Comparative investigation of the left ventricular pressure-volume relationship in rat models of type 1 and type 2 diabetes mellitus

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Radovits T, Korkmaz S, Loganathan S, Barnucz E, Bömicke T, Arif R, Karck M, Szabó G. Comparative investigation of the left ventricular pressure-volume relationship in rat models of type 1 and type 2 diabetes mellitus. Am J Physiol Heart Circ Physiol 297: H125–H133, 2009. First published May 8, 2009; doi:10.1152/ajpheart.00165.2009.—Diabetes mellitus (DM) is associated with characteristic structural and functional changes of the myocardium, termed diabetic cardiomyopathy. As a distinct entity independent of coronary atherosclerosis, diabetic cardiomyopathy is an increasingly recognized cause of heart failure. A detailed understanding of diabetic cardiac dysfunction, using relevant animal models, is required for the effective prevention and treatment of cardiovascular complications in diabetic patients. We investigated and compared cardiac performance in rat models of type 1 DM (streptozotocin induced) and type 2 DM (Zucker diabetic fatty rats) using a pressure-volume (P-V) conductance catheter system. Left ventricular (LV) systolic and diastolic function was evaluated in vivo at different preloads, including the slope of the end-systolic P-V relation (ESPVR) and end-diastolic P-V relationship (EDPVR), preload recruitable stroke work (PRSW), maximal slope of the systolic pressure increment (dP/dtmax), and its relation to end-diastolic volume (dP/dvmax-EDV) as well as the time constant of LV relaxation and maximal slope of the diastolic pressure decrement. Type 1 DM was associated with decreased LV systolic pressure, dP/dvmax, slope of ESPVR and dP/dvmax-EDV, PRSW, ejection fraction, and cardiac and stroke work indexes, indicating marked systolic dysfunction. In type 2 DM rats, systolic indexes were altered only to a lower extent and the increase of LV stiffness was more pronounced, as indicated by the higher slopes of EDPVR. Our data suggest that DM is characterized by decreased systolic performance and delayed relaxation (mainly in type 1 DM), accompanied by increased diastolic stiffness of the heart (more remarkably in type 2 DM). Based on the sophisticated method of P-V analysis, different characteristics of type 1 and type 2 diabetic cardiac dysfunction can be demonstrated.

systolic dysfunction; diastolic dysfunction; systolic function; diastolic function

CARDIOVASCULAR COMPLICATIONS are one of the main cause of mortality in diabetes mellitus (DM). Although much of the increased risk of cardiovascular diseases can be attributed to an increased atherosclerotic process, there is obvious evidence demonstrating that DM leads to structural and functional changes at the level of the myocardium. The development of diabetic cardiomyopathy has been described in both type 1 and type 2 DM and is suggested to be a consequence of altered cellular metabolism of the myocardium (3, 4).

Impaired cardiac performance in DM has been described since the 1970s (25), and diabetic cardiomyopathy has been intensively investigated. For many years, most of the experimental studies on diabetic cardiovascular dysfunction have been performed using streptozotocin (STZ)-induced type 1 diabetic (insulin deficient) rats, the most widely used, “standard” animal model of DM (18). Nevertheless, the majority of patients have type 2 diabetes (hyperinsulinemia and insulin resistance). According to the different pathophysiologic background, a number of animal models of type 2 DM have been developed (24) and have become the object of cardiovascular investigations in recent years (8, 28, 11).

Despite the numerous studies in the field of diabetic cardiomyopathy, there are rather controversial data throughout the literature regarding the severity and characteristics of cardiac dysfunction in DM, depending on the experimental approach used (echocardiography, isolated hearts, in vivo catheterization, MRI, etc.), animal species used, type and duration of diabetes, or functional indexes assessed (2, 4, 6, 18, 19, 20, 28). The major limitation of the previously used invasive and noninvasive approaches to study cardiac function in small animal models is that the hemodynamic parameters measured were not objective enough (echocardiography) or were largely dependent on loading conditions. Therefore, appropriate, hemodynamically validated standards of in vivo experimental models of type 1 and type 2 diabetic cardiac dysfunction are required for the reliable investigation of diabetic cardiomyopathy.

