Ventricular Relaxation of Diabetic Spontaneously Hypertensive Rat

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Diabetes, and possibly the hypothyroidism that 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 units/kg/day) or triiodothyronine (8–10 μg/kg/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 indexes of relaxation. The results suggest that 1) hypertrophic ventricles of spontaneously hypertensive rats are more susceptible to the detrimental effects of diabetes on relaxation indexes than are the nonhypertrophic Wistar-Kyoto rat ventricles, and 2) the hypothyroidism that attends diabetes may contribute to the impaired relaxation of diabetic spontaneously hypertensive rat left ventricle. (Hypertension 1990;15:643–651)

Impaired mechanical relaxation is an important component of the adverse effects of diabetes on cardiac performance.\(^1\)--\(^3\) 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 muscle\(^1\) 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 relaxation.\(^6\) Diabetes often depresses SR calcium uptake and the activity of the SR Ca\(^{2+}\)–Mg\(^{2+}\) adenosine triphosphatase (ATPase) in rat ventricle.\(^7\)--\(^10\)

Recent evidence suggests that left ventricular hypertrophy (LVH) exacerbates functional defects of diabetic myocardium,\(^1,11--12\) including those that may have an impact on ventricular relaxation. The presence of LVH alone may or may not be associated with impaired relaxation.\(^14\)--\(^17\) 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 with the effects of diabetes in normotensive rat strains.\(^11\)--\(^13\) Isometric relaxation is also prolonged in hypertrophic papillary muscles from the diabetic renovascular hypertensive rat.\(^12\) 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 relation 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 ventricles.\(^20\) Treatment of the diabetic SHR with triiodothyronine (T\(_3\)) effectively prevents the resultant cardiac dysfunction,\(^21,22\) but the influence of T\(_3\) treatment in nonhypertrophic diabetic rat models is inconsistent.\(^23--25\)
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 indexes 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 Labs., Inc. (Wilmington, Massachusetts) and then housed communally by strain and fed ad libitum. At 15 weeks of age, rats were weight matched and divided into either diabetic or nondiabetic groups. Diabetes was induced in lightly etherized rats by a single tail vein injection of streptozotocin (STZ) (Sigma Chemical Co., St. Louis, Missouri). The dose of STZ for each strain was adjusted to induce similar degrees of diabetes as assessed by serum glucose concentrations. Based on preliminary results, equieffective doses of STZ in the SHR and WKY rat strains, as indexed by serum glucose elevations, were determined to be 45 mg STZ/kg body wt and 50 mg STZ/kg body wt, respectively. The STZ was dissolved in citrate buffer (0.1 M at pH 4.5) just before use. The nondiabetic rats were also etherized and then injected with comparable volumes of the citrate buffer alone. Urinary glucose was detected in all rats of the diabetic groups at pH 4.5 just before use. The nondiabetic rats were killed 8 weeks later.

The diabetic SHR were subdivided into three subgroups corresponding to the treatment regimens initiated 3 days after STZ injection: 1) protamine zinc insulin (10 units/kg/day s.c.; 1) (Eli Lilly Co., Indianapolis, Indiana), 2) T$_3$ (8–10 µg/kg/day s.c.) (Sigma Chemical Co.), and 3) untreated. We observed initially that treatment of diabetic SHR with 10 µg/kg/day T$_3$ was sufficient to just reverse the bradycardia in vivo. After 6 weeks of T$_3$ treatment, the dose was reduced to 8 µg/kg/day to avoid a progressive T$_3$-induced tachycardia. The nondiabetic SHR were subdivided into two groups of untreated and T$_3$-treated (8–10 µg/kg/day s.c.) rats. Treatment of diabetic WKY rats with either insulin or T$_3$ was unnecessary because initial studies had shown that diabetes had little or no effect on mechanical indexes of ventricular relaxation in this strain (see Results).

