Effects of Moderate Diabetes on Cardiac Performance in Spontaneously Hypertensive and Wistar-Kyoto Rats

JIN YAMAMOTO AND MASATSUGU NAKAI

SUMMARY To assess the effects of imposition of moderate diabetes on in vivo cardiac performance in gradually proceeding hypertension, spontaneously hypertensive (SHR) and Wistar-Kyoto rats (WKY) were treated with streptozotocin (40 mg/kg) or vehicle at 8 weeks of age. Four and 20 weeks later, with the rats under ether anesthesia, peak cardiac output and stroke volume were measured during volume loading and peak left ventricular developed pressure and maximum rate of rise of pressure (dP/dt max) were determined during aortic occlusion. Additionally, passive pressure-volume relations were obtained during saline infusion in potassium-arrested hearts, and the chamber stiffness constant was derived from one exponential function. There was a mortality of 16.1% in the diabetic SHR only. While basal and stressed cardiac performance was unchanged despite the already decreased mean arterial pressure and left ventricular weight at 4 weeks, the diabetic SHR revealed significant decreases in peak cardiac pumping indexes, peak left ventricular developed pressure, and dP/dt max, with unchanged resting cardiac function, at 20 weeks. Changes seen in the diabetic WKY were reduced left ventricular weight at 4 weeks and reduced peak left ventricular dP/dt max at 20 weeks. The chamber stiffness was unaltered with strain or diabetes. These data show that imposition of even moderate diabetes substantially influences the stress-loaded in vivo cardiac performance in the SHR, whereas it produces only minor changes in the WKY. (Hypertension 11: 344-351, 1988)

KEY WORDS • cardiac pumping ability • left ventricular pressure generating capacity • maximum rate of rise of left ventricular pressure • chamber stiffness constant

A significant association between hypertension and diabetes mellitus has been long recognized.1 Since either disease is an important risk factor, this combination has a profound influence on cardiovascular mortality and morbidity. A series of studies by Factor et al.2-4 and Fein et al.5 demonstrated that combined hypertension and diabetes resulted in a congestive cardiomyopathy associated with a high mortality. There are at least two forms of experimental models: two-kidney, one clip Goldblatt3-5 rats and spontaneously hypertensive rats (SHR)6-10 with chemically induced severe diabetes. In both forms, largely identical cardiac histopathological alterations have been observed, including extensive myocytolysis, increased interstitial and replacement fibrosis, and microvascular abnormalities.3-7 Concurrently, studies of the isolated papillary muscle mechanics of diabetic Goldblatt rats revealed a marked depression of velocity of isometric and isotonic contraction and a slowing of the relaxation.5 The perfused working hearts isolated from diabetic SHR showed a marked decline of left ventricular (LV) pressure development and the maximum rate of rise of LV pressure (LV dP/dt max) at increasing filling pressure.8-10 However, these studies were performed in preparations exposed to artificial environments. Assessment of in vivo cardiac performance in environments of hypertensive diabetic states also should be made. The effects of a milder form of diabetes in hypertensive rats also need to be investigated. In addition, controversial results were presented as to whether diabetes produced depressed heart function in Wistar-Kyoto rats (WKY), the ancestor, control strain of SHR.8,10 Thus, we studied the in vivo cardiac

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performance in SHR and WKY with a moderate form of diabetes at two different intervals after streptozotocin (STZ) administration.

