OBJECTIVES We sought to evaluate in vivo and in vitro left ventricular (LV) geometry and function in streptozotocin-induced diabetic rats and the possible role of the nitric oxide (NO) pathway.

BACKGROUND Diabetes results in cardiac dysfunction; however, the specific abnormalities are unknown. Because decreased NO contributes to abnormal vascular function in diabetics, we hypothesized that NO pathway abnormalities may contribute to diabetic cardiomyopathy.

METHODS Control rats and those with non–insulin-dependent diabetes mellitus (NIDDM) underwent echocardiography, hemodynamic assessment, isolated heart perfusion and measurement of exhaled NO and LV endothelial constitutive nitric oxide synthase (ecNOS).

RESULTS Diabetic rats had increased LV mass (3.3 ± 0.6 vs. 2.6 ± 0.3 g/g body weight [BW], p < 0.001) and cavity dimensions (diastolic 2.0 ± 0.1 vs. 1.8 ± 0.2 cm/cm tibial length [TL], p < 0.05). Diabetic rats had prolonged isovolumic relaxation time (IVRT) (40 ± 8 vs. 26 ± 6 ms, p < 0.0001), increased atrial contribution to diastolic filling (0.47 ± 0.09 vs. 0.30 ± 0.08 m/s, p < 0.0001), and elevated in vivo LV end-diastolic pressure (7 ± 6 vs. 2 ± 1 mm Hg, p = 0.04). Diabetic rats had increased chamber stiffness. Shortening was similar in both groups, despite reduced meridional wall stress in diabetics, suggesting impaired systolic contractility. Exhaled NO was lower in diabetic rats (1.8 ± 0.2 vs. 3.3 ± 0.3 parts per billion, p < 0.01) and correlated with Doppler LV filling. The ecNOS was similar between the groups.

CONCLUSIONS Diabetic cardiomyopathy is characterized by LV systolic and diastolic dysfunction, the latter correlating with decreased exhaled NO. The NO pathway is intact, suggesting impaired availability of NO as contributor to cardiomyopathy. (J Am Coll Cardiol 1999;34:2111–9) © 1999 by the American College of Cardiology

Cardiovascular disease is the leading cause of death in diabetic patients (1). Both insulin-dependent diabetes mellitus (IDDM) and non–insulin-dependent diabetes mellitus (NIDDM) may result in myocardial dysfunction in the absence of coronary artery disease, valvular pathology or systemic hypertension (2,3). This diabetic cardiomyopathy has been postulated to contribute to both the higher incidence of congestive heart failure observed in longitudinal studies in diabetic patients without coronary artery disease and the higher in-hospital and long-term mortality after myocardial infarction in diabetic patients (3,4). However, the exact nature and mechanism(s) of cardiomyopathy are unknown.

Peripheral vascular endothelium-dependent vasodilation is impaired in patients with diabetes mellitus and experimentally induced hyperglycemia, probably as a result of the decreased availability of endothelial nitric oxide (NO) (5,6). Because NO also directly affects cardiac function (7), we hypothesized that reduced NO availability may also contribute to diabetic cardiomyopathy. Clinical studies in patients with diabetes have been limited by the influences of other coexisting cardiovascular diseases, such as hypertension and coronary artery disease. Therefore, experimentally induced animal models of diabetes have been developed to study cardiomyopathy (8). However, the evaluation of cardiac dysfunction has proven difficult in these small animal models of diabetes, primarily owing to the difficulty...
in obtaining invasive in vivo and in vitro hemodynamic studies. Previous studies have validated two-dimensional Doppler echocardiography in characterizing left ventricular (LV) geometry and function in a rat model of myocardial infarction (9,10). The aim of this study was therefore twofold: 1) to comprehensively evaluate in vivo LV geometry and function (systolic and diastolic) in the streptozotocin (STZ)-induced NIDDM rat utilizing Doppler echocardiography and to integrate this noninvasive assessment of cardiac function with data obtained from invasive in vivo hemodynamic assessment and in vitro isolated heart perfusion; and 2) to evaluate the contribution of the NO pathway to the abnormal cardiac function in the NIDDM rat.

