensive rats.

The results of this study also show that the cardiac impairments associated with diabetes in either the SHR or RVH can be prevented by T₃ treatment. This is the first known account of a moderate dose of T₃ successfully restoring cardiac function, including electrical, biochemical and mechanical events, in experimental diabetes. In addition, T₃ treatment, like insulin, prevents the fall in arterial pressure and bradycardia induced by diabetes in the SHR in vivo, and either reduces or delays mortality. The results suggest that attendant hypothyroidism may play a more significant role in the development of diabetic cardiomyopathy in the presence of LV hypertrophy than it does in nonhypertrophic muscle.

Diabetes mellitus is a heterogeneous disease and is subclassified by the National Diabetes Data Group as either one of the following: Type I (insulin-dependent diabetes mellitus or IDDM); Type II (non-insulin dependent diabetes mellitus or NIDDM); gestational diabetes; impaired glucose tolerance; or secondary diabetes. These results may have some clinical applicability particularly for IDDM patients. Hypertension apparently occurs more frequently among diabetic patients than in the general population (Fuller 1985; Sprafka et al. 1988; Bell 1989). It is often regarded as an important risk factor for the higher cardiovascular morbidity and mortality in this group (Kannel et al. 1974; Fuller 1985; Sprafka et al. 1988; Bell 1989). The incidence of impaired thyroid function among diabetics may be as high as 30%, is most severe during poor metabolic control, and is most evident in IDDM (Bagchi 1982). While diabetic patients with hypothyroidism may be at increased risk of cardiovascular morbidity and mortality (Whittingham et al. 1971),

this risk may be further intensified by hypertension. Supplemental thyroid hormone treatment in conjunction with insulin administration might provide additional therapeutic cardiovascular benefits, in patients with poorly controlled insulin-dependent diabetes complicated by attending hypertension and hypothyroidism.

Future studies designed to provide additional information about the adverse effects of diabetes on hypertrophic myocardium and the mechanisms by which the attending hypothyroidism influence this dysfunction may include:

Cellular mechanisms involved in the development of diabetic cardiomyopathy in the hypertrophic heart can be further characterized by studying, for example, ventricular myosin isozyme distributions, individual ionic currents in voltage-clamped isolated myocytes, and specific enzyme activities associated with sarcolemmal, mitochondrial and SR membranes. There are other experimental animal models that could be used to extend our knowledge about the adverse effects of diabetes on hypertrophic heart function (including mechanical, biochemical and electrophysiological indices of function). A few of these alternative models are described below.

Hyperlipidemia is another prominent cardiovascular risk factor in diabetic patients with hypertension (Tzagournis 1989). Abnormalities in lipid metabolism may account for depressed cardiac function in SHR and perhaps in RVH. Diabetes induces hyperlipidemia in serum of SHR without affecting serum lipid values of WKY rats (Rodrigues and McNeill 1986). Cardiac function, serum lipid profiles, as well as phospholipid content could be assessed in

diabetic hypertensive rats treated with lipolytic agents.

Antihypertensive therapy in diabetic patients and in diabetic SHR also reduces the accumulation of some plasma lipids which may account for improved cardiac performance (Tzagournis 1989; Rodrigues et al. 1986).

The cardiodepressive effects of diabetes may not be as severe in the SHR in the absence of preexisting hypertrophy. Antihypertensive treatment of SHR can reverse or prevent LV hypertrophy (Sen 1983). The cardiovascular effects of diabetes in nonhypertrophic SHR hearts could be studied by pretreating SHR with antihypertensive agents (Ruskoaho 1984). Chronic diabetes coupled with developing hypertension and LV hypertrophy may have different effects on cardiac function than when diabetes is superimposed on preexisting LV hypertrophy. Injection of streptozotocin in neonatal SHR causes diabetes mellitus without interfering with the onset of hypertension (Iwase et al. 1987a and 1987b). Simultaneous induction of diabetes and renovascular hypertension may be an alternative approach. Since hypertension often coincides with, but may not precede, diabetes, the use these experimental models could provide additional information about the cardiovascular manifestations characteristic of clinical diabetes.

Hyperglycemia is a predominant metabolic disturbance of diabetes which results in an increase in intracellular glucose concentration in tissues where glucose uptake is insulin independent (e.g., liver, brain and kidney). In the presence of too much intracellular glucose, aldose reductase metabolizes glucose, ultimately forming copious amounts of sorbitol which promotes hypertonicity. Clinicians

are presently investigating the therapeutic benefits of aldose reductase inhibitors (e.g., tolrestat and sorbinil) in order to minimize the accumulation of the toxic glucose metabolites (see Swonger and Matejski 1988). Diabetes reduces glucose transport into cardiac tissue (Segal 1989), therefore, treatment with an aldose reductase inhibitor would most likely not have direct beneficial effects on cardiac function. However, these drugs may provide additional indirect protection to the cardiovascular system during poorly controlled diabetes by improving hepatic, renal and neural functions.

APPENDIX A: Cardiac Performance of Diabetic RVH Rats

SUMMARY

Diabetes markedly depresses cardiac function in the spontaneously hypertensive rat (SHR). A low thyroid state appears to contribute to the impaired mechanical function of diabetic SHR hearts. In order to determine whether cardiac dysfunction is specific for the diabetic SHR, cardiac performance was assessed in diabetic renovascular hypertensive (RVH) rats. The 2-kidney, 1-clip procedure was used to produce hypertension in 11 week-old Wistar Kyoto (WKY) rats. Four weeks after surgery, chronic diabetes was chemically-induced by a single tail vein injection of streptozotocin (50 mg/kg). A subgroup of diabetic RVH were treated with either protamine zinc insulin (I; 10 U/kg/day) or triiodothyronine (T_3 ; 8-10 μ g/kg/day). Other control groups included; nondiabetic RVH treated with same T_3 dose as the diabetic group, food-restricted nondiabetic RVH, and diabetic and nondiabetic normotensive WKY rats. Weekly in vivo systolic arterial pressure (SAP) and heart rate (HR) were recorded throughout the study. The type of data collected and the manner in which it was analyzed was similar to the data presented in Davidoff and Rodgers. In brief, 8 weeks after the induction of diabetes animals were sacrificed, serum samples were collected and mechanical performance of isolated working hearts were assessed. Following the acquisition of cardiac performance data, wet heart weights, including whole heart (HW), left ventricular (LV) and right ventricular (RV) weights, were recorded. Hypertension in RVH was characterized by elevated SAP and HR when compared to WKY. Diabetes depressed HR only in RVH rat but had no effect on SAP in either RVH or WKY. Either insulin or T_3 treatment prevented the drop in HR in

diabetic RVH. Diabetes impaired LV pressure generation and rates of contraction and relaxation in RVH but it had no effect on these measurements in WKY. T_3 treatment was as effective as insulin was in preventing cardiac depression in diabetic RVH with one exception: The rate of LV pressure generation was significantly higher in T_3 -treated diabetic RVH than it was in untreated diabetic RVH, but the rate of LV pressure generation was also significantly lower in T_3 -treated diabetic RVH than it was in nondiabetic RVH. The cardiac performance in nondiabetic RVH was similar to nondiabetic WKY. The results provide additional evidence that hypertrophy, induced by 12 weeks of RVH does not adversely affect mechanical function in the isolated working heart. Diabetes impairs cardiac function in the hypertensive, hypertrophic RVH but it does not affect function in the nonhypertensive, nonhypertrophic WKY. Hypertrophy may predispose the myocardium to the adverse effects of diabetes. Furthermore, the attendant hypothyroidism may contribute to the functional deficits characteristic of the diabetic hypertrophic heart.