Materials and Methods

Six-week-old male SHR and WKY, obtained from Charles River Japan (Atsugi, Japan), were kept in a clean animal facility under standardized conditions. Food and tap water were given ad libitum throughout the study. At the age of 8 weeks, the rats were anesthetized with ether. To induce diabetes, STZ, 40 mg/kg (Sigma), which was dissolved in ice-chilled 0.02 M citrate buffer (pH 4.5) immediately before use, was given into the tail vein as a bolus injection to the nonfasted rats. Aron Alpha A (Sankyo Pharmaceuticals, Tokyo, Japan), an instantaneous adhesive agent, was used to prevent leakage of the drug. The 40 mg/kg dose was based on a previous dose-response study from this laboratory; STZ, 40 mg/kg, led to a moderate but distinct diabetes associated with hypoinsulinemia, but there were no remarkable toxic complications and little or only slight decreases in thyroxine (T₄; unpublished observations, 1987). A small decrease in T₃ and triiodothyronine was reported not to produce a depressed cardiac function. The rats injected with diluent alone were used as the controls. At selected intervals after STZ or vehicle injection, body weight was measured, and approximately 50 µl of blood was taken between 1500 and 1700 from the tail vein in nonfasted rats anesthetized lightly with ether. Blood glucose concentrations were determined using the glucose dehydrogenase method on an automatic analyzer (Model GA-1110, Kyoto Daiichi Kagaku, Kyoto, Japan), an instantaneous adhesive agent, which was dissolved in ice-chilled 0.02 M citrate buffer (pH 4.5) immediately before use, was given into the tail vein as a bolus injection to the nonfasted rats. Aron Alpha A (Sankyo Pharmaceuticals, Tokyo, Japan), an instantaneous adhesive agent, was used to prevent leakage of the drug. The 40 mg/kg dose was based on a previous dose-response study from this laboratory; STZ, 40 mg/kg, led to a moderate but distinct diabetes associated with hypoinsulinemia, but there were no remarkable toxic complications and little or only slight decreases in thyroxine (T₄; unpublished observations, 1987). A small decrease in T₃ and triiodothyronine was reported not to produce a depressed cardiac function. The rats injected with diluent alone were used as the controls. At selected intervals after STZ or vehicle injection, body weight was measured, and approximately 50 µl of blood was taken between 1500 and 1700 from the tail vein in nonfasted rats anesthetized lightly with ether. Blood glucose concentrations were determined using the glucose dehydrogenase method on an automatic analyzer (Model GA-1110, Kyoto Daiichi Kagaku, Kyoto, Japan). Diabetes was defined as a blood glucose level greater than 200 mg/dl; control rats seldom displayed a blood glucose greater than this level in our study. This diabetes was defined as mild to moderate, in relation to the severe diabetes induced by STZ, 60 mg/kg, that accompanied a blood glucose level greater than 350 mg/dl in a previous dose-response study (unpublished observations, 1987). This definition appeared to conform to a recent report on characterization of severe versus mild diabetes. Four and 20 weeks after STZ or vehicle treatment (at the ages of 12 and 28 weeks), the experiments were performed according to a slight modification of the procedure of Pfeffer and colleagues. The rats were anesthetized with ether, and a polyethylene catheter (PE-50) was placed in the femoral artery, through which blood for glucose and T₄ assay (0.2 ml) was withdrawn and replaced with fresh blood from donors. Blood T₄ concentrations were later determined by radioimmunoassay (Eiken Chemical, Tokyo, Japan). Further cannulation was made in the superior vena cava vein through the left jugular vein and in the femoral vein. Prethoracotomy mean arterial pressure (MAP), central venous pressure, and heart rate were recorded through transducers (Statham, Oxnard, CA) connected to a polygraph (San-ei, Tokyo, Japan). The parameters were determined as the average of five readings every 2 minutes for a 10-minute period. The trachea was then intubated, and ventilation was maintained with a rodent respirator (Harvard, Millis, MA, USA) connected in series to an ether-containing apparatus. A midsternal thoracotomy was performed, and a snare occluder was loosely placed around the ascending aorta. A high fidelity catheter-tip transducer (Model PR-249, Millar Instruments, Houston, TX, USA) was inserted into the left ventricle through the right carotid artery. The insertion of this microtip catheter was not accomplished in three each of diabetic SHR and WKY in the 4-week experiments because of narrow vessels; data on the hemodynamics of these diabetic rats were omitted, but the rats were included in calculating mortality. Baseline postthoracotomy hemodynamics were determined as already described after stabilization. Then, the ascending aorta was obstructed for 4 to 5 seconds with the occluder. Peak developed LV pressure was obtained as the difference between systolic and end-diastolic LV pressure seen with the first to sixth regular beats of this occlusion period. This measurement represented an index of LV pressure-generating capacity. Peak LV dP/dt was registered during this procedure as taken as an index of contractility-augmenting capacity. Our preliminary study showed that this brief aortic occlusion was repeatable even in diabetic SHR and that the reproducibility of these peak indexes was excellent. This part of the experiments was completed within 20 minutes of calibration, since a considerable time-dependent baseline drift occurred with this catheter, thereby making the pressure reading unreliable with time. The microtip catheter was then withdrawn, and the aortic occluder was detached.