METHODS

Study population and creation of diabetes. The experimental procedures were approved by the Beth Israel Deaconess Medical Center’s Institutional Animal Care and Use Committee (IACUC) and Taconic Farm’s (Germantown, New York) IACUC. The National Institutes of Health’s Guide for the Care and Use of Laboratory Animals was followed. Sixty-two Sprague-Dawley female rats weighing 140 to 189 g were assigned to two groups. Rats were anesthetized with ketamine and xylazine at the same doses or STZ, transthoracic echocardiograms were performed in control (n = 14) and diabetic (n = 29) animals as previously described (9). Rats were anesthetized with a combination of ketamine hydrochloride 50 mg/kg intraperitoneally (IP) (Parke Davis, Morris Plains, New Jersey) and xylazine 10 mg/kg IP (Lloyd Laboratories, Shenandoa, Iowa). Echocardiograms were performed with a Hewlett-Packard Sonos 2500 (Andover, Massachusetts) sector scanner equipped with a 7.5-MHz phased-array transducer. Animals were scanned from below, at 2-cm depth with focus optimized at 1 cm. Because the leading edge of the anterior wall was not identifiable consistently, in part secondary to chest wall artifact, the anterior edge of the anterior wall was measured by the trailing-edge method (13). The posterior edge and posterior wall thickness and LV internal dimensions were measured according to the leading-edge method of the American Society of Echocardiography (14). We have demonstrated previously the reproducibility and excellent correlation with ex vivo mass of the aforementioned digitizing protocol (9). All measurements were based on the average of three consecutive cardiac cycles. Two-dimensionally guided pulsed Doppler recordings of LV in-flow were obtained from the apical four-chamber view. Maximal early (E) and late (A) diastolic flow velocities were derived from mitral in-flow velocities. The LV outflow tract diameter was measured on a still-frame two-dimensional image at the base of the aortic leaflets in a parasternal long-axis view. The LV outflow tract velocity was measured just below the aortic valve, from an apical five-chamber view. Cardiac output (ml/kg per min) was calculated as previously described (9). The sample volume was then placed between the mitral valve and LV outflow tract so that the aortic valve closure line and the onset of mitral flow could be clearly identified. Isovolumic relaxation time (IVRT) was taken as the time from aortic valve closure to the onset of mitral flow.

Allometric relations exist between cardiac and body size measurements. However, the correct method to use in the rat is unknown. The LV mass was normalized to body weight (BW) (9,15). The linear measurements of LV wall thicknesses and chamber dimensions were normalized to the linear measurement of tibial length (TL), an index of growth that is independent of body fat or fluid homeostasis (16). Tibial length was measured by radiography after rat death, as previously described (10).

Measurement of exhaled NO. Between 12 and 24 h after echocardiography, five control and eight diabetic rats were anesthetized with ketamine and xylazine at the same doses used for echocardiography. The animals were intubated with an endotracheal tube through a small midline trache-
ostomy and mechanically ventilated with a rodent volume ventilator (Harvard Apparatus, South Natick, Massachusetts) at a tidal volume of 20 ml/kg, ratio of inspiratory to expiratory time 1.0 and respiratory rate 52 breaths/min. The inspired oxygen fraction was 0.21 from a medical air cylinder containing only oxygen and nitrogen (Airco/New England, Hingham, Massachusetts). After a 2-min stabilization period, gas was collected from the expiratory port of the ventilator for three 2-min periods, and the results were averaged. The NO content was immediately analyzed by chemiluminescence (NO analyzer model 42S [low level], Thermo Environmental Instruments Inc., Franklin, Massachusetts). Briefly, the measurement of NO by chemiluminescence involves light generation from a chemical reaction between NO in the sample and ozone in the reaction chamber (17). A photomultiplier tube produces an electrical signal proportional to the light generated from the reaction between NO in the sample and ozone. The NO analyzer was calibrated using a reference gas of known NO concentration. Expired NO is expressed as the concentration of NO in expired gas in parts per billion (ppb). The minimal detectable limit is 50 ppb (data on file from Thermo Environmental Instruments Inc.).