**In Vivo Measurements**

Systolic arterial pressure, heart rate, and body weight were taken just before the tail vein injections and then 4 and 8 weeks later. A standard tail-cuff sphygmomanometer in a temperature-controlled chamber (34° C) was used to measure systolic arterial pressure and heart rate, after the rats were prewarmed for 10–15 minutes. All measurements were recorded before the daily injections of T$_3$ or insulin.

**Serum Assays**

Eight weeks after they were injected with STZ or citrate buffer, the rats were rapidly decapitated, and their serum was collected and stored at -80° C. The rats received the final injection on the day before they were killed. The degree of diabetes was evaluated by serum glucose concentration with a glucose oxidase assay (Sigma Chemical Co.). Rats were classified as diabetic if serum glucose values exceeded 300 mg/dl. Radioimmunoassays were used to determine serum insulin (Micromedic Systems Inc., Horsham, Pennsylvania) and total T$_3$ and thyroxine (T$_4$) concentrations (Cambridge Medical Diagnostics, Billerica, Massachusetts). Rat insulin was generously supplied by Dr. R. Chance (Eli Lilly Co., Indianapolis, Indiana) and was used as the standard for the insulin assays.

**Heart Perfusion**

A subset of each rat group was used to assay left ventricular relaxation of isolated perfused working hearts. The hearts were quickly excised and perfused as described previously. In brief, the perfusion apparatus was designed to manipulate left ventricular 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, Puerto Rico). The aorta was tied to a 14 gauge cannula and the heart was first perfused in a retrograde fashion for 3–5 minutes, 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 (mM) was NaCl 120, KC1 5.6, MgSO$_4$ 0.65, NaH$_2$PO$_4$ 1.21, NaHCO$_3$ 25, CaCl$_2$ 2.4, EDTA 0.2, and glucose 10 (pH 7.4 when warmed and gassed with 95% O$_2$ and 5% CO$_2$). Hearts were paced at 300 beats/min with a bipolar stimulating electrode placed on the right atrium. Before data collection, LVP was monitored in the working heart continually for 10–15 minutes on a Narcotrace Model 80 chart recorder (Narco Biosystems, Houston, Texas) interfaced with a dedicated digital computer (Buxco Corp., Sharon, Connecticut) to ensure stability to each preparation. During this time, left atrial filling pressure (LAFP) was fixed at 15 cm H$_2$O and resistance to aortic ejection was set at 1.54 kPa/cm$^3$/min. Aortic flow could be directed through one of four segments of PE tubing, the length and diameter of which was varied to impose a final resistance of either 0.19, 0.41, 1.54, or 3.13 kPa/cm$^3$/min (calibrated at constant flow).
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 relaxation. 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 400.25 _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, left ventricular including the septum, and right ventricular. These heart weights were used to assess the degree of ventricular hypertrophy.

**Sarcoplasmic Reticulum 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. 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 2,000g. The supernatant was then centrifuged at 8,700g for

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**TABLE 1. Serum Glucose, Insulin, Triiodothyronine, and Thyroxine Values of Nondiabetic and Diabetic Rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Glucose (mg/dl)</th>
<th>Insulin (µunits/ml)</th>
<th>T$_3$ (ng/dl)</th>
<th>T$_4$ (µg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR Nondiabetic</td>
<td>20</td>
<td>124±20</td>
<td>68±26</td>
<td>99±19*</td>
<td>5.52±1.93*</td>
</tr>
<tr>
<td>Diabetic</td>
<td>18</td>
<td>580±65†</td>
<td>16±6†</td>
<td>47±13†</td>
<td>2.12±0.76†</td>
</tr>
<tr>
<td>Diabetic+T$_3$</td>
<td>18</td>
<td>615±77†</td>
<td>25±14†</td>
<td>118±64</td>
<td>0.21±0.12‡</td>
</tr>
<tr>
<td>Diabetic+I</td>
<td>16</td>
<td>542±196†</td>
<td>287±165‡</td>
<td>71±23‡</td>
<td>4.12±1.60‡</td>
</tr>
<tr>
<td>Nondiabetic+T$_3$</td>
<td>15</td>
<td>132±27</td>
<td>69±15</td>
<td>109±50</td>
<td>0.27±0.15‡</td>
</tr>
<tr>
<td>WKY Nondiabetic</td>
<td>21</td>
<td>149±34</td>
<td>77±21</td>
<td>86±13</td>
<td>4.62±1.12</td>
</tr>
<tr>
<td>Diabetic</td>
<td>16</td>
<td>567±118†</td>
<td>22±9†</td>
<td>41±11†</td>
<td>2.60±0.68†</td>
</tr>
</tbody>
</table>