An electromagnetic flow probe (inside diameter, 1.5, 2.0, or 2.5 mm; Nihon Kohden, Tokyo, Japan) was placed around the ascending aorta, whereby mean aortaloc flow was determined as an estimate of cardiac output (actually, cardiac output minus coronary flow). Stroke volume was calculated as the quotient of cardiac output and heart rate. Cardiac and stroke indexes (ml/min/kg of body weight) were reported. The simultaneous measurements of aortic blood flow and LV pressure were withheld for the following reasons. First, the placement of the aortic flow probe around the catheterized aorta was found to exert a considerable constrictor effect on the aorta, thereby leading to an artificially increased LV pressure. Second, our preliminary experiments showed that direct puncture of the LV apex with this microtip catheter inevitably caused leakage of blood. After restabilization, fresh heparinized blood obtained from donor rats was infused into the femoral vein with a Harvard 944D pump at a rate of 40 ml/kg/min for 45 seconds. A plateau value in aortic flow reached during this rapid volume loading was regarded as an estimate of peak cardiac pumping ability with maximum preload stress. Expanded blood volume was then reduced by bleeding of about half the amount of blood infused through the femoral artery catheter, and cardiovascular parameters were restored.
toward preexisting levels. During the experimental period, blood gas was monitored and ventilation was adjusted. Oxygen was supplied when necessary; in the great majority of cases, oxygen was required after completion of the blood volume loading, and the number of rats given oxygen did not differ among the groups.

The hearts were arrested in diastole by an intravenous overdose of potassium chloride. A double-lumen catheter (14 gauge, Argyle, Tokyo, Japan) was advanced into the left ventricle by way of the incised aorta. The left ventricle was isolated from the atria along the atrioventricular groove by ligation with thread. The right ventricle was cut open. LV pressure was registered continuously over a physiologic range of up to 20 mm Hg. The passive pressure-volume relation of the left ventricle was calculated as an average of three measurements obtained within 10 minutes of cardiac arrest. At the end of the experiments, the heart was removed with the double-lumen catheter, completeness and appropriateness of the preparation were checked, and a few experiments were rejected. For analysis, this LV pressure-volume curve above 5 mm Hg was fitted to one exponential function, $P = b e^{uV}$, the overall LV ventricular chamber stiffness constant $K$ was derived as the slope of the log pressure versus volume per kilogram relation. The log pressure versus volume per kilogram relation was used because of the considerable variability of body weight present between diabetic and control animals and between strains.

Statistical analysis was performed initially by two-way analysis of variance and subsequently by the Bonferroni method using a PDP 11/44 computer (Digital Equipment, Maynard, MA, USA). A $p$ level below 0.05 was considered statistically significant. All values are expressed as means ± SE.

### Results

None of the STZ-treated SHR died in the first 4 weeks, but five died during the ensuing 16-week observation period. There was no mortality in the control groups of SHR and WKY or in the STZ-treated WKY. The total death rate during a 20-week period, expressed as the number of dead rats per number of rats at entry, was 0 of 28 in control SHR, 0 of 30 in control WKY, 5 of 31 (16.1%) in the STZ-treated SHR, and 0 of 31 in the STZ-treated WKY, respectively. Gross postmortem inspection disclosed no sign of heart failure, such as pleural effusion and increased lung weight, in any of the dead rats. Two rats were markedly emaciated and eventually moribund. The remaining three rats were much less emaciated and died unexpectedly. The causes of the deaths were not identified.

As shown in Table 1, the 40 mg/kg dose of STZ led to hyperglycemia and retarded weight gain in SHR and WKY 4 and 20 weeks after administration. This magnitude of alteration was, on the whole, indicative of the induction of a relatively moderate form of diabetes in both strains, in agreement with earlier reports. Blood glucose levels in the STZ-treated SHR did not significantly differ from, but tended to be higher than, those in the STZ-treated WKY at both time periods. Blood T levels were slightly but significantly decreased in the STZ-treated SHR compared with control SHR at 4 weeks, while no significant decrease was reached at 20 weeks. Yet these levels did not differ between the STZ-treated SHR and WKY. LV weight was significantly greater in control SHR than in control WKY 20 weeks after vehicle treatment; the LV weight to body weight ratio was already greater in control SHR than in control WKY 4 weeks, while no significant decrease was reached at 20 weeks.