In vivo hemodynamic studies. In a subgroup of 17 animals (6 control, 11 diabetic) a calibrated 2F micromanometer-tipped catheter (Millar Instruments, Houston, Texas) was passed through the right carotid artery into the LV under constant pressure monitoring. Before instrumentation, rats were anesthetized with ketamine and xylazine at the same doses used for echocardiography. The LV end-diastolic pressure was recorded with an expanded scale. An estimation of vascular resistance was calculated as mean arterial blood pressure/cardiac output normalized to BW. Midwall fractional shortening was calculated as described by de Simone et al. (18). The meridional wall stress was calculated as described by Litwin et al. (13).

Blood analysis. Immediately after cardiac catheterization, 1 ml of blood was collected through direct cardiac puncture for measurement of serum fructosamine levels by a reduction method with nitroblue tetrazolium (19). Serum fructosamine levels give an indication of fluctuations in blood glucose levels that occur over a two- to three-month period (19).

In vitro isolated heart perfusion. A separate subgroup of six control and nine diabetic animals underwent isolated heart perfusion. Isolated hearts were placed in an isovolumic, buffer-perfused rat heart preparation according to the Langendorff technique, as previously described (10). Retrograde aortic perfusion of the coronary arteries was performed through a constant flow of 10 ml/min per g heart weight (20). The composition of the perfusate was as follows (mmol/liter): NaCl 118, KCl 4.7, KH2PO4 1.2, CaCl2 1.0, MgCl2 1.2, NaHCO3 23 and dextrose 10.0, and was saturated with a 95% O2 and 5% CO2 gas mixture to a pH of 7.4 ± 0.2. The LV pressure was measured using a fluid-filled latex balloon, and hearts were paced at 2.5 Hz. Measurements of LV function were obtained when the preparation achieved a steady state (~15 min); the balloon volume was then set to the lowest volume at which minimal LV pressure tracings (~1 mm Hg) could be recorded. This volume was defined as the zero volume (V0). The LV volume was then further increased in steps of 10 to 20 µl. The LV functional variables were obtained 1 to 2 min after each increment of volume when a new steady state was reached. The LV volume was increased up to a value (volmax) at which maximal peak-developed pressure was reached and a further increase in balloon volume led to a decrease of developed pressure. To achieve comparable loading conditions (i.e., balloon volumes) in hearts of different sizes, the LV variable of interest was plotted versus vol/volmax. This method of normalization has recently been validated in our laboratory by showing that control hearts of different sizes reveal superimposable pressure-volume curves, as well as superimposable relaxation and contractility curves once the intracardiac balloon volume is normalized by volmax (21). Differences in LV functional variables at balloon volume normalized by volmax can therefore be ascribed to intrinsic changes of cardiac properties. Because studying the whole heart function at 100% of volmax would represent a situation of unphysiologic preload, we compared function at 50% volmax (22).

Biochemistry. Immediately after catheterization and blood collection, five control and five diabetic hearts were excised and arrested in ice-cold Krebs-Henselte solution. The LV tissue was rapidly placed into liquid nitrogen and stored at −70°C. To obtain total muscle cell protein, the frozen LVs were ground to a fine powder and resuspended in a modified RIPA buffer (9.1 mmol/liter dibasic sodium phosphate, 1.7 mmol/liter monobasic sodium phosphate, 150 mmol/liter sodium chloride, pH 7.4, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, phenylmethylsulfonylfluoride at 10 µg/ml, pepstatin A at 1 µg/ml, leupeptin at 1 µg/ml and dithiothreitol at 1 µmol/liter). Samples were centrifuged at 14,000 rpm. Determination of the protein concentrations of the resultant supernatant were made by the method of Bradford using bovine serum albumin as a standard (23) or by the biuret reaction (24). The LV control and diabetic total cell lysates (either 50 or 100 µg) were electrophoresed in 7.5% or 4% to 20% gradient polyacrylamide/sodium dodecyl sulfate gels (25). Gels were then either stained with Coomassie brilliant blue R-250 or transferred onto nitrocellulose electrophoretically using a minitransblot electrophoretic transfer cell (Biorad, Hercules, California). Western blotting was performed by standard procedures (as described in Biorad). A polyclonal antibody for endothelial constitutive nitric oxide synthase (ecNOS) was obtained from Affinity Bioreagents, Inc. (Golden, Colorado). The secondary antibody (goat anti-rabbit) conjugated to horse radish peroxidase allowed de-
In both groups despite a lower wall stress in the diabetic,

Because both midwall and fractional shortening were similar

the diabetic animals as compared with the control animals.