Pressure-volume (P-V) analysis is a useful approach for examining in vivo intact ventricular function independently of loading conditions. This analysis has been widely used in large animal studies and in humans since the mid-1980s (16). Advances of the last decade in the development and validation of miniature P-V catheters have made it possible to use this approach for studies in small animals (9). The technique of left ventricular (LV) P-V analysis in rats has been introduced only recently, and very limited normative data are available (22).

In the present study, we provide a detailed characterization of the systolic and diastolic function of the LV in rat models of type 1 (STZ induced) and type 2 [Zucker diabetic fatty (ZDF) rats] DM using a P-V conductance catheter system. We aimed to reliably assess the severity and characteristics of type 1 and type 2 diabetic cardiac dysfunction and to compare the two models.

MATERIALS AND METHODS

Animals

This investigation conformed with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1996). All procedures and handling of the animals...
Table 1. Body weights, blood glucose, and urine glucose values of control SD, STZ-induced diabetic SD, ZDF lean, and ZDF diabetic rats

<table>
<thead>
<tr>
<th></th>
<th>Control SD</th>
<th>STZ-Induced Diabetic SD Rats</th>
<th>ZDF Lean</th>
<th>ZDF Diabetic Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>13</td>
<td>6</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>423.9±5.3</td>
<td>340.0±12.9*</td>
<td>435.0±6.3</td>
<td>357.9±9.8*</td>
</tr>
<tr>
<td>Blood glucose, mmol/l</td>
<td>6.69±0.19</td>
<td>24.08±0.97*</td>
<td>6.49±0.33</td>
<td>23.61±2.88*</td>
</tr>
<tr>
<td>Urine glucose, qualitative</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Values are means ± SE; N, no. of rats/group. SD, Sprague-Dawley; STZ, streptozotocin; ZDF, Zucker diabetic fatty. *P < 0.05 vs. the corresponding nondiabetic group.

During the study were reviewed and approved by the local Ethical Committee for Animal Experimentation.

Rat model of type 1 DM. Young adult (12 wk old) male Sprague-Dawley rats (Charles River, Sulzfeld, Germany) were housed at one rat per cage in a room at a constant temperature of 22 ± 2°C with a 12:12-h light-dark cycle and fed a standard laboratory rat diet and water ad libitum. After acclimatization, type 1 DM was induced in rats with a single dose of STZ at a dose of 60 mg/kg ip. STZ was freshly dissolved in citrate buffer (0.1 mol/l). Control animals received only the buffer (control Sprague-Dawley group). Seventy-two hours after the injection of STZ, a drop of blood was collected from the tail vein, and the blood glucose concentration was determined using a digital blood glucose meter and test strips (Accu-Chek Sensor, Roche, Mannheim, Germany). Animals with a random blood glucose level of >15 mmol/l were considered as diabetic and were included into the study (STZ-induced diabetic Sprague-Dawley group). Experiments on the rats were performed 8 wk after the confirmation of diabetes (this time point was chosen on the basis of previous pilot studies).

Rat model of type 2 DM. The ZDF rat is an inbred rat model that through genetic mutation and a special diet develops type 2 diabetes and related complications. Homozygous recessive males (fa/fa) develop obesity, fasting hyperglycemia, and type 2 diabetes. Homozygous dominant (+/+ ) and heterozygous (fa/+ ) lean genotypes remain normoglycemic. Young adult (12 wk old) male ZDF diabetic (fa/fa) and ZDF lean (+/+ ) rats (Charles River, Kingston, NY) were housed at one rat per cage in a room at a constant temperature of 22 ± 2°C with a 12:12-h light-dark cycle and fed a diet of Purina 5008 as recommended by the supplier and water ad libitum. Experiments on the rats were performed at the age of 30–32 wk (this time point was chosen on the basis of previous pilot studies).

Blood and Urine Glucose Measurements

Before the hemodynamic measurements, blood samples were collected from the tail vein. Urine samples were collected by punctation of the urine bladder after the hemodynamic measurements had been completed. Blood and urine glucose levels were determined by a digital blood glucose meter and test strips (Accu-Chek Sensor, Roche).