The development of STZ diabetes tended to decrease or did decrease LV weight in both strains. However, the LV weight to body weight ratio was already greater in control SHR than in control WKY at 4 weeks and was more so at 20 weeks. The development of STZ diabetes tended to decrease or did decrease LV weight in both strains. However, the LV weight to body weight ratio did not differ between the STZ-treated and control rats from the same strain at either period.

As shown in Table 2, prethoracotomy MAP was decreased in diabetic SHR compared with control SHR

### Table 1. Body and Left Ventricular Weights and Blood Glucose and Thyroxine Levels in SHR and WKY 4 and 20 Weeks After Streptozotocin or Vehicle Treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Body wt (g)</th>
<th>LV wt (mg)</th>
<th>LV wt/body wt ratio (mg/g)</th>
<th>Glucose (mg/dl)</th>
<th>Thyroxine (μg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>4 wk after treatment</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control SHR ($n=16$)</td>
<td>256 ± 4</td>
<td>663 ± 13</td>
<td>2.59 ± 0.05*</td>
<td>155 ± 4</td>
<td>4.4 ± 0.2</td>
</tr>
<tr>
<td>Control WKY ($n=15$)</td>
<td>282 ± 4</td>
<td>658 ± 18</td>
<td>2.34 ± 0.05</td>
<td>149 ± 4</td>
<td>4.2 ± 0.2</td>
</tr>
<tr>
<td>Diabetic SHR ($n=15$)</td>
<td>217 ± 7†</td>
<td>539 ± 26†</td>
<td>2.48 ± 0.08</td>
<td>395 ± 14†</td>
<td>3.5 ± 0.2†</td>
</tr>
<tr>
<td>Diabetic WKY ($n=15$)</td>
<td>250 ± 8†</td>
<td>566 ± 18†</td>
<td>2.28 ± 0.04</td>
<td>351 ± 18†</td>
<td>3.7 ± 0.2</td>
</tr>
<tr>
<td><strong>20 wk after treatment</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control SHR ($n=14$)</td>
<td>378 ± 4*</td>
<td>1020 ± 30*</td>
<td>2.70 ± 0.06*</td>
<td>148 ± 6</td>
<td>4.2 ± 0.2</td>
</tr>
<tr>
<td>Control WKY ($n=13$)</td>
<td>421 ± 5</td>
<td>916 ± 16</td>
<td>2.18 ± 0.30</td>
<td>146 ± 4</td>
<td>4.1 ± 0.2</td>
</tr>
<tr>
<td>Diabetic SHR ($n=13$)</td>
<td>320 ± 11†‡</td>
<td>846 ± 21†</td>
<td>2.69 ± 0.06</td>
<td>345 ± 18†</td>
<td>3.6 ± 0.8‡</td>
</tr>
<tr>
<td>Diabetic WKY ($n=13$)</td>
<td>380 ± 15†</td>
<td>866 ± 31</td>
<td>2.30 ± 0.04</td>
<td>314 ± 18†</td>
<td>3.8 ± 0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. LV = left ventricular.

* $p < 0.05$, compared with control WKY.
† $p < 0.05$, compared with control rats in the same strain.
‡ $p < 0.05$, compared with diabetic WKY.
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Table 2. Prethoracotomy Hemodynamic Data in SHR and WKY 4 and 20 Weeks After Streptozotocin or Vehicle Treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>CVP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 wk after treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control SHR</td>
<td>138 ± 2*</td>
<td>399 ± 7*</td>
<td>2.6 ± 0.4</td>
</tr>
<tr>
<td>Control WKY</td>
<td>105 ± 2</td>
<td>369 ± 9</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>Diabetic SHR</td>
<td>122 ± 3t‡</td>
<td>380 ± 7</td>
<td>2.9 ± 0.3</td>
</tr>
<tr>
<td>Diabetic WKY</td>
<td>106 ± 2</td>
<td>358 ± 8†</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td>20 wk after treatment</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control SHR</td>
<td>144 ± 3*</td>
<td>397 ± 10*</td>
<td>2.9 ± 0.3</td>
</tr>
<tr>
<td>Control WKY</td>
<td>105 ± 3</td>
<td>346 ± 9</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>Diabetic SHR</td>
<td>136 ± 2t‡</td>
<td>387 ± 7‡</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>Diabetic WKY</td>
<td>107 ± 2</td>
<td>330 ± 8</td>
<td>3.0 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. Numbers of rats are shown in Table 1. MAP = mean arterial pressure; HR = heart rate; CVP = central venous pressure.