Table 1. Serum glucose, insulin, triiodothyronine and thyroxine levels of nondiabetic and diabetic RVH and WKY rats.

Group	n	Glucose (mg/dl)	Insulin (μ U/ml)	T ₃ (ng/dl)	T ₄ (μ g/dl)
RVH					
Nondiabetic	11	127 \pm 20	77 \pm 56	72 \pm 13 ^Y	5.09 \pm 1.44
Diabetic	9	500 \pm 44 [*]	10 \pm 8 [*]	38 \pm 10 [*]	2.98 \pm 1.02 [*]
Diabetic + T ₃	7	693 \pm 150 ^{**}	26 \pm 12 [*]	116 \pm 76	0.42 \pm 0.41 ^{**}
Diabetic + I	8	176 \pm 76	273 \pm 86	86 \pm 12	5.36 \pm 1.38
Nondiabetic + T ₃	5	168 \pm 33	84 \pm 25	397 \pm 258 [*]	0.33 \pm 0.10 ^{**}
Nondiabetic FR	5	172 \pm 22	36 \pm 8 [*]	74 \pm 13	3.63 \pm 1.04 [*]
WKY					
Nondiabetic	13	140 \pm 40	74 \pm 22	87 \pm 12	5.19 \pm 1.05
Diabetic	9	499 \pm 103 [*]	18 \pm 9 [*]	39 \pm 13 [*]	2.35 \pm 0.78 [*]

Values are means \pm SD, obtained 8 weeks after initiation of diabetes or treatment.

T₃, triiodothyronine; T₄, thyroxine; I, insulin; FR, food restricted.

* Significantly different from the nondiabetic group (within group).

** Significantly different from the nondiabetic and diabetic groups.

^Y Significantly different from the nondiabetic WKY.

TABLE 2. Body and heart weights of nondiabetic and diabetic RVH and WKY rats.

Group	n	BW (g)	HW (mg)	LV/BW (mg/g)	LV/RV (mg/mg)
<u>RVH</u>					
Nondiabetic	11	329 ± 53 ^Y	1556 ± 195 ^Y	3.77 ± 0.57 ^Y	5.07 ± 0.62 ^Y
Diabetic	9	266 ± 27 [*]	1219 ± 88 [*]	3.57 ± 0.35	5.02 ± 0.39
Diabetic + T ₃	7	225 ± 50 [*]	1258 ± 209 [*]	4.48 ± 0.52 [*]	4.92 ± 0.90
Diabetic + I	8	356 ± 48	1508 ± 125	3.44 ± 0.55	5.17 ± 0.63
Nondiabetic + T ₃	5	353 ± 24	1628 ± 146	3.59 ± 0.46	4.52 ± 0.86 [*]
Nondiabetic FR	5	294 ± 38	1182 ± 59 [*]	3.29 ± 0.48	5.27 ± 0.48
<u>WKY</u>					
Nondiabetic	13	376 ± 28	1428 ± 182	2.92 ± 0.15	4.57 ± 0.76
Diabetic	9	290 ± 34 [*]	1070 ± 75 [*]	2.91 ± 0.22	4.58 ± 0.24

Values are means ± SD, obtained 8 weeks after initiation of diabetes or treatment.

T₃, triiodothyronine; I, insulin; FR, food restricted.

* Significantly different from nondiabetic group (within group).

^Y Significantly different from the nondiabetic WKY.

TABLE 3. Systolic arterial pressure (SAP) of nondiabetic and diabetic RVH and WKY rats.

Group	n	SAP (mmHg)		
		Week 0	Week 4	Week 12
<u>RVH</u>				
Nondiabetic	11	136 ± 3	201 ± 6 ^Y	219 ± 10 ^Y
Diabetic	9	126 ± 4	194 ± 12	191 ± 14
Diabetic + T ₃	7	127 ± 4	194 ± 6	243 ± 8
Diabetic + I	8	126 ± 4	204 ± 6	196 ± 7
Nondiabetic + T ₃	5	126 ± 10	194 ± 9	215 ± 12
Nondiabetic PR	5	118 ± 4	191 ± 6	203 ± 18
<u>WKY</u>				
Nondiabetic	13	131 ± 4	133 ± 3	140 ± 3
Diabetic	9	144 ± 3	123 ± 4	140 ± 7

Values are means ± SE, obtained before renovascular constriction (week 0), 4 weeks after surgery and just before induction of diabetes (week 4), and 8 weeks after initiation of diabetes or treatment (week 12). T₃, triiodothyronine; I, insulin; PR, food restricted.

^Y Significantly different from the nondiabetic WKY.

TABLE 4. Heart rate (HR) of nondiabetic and diabetic RVH and WKY rats.

Group	n	HR (beats/min)		
		Week 0	Week 4	Week 12
<u>RVH</u>				
Nondiabetic	11	312 ± 13	381 ± 20	364 ± 15 ^Y
Diabetic	9	339 ± 16	382 ± 22	310 ± 14 [*]
Diabetic + T ₃	7	295 ± 5	366 ± 26	386 ± 20
Diabetic + I	8	313 ± 8	364 ± 22	325 ± 19
Nondiabetic + T ₃	5	297 ± 6	358 ± 20	324 ± 8
Nondiabetic FR	5	302 ± 5	386 ± 25	310 ± 34 [*]
<u>WKY</u>				
Nondiabetic	13	330 ± 19	316 ± 7	304 ± 9
Diabetic	9	285 ± 8	311 ± 7	282 ± 7

Values are means ± SE, obtained before renovascular constriction (week 0), 4 weeks after surgery and just before induction of diabetes (week 4), and 8 weeks after initiation of diabetes or treatment (week 12). T₃, triiodothyronine; I, insulin; FR, food restricted.

*Significantly different from nondiabetic group (within group).

^Y Significantly different from the nondiabetic WKY.

Table 5. LVPP stroke work, left ventricular contractility (LV+dP/dt) and relaxation (LV-dP/dt) of nondiabetic and diabetic RVH and WKY hearts.

Group	n	LVPP (kPa)	Stroke Work (mJ/g)	LV+dP/dt (kPa/sec)	LV-dP/dt (kPa/sec)
<u>RVH</u>					
Nondiabetic	11	22.38 ± 1.91	2.44 ± 0.34	991 ± 139	666 ± 108
Diabetic	9	17.72 ± 2.29*	1.90 ± 0.43	651 ± 110*	529 ± 99*
Diabetic + T ₃	7	21.51 ± 3.34	2.23 ± 0.72	810 ± 154**	645 ± 112
Diabetic + I	8	24.86 ± 2.13	2.33 ± 0.54	914 ± 81	726 ± 128
Nondiabetic + T ₃	5	24.76 ± 2.38	2.32 ± 0.53	1029 ± 130	741 ± 96
Nondiabetic FR	5	23.23 ± 1.87	2.25 ± 0.37	902 ± 72	689 ± 108
<u>WKY</u>					
Nondiabetic	13	20.40 ± 2.74	2.30 ± 0.59	884 ± 114	582 ± 130
Diabetic	9	17.93 ± 1.88	2.25 ± 0.69	638 ± 90	547 ± 115

Values are means ± SD, obtained 8 weeks after initiation of treatment or diabetes. Left atrial filling pressure was fixed at 20 cm H₂O and resistance to aortic ejection was fixed at 1.54 kPa/cm³/min. Same values are also represented in Figures 1 and 3. LVPP, left ventricular pulse pressure; T₃, triiodothyronine; I, insulin; FR, food restricted.