However, meridional wall stress was significantly lower in

tional shortening were not different between the two groups.

pattern of eccentric hypertrophy in diabetic animals (27).

was not different between the two groups, indicating a

significantly greater in diabetic animals as compared with con-

The maintenance of immobilized ecNOS by a chemiluminescent

system (ECL, Amersham Life Sciences, Buckinghamshire, United Kingdom). Human endothelial cell lysates (Transduction Laboratories, Lexington, Kentucky) were used as a positive control. Band densities on the exposed films were then quantitated on a gel scanner using Molecular Dynamics Image Quant Version 3.0 software.

Statistics. Data are expressed as the mean value ± SEM in the figures and tables. Parametric data were compared using the Student t test or paired t-test as appropriate. Nonparametric data were compared using the Wilcoxon two-sample test. Pressure–volume data may be fitted to a monoexponential function to derive chamber stiffness. However, the real relation is frequently curvilinear (26). Therefore, the overall difference between the diabetic and control curves was analyzed by repeated-measures analysis of variance. A p value <0.05 was considered significant. Data were analyzed using Microsoft Excel, SPSS and SAS statistical packages.

RESULTS

Body Weight, Blood Glucose and Serum Fructosamine Levels

Diabetic animals gained significantly less weight than con-

control animals. Blood glucose and serum fructosamine levels were significantly elevated in diabetic animals as compared with control animals (Table 1).

Echocardiography

M-mode measurements. Diabetic animals had a signifi-

antly greater LV mass per BW as compared with control

animals. This finding suggests that the diabetic animals

exhibited impaired myocardial systolic function.

Doppler measurements. Systolic variables. Heart rates were significantly lower in diabetic animals (Table 3). Stroke volumes were higher in diabetic animals, resulting in a higher cardiac output in the diabetic group. Peripheral vascular resistance was similar between the two groups, making it unlikely that a reduced peripheral vascular resis-
tance resulted in the higher cardiac output observed in the diabetic group.

DIASTOLIC VARIABLES. Diabetic animals were character-

ized by impaired LV relaxation and filling (Table 4). This included significantly longer IVRT and an increased dependence on the atrial contribution to diastolic filling (greater peak A and reduced E/A ratio) as compared with control animals. Impaired LV relaxation and filling were not correlated with heart rate in the control or diabetic groups.

Table 1. Body Weight, Blood Glucose and Serum Fructosamine Levels

<table>
<thead>
<tr>
<th></th>
<th>Control Group (n = 14)</th>
<th>Diabetic Group (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final BW (g)</td>
<td>285 ± 6</td>
<td>188 ± 5*</td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>108 ± 6</td>
<td>32 ± 5*</td>
</tr>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>81 ± 2</td>
<td>267 ± 5*</td>
</tr>
<tr>
<td>Final fructosamine (mg/dl)</td>
<td>139 ± 7</td>
<td>245 ± 22*</td>
</tr>
</tbody>
</table>

*p < 0.0001 versus control group. Data are presented as mean value ± SEM. BW = body weight.