Values represent mean±SD obtained 8 weeks after initiation of diabetes or treatment. T$_3$, triiodothyronine; T$_4$, thyroxine; SHR, spontaneously hypertensive rats; I, protamine zinc insulin; WKY, Wistar-Kyoto rats.

*Significantly different from the nondiabetic WKY rats.
†Significantly different from the nondiabetic group (within strain).
‡Significantly different from the nondiabetic and diabetic groups (within strain).
The pellet was resuspended in 10 ml of 0.6 M KC1 and centrifuged a third time at 50,000g for 30 minutes. Another supernatant was centrifuged a third time at 50,000g for 15 minutes. The second supernatant was used to characterize in the presence and absence of sodium azide, KC1, adenosine triphosphate (ATP), and nonradioactive CaCl2. The final concentration of free calcium concentrations were calculated to range between 0.18 and 0.57 μM.

Calcium uptake was determined by using the Millipore filtration technique. Cardiac microsomes were preincubated for 3 minutes with 2-3 ml of 50 mM KC1 and 10 mM imidazole (pH 6.8) and centrifuged at 120,000g. Filters were dried and then counted in a liquid scintillation counter in 5 ml scintillation fluid (BetaBlend, ICN Radiochemicals, Irvine, California). Protein concentrations were determined by the method of Lowry et al.27 Rates of SR calcium uptake were expressed as micromoles per milligram protein per minute. 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, North Carolina). 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 terms 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, that was equivalent in the SHR and WKY rat strains (Table 1). The diabetic rats were characterized by depressed serum T3 and T4 levels. The magnitudes of the depressions in thyroid hormone concentrations were about the same in the two strains, although both T3 and T4 levels of nondiabetic SHR were slightly higher than those of the nondiabetic WKY rats.

Treatment of diabetic SHR with T3 prevented the decline in serum T3 levels but had no effect on the elevated serum glucose or depressed insulin values (Table 1). It also exacerbated the decline in serum T3 levels, probably by inhibiting thyroid-stimulating hormone production and glandular T4 secretion. T3 treatment of nondiabetic SHR had the same effect on T3 levels but otherwise did not alter glucose, insulin, or T3 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 T3 and T4 to normal.

**Body and Heart Weights**

As expected, nondiabetic SHR had lower body weight, but higher ratios of left ventricular weight to either body weight or right ventricular weight, than did nondiabetic WKY rats (Table 2). Thus, the left ventricles of SHR were both relatively (LV/BW) and absolutely (LV/RV) hypertrophic; where LV is left ventricular weight; LV, left ventricular weight; RV, right ventricular weight; SHR, spontaneously hypertensive rats; T3, triiodothyronine; I, protamine zinc insulin; WKY, Wistar-Kyoto rats.

