EXPERIMENTAL PAPER

Doppler myocardial imaging in the diagnosis of early systolic left ventricular dysfunction in diabetic rats

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Aim To find out if Doppler myocardial imaging (DMI) can detect early signs of left ventricular (LV) dysfunction in a rat model of diabetic cardiomyopathy.

Methods Eight control and 12 Streptozotocin (STZ)-induced diabetic rats underwent transthoracic echocardiography with high-resolution technology at baseline and 2, 4, 8, 12, and 16 weeks after STZ injection. Radial function was analysed using conventional M-mode, and velocity, strain and strain rate imaging. Longitudinal function was analysed using pulsed Doppler imaging of the mitral annulus.

Results In the diabetic rats, a significant increase in LV end diastolic and end systolic diameter was measured when compared with controls ($P < 0.001$). Fractional shortening and LV ejection fraction remained unchanged in both groups. Using DMI, diabetic rats demonstrated a decrease in radial systolic velocity (rate of change: $+0.01$ vs. $-0.003$ week$^{-1}$; $P = 0.01$) and radial systolic strain rate ($+0.003$ vs. $-0.205$ week$^{-1}$; $P = 0.08$) of the anteroseptal wall. Histologic examination revealed dilated cardiomyopathy with no signs of fibrosis.

Conclusion Although LV ejection fraction remained preserved, velocity and strain rate imaging was able to detect radial systolic dysfunction in diabetic rats. The absence of histological signs of fibrosis suggests that other mechanisms play a role in the development of diabetic cardiomyopathy.

KEYWORDS
Diabetic cardiomyopathy; Small animals; Tissue Doppler imaging; Echocardiography; Diabetes; Left ventricular function; Strain rate imaging

Introduction

Diabetic patients have a higher prevalence of heart failure when compared with the general population. The most important factors contributing to the development of left ventricular (LV) dysfunction are the presence of coronary artery disease (CAD) and hypertension. In diabetic patients, however, LV dysfunction can be demonstrated even in the absence of hypertension or CAD. For instance, LV diastolic dysfunction is frequently present in young Type I diabetic patients without concomitant risk factors.1–3 Moreover, several studies have demonstrated the presence of subclinical LV systolic dysfunction in Types I and II diabetic patients using Doppler myocardial imaging (DMI).4–8

Streptozotocin (STZ)-induced (Type I) diabetes is a quite well-established model to investigate diabetic cardiomyopathy in small animals. Abnormal diastolic and systolic LV function could be demonstrated using standard echocardiographic techniques after a few weeks of diabetes9–13 and were correlated to invasive measurements.13,14

DMI is a sensitive tool to measure objectively regional myocardial dysfunction and the technique has been validated in large animals and in mice using sonomicrometry.15,16 Our group recently showed that application of this tool in small animals was feasible and reproducible in normal rats.17 Previous authors demonstrated that changes in deformation parameters could be detected in healthy rats in response to dobutamine and in rats with infarcted myocardium.18 This technique has not yet been used for the detection of early myocardial dysfunction as in subclinical diabetic cardiomyopathy.

Therefore, the purpose of our study was to evaluate regional myocardial function using DMI in a Type I diabetic rat model treated with a moderate dose of STZ (45 mg/kg). We intent to study radial velocity, strain and strain rate and longitudinal velocity of the LV over a period of 16 weeks in normal and diabetic rats.

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Methods

Animals
A total of 20 adult male Wistar rats (12-week-old, 410 ± 20 g) were studied. The study conformed to the guidelines of the American Heart Association on research animal use and was approved by the Animal Research Committee of our institution. Diabetes mellitus was induced in 12 rats by a single intravenous injection of 45 mg/kg STZ in a 0.1 mol/L citrate buffer solution. In the remaining eight control rats, only the buffer solution was injected. Three days after treatment with STZ, tail vein blood glucose samples were measured with an Onetouch® glucometer (Johnson & Johnson) to ensure induction of diabetes.

All rats had unlimited access to food and water during follow-up.

Echocardiogram protocol
Echocardiograms were performed repeatedly at baseline, 2, 4, 8, 12, and 16 weeks of follow-up. All echocardiograms were performed under anaesthesia with pentobarbital 50 mg/kg administered intraperitoneally and allowing spontaneous respiration.

Images were acquired using a Vivid 7 (GE Vingmed, Horten, Norway) with a linear 13 MHz probe (i13L) for grey-scale and tissue Doppler images and with a spectral 10 MHz probe for pulsed wave Doppler traces. The anterior chest hair was removed using a shaver and the rats were positioned in left lateral decubitus on a wooden table. Recordings were made simultaneously with electrocardiography by fixing the electrodes on the paws.