Table 2. M-Mode Measurements

<table>
<thead>
<tr>
<th></th>
<th>Control Group (n = 14)</th>
<th>Diabetic Group (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV mass/BW (mg/g)</td>
<td>2.6 ± 0.3</td>
<td>3.3 ± 0.6*</td>
</tr>
<tr>
<td>AWTd/TB (cm/cm)</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>AWTs/TB (cm/cm)</td>
<td>0.7 ± 0.1</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>PWTd/TB (cm/cm)</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.7</td>
</tr>
<tr>
<td>PWTs/TB (cm/cm)</td>
<td>0.7 ± 0.1</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>LVd/TB (cm/cm)</td>
<td>1.8 ± 0.2</td>
<td>2.0 ± 0.1†</td>
</tr>
<tr>
<td>LVS/TB (cm/cm)</td>
<td>0.5 ± 0.0</td>
<td>0.6 ± 0.0‡</td>
</tr>
<tr>
<td>RWT</td>
<td>0.4 ± 0.1</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Endocardial FS (%)</td>
<td>50 ± 6</td>
<td>50 ± 7</td>
</tr>
<tr>
<td>Midwall FS (%)</td>
<td>11 ± 5</td>
<td>11 ± 4</td>
</tr>
<tr>
<td>Meridional stress (kdyne/cm²)</td>
<td>37 ± 4</td>
<td>25 ± 8§</td>
</tr>
</tbody>
</table>

*p < 0.001; †p < 0.05; ‡p < 0.0001; §p < 0.01. Data are presented as the mean value ± SEM. AWTd = anterior wall thickness in diastole; AWTs = anterior wall thickness in systole; BW = body weight; FS = fractional shortening; LVd = left ventricular internal dimension in diastole; LVS = left ventricular internal dimensions in systole; PWTd = posterior wall thickness in diastole; PWTs = posterior wall thickness in systole; RWT = relative wall thickness; TB = tibial length.

Table 3. Systolic Doppler Measurements

<table>
<thead>
<tr>
<th></th>
<th>Control Group (n = 14)</th>
<th>Diabetic Group (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>250 ± 16</td>
<td>212 ± 25*</td>
</tr>
<tr>
<td>Acceleration time (ms)</td>
<td>20 ± 10</td>
<td>24 ± 10</td>
</tr>
<tr>
<td>Deceleration time (ms)</td>
<td>80 ± 10</td>
<td>113 ± 20*</td>
</tr>
<tr>
<td>Stroke volume/BW (ml/kg)</td>
<td>0.92 ± 0.17</td>
<td>1.56 ± 0.41*</td>
</tr>
<tr>
<td>Cardiac output/BW (ml/kg per min)</td>
<td>231 ± 45</td>
<td>327 ± 81*</td>
</tr>
<tr>
<td>PVR/BW (arbitrary units)</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.2</td>
</tr>
</tbody>
</table>

*n = 6; (n = 11)

*p < 0.0001. Data are presented as the mean value ± SEM. BW = body weight; PVR = peripheral vascular resistance.
Table 4. Diastolic Doppler Measurements

<table>
<thead>
<tr>
<th></th>
<th>Control Group (n = 14)</th>
<th>Diabetic Group (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVRT (ms)</td>
<td>26 ± 6</td>
<td>40 ± 8*</td>
</tr>
<tr>
<td>Peak E (m/s)</td>
<td>0.70 ± 0.07</td>
<td>0.75 ± 0.11</td>
</tr>
<tr>
<td>Peak A (m/s)</td>
<td>0.30 ± 0.08</td>
<td>0.47 ± 0.09*</td>
</tr>
<tr>
<td>Peak E/A</td>
<td>2.52 ± 0.81</td>
<td>1.64 ± 0.36†</td>
</tr>
<tr>
<td>VTIE (cm/s)</td>
<td>0.03 ± 0.00</td>
<td>0.03 ± 0.00</td>
</tr>
<tr>
<td>VTIA (cm/s)</td>
<td>0.01 ± 0.00</td>
<td>0.02 ± 0.01*</td>
</tr>
</tbody>
</table>

*p < 0.0001; †p < 0.03; ‡p < 0.001. Data are presented as the mean value ± SEM. IVRT = isovolumic relaxation time; Peak A = peak velocity of late filling; Peak E = peak velocity of early filling; VTIE = velocity time integral of early filling; VTIA = velocity time integral of late filling.

Animals other than control and diabetic (peak A, E/A, IVRT: all r < 0.40, p > 0.05), excluding an influence of heart rate on the impaired diastolic variables.