*p < 0.05, compared with control WKY.
†p < 0.05, compared with control rats in the same strain.
‡p < 0.05, compared with diabetic WKY.

at both periods, while there was no such influence of STZ diabetes in WKY. Heart rate was faster in SHR than in WKY regardless of the presence or absence of diabetes; heart rate showed no significant difference between diabetic and control groups in either strain.

As shown in Table 3, though thoracotomy apparently decreased MAP in all groups, the relationship of postthoracotomy baseline MAP and heart rate among the groups was the same as that seen before thoracotomy 4 and 20 weeks after STZ or vehicle treatment; diabetic SHR had a lower postthoracotomy baseline MAP than did the control SHR. No such changes were seen in the diabetic WKY. Postthoracotomy MAP of diabetic SHR was still higher than that of diabetic WKY. Central venous pressure was not significantly altered between strains, nor with STZ diabetes, at 4 and 20 weeks, though the diabetic SHR tended to show a somewhat higher central venous pressure 20 weeks after STZ injection. LV end-diastolic pressure was comparable in the diabetic and control SHR at 4 weeks, while there was a tendency toward an increase in LV end-diastolic pressure in diabetic SHR at 20 weeks. No significant difference was noted in baseline LV dP/dtmax between control groups of either strain, or between diabetic and control groups of the same strain at either period. The baseline cardiac index did not differ with the strain or with STZ diabetes, at either period. Control SHR tended to show a lesser baseline stroke index than did control WKY at both intervals. Either diabetic group of both strains tended to have a larger stroke index compared with the respective control group. Therefore, the unchanged cardiac index of the SHR, in relation to WKY, either diabetic or non-diabetic, appeared to relate to their faster heart rate in these experiments.

Figures 1 and 2 illustrate pressure-generating and cardiac pumping abilities with short-term stress loading. Peak LV pressure developed during aortic occlusion was significantly higher in control SHR than in control WKY 4 weeks (268 ± 5 vs 211 ± 5 mm Hg; p < 0.01) and 20 weeks (281 ± 6 vs 230 ± 3 mm Hg; p < 0.01) after treatment. Peak developed LV pressure was somewhat lower in diabetic SHR than in control SHR at 4 weeks (253 ± 5 vs 268 ± 5 mm Hg; p = NS), and this pressure was significantly lower in diabetic SHR than in control SHR at 20 weeks (234 ± 4 vs 281 ± 6 mm Hg; p < 0.05). In contrast, there were no changes of peak developed LV pressure between diabetic and control groups of the WKY strain at either interval. Peak developed LV pressure remained significantly (p < 0.05) higher in diabetic SHR than in diabetic WKY. Peak LV dP/dtmax attained with this aortic occlusion was significantly (p < 0.05) greater in control SHR than in control WKY 4 weeks and 20 weeks after treatment. This peak LV dP/dtmax tended to be lower in diabetic SHR than in control SHR at 4 weeks (4953 ± 187 vs 5487 ± 171 mm Hg/sec; p = NS) and was significantly lower in diabetic SHR at 20 weeks (4992 ± 179 vs 6130 ± 160 mm Hg/sec; p < 0.01). In WKY, a similar decrease in peak LV dP/dtmax was noted in the diabetic group compared with the control group only at 20 weeks after treatment (4562 ± 169 vs 5441 ± 151 mm Hg/sec; p < 0.05).