* Significantly different from nondiabetic group (within group).

** Significantly different from nondiabetic and diabetic groups (within group).

TABLE 6. Myocardial oxygen consumption (VO_2), cardiac output (CO), power and efficiency of contraction (E) of nondiabetic and diabetic RVH and WKY.

Group	n	VO_2 (μLO_2 /Sec/g)	CO (cm^3 /sec/g)	Power (mW/g)	E (mJ/ μLO_2)
<u>RVH</u>					
Nondiabetic	11	5.06 \pm 1.04	0.48 \pm 0.07	9.99 \pm 2.01	2.00 \pm 0.31
Diabetic	9	4.09 \pm 1.34	0.46 \pm 0.09	7.72 \pm 2.22	1.92 \pm 0.54
Diabetic + T_3	7	5.30 \pm 1.31	0.47 \pm 0.10	9.93 \pm 3.11	1.84 \pm 0.24
Diabetic + I	8	4.83 \pm 1.38	0.42 \pm 0.08	9.78 \pm 2.62	2.06 \pm 0.33
Nondiabetic + T_3	5	5.00 \pm 0.85	0.40 \pm 0.06	8.85 \pm 2.15	1.76 \pm 0.26
Nondiabetic FR	5	4.41 \pm 1.09	0.44 \pm 0.07	9.87 \pm 1.64	2.30 \pm 0.47
<u>WKY</u>					
Nondiabetic	13	4.98 \pm 0.94	0.48 \pm 0.07	9.19 \pm 2.04	1.86 \pm 0.36
Diabetic	9	4.96 \pm 1.02	0.56 \pm 0.14	9.71 \pm 3.45	1.97 \pm 0.57

Values are means \pm SD, obtained 8 weeks after initiation of treatment or diabetes. Left atrial filling pressure was fixed at 15 cm H_2O and resistance to aortic ejection was fixed at 1.54 kPa/ cm^3 /min. T_3 , triiodothyronine; I, insulin; FR, food restricted.

TABLE 7. Coronary flow rate (CFR) and coronary vascular resistance (CVR) of nondiabetic and and diabetic RVH and WKY.

Group	n	CFR (cm ³ /sec)	CFR (cm ³ /sec/g)	CVR (kPa/cm ³ /sec/g)
<u>RVH</u>				
Nondiabetic	11	0.45 ± 0.13	0.29 ± 0.07	70.55 ± 5.46 ^Y
Diabetic	9	0.31 ± 0.10 [*]	0.26 ± 0.08	62.92 ± 4.67
Diabetic + T ₃	7	0.37 ± 0.11	0.30 ± 0.08	65.64 ± 8.83
Diabetic + I	8	0.40 ± 0.12	0.27 ± 0.08	72.64 ± 3.68
Nondiabetic + T ₃	5	0.42 ± 0.10	0.26 ± 0.05	79.19 ± 5.56
Nondiabetic FR	5	0.28 ± 0.09 [*]	0.24 ± 0.08	97.02 ± 7.31 [*]
<u>WKY</u>				
Nondiabetic	13	0.44 ± 0.08	0.31 ± 0.05	59.70 ± 4.32
Diabetic	9	0.40 ± 0.10	0.38 ± 0.11	45.05 ± 2.65 [*]

CFR values are means ± SD and CVR values are means ± SE, obtained 8 weeks after initiation of diabetes or treatment. CFR was measured when left atrial filling pressure was fixed at 15 cm H₂O and resistance to aortic ejection was fixed at 1.54 kPa/cm³/min. CVR (normalized for wet heart weight), when hearts were pressure loaded. T₃, triiodothyronine; I, insulin; FR, food restricted.

*Significantly different from nondiabetic group (within group).

^YSignificantly different from the nondiabetic WKY.

FIGURE 1. Cardiac performance ex vivo as measured by left ventricular pulse pressure (LVPP) of nondiabetic (○), diabetic (●), T₃-treated diabetic (▲) and insulin-treated diabetic (■), T₃ treated nondiabetic (△) RVH. The hearts were pressure loaded by varying the resistance to aortic ejection between 0.19 and 3.13 kPa/cm³.min⁻¹. Left atrial filling pressure was fixed at 15 cm H₂O. The number of animals in each group is shown in parenthesis. Values are means ± SD, obtained 8 weeks after initiation of diabetes or treatment.

*Significantly different from the nondiabetic group.