Expired NO Levels

Mixed expired NO levels were lower in the diabetic group than in the control group (1.78 ± 0.16 vs. 3.26 ± 0.30 ppb, p < 0.01). Mixed expired NO levels were correlated with a greater dependence of atrial filling (greater peak A velocity) to total diastolic filling (r = −0.7, p = 0.08 [borderline significance]), a reduced E/A ratio (r = 0.7, p < 0.01) and prolonged IVRT (r = −0.5, p < 0.05) in all animals (Fig. 1). There was no significant correlation within each group between expired NO and the echocardiographic variables of cardiac function.

In Vivo and In Vitro Hemodynamic Studies

Peak LV systolic pressure, aortic diastolic pressure and mean arterial pressure were significantly lower in diabetic animals (Table 5). The mean LV end-diastolic pressure was significantly higher in diabetic animals than in control animals.

Fig. 1. Exhaled NO versus diastolic filling pattern. Mixed expired NO levels were strongly correlated with a greater atrial contribution to diastolic filling (reduced E/A ratio) in all animals. Circles = diabetic animals; squares = control animals.

Table 5. Mechanical Variables In Vivo and in the Isolated Whole Heart

<table>
<thead>
<tr>
<th></th>
<th>Control Group (n = 6) In Vivo</th>
<th>Diabetic Group (n = 11) In Vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak LV systolic pressure (mm Hg)</td>
<td>139 ± 14</td>
<td>92 ± 23*</td>
</tr>
<tr>
<td>Aortic diastolic pressure (mm Hg)</td>
<td>105 ± 5</td>
<td>65 ± 21†</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>116 ± 8</td>
<td>74 ± 21*</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>2 ± 1</td>
<td>7 ± 6*</td>
</tr>
</tbody>
</table>

Control Group (n = 9) In Vivo

<table>
<thead>
<tr>
<th></th>
<th>Control Group (n = 6) In Vitro</th>
<th>Diabetic Group (n = 11) In Vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPP (mm Hg)</td>
<td>133 ± 52</td>
<td>135 ± 31</td>
</tr>
<tr>
<td>Peak LV pressure (mm Hg)</td>
<td>112 ± 36</td>
<td>73 ± 19‡</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>4 ± 5</td>
<td>5 ± 4</td>
</tr>
<tr>
<td>Peak developed pressure (mm Hg)</td>
<td>109 ± 36</td>
<td>68 ± 19‡</td>
</tr>
<tr>
<td>90% Relaxation time (ms)</td>
<td>139 ± 11</td>
<td>142 ± 18</td>
</tr>
<tr>
<td>Peak +dP/dt (mm Hg/s)</td>
<td>2,517 ± 672</td>
<td>1,461 ± 428‡</td>
</tr>
<tr>
<td>Peak −dP/dt (mm Hg/s)</td>
<td>1,374 ± 391</td>
<td>843 ± 223‡</td>
</tr>
</tbody>
</table>

*p < 0.0001; †p < 0.03; ‡p < 0.05 control versus diabetic group. Data are presented as the mean value ± SEM.

CPP = coronary perfusion pressure; dP/dt = rate of change in left ventricular pressure; LV = left ventricular; LVEDP = left ventricular end-diastolic pressure; MAP = mean arterial pressure.

In vitro coronary perfusion pressure was not different between the two groups (Table 5). Peak LV systolic pressure, developed pressure and +dP/dtmax were significantly lower in the diabetic animals than in the control animals. The LV end-diastolic pressure did not differ between the diabetic and control animals. The −dP/dtmin was significantly lower in the diabetic group than in the control group. The diabetic diastolic pressure–volume relation was shifted to the left, with a significant change in the shape of the curve, indicating increased chamber stiffness (Fig. 2).

Comparison of ecNOS Expression

Western blots of cell lysates from control and diabetic LVs were performed to determine if any differences in the relative expression of the ecNOS protein existed between control (n = 4) and diabetic hearts (n = 4) (Fig. 3). There was no difference in relative ecNOS protein levels (control = 129,529 ± 9,275, diabetic = 129,222 ± 14,662, p = NS; expressed as a percentage: control 100%, diabetic 99.7%).