**Table 2. Body and Heart Weights of Nondiabetic and Diabetic Rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>BW (g)</th>
<th>HW (mg)</th>
<th>LV/BW (mg/g)</th>
<th>LV/RV (mg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondiabetic</td>
<td>20</td>
<td>328±25*</td>
<td>1,370±132</td>
<td>3.20±0.40*</td>
<td>5.37±0.72*</td>
</tr>
<tr>
<td>Diabetic</td>
<td>18</td>
<td>196±21†</td>
<td>907±85†</td>
<td>3.04±0.51†</td>
<td>4.42±1.04†</td>
</tr>
<tr>
<td>Diabetic+T3</td>
<td>18</td>
<td>174±21‡</td>
<td>929±146‡</td>
<td>3.68±0.63†</td>
<td>4.41±0.73†</td>
</tr>
<tr>
<td>Diabetic+I</td>
<td>16</td>
<td>305±25‡</td>
<td>1,371±83</td>
<td>3.00±0.59†</td>
<td>4.84±1.11</td>
</tr>
<tr>
<td>Nondiabetic+T3</td>
<td>15</td>
<td>322±35</td>
<td>1,406±75</td>
<td>3.36±0.58</td>
<td>4.86±0.90</td>
</tr>
<tr>
<td>WKY</td>
<td>21</td>
<td>386±31</td>
<td>1,428±182</td>
<td>2.66±0.37</td>
<td>4.31±0.80</td>
</tr>
<tr>
<td>Diabetic</td>
<td>16</td>
<td>306±37†</td>
<td>1,070±75†</td>
<td>2.75±0.50</td>
<td>4.13±0.66</td>
</tr>
</tbody>
</table>

Values represent mean±SD obtained 8 weeks after initiation of diabetes or treatment. BW, body weight; LV, left ventricular weight; RV, right ventricular weight; SHR, spontaneously hypertensive rats; T3, triiodothyronine; I, protamine zinc insulin; WKY, Wistar-Kyoto rats.

*Significantly different from the nondiabetic WKY rat.
†Significantly different from the nondiabetic group (within strain).
‡Significantly different from the nondiabetic and diabetic groups (within strain).
ventricular weight, BW is body weight, and RV is right ventricular weight. Diabetes reduced body weight and heart weight 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 ventricles.¹¹

Treatment of diabetic SHR with T₃ caused a further decline in body weight but had no effect on heart weight; thus it increased LV/BW (Table 2). However, T₃ had no effect on LV/RV and therefore did not affect the reversal of hypertrophy induced by diabetes. Treatment of nondiabetic SHR with T₃ had no effect on any of the body or heart weight measurements.

Insulin treatment (Table 2) nearly restored body weight to normal and prevented the declines in heart weight and LV/BW in diabetic SHR in spite of its failure to correct the hyperglycemia.¹

### Arterial Pressure and Heart Rate In Vivo

As expected, nondiabetic SHR were hypertensive and tachycardic compared with the WKY rats (Table 3). The diabetic SHR were characterized by depressed systolic arterial pressure and heart rate after 8 weeks, confirming previous observations.¹¹ However, diabetes had no effect on these measurements in the WKY rat strain.

Treatment of diabetic SHR with T₃, at a dose that was adjusted to prevent the bradycardia, only partially reversed the depressor effect of diabetes (Table 3). In contrast, insulin treatment prevented the decline in systolic arterial pressure but did not fully restore heart rate to normal. Treatment of nondiabetic SHR with T₃ had no significant effect on either systolic arterial pressure or heart rate.

### Left Ventricular Pressure Wave Characteristics

Perfused hearts from nondiabetic SHR had a shorter Tₚ but otherwise did not differ from those of the WKY rat strain with regard to Pₘₐₓ, Tₚ, and LV-dP/dt (Table 4). Diabetes increased Tₚ of both SHR and WKY rat hearts but increased Tₛ and reduced Pₘₐₓ 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 observations⁹,²² (data not shown).

All of the effects of diabetes on LVP wave characteristics in the SHR strain were prevented by either T₃ or insulin treatment (Table 4). However, none of these measurements was altered by T₃ treatment of nondiabetic SHR. The effects of diabetes and treatment with either insulin or T₃ on LVP waveforms are illustrated in Figure 1.