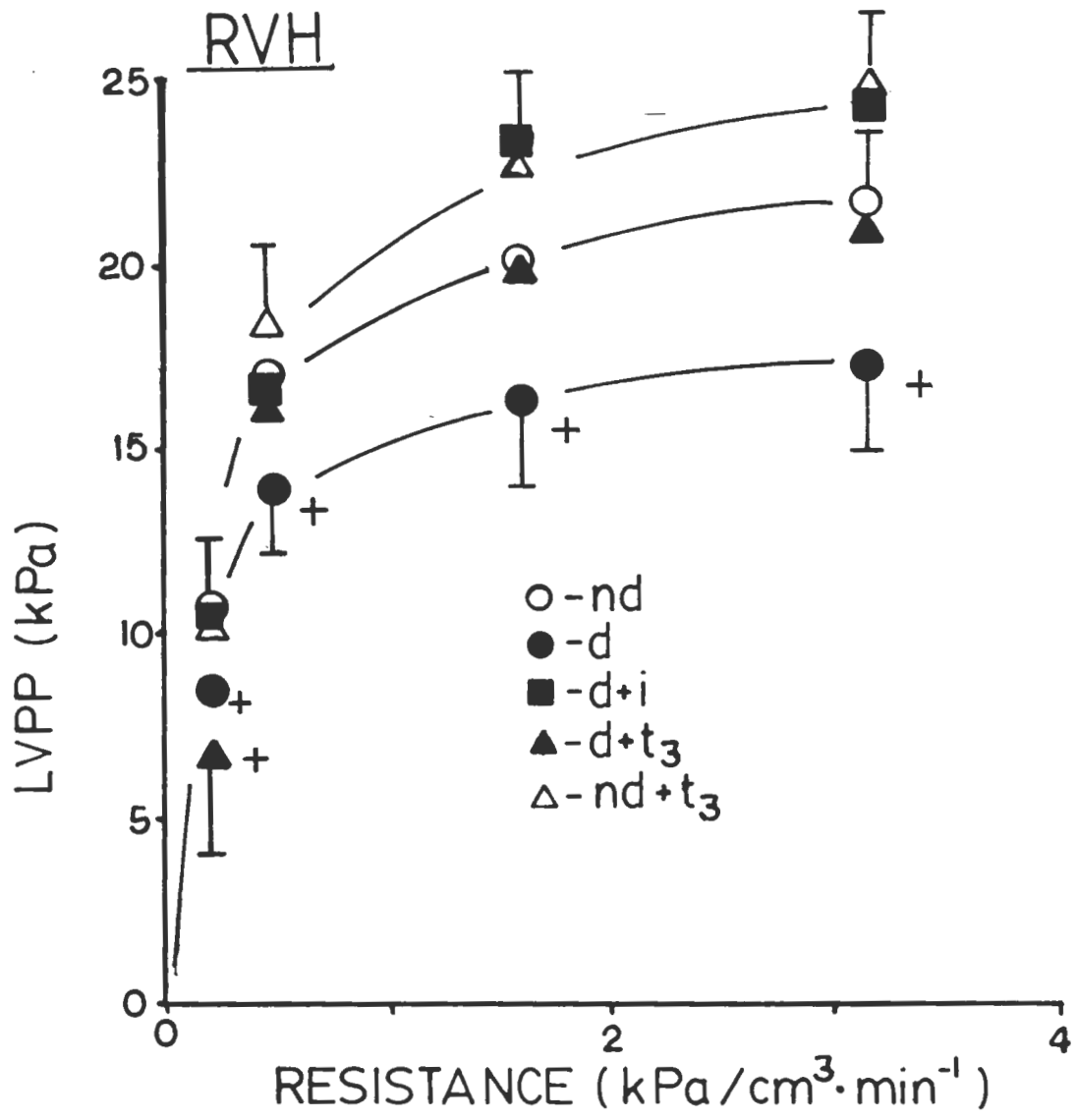
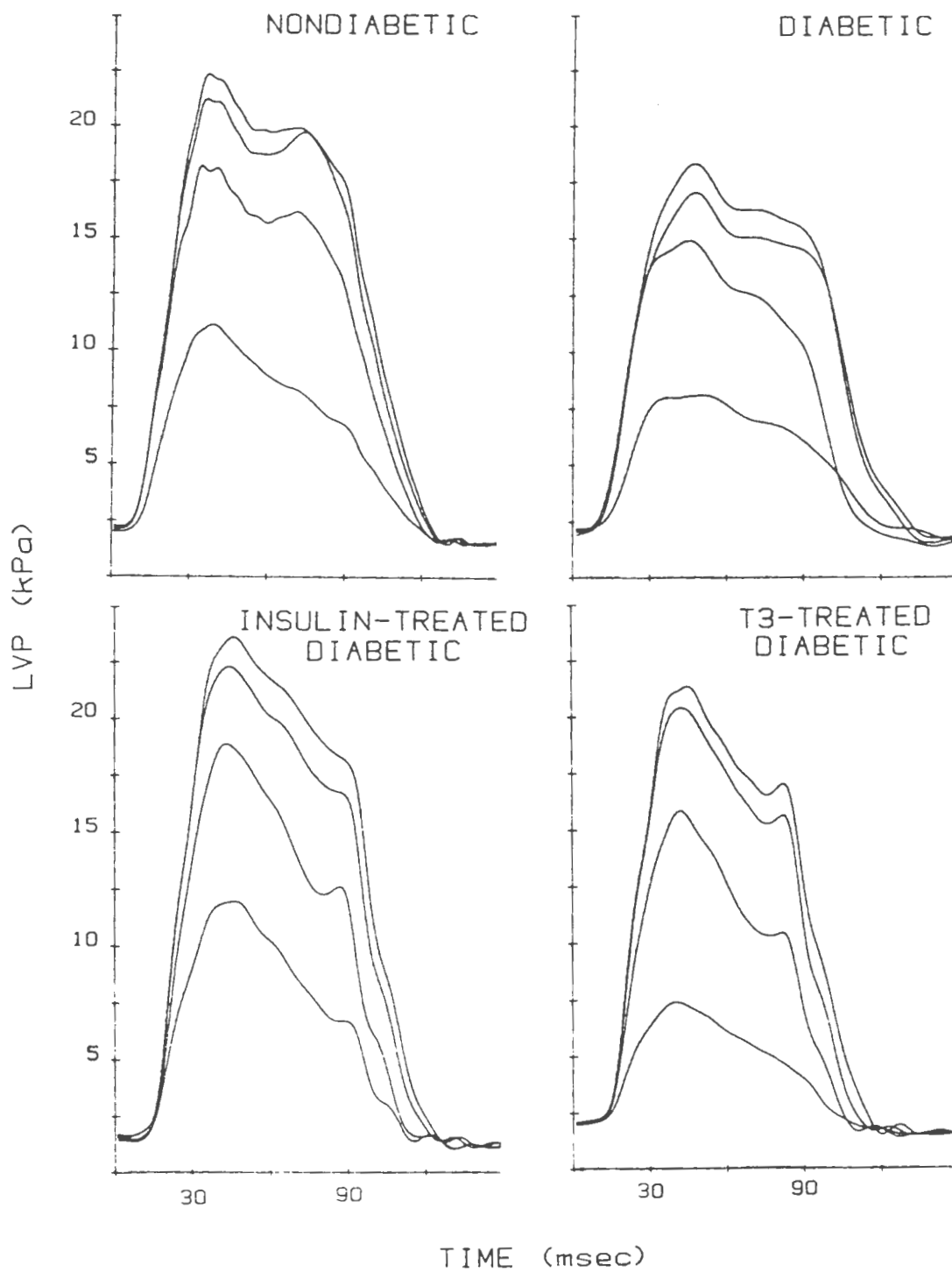


FIGURE 2. Left ventricular pressure (LVP) waves from perfused working hearts of nondiabetic, diabetic, insulin-treated diabetic and T₃-treated diabetic RVH, under increasing pressure-loading conditions. Each of the four superimposed LVP waves was obtained at one of four resistances to aortic ejection: 0.19, 0.41, 1.54 and 3.13 kPa/cm³.min⁻¹. Left atrial filling pressure was fixed at 15 cm H₂O. LVP waves were recorded from a single heart in each group and were obtained eight weeks after initiation of diabetes or treatment. Analog signals were digitized and stored on a diskette and regenerated using an IBM PC II with ASYST software and an HP7470 plotter. Individual records were selected with the aim of approximating, as closely as possible, the mean maximum LVP values for each group as shown in Figure 1.