Table 3. Postthoracotomy Baseline Hemodynamics in SHR and WKY 4 and 20 Weeks After Streptozotocin or Vehicle Treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>CVP (mm Hg)</th>
<th>LVEDP (mm Hg)</th>
<th>dP/dtmax (mm Hg/sec)</th>
<th>CI (ml/min/kg)</th>
<th>SI (ml/min/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 wk after treatment</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control SHR</td>
<td>115 ± 3*</td>
<td>423 ± 7*</td>
<td>3.6 ± 0.2</td>
<td>3.7 ± 0.2</td>
<td>4084 ± 151</td>
<td>261 ± 9</td>
<td>0.616 ± 0.019</td>
</tr>
<tr>
<td>Control WKY</td>
<td>91 ± 2</td>
<td>383 ± 10</td>
<td>2.9 ± 0.2</td>
<td>3.4 ± 0.2</td>
<td>3764 ± 118</td>
<td>252 ± 8</td>
<td>0.653 ± 0.022</td>
</tr>
<tr>
<td>Diabetic SHR</td>
<td>101 ± 2t†</td>
<td>404 ± 6</td>
<td>3.7 ± 0.3</td>
<td>4.2 ± 0.3</td>
<td>3958 ± 137</td>
<td>264 ± 8</td>
<td>0.654 ± 0.018</td>
</tr>
<tr>
<td>Diabetic WKY</td>
<td>90 ± 2</td>
<td>364 ± 7</td>
<td>3.3 ± 0.2</td>
<td>4.1 ± 0.3</td>
<td>3617 ± 188</td>
<td>260 ± 11</td>
<td>0.712 ± 0.029</td>
</tr>
<tr>
<td>20 wk after treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control SHR</td>
<td>117 ± 3*</td>
<td>415 ± 7*</td>
<td>3.7 ± 0.2</td>
<td>4.1 ± 0.2</td>
<td>4559 ± 158</td>
<td>190 ± 8</td>
<td>0.455 ± 0.018</td>
</tr>
<tr>
<td>Control WKY</td>
<td>88 ± 2</td>
<td>352 ± 10</td>
<td>3.9 ± 0.2</td>
<td>3.7 ± 0.2</td>
<td>4114 ± 124</td>
<td>178 ± 5</td>
<td>0.506 ± 0.022</td>
</tr>
<tr>
<td>Diabetic SHR</td>
<td>107 ± 3†</td>
<td>399 ± 10†</td>
<td>4.8 ± 0.3</td>
<td>4.8 ± 0.3</td>
<td>4299 ± 142</td>
<td>196 ± 8</td>
<td>0.499 ± 0.024</td>
</tr>
<tr>
<td>Diabetic WKY</td>
<td>87 ± 2</td>
<td>336 ± 10</td>
<td>4.1 ± 0.2</td>
<td>4.2 ± 0.3</td>
<td>3976 ± 193</td>
<td>187 ± 5</td>
<td>0.566 ± 0.021</td>
</tr>
</tbody>
</table>

Values are means ± SE. Numbers of rats are shown in Table 1. MAP = mean arterial pressure; HR = heart rate; CVP = central venous pressure; LVEDP = left ventricular end-diastolic pressure; dP/dtmax = maximum rate of rise of LV pressure; CI = cardiac index; SI = stroke index.

*p < 0.05, compared with control WKY.
†p < 0.05, compared with control SHR.
‡p < 0.05, compared with diabetic WKY.
The peak cardiac index attained with short-term volume loading did not differ between control groups of SHR and WKY 4 and 20 weeks after streptozotocin or vehicle treatment (see Figure 2). In SHR, this peak cardiac index tended to be smaller in the diabetic group than in the control group at 4 weeks (410 ± 13 vs 477 ± 14 ml/min/kg; \( p = \text{NS} \)), and this value was significantly smaller at 20 weeks (306 ± 9 vs 360 ± 7 ml/min/kg; \( p < 0.05 \)). There was no significant influence of diabetes on the peak cardiac index in the WKY strain. Peak stroke index tended to be lower in either group of SHR than in the corresponding group of WKY at 4 weeks. At 20 weeks, diabetic SHR showed a significantly decreased peak stroke index compared with diabetic WKY (0.865 ± 0.024 vs 1.070 ± 0.025 ml/min/kg; \( p < 0.05 \)) and also compared with control SHR (0.865 ± 0.024 vs 0.972 ± 0.023 ml/min/kg; \( p < 0.05 \)).

Table 4 shows the LV chamber stiffness constant \( K \) derived from the passive pressure-volume relation of potassium-arrested hearts. This constant was not significantly altered with strain or with STZ diabetes 4 and 20 weeks after STZ or vehicle treatment.