DISCUSSION

The present study provides an integrated approach to the assessment of cardiac function in the diabetic rat, utilizing both Doppler echocardiographic and invasive hemodynamic techniques.
data, and it provides important new insights into the pathophysiology of diabetic cardiomyopathy as it relates to the NO pathway. The Doppler echocardiographic findings in the STZ-induced diabetic animals showed evidence of a cardiomyopathy characterized by eccentric hypertrophy, abnormal LV filling and systolic dysfunction. In vivo and in vitro hemodynamic data revealed impaired systolic and diastolic function. Diabetic animals were characterized by lower exhaled NO levels, which were correlated with abnormal LV filling. We therefore postulate that a defect in the NO pathway contributes to the cardiac dysfunction in experimental diabetes mellitus, probably as a result of the decreased availability of endothelial NO, as cardiac ecNOS protein levels were similar between the two groups. In addition, exhaled NO may serve as a noninvasive marker for this myocardial dysfunction.

Doppler echocardiographic findings. The echocardiographic finding of eccentric hypertrophy provides new morphologic data not obtained previously from conventional invasive hemodynamic data in animal models (8). By integrating this morphologic data with the physiologic data obtained in our study, it is apparent that a thickened and stiff myocardium results in diastolic dysfunction characterized by elevated LV filling pressures. Extending this concept of diastolic dysfunction to clinical practice provides a firm basis for the hypothesis that diastolic dysfunction is one mechanism by which diabetic patients have a higher incidence of congestive heart failure in the absence of coronary artery disease and in the post–myocardial infarction period, the latter despite similar infarct sizes and ejection fractions, as compared with nondiabetic patients (28).

Diastolic dysfunction. In our study, diastolic dysfunction was characterized by impaired LV relaxation and increased chamber stiffness. The finding of increased chamber stiffness has not been a consistent finding in animal models (12) and may relate to methodologic issues in calculating stiffness or compliance (26). Some hemodynamic studies reporting pressure–volume relations have noted upward or downward shifts in response to a specific intervention (26). However, because neither the slope nor the shape of the pressure–volume relation was altered, this may not be due to a change in chamber stiffness or compliance, and the term "distensi-
LV variables of interest were plotted versus vol/volmax. This change in stiffness. To achieve comparable loading conditions (i.e., balloon volumes) in hearts of different sizes, the LV variables of interest were plotted versus vol/volmax. This method of normalization has not been used in previous studies and may also explain the discrepancies noted in these studies as compared with ours. Although we did not measure myocardial and interstitial collagen, they do not appear to be consistently increased (8), suggesting that metabolic or myocardial factors may play a significant role in the increased chamber stiffness noted. This concept is strengthened by the observation that insulin therapy corrected the hemodynamic and mechanical abnormalities in a rat model of diabetes mellitus, unassociated with histologic changes (8). The LV end-diastolic pressure was elevated in vivo but not in vitro assessment. This may relate to the influence of the constraining effect of the pericardium in the closed-chest animal as opposed to the in vitro isolated perfused heart (29).

Systolic dysfunction. Diabetic animals were characterized by impaired systolic performance. This included a lower peak-developed pressure and +dP/dt in vitro, whereas endocardial and midwall shortening did not differ between diabetic and control animals, despite a lower wall stress in the diabetic animals. These findings suggest that intrinsic myocardial contractility was reduced in the diabetic animals. The bradycardia noted in the diabetic group has been a consistent finding (8,30). We excluded an influence of bradycardia on the echocardiographic variables of LV diastolic function by showing no correlation between heart rate and diastolic relaxation and filling. In addition, slower heart rates result in a smaller atrial contribution to ventricular filling (31), yet despite bradycardia, we found a greater atrial contribution to ventricular filling in the diabetic animals.