APPENDIX B: Data Acquisition Programs

```

\ TRIG.GER

\ The following program allows data acquisition to be
\ sampled from channel #1 when the external trigger
\ button on the Narcotrace recorder is pressed.

DT2800
INTEGER SCALAR T.RATE
    20 T.RATE :=

INTEGER DIM[ 250 ] ARRAY DATA.BUF.1 INTEGER DIM[ 250 ] ARRAY
DATA.BUF.2

1 1 A/D.TEMPLATE CHNL.1.IN
    DATA.BUF.1 DATA.BUF.2 CYCLIC DOUBLE.TEMPLATE.BUFFERS

: START.ACQ                                \ Starts data acquisition,
    CHNL.1.IN A/D.INIT                      \ input on channel 1
    3 A/D.GAIN A/D.INIT
    CLEAR.TASKS
    1 TASK A/D.IN>ARRAY
    T.RATE TASK.PERIOD
    PRIME.TASKS
    TRIGGER.TASKS ;

: STOP.ACQ                                  \ Stops data acquisition
    STOP.TASKS
    CLEAR.TASKS ;

: PLOT.IT                                   \ Plots data continually until
    BEGIN                                    \ a key is pressed. This is
    ERASE.LINES                              \ used for checking calibration
    BEGIN ?BUFFER.SWITCH UNTIL              \ of machines
        ?BUFFER.A/B
    IF DATA.BUF.1 ELSE DATA.BUF.2
    THEN 2 217 A/D.SCALE Y.DATA.PLOT
    ?KEY UNTIL ;

REAL DIM[ 5000 ] ARRAY PIXBUF

: SET.GRAPHICS                              \ Sets graphics display for
    GRAPHICS.DISPLAY                        \ checking calibration
    AXIS.FIT.OFF
    HORIZONTAL 0. 250. WORLD.SET
    VERTICAL 0. 150. WORLD.SET
    OUTLINE
    XY.AXIS.PLOT
    PIXBUF LINE.BUFFER.ON ;

: SET.GRAPHICS                              \ Sets graphics display for
    GRAPHICS.DISPLAY                        \ acquired data points
    AXIS.FIT.OFF
    HORIZONTAL 0. 1000. WORLD.SET

```

```

AXIS.FIT.OFF
VERTICAL 0. 250. WORLD.SET
OUTLINE
XY.AXIS.PLOT          \ Show axis and grid lines
PIXBUF LINE.BUFFER.ON ;

: CHECK.IT            \ Allows continual monitoring
  SET.GRAPHICS        \ of input for checking
  START.ACQ           \ calibration
  PLOT.IT             \ DOES NOT STORE DATA!!!!
  STOP.ACQ ;

1 1 a/d.template 5PP EXT.TRIG A/D.INIT
integer dim[ 1000 ] array BEAT
BEAT template.buffer A/D.INIT \ Defines the input channel #1
2. conversion.delay a/d.init \ to be activated by an external
INTEGER SCALAR COUNT        \ trigger signal. Sets array size 0
COUNT :=                   \ (how many data points to be
                             \ collected and stored)

: YUP 5PP EXT.TRIG A/D.INIT
  3 A/D.GAIN A/D.INIT      \ Waits for external trigger to
  STACK.CLEAR              \ be pressed before acquiring data
  BEGIN BELL A/D.IN>ARRAY  \ BELL rings before data is
  ?BUFFER.FULL             \ collected then another BELL after
  UNTIL BELL               \ data collection is complete
  COUNT SUBFILE BEAT ARRAY>FILE
  COUNT . ;

: GRAPHS              \ Graphs acquired data
  BEAT 2 217 A/D.SCALE
  Y.DATA.PLOT ;

14  STRING FILENAME

: CREATE.DATA.FILE    \ Creates a file on a disk (be
  FILE.TEMPLATE       \ sure to specify B:FILENAME)
  15 COMMENTS         \ 15 subfiles are available,
  BEAT []FORM.SUBFILE \ therefore, 15 separate arrays
  15 TIMES            \ can be acquired and stored
  END
  CR ." NAME OF FILE TO CREATE? "
  "INPUT FILENAME " :=
  FILENAME DEFER> FILE.CREATE
  FILE.OPEN ;

: RESTART.IT         \ Adds sequential numbers to number
  BEGIN              \ stack so that subsequent data
  COUNT 1 + COUNT := \ points (arrays) are put in
  YUP                \ consecutive subfiles
  ERASE.LINES
  GRAPHS
  ?KEY UNTIL ;

```



```
: CLEAN.UP
  LINE.BUFFER.OFF
  AXIS.DEFAULTS
  SCREEN.CLEAR
  CR ." Data acquisition finished"
  CR
  BELL ;
```

```
: END.DATA.ACQ
  FILE.CLOSE
  0 COUNT :=
  CLEAN.UP ;
```

F9 FUNCTION.KEY.DOES END.DATA.ACQ

```
: GO.TEAM                                \ Initiates program to create
  CREATE.DATA.FILE                        \ a data file and collect data
  SET.GRAPHS
  RESTART.IT ;
```

```
\ To check machine calibration, type "CHECK.IT (return)".
\ To initiate the program to store data, type "GO.TEAM (return)".
\ To end data acquisition and close data file, press
\   F9 and HIT the external trigger one more time.
\   "OH BOY!"
```

BELL BELL

```

\ EXT.TRG
\ This program allows user to monitor and store
\ electrophysiological data from channel #2
\ (e.g., cardiac action potentials)
\ Data acquisition is controlled by an external trigger signal
\ or continually monitored for calibration

```

DT2800

```

: INSTRUCTIONS
CR ." <F3> End data acquisition      <F4> Continual display "
CR ." <F5> Create a data file        <F6> Instructions      "
CR ." <F7> Change sensitivity        <F8> Set graphics      "
CR ." <F9> View potentials           <F10> Add to data file " ;

```

REAL SCALAR DEFAULT.NUMBER

```

: SENSITIVITY
CR ." Select sensitivity, e.g., .2 or .5 (else .125) "
.125 DEFAULT.NUMBER :=
#INPUT                \ Asks user for the desired
IF                    \ sensitivity, default value
  DEFAULT.NUMBER :=   \ was good for AJD (8/89)
THEN
INSTRUCTIONS ;

```

REAL DIM[5000] ARRAY PIXBUF

```

: SET.GRAPHICS          \ Set axes to plot acquired
  VUPORT.CLEAR          \ data points (arrays)
  0. 200.  HORIZONTAL WORLD.SET
 -.125 .025 VERTICAL   WORLD.SET
  XY.AXIS.PLOT
  PIXBUF LINE.BUFFER.ON ;

```

```

2 2 A/D.TEMPLATE CHNL2 EXT.TRIG  \ Define input channel #2
INTEGER DIM[ 200 ] ARRAY APS     \ controlled by external
APS TEMPLATE.BUFFER             \ trigger signal

```

INTEGER SCALAR COUNT

0 COUNT :=

14 STRING FILENAME

```

: CREATE.DATA.FILE      \ Create a data file to store
  FILE.TEMPLATE         \ acquired data points
  APS [ ]FORM.SUBFILE   \ Prompts user for file name
  30 TIMES              \ BE SURE to specify B:FILENAME!
  END                  \ Allows 30 ARRAYS to be collected
CR ." Name of file to create? "
"INPUT FILENAME " :=
FILENAME DEFER> FILE.CREATE
FILE.OPEN ;