Hemodynamic Measurements

Rats were anesthetized with a mixture of ketamine (100 mg/kg ip) and xylazine (3 mg/kg ip), tracheotomized, and intubated to facilitate breathing. Animals were placed on controlled heating pads, and the core temperature, measured via a rectal probe, was maintained at 37°C. A polyethylene catheter was inserted into the left external jugular vein for fluid administration. A 2-Fr microtip P-V catheter (SPR-838, Millar Instruments, Houston, TX) was inserted into the right carotid artery and advanced into the ascending aorta. After stabilization for 5 min, arterial blood pressure was recorded. After that, the catheter was advanced into the LV under pressure control. After stabilization for 5 min, signals were continuously recorded at a sampling rate of 1,000 samples/s using a P-V conductance system (MPVS-400, Millar Instruments), stored, and displayed on a personal computer by the PowerLab Chart5 Software System (AD Instruments, Colorado Springs, CO).

With the use of a special P-V analysis program (PVAN, Millar Instruments), heart rate (HR), mean arterial pressure (MAP), maximal LV systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), the maximal slope of the systolic pressure increment (dP/dmax) and the diastolic pressure decrement (dP/dmin) and constant time of LV pressure decay [τ, according to the Weiss method and Glantz method (22)], ejection fraction (EF), and stroke work (SW) were computed and calculated. Stroke volume (SV) and cardiac output (CO) were calculated and calculated according to in vitro and in vivo volume calibrations using PVAN software. To exclude the influence of body weight differences, CO and SW were normalized to body weight [cardiac index (CI) and SW index (SWI)]. The total peripheral resistance index (TPRI) was calculated by the following equation:

\[ TPRI = \frac{MAP}{CI} \]

In addition to the above parameters, P-V loops recorded at different preload can be used to derive other useful systolic function indexes that may be less influenced by loading conditions and cardiac mass (13, 22). Therefore, LV P-V relations were measured by transiently compressing the inferior vena cava (reducing preload) under the diaphragm with a cotton-tipped applicator. The slope (E\text{max}) of the LV end-systolic P-V relationship [ESPVR; according to the parabolic curvilinear model (14)], preload recruitable SW (PRSW), and the dP/d\text{max}-end-diastolic volume relation (dP/d\text{max}-EDV) were calculated as load-independent indexes of LV contractility. The slope of the LV end-diastolic P-V relationship (EDPVR) was calculated as a reliable index of LV stiffness (22).

At the end of each experiment, 100 μl of hypertonic saline were injected intravenously, and from the shift of P-V relations, parallel conductance volume (V\text{p}) was calculated by the software and used for the correction of the cardiac mass volume. The volume calibration of the conductance system was performed as previously described (22). Briefly, nine cylindrical holes in a block 1 cm deep and with known
Cardiac Function

Baseline hemodynamic data. Figure 1 shows representative LV blood pressure and dP/dt signals obtained from diabetic and control animals in both models. The decreased amplitude of these signals in diabetic rats indicates a decrease in cardiac contractility. As shown in Table 2, type 1 DM was associated with significantly decreased HR, LVSP, dP/dt max, dP/dt min, EF, SV, CI, and SWI compared with nondiabetic controls. τ and TPRI were increased in STZ-induced diabetic animals. MAP and LVEDP were not significantly altered (Table 2).

Compared with the corresponding control group (ZDF lean), type 2 DM was associated with significantly decreased HR and CO, and there was a very strong tendency toward decreased MAP, CI, SW, dP/dt max, and dP/dt min; however, these differences did not reach the level of statistical significance. Increased τ and LVEDP were also detected in diabetic rats. LVSP, EF, and TPRI were not altered in this model (Table 3).

Functional indexes derived from P-V analysis at different preloads. Figure 2 shows representative original P-V loops registrated during transient occlusion of the inferior vena cava in rat models of type 1 and type 2 DM. Overall, the results of ESPVR (Emax) and EDPRV are shown in Tables 2 and 3, respectively. As shown in Fig. 2 and Table 2, ESPVR was steeper in nondiabetic animals than in STZ-induced diabetic animals, suggesting decreased systolic performance in type 1 DM. In contrast, EDPRV had a tendency (not statistically significant) to be increased in type 1 diabetic rats. Interestingly, compared with the corresponding control, type 2 diabetic ZDF rats showed only slightly decreased E max but significantly increased EDPRV, indicating a marked increase in end-diastolic stiffness (Fig. 2 and Table 3).