### Pressure Load–Dependent Relaxation and Sarcoplasmic Reticulum Ca²⁺ Uptake

Pressure load–dependent relaxation of SHR ventricles was impaired by diabetes, but that of the WKY rat 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 T₃ treatment (Table 5, Figures 1–3). Therefore, T₃ 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 T₃ 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 microsomes.⁷ Proposed mechanisms include reductions in either the density or the activity of Ca²⁺-Mg²⁺ ATPase,⁶,⁸,¹⁸ alterations in membrane
TABLE 4. Left Ventricular Pressure Wave Characteristics of Nondiabetic and Diabetic Rat Hearts Ex Vivo

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>P&lt;sub&gt;max&lt;/sub&gt; (kPa)</th>
<th>T&lt;sub&gt;p&lt;/sub&gt; (msec)</th>
<th>T&lt;sub&gt;r&lt;/sub&gt; (msec)</th>
<th>LV-dP/dt (kPa/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nondiabetic</td>
<td>12</td>
<td>19.1±0.9</td>
<td>38±3</td>
<td>83±5</td>
<td>600±92</td>
</tr>
<tr>
<td>Diabetic</td>
<td>9</td>
<td>14.7±1.9†</td>
<td>42±6†</td>
<td>104±8†</td>
<td>416±113†</td>
</tr>
<tr>
<td>Diabetic+T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>9</td>
<td>17.9±0.7</td>
<td>34±5</td>
<td>82±8</td>
<td>549±76</td>
</tr>
<tr>
<td>Diabetic+I</td>
<td>7</td>
<td>20.0±1.5</td>
<td>41±3</td>
<td>80±6</td>
<td>601±70</td>
</tr>
<tr>
<td>Nondiabetic+T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>7</td>
<td>20.9±1.6</td>
<td>34±6</td>
<td>78±5</td>
<td>645±105</td>
</tr>
<tr>
<td>WKY</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nondiabetic</td>
<td>13</td>
<td>18.9±2.7</td>
<td>36±4</td>
<td>99±9</td>
<td>541±134</td>
</tr>
<tr>
<td>Diabetic</td>
<td>9</td>
<td>17.1±2.0</td>
<td>45±5†</td>
<td>98±12</td>
<td>517±108</td>
</tr>
</tbody>
</table>

Values represent mean±SD obtained 8 weeks after initiation of diabetes or treatment. P<sub>max</sub>, maximum left ventricular developed pressure (LVDP); T<sub>p</sub>, time to P<sub>max</sub>; T<sub>r</sub>, time from P<sub>max</sub> to minimum LVDP; LV-dP/dt, maximum rate of left ventricular pressure decline; SHR, spontaneously hypertensive rats; T<sub>3</sub>, triiodothyronine; I, protamine zinc insulin; WKY, Wistar-Kyoto rats. Left ventricular filling pressure was fixed at 15 cm H<sub>2</sub>O and resistance to aortic ejection was fixed at 1.54 kPa/cm<sup>3</sup>/min.

•Significantly different from the nondiabetic WKY rats.
†Significantly different from the nondiabetic group (within strain).

lipid profiles,<sup>6,28,29</sup> and changes in the degree of membrane phosphorylation.<sup>30</sup> 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<sup>2+</sup> uptake of SHR left ventricles but had no effect in the nonhypertensive, nonhypertrophic WKY rat strain. These results extend those of earlier studies that had demonstrated more pronounced effects of diabetes on hypertrophic ventricle of either SHR or renovascular hypertensive rats when compared with the effects in normotensive rats.<sup>11-13</sup> The WKY rat seems to be more resistant to the cardiodepressive effects of diabetes than are other normotensive rat strains.<sup>11-13</sup> Nevertheless, the exacerbating influences of hypertension on mechanical function and histopathology are apparent whether the diabetic normotensive control is the WKY,<sup>11,13</sup> the Sprague-Dawley,<sup>11</sup> or the Wistar strain.<sup>12,13</sup>