```

```

: CLEAN.UP                                \ Resets default values
  LINE.BUFFER.OFF
  AXIS.DEFAULTS
  SCREEN.CLEAR
  VUPORT.CLEAR
  CR ." Data acquisition finished, YEAH!?"
  CR
  BELL ;

: END.DATA.ACQ                             \ Ends data acquisition,
  FILE.CLOSE                               \ closes file and resets
  0 COUNT :=                               \ count to equal 0
  CLEAN.UP ;

: START.AGAIN                              \ Initiates data collection
  CHNL2                                     \ BELL rings when complete
  3 A/D.GAIN A/D.INIT
  BEGIN                                     \ DOES NOT STORE DATA!
  A/D.IN>ARRAY
  ?BUFFER.FULL
  UNTIL BELL ;

: GRAPH.IT                                 \ Scales and plots acquired data
  APS DEFAULT.NUMBER -1 *
  DEFAULT.NUMBER A/D.SCALE
  Y.DATA.PLOT ;

: ADD.TO.DATAFILE                          \ Collects, STORES and graphs data
  COUNT 1 + COUNT :=
  START.AGAIN
  COUNT SUBFILE APS ARRAY>FILE BELL
  COUNT .
  GRAPH.IT ;

: VIEW.IT                                  \ Collects data (without storing)
  START.AGAIN                               \ and graphs it
  GRAPH.IT ;

: GET.IT                                    \ Used to check stored data
  SUBFILE APS FILE>ARRAY                   \ Put number on number stack
  APS DEFAULT.NUMBER -1 *                 \ (type a number and return)
  DEFAULT.NUMBER A/D.SCALE               \ then GET.IT
  Y.DATA.PLOT ;

INTEGER DIM[ 200 ] ARRAY APS.1

2 2 A/D.TEMPLATE CHNL1                    \ Defines channel #2 as cyclical APS.1
CYCLIC TEMPLATE.BUFFER                   \ in order to continually plot
                                           \ BUT NOT STORE data
INTEGER SCALAR T.RATE                     \ Use to check calibration
  2. T.RATE :=

: SET.UP
  CHNL1

```

```

3 A/D.GAIN A/D.INIT
CLEAR.TASKS
1 TASK A/D.IN>ARRAY
T.RATE TASK.PERIOD
PRIME.TASKS
TRIGGER.TASKS ;

: START.ACQ                                \ Starts data acquisition
BEGIN                                      \ continually plots input
  ERASE.LINES                              \ data until a key is pressed
  BEGIN ?BUFFER.SWITCH UNTIL
  APS.1 DEFAULT.NUMBER -1 *
  DEFAULT.NUMBER A/D.SCALE
  Y.DATA.PLOT
?KEY UNTIL BELL ;

: STOP.ACQ                                  \ Stops data acquisition
STOP.TASKS
CLEAR.TASKS ;

: CONTINUOUS                               \ Initiates program to continually
SET.UP                                     \ monitor input, WITHOUT storing
START.ACQ                                  \ the data
STOP.ACQ
CLEAR.TASKS ;

20 0 24 79 WINDOW {BOT}                   \ Defines vuport space for
                                           \ instructions

: GO.TEAM                                   \ Initiates program to create a
CREATE.DATA.FILE                           \ data file, set sensitivity, set
SENSITIVITY                                 \ graphics display, present
DEFAULT.NUMBER                              \ instructions
GRAPHICS.DISPLAY
SCREEN.CLEAR
{BOT}
0 .20 VUPORT.ORIG                           \ Save bottom four lines
1 .80 VUPORT.SIZE                            \ for text
INSTRUCTIONS ;

F3 FUNCTION.KEY.DOES END.DATA.ACQ
F4 FUNCTION.KEY.DOES CONTINUOUS
F5 FUNCTION.KEY.DOES CREATE.DATA.FILE
F6 FUNCTION.KEY.DOES INSTRUCTIONS
F7 FUNCTION.KEY.DOES SENSITIVITY
F8 FUNCTION.KEY.DOES SET.GRAPHICS
F9 FUNCTION.KEY.DOES VIEW.IT
F10 FUNCTION.KEY.DOES ADD.TO.DATAFILE

```

BELL BELL

```

\ To initiate program, type "GO.TEAM (return)".
\ If you are not sure what to do, type "INSTRUCTIONS (return)"
\ at any time to refresh instruction screen

```

\ If you ever want to overwrite collected data, type "# COUNT :=
\ (return)". Specify the number (#) corresponding to the subfile
\ PRIOR TO the one you want to write over (e.g., 3 COUNT := will
\ set count to 3, when EXT TRIG is pressed, data will be stored
\ in subfile #4
\ To end data acquisition and close file press (F3)
\ Program allows for 30 arrays to be stored
\ GOOD LUCK MR/MS PHELPS!

\ ANALYZE.LVP

\ Program allows user to look at stored data points (arrays)
\ within a file, plot the arrays, extract a portion (300 msec),
\ plot subset on HP plotter, and store that subset in a
\ SEPARATE file

DT2800

```
: LOOK.SEE                \ Sets up axes to view stored
  GRAPHICS.DISPLAY        \ LVPs (arrays) in data file
  AXIS.DEFAULTS
  HORIZONTAL 0 1000 WORLD.SET
  VERTICAL 0 180 WORLD.SET
  GRAPHICS.READOUT
  NORMAL.COORDS
  .7 .975 READOUT>POSITION
  XY.AXIS.PLOT ;

: SUBPLOT.AXES            \ Sets up axes to view subsets
  VUपोर्ट.CLEAR          \ of LVPs in data file
  HORIZONTAL 0 270 WORLD.SET
  VERTICAL 0 180 WORLD.SET
  XY.AXIS.PLOT ;

: LABEL.PLOT             \ Defines coordinates, axes and
  NORMAL.COORDS          \ labels axes on HP plotter
  .5 .05 POSITION
  "   TIME (msec)" LABEL
  90 LABEL.DIR
  .025 .4 POSITION
  "   LVP (mmHg)" LABEL
  WORLD.COORDS ;

45 STRING ANIMAL

: HEADER                 \ Asks for title name for the
  NORMAL.COORDS         \ graph to be plotted
  0 LABEL.DIR
  .4 .95 POSITION
  CR ." Animal number and group? "
  "INPUT ANIMAL " :=
  ANIMAL LABEL ;

: HP.PLOTTER             \ Plots axes, labels and title
  HP7470
  PLOTTER.DEFAULTS
  SUBPLOT.AXES
  LABEL.PLOT
  HEADER ;

: PLOT.IT                \ Plot a named array (LVP) either
  Y.DATA.PLOT           \ on CRT screen or on HP plotter
  STACK.CLEAR ;
```

```
INTEGER DIM[ 1000 ] ARRAY LVP
```

```
: GET.IT                                \ Waits for a number on number  
  SUBFILE LVP FILE>ARRAY                \ stack (e.g., 1 for subfile 1  
  LVP 0 200 A/D.SCALE                    \ which contains FP15), reads  
;                                          \ the array and plots it
```

```
SCALAR RANGES
```

```
: RESCALE.IT                            \ Allows user to rescale raw  
  ." Range for scaling? "                \ data stored in files, if  
  #INPUT RANGES :=                       \ calibration was incorrect  
  SUBFILE LVP FILE>ARRAY  
  LVP 0 RANGES A/D.SCALE ;
```

```
INTEGER DIM[ 300 ] ARRAY FP15  
INTEGER DIM[ 300 ] ARRAY FP20  
INTEGER DIM[ 300 ] ARRAY FP10  
INTEGER DIM[ 300 ] ARRAY FP5  
INTEGER DIM[ 300 ] ARRAY R3  
INTEGER DIM[ 300 ] ARRAY R4  
INTEGER DIM[ 300 ] ARRAY R2  
INTEGER DIM[ 300 ] ARRAY R1
```

```
SCALAR STARTING.PT
```

```
: DEFINE.IT                              \ Defines subset of arrays in  
  CR ." Starting point? "                \ data file (for area analysis)  
  #INPUT STARTING.PT :=                  \ specify starting point (X-axis)  
  SUB[ STARTING.PT , 300 ]  
  CR ." Array Name? "                    \ Name the subset (e.g., FP15 or  
;                                          \ R3)
```

```
14 STRING FILENAME
```

```
INTEGER DIM[ 300 ] ARRAY LVPP
```

```
: CREATE.SUB.FILE                        \ Create a file to store the  
  FILE.TEMPLATE                          \ defined subsets  
  8 COMMENTS  
  LVPP [ ]FORM.SUBFILE  
  8 TIMES  
  END CR ." Name of file to create? "  
  "INPUT FILENAME " :=  
  FILENAME DEFER> FILE.CREATE FILE.OPEN  
  " FP15" 1 >COMMENT  
  " FP20" 2 >COMMENT  
  " FP10" 3 >COMMENT  
  " FP5" 4 >COMMENT  
  " R3" 5 >COMMENT  
  " R4" 6 >COMMENT  
  " R2" 7 >COMMENT  
  " R1" 8 >COMMENT  
  BELL ;
```

```
: WRITE.IT          \ Stores defined subsets into
1 SUBFILE FP15 ARRAY>FILE \ newly created file
2 SUBFILE FP20 ARRAY>FILE
3 SUBFILE FP10 ARRAY>FILE
4 SUBFILE FP5  ARRAY>FILE
5 SUBFILE R3   ARRAY>FILE
6 SUBFILE R4   ARRAY>FILE
7 SUBFILE R2   ARRAY>FILE
8 SUBFILE R1   ARRAY>FILE
BELL ;
```

```
F2 FUNCTION.KEY.DOES RESCALE.IT
F3 FUNCTION.KEY.DOES PLOT.IT
F4 FUNCTION.KEY.DOES CREATE.SUB.FILE
F5 FUNCTION.KEY.DOES SUBPLOT.AXES
F6 FUNCTION.KEY.DOES LOOK.SEE
F7 FUNCTION.KEY.DOES GET.IT
F8 FUNCTION.KEY.DOES DEFINE.IT
F9 FUNCTION.KEY.DOES HP.PLOTTER
F10 FUNCTION.KEY.DOES WRITE.IT
```