**Discussion**

Hypertension develops gradually according to a genetically programmed mode in the SHR; therefore, these rats are considered an excellent model of human essential hypertension. In the present work, treatment with STZ, 40 mg/kg, and associated moderate diabetes exerted a substantial influence not only on in vivo cardiac performance but also on mortality in the SHR, whereas there were minor changes with no deaths in the WKY. In the diabetic SHR, basal and stressed cardiac performance was minimally altered, despite an already decreased LV weight and MAP 4 weeks after injection of STZ. At 20 weeks, while changes in basal parameters were a decreased MAP and a tendency toward increased LV end-diastolic pressure only, changes observed with short-term cardiac preload and afterload stresses were remarkable and included decreases in peak LV developed pressure and peak LV dP/dt\( _{\text{max}} \) during aortic occlusion and decreases in peak cardiac and stroke indexes during blood infusion. Despite administration of a moderate dose of STZ and the resultant development of moderate diabetes, there was a mortality of 16.1% during a 20-week period. Earlier studies reported a mortality of 43 or 55% in severely diabetic Goldblatt rats (STZ, 60 mg/kg) over 5 to 6 or 7 months and of 46% in severely diabetic SHR (STZ, 55 mg/kg) over 12 weeks. Thus, the death rate in hypertensive rats with STZ diabetes seems to be dose-dependent. Since we have no information on the cause of death, the possibility cannot be ruled out that the SHR died of effects of STZ treatment that were independent of diabetes or hypertension.

Cardiac responses to stress are determined by multi-

<table>
<thead>
<tr>
<th>Period</th>
<th>Control SHR</th>
<th>Control WKY</th>
<th>Diabetic SHR</th>
<th>Diabetic WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 wk</td>
<td>2.33 ± 0.17</td>
<td>2.42 ± 0.14</td>
<td>2.16 ± 0.15</td>
<td>2.28 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>(n = 12)</td>
<td>(n = 10)</td>
<td>(n = 12)</td>
<td>(n = 11)</td>
</tr>
<tr>
<td>20 wk</td>
<td>2.51 ± 0.17</td>
<td>2.62 ± 0.18</td>
<td>2.25 ± 0.16</td>
<td>2.53 ± 0.18</td>
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<tr>
<td></td>
<td>(n = 12)</td>
<td>(n = 11)</td>
<td>(n = 11)</td>
<td>(n = 10)</td>
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Values are means ± SE. No significant \( p < 0.05 \) difference was reached with strain or diabetes at either period.
CARDIAC PERFORMANCE IN MODERATELY DIABETIC SHR/Yamamoto and Nakai

ple factors. Peak cardiac and stroke indexes are influenced by cardiac contractility (intrinsically sympatho-
adrenergically mediated), cardiac muscle mass, intraventricular volume to ventricular mass ratio, aortic pressure, heart rate, coronary flow reserve capacity, and so on. Cardiac contractile function in diabetes or hypertension will be discussed in detail. Data on sympatho-adrenergic support to the heart in SHR and diabetes are controversial. Aortic pressure, which differed between strains and between diabetic and control SHR, was described as unimportant. Considering the variability of heart rate with strain and diabetes in our study, peak stroke index may be a better reflection of cardiac performance, but both indexes did show consistent changes. These peak indexes probably are not much influenced by preexisting blood volume and systemic venous tone because these values were obtained as prepeak levels of cardiac function or function curves achieved with volume loading. Our finding of no difference in the LV chamber stiffness constant \( K \) may suggest the unimportance of LV diastolic properties. Peak LV pressure and peak LV \( dp/dt_{max} \) are influenced mainly by contractility and cardiac mass. The differences in these parameters seen between diabetic and control SHR and between control SHR and WKY at 20 weeks may be ascribed in part to differences in cardiac mass (LV weight) and, hence, in cardiac contractile units.

Severe experimental diabetes alone results in cardiac functional changes, including a slowing of myocardial contraction and relaxation, and a decreased resistance to global ischemia. These events are attributed to alterations in biochemical factors determining intrinsic cardiac contractility; that is, a reduction in myosin adenosine triphosphatase activity and sarcolemmal reticular function and a transformation of myosin isoenzymes from \( V_1 \) to \( V_2 \). In experimental hypertension, biochemical contractile protein changes and reduced cardiac resistance to ischemia, similar to findings in the presence of diabetes, are usually noted. However, there is some controversy regarding mechanical functions; most studies of the established phases of hypertension reported largely unaltered, sometimes improved, cardiac function, whereas others found depressed function. In contrast to Goldblatt hypertension, the emergence of hypertension-related cardiac functional abnormalities is of late onset in SHR. This observation is confirmed by the present finding that cardiac pumping ability and pressure-generating capacity were well preserved in SHR at both periods (i.e., at 12 and 28 weeks of age).