NO and diabetes. Nitric oxide is synthesized from L-arginine by three different isoforms of the enzyme NO synthase (ecNOS, a neuronal type [nNOS] and an inducible enzyme iNOS), and this pathway is inhibited by L-arginine analogues (32). The cardiac effects of NO are complex and species-dependent, and the results have been conflicting in terms of a beneficial or deleterious effect of NO. The direct NO donor, nitroprusside, increased myocardial relaxation in the isolated ejecting heart (33). Data from peripheral vascular endothelial function in diabetic patients suggest that endothelium-dependent vasodilatation is impaired in patients with diabetes mellitus and experimentally induced hyperglycemia, and function can be restored by NO donors, suggesting decreased availability of endothelial NO as a contributor to endothelial dysfunction (5,6). However, in direct contradistinction to these results, NO donors reduce peak contractile performance and shorten the myocardial contraction phase in humans (34). L-NAME, a nonselective inhibitor of NOS, improves ventricular performance in isolated perfused diabetic hearts, suggesting a role for increased NO in diabetic cardiomyopathy (35). Chronic administration of the inducible NO synthase (iNOS) inhibitor, aminoguanidine, in diabetic rats improved the decreased myocardial compliance (19).

Exhaled NO. The development of chemiluminescence analyzers has allowed the accurate measurement of NO in exhaled air. The major sources of NO in expired air are the nose and sinuses, with a lesser contribution from NO produced in the lungs and airways (36). Pulmonary production of NO probably arises from both pulmonary vascular endothelium and pulmonary epithelial cells, as both constitutive and inducible forms of NO synthase have been identified in cells representative of bronchial epithelium and type II alveolar pneumocytes (37). Nitric oxide produced by the pulmonary vascular endothelium appears to function as a pulmonary vasodilator (38). Nasal breathing leads to higher concentrations of exhaled NO owing to contamination from NO produced in the paranasal sinuses and nasopharynx (39). We eliminated upper airway contamination by sampling NO from tracheal intubation. It is unlikely that NO produced by the peripheral circulation is eliminated through the lungs, as NO is avidly bound to hemoglobin, which results in its inactivation on reaching the intravascular space (40). More specifically, we showed that the decreased expired NO is not due to a lesser amount of the enzyme ecNOS, but is more likely limited by substrate availability. Although we did not measure ecNOS in the lung, the reduced exhaled NO in the diabetic animals probably is a marker for the generalized reduced availability of NO. However, we cannot exclude lower lung levels of ecNOS in the diabetic animals.

Study limitations. Although IVRT and filling patterns can assess LV diastolic chamber function, under some circumstances they lack specificity (26). If the systolic pressure is high, the IVRT can be prolonged, even if the relaxation process is unchanged. The E/A ratio can be reduced despite no change in relaxation and a decline in chamber stiffness purely by lowering chamber preload. This would result in a lower E wave velocity (reduced early filling pressure gradients between the left atrium and ventricle), but have relatively less effect on the A wave. These factors do not appear to play a major role in our study, as the systolic pressure was lower in the diabetic animals and LV filling showed a greater A wave with little effect on the E wave. These factors do not appear to play a major role in our study, as the systolic pressure was lower in the diabetic animals and LV filling showed a greater A wave with little effect on the E wave. Although protein levels of ecNOS were similar in both groups, we did not measure ecNOS function or messenger ribonucleic acid expression. The localization of ecNOS was not determined, but studies have reported ecNOS in coronary endocardial endothelium (41,42) and cardiac myocytes (43). Accelerated inactivation of NO is another potential mechanism for decreased NO availability in diabetic rats (44).

Clinical implications. Cardiovascular disease is a major cause of morbidity and mortality in diabetic patients. In the
absence of coronary artery disease, diabetic patients may exhibit evidence of a cardiomyopathy (2). However, the specific abnormality and how this cardiomyopathy relates to the higher postinfarction mortality observed in diabetic patients has not been well defined. We have shown in the STZ-induced diabetic rat that the cardiomyopathy is characterized by eccentric hypertrophy and both diastolic and systolic dysfunction. Further, this observed diastolic dysfunction is correlated with reduced NO availability. This constellation of pathologic findings elucidated by our study represents several mechanisms by which diabetic patients have a higher incidence of congestive heart failure in the absence of coronary artery disease, and also in the post-myocardial infarction period. Our data suggest that exhaled NO may serve as a useful noninvasive marker for diabetic myocardial dysfunction, and if validated in the clinical setting, may be used both as an early marker for myocardial dysfunction and to assess the effects of various therapeutic strategies.

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