BELL BELL

```
\ To initiate program, open data file in drive B:
\ (FILE.OPEN B:filename).
\ Plot LOOK.SEE axes (F6), type a number corresponding to desired
\ subfile, and plot it or GET.IT (F7).
\ Cursor can be used to determine starting point for subset
\ (position cursor and press HOME KEY for read out - see ASYST
\ manual for more detail on cursor movement).
\ Before creating a new file for the subset arrays, be sure to
\ close the parent file (FILE.CLOSE), then CREATE.SUB.FILE
\ (F4).
```


\ AREA.PLT

\ Program allows user to call up (GET.IT) stored subfiles,
\ graph the arrays, and move cursor around to outline LVP wave
\ (e.g., from Pmax to max EDP), in order to calculate the
\ area defined

DT2800

COMPLEX SCALAR Z0 REAL
SCALAR AREA AXIS.DEFAULTS

```
: NEXT.POINT
  SWAP CONJ
  OVER *
  ZIMAG
  2. /
  AREA + AREA := ;
```

```
: POINT
  ?CURSOR
  3 PICK
  ZRE&IM POSITION
  DRAW.TO
  ?CURSOR
  Z=X+IY
  NEXT.POINT ;
```

```
: FIRST.POINT
  ?CURSOR
  Z=X+IY
  DUP Z0 :=
  0. AREA :=
  BELL ;
```

```
: LAST.POINT
  POINT
  Z0 ZRE&IM DRAW.TO
  Z0 NEXT.POINT DROP
  GRAPHICS.READOUT
  CR AREA .
  BELL
  STACK.CLEAR ;
```

```
: SUBPLOT.AXES
  GRAPHICS.DISPLAY
  HORIZONTAL 0 270 WORLD.SET
  VERTICAL 0 180 WORLD.SET
  GRAPHICS.READOUT
  NORMAL.COORDS
  .7 .975 READOUT>POSITION
  WORLD.COORDS
  XY.AXIS.PLOT ;
```

```
: PLOT.IT
```

```
Y.DATA.PLOT  
ARRAY.READOUT ;
```

```
INTEGER DIM[ 300 ] ARRAY WAVES
```

```
: GET.IT                \ Reads the subfile specified by  
  SUBFILE WAVES FILE>ARRAY \ the number placed on number stack  
  WAVES  
  PLOT.IT ;
```

```
F1 FUNCTION.KEY.DOES FIRST.POINT  
F2 FUNCTION.KEY.DOES POINT  
F3 FUNCTION.KEY.DOES LAST.POINT  
F5 FUNCTION.KEY.DOES SUBPLOT.AXES  
F7 FUNCTION.KEY.DOES GET.IT  
F8 FUNCTION.KEY.DOES PLOT.IT
```

```
BELL BELL
```

```
\ NOTE: See ASYST manual for more detailed instruction about  
\ cursor movement.  
\ ARROWS on key pad moves cursor.  
\ HOME KEY gives read out of present cursor position.  
\ PG UP changes the increment of cursor movement, e.g., strike  
\ once = slowest or smallest distance moved, strike multiple  
\ times in succession = larger increments.
```

\ ANALYZE.APS

\ This program allows user to analyze the area of AP records,
\ AP durations, RMPs, dV/dt's, and plot APs on HP plotter
\ Type the word "INSTRUCTIONS" to list function key assignments

DT2800

COMPLEX SCALAR Z0 REAL SCALAR AREA

```
: NEXT.POINT
  SWAP CONJ
  OVER *
  ZIMAG
  2. /
  AREA + AREA := ;

: POINT
  ?CURSOR
  3 PICK
  ZRE&IM POSITION
  DRAW.TO
  ?CURSOR
  Z=X+IY
  NEXT.POINT ;

: FIRST.POINT
  ?CURSOR
  Z=X+IY
  DUP Z0 :=
  0. AREA :=
  BELL ;

: LAST.POINT
  POINT
  Z0 ZRE&IM DRAW.TO
  Z0 NEXT.POINT DROP
  GRAPHICS.READOUT
  CR AREA .
  BELL
  STACK.CLEAR ;

: GRAPHS
  VUPOINT.CLEAR
  AXIS.DEFAULTS
  HORIZONTAL 0 200 WORLD.SET
  VERTICAL -125 25 WORLD.SET
  GRAPHICS.READOUT
  NORMAL.COORDS
  .7 .975 READOUT>POSITION
  XY.AXIS.PLOT ;

: LABEL.PLOT
  NORMAL.COORDS
```

```
.5 .05 POSITION
" TIME (msec)" LABEL
90 LABEL.DIR
.025 .4 POSITION
" MP (mV)" LABEL
WORLD.COORDS ;
```

45 STRING ANIMAL

```
: HEADER
NORMAL.COORDS
0 LABEL.DIR
.4 .95 POSITION
CR ." Animal number and group? "
"INPUT ANIMAL " :=
ANIMAL LABEL
WORLD.COORDS ;
```

```
: HP.PLOTTER
HP7470
PLOTTER.DEFAULTS
VUPORT.CLEAR
HORIZONTAL 0 200 WORLD.SET
VERTICAL -125 25 WORLD.SET
XY.AXIS.PLOT
LABEL.PLOT
HEADER ;
```