The additive or incremental influences of the combination of hypertension and severe diabetes on in vitro cardiac function and histopathology have been noted in both Goldblatt and SHR models. Our in vivo cardiac performance studies also revealed a greater functional depression in diabetic SHR than in either diabetic WKY or control SHR. Combined Goldblatt hypertension and severe diabetes brought about a more prominent delay of peak velocity of isometric contraction than did either disorder alone, but with no changes in peak developed tension and peak shortening. Directly correlating these data on papillary muscle mechanics to our in vivo data shows a similarity and a dissimilarity. Studies of the perfused hearts isolated from severely diabetic SHR showed a more blunted response of cardiac output, LV pressure development, and \( dp/dt_{max} \) to increasing preload. It is not surprising that our 4-week experiments revealed no notable cardiac functional disturbances. However, our observation of a significant reduction in MAP in SHR 4 weeks after induction of diabetes indicates some changes are already occurring in blood pressure and, hence, in cardiovascular regulation.

It is reasonable to speculate that altered cardiac performance was a combined consequence of moderate diabetes and hypertension in the STZ-treated SHR. Although the SHR tended to show a somewhat greater hyperglycemia with STZ treatment, the blood glucose concentrations were not significantly different between the diabetic SHR and WKY. We found a slight decrease in the blood T4 levels in the STZ-treated SHR compared with control SHR, but there also was no significant difference between STZ-treated SHR and WKY. Although triiodothyronine levels were not measured in this study, previous studies indicated that this extent of STZ-induced hypothyroidism played a negligible role in the cardiac impairment. Factors such as the cardiotoxicity of STZ and malnutrition seem to have been ruled out, though there may be a different susceptibility to STZ in SHR than in WKY. In view of the nature of our in vivo experiments, environmental factors may have influenced the evaluation. However, hyperlipidemia, ketoadiposis, elevated blood urea nitrogen, and other metabolic abnormalities were only slight and did not differ between SHR and WKY with the dose of STZ used herein (unpublished observations, 1987). Considering all data together, the more impaired cardiac performance observed in diabetic SHR compared with diabetic WKY is likely to result not from a more severe diabetes but from a combination of a similar degree of diabetes and hypertension.

Regarding the underlying mechanisms for the strikingly impaired cardiac function in hypertensive diabetic states, of particular interest is the coronary microangiopathic theory of Factor and colleagues. They suggested that microvascular spasm and consequent reperfusion injury associated with increased leakage of the microvasculature may cause extensive focal myocardial necrosis and scar formation, leading to myocardial degeneration and, ultimately, cardiomyopathy. Much earlier, Hashimoto and Wexler, noted a similar microangiopathy and microangiopathy with frequent myocardial necrosis in the diabetic SHR model.
The present result of no significant alterations, except for reduced peak LV dp/dt max and LV weight, in diabetic WKY in comparison with control WKY shows that a moderate form of diabetes had only a minor influence on cardiac performance in normotensive rats, a notion that is in accord with earlier data. With regard to WKY, Rodrigues and McNellis found that SHR and another control strain of Wistar rats, but not WKY, manifested diminished cardiac function in the presence of similarly severe hyperglycemia and reduced LV weight. Those workers studied the isolated perfused hearts from these three strains 12 weeks after injection of STZ, 55 mg/kg. In contrast, using a similar technique, Rodgers reported that the WKY exhibited cardio depression 8 weeks after STZ, 50 mg/kg. Our evidence of a diminution in peak LV dp/dt max during afterload stress in the diabetic WKY 20 weeks after STZ injection indicates that changes, though less extensive than those in the SHR, may occur in the WKY with STZ diabetes.

In this study, the LV chamber stiffness constant K was not altered with diabetes or with strain. Since uncertainty remained with the assumption of ~5 mm Hg at zero volume in the in vivo left ventricle, we did not construct the pressure-volume curves; however, other workers found that the constant K calculated was only slightly or not at all affected. Most investigators dealing with diastolic pressure-volume relationships of isolated hearts from pure diabetic rats and from SHR reported the slope, and hence, the stiffness, to be largely unaltered, compared with findings in respective controls, though increased stiffness was noted in the SHR by some investigators. There is evidence of a rightward shift of the pressure-volume curves with a prolongation of diabetes or hypertension, a finding indicative of geometrically dilated hearts.

Collectively, our findings show that STZ treatment and associated moderate diabetes resulted in depressed in vivo cardiac performance in the SHR, whereas there were relatively minor changes in the WKY. These data suggest a basis for the clinically held contention that hypertension combined with even moderate diabetes leads to a greater risk of cardiovascular disorders.

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