Table 2. Hemodynamic parameters in the rat model of type 1 diabetes mellitus

<table>
<thead>
<tr>
<th></th>
<th>Control SD Rats</th>
<th>STZ-Induced Diabetic SD Rats</th>
<th>P Value Versus Control SD Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>277.2±8.6</td>
<td>213.3±23.1*</td>
<td>0.0025</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>77.8±3.4</td>
<td>72.8±2.5</td>
<td>0.2903</td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>106.2±2.8</td>
<td>95.6±5.2*</td>
<td>0.0003</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>8.1±0.3</td>
<td>8.4±0.2</td>
<td>0.4139</td>
</tr>
<tr>
<td>CO, ml/min</td>
<td>48.23±4.44</td>
<td>24.11±5.23*</td>
<td>0.0011</td>
</tr>
<tr>
<td>CI, ml·min⁻¹·1·100 g body wt⁻¹</td>
<td>14.00±0.98</td>
<td>7.21±1.92*</td>
<td>0.0005</td>
</tr>
<tr>
<td>EF, %</td>
<td>64.8±2.3</td>
<td>55.3±1.9*</td>
<td>0.0305</td>
</tr>
<tr>
<td>SW, mmHg·ml·100 g body wt⁻¹</td>
<td>13.21±1.56</td>
<td>8.97±1.12*</td>
<td>0.0571</td>
</tr>
<tr>
<td>SWI, mmHg·ml·100 g body wt⁻¹</td>
<td>4.03±0.43</td>
<td>2.65±0.32*</td>
<td>0.0321</td>
</tr>
<tr>
<td>dP/dt max, mmHg/s</td>
<td>730.2±242</td>
<td>596.8±176*</td>
<td>0.0006</td>
</tr>
<tr>
<td>dP/dt min, mmHg/s</td>
<td>768.1±297</td>
<td>4815.8±183*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>τ (Weiss), ms</td>
<td>9.4±0.4</td>
<td>13.3±0.8*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>τ (Glantz), ms</td>
<td>10.6±0.5</td>
<td>14.1±1.2*</td>
<td>0.0006</td>
</tr>
<tr>
<td>TPRI, mmHg·ml⁻¹·min⁻¹·1·100 g body wt⁻¹</td>
<td>5.67±0.51</td>
<td>11.97±2.39*</td>
<td>0.0002</td>
</tr>
<tr>
<td>SV, ml</td>
<td>160.4±10.4</td>
<td>118.8±12.8*</td>
<td>0.0136</td>
</tr>
<tr>
<td>E max, mmHg/μl</td>
<td>2.23±0.20</td>
<td>1.04±0.07*</td>
<td>0.0002</td>
</tr>
<tr>
<td>Slope of EDPVR, mmHg/μl</td>
<td>0.037±0.002</td>
<td>0.054±0.013</td>
<td>0.1558</td>
</tr>
<tr>
<td>PRSW, mmHg</td>
<td>76.44±4.06</td>
<td>45.39±2.45*</td>
<td>0.0002</td>
</tr>
<tr>
<td>Slope of dP/dt max-EDV, mmHg·s⁻¹·μl⁻¹</td>
<td>36.64±2.68</td>
<td>24.37±4.25*</td>
<td>0.0201</td>
</tr>
</tbody>
</table>

Values are means ± SE. Hemodynamic parameters were measured by the Millar-pressure-volume conductance catheter system. *Statistically significant value vs. control SD rats.

Statistical Analysis

All data are expressed as means ± SE. An unpaired two-sided Student’s t-test was used to compare parameters of diabetic and control rats in both models. To compare diabetes-induced changes of cardiac function between the two DM models, individual data of the diabetic animals were normalized to the mean value of the corresponding control group. An unpaired two-sided Student’s t-test was used to compare these diabetes-associated percent changes of each parameter between the two models. Differences were considered statistically significant when P < 0.05.

RESULTS

Body Weight and Glucose Levels

Compared with the corresponding control group, DM was associated with decreased body weight and increased blood and urine glucose levels in both models (Table 1). Blood glucose levels did not significantly differ between the two diabetic groups.
Figure 3, bottom, shows PRSW (the slope of the linear relation between SW and EDV) in a representative diabetic and control animal in both models. The slope was steeper in nondiabetic control rats than in diabetic rats, indicating decreased systolic performance in DM. Compared with the corresponding control animals, the overall PRSW values were significantly lower in diabetic rats in the type 1 DM model and showed a strong tendency toward impaired values in type 2 DM (Fig. 3, top, and Tables 2 and 3).