INTEGER DIM[200] ARRAY APS INTEGER DIM[200] ARRAY AP

```
: GET.IT
0 AP :=
SUBFILE APS FILE>ARRAY
APS -125 125 A/D.SCALE
AP := ;
```

.5 SET.CUTOFF.FREQ 1 SET.#.OPTIMA

```
: VMAX
DIFFERENTIATE.DATA ;
```

```
INTEGER DIM[ 200 ] ARRAY AP1
INTEGER DIM[ 200 ] ARRAY AP2
INTEGER DIM[ 200 ] ARRAY AP3
INTEGER DIM[ 200 ] ARRAY AP4
INTEGER DIM[ 200 ] ARRAY AP5
INTEGER DIM[ 200 ] ARRAY AP6
INTEGER DIM[ 200 ] ARRAY AP7
INTEGER DIM[ 200 ] ARRAY AP8
INTEGER DIM[ 200 ] ARRAY AP9
INTEGER DIM[ 200 ] ARRAY AP10
INTEGER DIM[ 200 ] ARRAY AP11
INTEGER DIM[ 200 ] ARRAY AP12
```

```
INTEGER DIM[ 200 ] ARRAY AP13
INTEGER DIM[ 200 ] ARRAY AP14
INTEGER DIM[ 200 ] ARRAY AP15
INTEGER DIM[ 200 ] ARRAY AP16
INTEGER DIM[ 200 ] ARRAY AP17
INTEGER DIM[ 200 ] ARRAY AP18
INTEGER DIM[ 200 ] ARRAY AP19
INTEGER DIM[ 200 ] ARRAY AP20
INTEGER DIM[ 200 ] ARRAY AP21
INTEGER DIM[ 200 ] ARRAY AP22
INTEGER DIM[ 200 ] ARRAY AP23
INTEGER DIM[ 200 ] ARRAY AP24
INTEGER DIM[ 200 ] ARRAY AP25
INTEGER DIM[ 200 ] ARRAY AP26
INTEGER DIM[ 200 ] ARRAY AP27
INTEGER DIM[ 200 ] ARRAY AP28
INTEGER DIM[ 200 ] ARRAY AP29
INTEGER DIM[ 200 ] ARRAY AP30
```

```
: GET.THEM
```

```
1  SUBFILE AP1  FILE>ARRAY
   AP1 -125 125 A/D.SCALE AP1 :=
2  SUBFILE AP2  FILE>ARRAY
   AP2 -125 125 A/D.SCALE AP2 :=
3  SUBFILE AP3  FILE>ARRAY
   AP3 -125 125 A/D.SCALE AP3 :=
4  SUBFILE AP4  FILE>ARRAY
   AP4 -125 125 A/D.SCALE AP4 :=
5  SUBFILE AP5  FILE>ARRAY
   AP5 -125 125 A/D.SCALE AP5 :=
6  SUBFILE AP6  FILE>ARRAY
   AP6 -125 125 A/D.SCALE AP6 :=
7  SUBFILE AP7  FILE>ARRAY
   AP7 -125 125 A/D.SCALE AP7 :=
8  SUBFILE AP8  FILE>ARRAY
   AP8 -125 125 A/D.SCALE AP8 :=
9  SUBFILE AP9  FILE>ARRAY
   AP9 -125 125 A/D.SCALE AP9 :=
10 SUBFILE AP10 FILE>ARRAY
   AP10 -125 125 A/D.SCALE AP10 :=
11 SUBFILE AP11 FILE>ARRAY
   AP11 -125 125 A/D.SCALE AP11 :=
12 SUBFILE AP12 FILE>ARRAY
   AP12 -125 125 A/D.SCALE AP12 :=
13 SUBFILE AP13 FILE>ARRAY
   AP13 -125 125 A/D.SCALE AP13 :=
14 SUBFILE AP14 FILE>ARRAY
   AP14 -125 125 A/D.SCALE AP14 :=
15 SUBFILE AP15 FILE>ARRAY
   AP15 -125 125 A/D.SCALE AP15 :=
16 SUBFILE AP16 FILE>ARRAY
   AP16 -125 125 A/D.SCALE AP16 :=
17 SUBFILE AP17 FILE>ARRAY
   AP17 -125 125 A/D.SCALE AP17 :=
```

```

18 SUBFILE AP18 FILE>ARRAY
    AP18 -125 125 A/D.SCALE AP18 :=
19 SUBFILE AP19 FILE>ARRAY
    AP19 -125 125 A/D.SCALE AP19 :=
20 SUBFILE AP20 FILE>ARRAY
    AP20 -125 125 A/D.SCALE AP20 :=
21 SUBFILE AP21 FILE>ARRAY
    AP21 -125 125 A/D.SCALE AP21 :=
22 SUBFILE AP22 FILE>ARRAY
    AP22 -125 125 A/D.SCALE AP22 :=
23 SUBFILE AP23 FILE>ARRAY
    AP23 -125 125 A/D.SCALE AP23 :=
24 SUBFILE AP24 FILE>ARRAY
    AP24 -125 125 A/D.SCALE AP24 :=
25 SUBFILE AP25 FILE>ARRAY
    AP25 -125 125 A/D.SCALE AP25 :=
26 SUBFILE AP26 FILE>ARRAY
    AP26 -125 125 A/D.SCALE AP26 :=
27 SUBFILE AP27 FILE>ARRAY
    AP27 -125 125 A/D.SCALE AP27 :=
28 SUBFILE AP28 FILE>ARRAY
    AP28 -125 125 A/D.SCALE AP28 :=
29 SUBFILE AP29 FILE>ARRAY
    AP29 -125 125 A/D.SCALE AP29 :=
30 SUBFILE AP30 FILE>ARRAY
    AP30 -125 125 A/D.SCALE AP30 :=
BELL ;

: INSTRUCTIONS
CR ."                                <F4> Plot array          "
CR ." <F5> Get one array from file   <F6> Vmax for array     "
CR ." <F7> Plotter                   <F8> Set graphics      "
CR ." <F9> Get all arrays from file  <F10> Locate Vmax     "
;

: PLOT.IT
Y.DATA.PLOT
STACK.CLEAR ;

: LOCATE.IT
LOCAL.MAXIMA
POSITION ;

20 0 24 79 WINDOW {BOT}

: GO.TEAM
GRAPHICS.DISPLAY
SCREEN.CLEAR
{BOT}
0 .20 VUPORT.ORIG
1 .80 VUPORT.SIZE
INSTRUCTIONS ;

F1 FUNCTION.KEY.DOES FIRST.POINT

```

F2 FUNCTION.KEY.DOES POINT
F3 FUNCTION.KEY.DOES LAST.POINT
F4 FUNCTION.KEY.DOES PLOT.IT
F5 FUNCTION.KEY.DOES GET.IT
F6 FUNCTION.KEY.DOES VMAX
F7 FUNCTION.KEY.DOES HP.PLOTTER
F8 FUNCTION.KEY.DOES GRAPHS
F9 FUNCTION.KEY.DOES GET.THEM
F10 FUNCTION.KEY.DOES LOCATE.IT

\ Type GO.TEAM to initiate program or to return graphics to
\ the CRT rather than the plotter

BELL BELL

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