The mechanism for the apparent interaction between diabetes and LVH on SR Ca<sup>2+</sup> uptake is a matter of speculation. In nondiabetic hypertrophic ventricle, SR Ca<sup>2+</sup> uptake might be impaired<sup>20,31</sup> or normal<sup>32</sup> depending on either the experimental model or the duration of hypertension.<sup>33</sup> Like diabetes, LVH is associated with alterations in lipid metabolism<sup>34,35</sup> and restricted fuel source availability.<sup>36,37</sup> Elevated workloads, reduced coronary reserve,<sup>38</sup> and possibly increased metabolic demand for carnitine<sup>34</sup> (see below) may combine to predispose the hypertrophic ventricle to membrane-disrupting effects of diabetes that might otherwise be less apparent in the absence of preexisting LVH.

Our results also show that the activity of SR Ca<sup>2+</sup> uptake was well correlated with indexes of mechanical relaxation. Both measurements were depressed in the diabetic SHR, but neither one was significantly affected in any other experimental group (Table 5,
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Four pressure loads (see Figure 1). Loading was altered diabetic insulin-treated relaxation of nondiabetic (d), diabetic (d+i), triiodothyronine (T3)-treated diabetic (d+t3), and T3-treated nondiabetic (nd+t3) spontaneously hypertensive rat (SHR) hearts ex vivo. Four points within each group represent maximum left ventricular pulse pressure (P^A) vs. mean slope is depicted for remaining experimental groups. Whether diabetes can specifically depress load-dependent relaxation of nonhypertrophic myocardium remains to be determined.

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 mechanisms. Load-dependent relaxation is most often demonstrated in isolated ventricular muscle preparations. A recent report from this laboratory suggested that the ratio P_avg/A_r can serve as an index of load-dependent relaxation in the ejecting ventricle.16 As expected, the value of P_avg/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 rat groups, whereas the SR Ca^2+ uptake activity did not (Tables 4 and 5). In contrast to the results of the previous report,16 in the present study LV-dP/dt varied with P_avg/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.1,12,39

The effectiveness of T3 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 levels.1 The dose of T3 used in the current study (8–10 µg/kg/day) was selected on the basis of its ability to restore the depressed heart rate 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 T3 levels above those of the nondiabetic controls, although it apparently did inhibit T4 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 systolic arterial pressure or heart rate in vivo (Tables 2 and 3); 3) T3 treatment of nondiabetic SHR also had no significant effect on either serum T3 levels (Table 1), indexes 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 that had been shown to be sufficient for replacement therapy of simple hypothyroidism) were only partially effective in restoring cardiac function of diabetic normotensive rats.8,23–25 Interestingly, however, T3 (10 µg/kg b.i.d.) 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 atria.40

Our results suggest that hypertension, and probably LVH, increase the responsiveness of diabetic myocardium to the ameliorative effects of T3 therapy,
at least with regard to mechanical and biochemical indexes of relaxation. The mechanism may involve, in part, differential effects of diabetes on membrane lipid profiles and other aspects of lipid metabolism, of hypertrophic versus nonhypertrophic ventricle. The acute effects of T3 on Ca\(^{2+}\)-Mg\(^{2+}\) ATPases are profoundly influenced by the lipid environment.\(^4\) Because hypertrophy,\(^4\) hypothyroidism,\(^4\) and diabetes\(^2\) all affect membrane lipid composition, it seems reasonable to hypothesize that different combinations of these pathophysiological conditions would have variable influences on the subsequent effects of T3 administration. In normotensive rats, myoinositol coadministration increases the therapeutic efficacy of T3 in diabetic cardiopathy.\(^4\) 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 diabetes,\(^28\) and T3 treatment decreases LCAC levels in SR membranes.\(^4\) Another potential mechanism undoubtedly involves the regulation of SR calcium pump activity by thyroid hormone. Chronically, T3 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 measurements.\(^4\) The data from our study (Figure 3) and those of a previous report\(^19\) 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 LVH 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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