Figure 4, bottom, shows the relation between dP/dt max and EDV in a representative diabetic and control animal in both models. The slope was steeper in nondiabetic control rats than in diabetic rats, indicating decreased systolic performance in DM. Compared with the corresponding control animals, the overall PRSW values were significantly lower in diabetic rats in the type 1 DM model and showed a strong tendency toward impaired values in type 2 DM (Fig. 4, top, and Tables 2 and 3).

Diabetes-associated changes in systolic and diastolic functional parameters: a comparison of the two models. Impairment of the load-dependent contractility parameters EF and dP/dt max was not significantly different between the two DM models, whereas the impairment of the P-V loop-derived, load-independent contractility indexes $E_{\text{max}}$ and PRSW was found to be significantly higher in type 1 DM compared with type 2 DM (Fig. 5A), indicating a significantly more severe decrease in LV contractile function in type 1 DM.

Diabetes-associated changes of the LV relaxation parameters dP/dt min and $\tau$ showed a tendency toward higher values in the type 1 DM model; however, the level of statistical significance was not reached. In turn, parameters of LV stiffness (LVEDP and slope of EDPVR) changed to a significantly higher extent in diabetic animals in the type 2 DM model than in those in the model of type 1 DM (Fig. 5B), indicating a significantly more severe increase in LV stiffness (reduction of LV compliance) in type 2 DM.
DISCUSSION

Based on former observations of structural alterations in diabetic hearts, numerous clinical and experimental studies have aimed to characterize cardiac function in DM. Systolic and/or diastolic dysfunction of different severity have been described inconsistently in patients and experimental animals with type 1 and type 2 DM using invasive and noninvasive methods (3, 4, 7).

To our knowledge, this is the first study to reliably characterize and compare systolic and diastolic cardiac function in rat models of type 1 and type 2 DM using a P-V conductance catheter system and measuring load-independent functional indexes. Here, we show that diabetic cardiomyopathy is characterized by decreased systolic performance (more pronounced in type 1 DM) accompanied by delayed relaxation and increased diastolic stiffness of the heart (predominantly in type 2 DM), indicating different characteristics and severity of diabetic cardiac dysfunction depending on the type of DM.

Our baseline hemodynamic data in the type 1 diabetic rat model are in good agreement with the results of Litwin et al. (18). In that study, Litwin et al. described, for the first time, abnormal cardiac function in STZ-induced diabetic rats by measurements of LV pressure-derived parameters (decreased LVSP, dP/dt\text{max}, and dP/dt\text{min} and prolonged $\tau$). Similarly, recent experimental studies on the same rat model reported systolic and diastolic dysfunction in type 1 DM as assessed by echocardiographic and LV pressure-derived indexes (3) as well as by high-resolution MRI (19). In accordance with literature data (18, 27), we detected decreased HRs in diabetic animals of both models compared with control animals. Considering the Bowditch effect, this fact may also contribute to alterations in cardiac contractility. To exclude the influence of HR differences on contractility, adjustment of HR by pacing should be performed, which was unfortunately not possible in the present closed-chest model. Furthermore, in the relatively small rat heart, the sensitive sensors of the conductance catheter might be disturbed by the electric stimuli of the very close pacing electrodes.

Systolic function and cardiac contractility

We found in our rat model that type 1 DM was associated with significantly decreased dP/dt\text{max} and EF (Table 2). Accordingly, several studies have shown impaired dP/dt\text{max} and/or EF in type 1 diabetic rats (3, 10, 18). Although dP/dt\text{max} has been widely used as a cardiac contractility parameter, it is well recognized that it is dependent on loading conditions,
especially on changes in preload (13, 15). EF is also known to
be influenced by both preload and afterload and, therefore,
cannot reliably be used to assess contractile function in models
where loading is altered.

There are previous works from a research group that have
described impaired LV contractility in STZ-induced diabetic
rats by measuring LV pressure and aortic flow and using a
mathematical model (elastance-resistance LV model) (5, 31).
The method of simultaneous LV pressure and volume analysis
by the miniaturized pressure-conductance catheters allowed us
to calculate highly sensitive and reliable load-independent
indexes of LV contractility.

Historically, ESPVR ($E_{\text{max}}$) has been proposed as a fairly
load-insensitive index of ventricular contractility. According to
the present results, $E_{\text{max}}$ was significantly decreased in type 1
diabetic animals, indicating an impaired contractile state of the
LV myocardium (Fig. 2 and Table 2). Correspondingly, de-
creased $E_{\text{max}}$ has been recently reported in STZ-induced dia-
betic mice (29). However, because this relation can be altered
not only by changes in the inotropic state but also by changes
in chamber geometry and other diastolic factors, we also
calculated other parameters (13). PRSW represents the slope of
the linear relation between SW and EDV. It has been described
as a parameter independent of chamber size and mass, and it is
sensitive to the contractile function of the ventricle. Although
it integrates data from the entire cardiac cycle, it is influenced
most of all by the systole (13). PRSW was also significantly
decreased in type 1 diabetic animals compared with control
animals (Fig. 3 and Table 2). A previous investigation (17) has
demonstrated that the slope of the relation between $dP/dr_{\text{max}}$
and EDV, another P-V loop-derived index, represents a sensi-
tive but less load-dependent parameter of LV contractility. We
found that this index was also depressed in type 1 diabetic rats
compared with control rats (Fig. 4 and Table 2).

In contrast with our present findings and many previous
results, Connelly et al. (6) reported in a recent work about
completely unaltered cardiac function (no changes in baseline
hemodynamics and contractility parameters) in STZ-induced
diabetic rats. However, their diabetic rats were treated with
insulin; therefore, the use of these animals was not reliable for
investigating the disease (diabetic cardiovascular dysfunction)
itself.

Regarding the model of type 2 DM, we have shown that LV
systolic function, as characterized by both classical ($dP/dr_{\text{max}}$
and EF) and sensitive, P-V loop-derived, load-independent
functional parameters ($E_{\text{max}},$ PRSW, and $dP/dr_{\text{max}}$-EDV), was
not significantly impaired in type 2 diabetic animals. However,
there was a strong tendency toward impaired load-independent
contractility indexes in diabetic ZDF rats. Echocardiographic
and MRI studies in experimental type 2 DM have revealed discrepant results on cardiac dysfunction. Previous works that
reported impaired systolic function in ZDF (32) and Goto-
Kakizaki rats (12) could not be reproduced by another group
(8). Moreover, there are experimental results showing en-
hanced LV systolic function in an early stage of type 2 DM (2,
8). Recently, a reliable LV P-V analysis showed preserved

Fig. 4. Maximal slope of the systolic pressure increment ($dP/dr_{\text{max}}$)-EDV relationship. Slope values of the relationship between $dP/dr_{\text{max}}$ and EDV are shown from the groups of control SD, STZ-induced diabetic SD, ZDF lean, and ZDF diabetic rats (top). The relation between $dP/dr_{\text{max}}$ and EDV in one representative rat from each group of both models is shown at the bottom. *$P < 0.05$ vs. the corresponding nondiabetic group. Note that the slope values were lower in diabetic rats than in nondiabetic rats, suggesting that systolic performance is decreased in DM.

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systolic function in type 2 diabetic ZDF rats at the age of 37 wk (27), which is in line with our present findings. In another recent study, Marsh et al. (20) found a markedly increased prevalence of hydronephrosis in diabetic ZDF rats (20), which could be associated with LV dysfunction and therefore might represent a limitation of the ZDF model for the investigation of diabetic cardiomyopathy. Nevertheless, in the present study, we observed hydronephrosis only in one animal (the contractility data of which has been excluded); thus, hydronephrosis could not be a factor influencing LV function in our experiments.

When the two models were compared, contractile function showed a significantly higher deterioration in type 1 DM if assessed by P-V loop derived, load-independent contractility indexes ($E_{\text{max}}$ or PRSW) but not by load-dependent classical contractility parameters (EF and $dP/dt_{\text{max}}$; Fig. 5A). This emphasizes the importance of P-V analysis as the most reliable method for the investigation of LV function in diabetic cardiomyopathy.

**Diastolic Function**

Ventricular relaxation, an ATP-dependent active process, depends mostly on $Ca^{2+}$ uptake by the sarcoplasmic reticulum during the first third of diastole. End-diastolic stiffness is predominantly affected by alterations in myocardial structural components. Prolonged ventricular relaxation has been observed in our type 1 diabetic rats (as indicated by decreased $dP/dt_{\text{min}}$ and increased $\tau$; Table 2), which is in line with previous results (3, 18). Interestingly, indexes of LV compliance (LVEDP and slope of EDPVR) were not significantly different between STZ-induced diabetic and control animals.

Based on the results of the present P-V analysis, the predominant feature in type 2 diabetic cardiomyopathy seems to be the LV diastolic dysfunction. In accordance with a recent work (27), we observed markedly impaired ventricular relaxation and significantly increased end-diastolic stiffness in type 2 diabetic animals, as reflected by the prolonged $\tau$ and by the increased LVEDP and steeper slope of EDPVR, respectively (Fig. 2 and Table 3).

When diastolic dysfunction was compared between the type 1 and type 2 DM models, we detected no significant differences regarding indexes of active LV relaxation ($dP/dt_{\text{min}}$ and $\tau$); however, there was a tendency toward a higher impairment of these parameters in diabetic rats of the type 1 DM model. In contrast, diabetes-associated changes of indexes of LV diastolic stiffness (LVEDP and slope of EDPVR) were significantly higher in type 2 DM compared with type 1 DM (Fig. 5B). These data indicate that diabetic diastolic dysfunction is characterized mainly by impaired LV relaxation in type 1 DM and, more remarkably, by decreased LV compliance in type 2 DM in rats. The detected functional differences might reflect marked differences regarding $Ca^{2+}$ homeostasis and myocardial structural alterations between type 1 and type 2 diabetic animals.

**Limitations**

P-V loop analysis, which is widely used in large animals and mice, has become a prerequisite for the assessment of LV function because it is the only technique that allows the measurement of LV performance independent from loading conditions. The combined pressure-conductance microcatheter for rats has been introduced only a few years ago, and limited normative data are available (22). The proper measurement of absolute volumes is the most vulnerable part of the P-V technique. Nevertheless, as our results demonstrate, the baseline P-V data of control rats were very similar to those reported.
in a recent methodological study (22). Furthermore, importantly, our SV data in STZ-induced diabetic rats were very similar to data recently obtained in the same model by MRI (1).

Because both types of DM are associated with vascular dysfunction and remodeling (21, 23, 26), it is conceivable that the impairment of vascular function and the depressed cardiac performance are interrelated. Diabetes-induced endothelial dysfunction (i.e., impairment of endothelium-dependent vasodilation) might lead to global or regional myocardial ischemia, which may secondarily impair cardiac performance. Therefore, direct effects of diabetes on myocardial tissue interact with diabetic endothelial dysfunction, which together define the phenotype of diabetic cardiomyopathy.

Unlike the majority of type 2 diabetic patients, ZDF diabetic rats at an older age show lower body weight compared with ZDF lean animals (as demonstrated in the present study). Although during the first months of diabetes duration (when hyperinsulinemia is dominating) ZDF diabetic rats have been reported to have significantly higher body weights compared with lean controls (8, 28, 32), the lower body weight of ZDF diabetic compared with ZDF lean rats at an older age is a common finding that can be attributed to the excessive loss of calories (glucosuria and proteinuria), which cannot be compensated for by an increased food intake (27). The possible influence of body weight differences on hemodynamic parameters was excluded in the present study by normalizing various indexes to body weight or by calculating load-independent (and body weight independent) contractility indexes.

The present work was primarily designed as a functional study aiming to characterize and compare LV dysfunction in rat models of type 1 and type 2 DM with the use of the highly sophisticated method of LV P-V analysis. Investigations of diabetes-induced metabolic, molecular, and/or cellular changes and pathomechanisms underlying the functional changes observed here were not among the goals of this work. However, based on the detected interesting differences regarding the severity and characteristics of diabetic cardiomyopathy between the two DM models, further studies investigating possible pathomechanisms are warranted.

Conclusions

In conclusion, we characterized and compared the cardiac dysfunction in two standard rat models of type 1 and type 2 DM using the P-V conductance catheter system. Based on reliable load-independent indexes of LV performance, in the present study, we demonstrated both systolic (impaired contractility) and diastolic dysfunction (prolonged ventricular relaxation) in type 1 diabetic rats with the former dominating. In contrast, the functional impairment of the myocardium of type 2 diabetic ZDF rats was characterized by a predominant diastolic dysfunction (prolonged relaxation and markedly increased LV stiffness) and a relatively preserved systolic performance. The P-V methodology could be a very useful and reliable approach for the assessment of LV dysfunction in DM in rats; furthermore, it could be successfully applied to evaluate potential pharmacotherapies against diabetic cardiomyopathy.

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REFERENCES