Grey-scale imaging for the evaluation of global systolic function
Grey-scale images were recorded in a parasternal short-axis view at a depth of 1.5–2 cm. M-mode tracings were recorded at the level of the papillary muscles at a speed of 200 mm/s. Measurements were done offline using EchoPAC PC (GE Vingmed, version 3.1.3). The thickness of the inter-ventricular septum, posterior wall, and LV dimensions were measured from three consecutive cardiac cycles on the M-mode tracings. In order to calculate LV volumes and EF, the formula \( \text{EF} = \frac{1}{\frac{a}{D}} \frac{L}{a} \) was used, assuming the LV as an ellipsoid (a = ellipticity factor). We used an ellipticity factor of 1/3 as measured from five parasternal long-axis views: \( D \) = diameter of LV and \( 'L' \) the length of the 'long axis of the ellipsoid').

Doppler myocardial imaging for the evaluation of radial left ventricular function
Tissue Doppler images were recorded in a parasternal short-axis view at the mid-ventricular level at a depth of 2 cm. The tissue Doppler frequency was 6.4 MHz. The Nyquist limit was set as low as possible avoiding aliasing. High frame rate was obtained by reducing sector width.

All measurements were performed offline using dedicated software (SPEQLE, Catholic University Leuven, Belgium). A total of 10 ± 3 consecutive cycles were averaged during analysis. A strain estimation length of 0.8 mm was used. Timings of the beginning and ending of the ejection phase were obtained using the ECG and the velocity trace as previously demonstrated in humans. Reproducibility and repeatability of the deformation parameters was recently assessed and published by our group.

Pulsed doppler imaging for the evaluation of longitudinal left ventricular function
To evaluate systolic longitudinal function, peak systolic velocities were measured with pulsed Doppler from the septal mitral annulus in apical four-chamber view. Three consecutive cycles were used for averaging.

Histology
At Week 20, all control rats (n = 8) and surviving diabetic rats (n = 8) were euthanized with high-dose pentobarbital for histological studies. The hearts were immediately removed and fixed in 4% neutral formalin for 2 h. Three short-axis slices of myocardial tissue were obtained and embedded in paraffin. Haematoxylin-eosin and Masson’s trichrome staining were performed. Microscopy was done at ×400 enlargement. The thickness of the muscular fibres was measured in every sample from digital images (10 measurements per sample).

Statistical analysis
Data were averaged and presented as mean ± SD.

Differences between groups were evaluated using a ‘two-step procedure’ to account for repeated measures. This means that for each parameter and each rat a model was fitted to the data \( y = \alpha x + \beta \) using all time measurements (baseline, 2, 4, 8, 12, 16 weeks). Baseline values and slopes (a) were compared between control and diabetic rats using an unpaired t-test.

Correlation between results and animal characteristics like heart rate and body weight were compared using spearman correlation. A value of \( P < 0.05 \) was considered statistically significant.

Results
Three diabetic rats died before the end of the 16 week echocardiogram protocol and another rat died before the histology study was performed.

As all rats survived the 12-week echocardiogram protocol, the results of baseline and 12 weeks follow-up will be demonstrated in the tables. All rats survived the 12-week protocol, but four rats died before the histological examination.

Mean glycaemia was 99 ± 12 mg/dL in the control rats and 391 ± 37 mg/dL in the diabetic rats 3 days after administration of STZ. Glycaemia remained high (>300 mg/dL) in all diabetic rats during follow-up.

After the administration of STZ, there was a significant decrease in heart rate and body weight in the diabetic rats (Table 1).

Table 1 Characteristics of control (n = 8) and diabetic (n = 12) rats

<table>
<thead>
<tr>
<th></th>
<th>Baseline value</th>
<th>P</th>
<th>Rate of change/week</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Diabetic</td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>404 ± 20</td>
<td>416 ± 20</td>
<td>NS</td>
<td>+8.7</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>316 ± 25</td>
<td>335 ± 21</td>
<td>NS</td>
<td>−1.3</td>
</tr>
</tbody>
</table>

Baseline values and slopes of the regression lines (rate of change/week).
The quality of the M-mode images was excellent in all rats. Results of the M-mode measurements are given in Table 2, which shows the baseline values and rate of change per week in both groups.

A significant increase in LV end diastolic diameters (LVEDDs) and end systolic diameters (LVESDs) could be detected in the diabetic rats when corrected for LV mass ($LV mass = 1.04^{*}(IVS + LVEDD + PW)^{3} - (LVESD)^{3} + 0.8 + 0.14$) (Figure 1). Fractional shortening and LV ejection fraction did not change significantly.

**Table 2** M-mode parameters of the LV (parasternal short-axis view) in normal ($n = 8$) and diabetic rats ($n = 12$)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Diabetic</th>
<th>$P$</th>
<th>Rate of change/week</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS WT (mm)</td>
<td>$1.8 \pm 0.2$</td>
<td>$1.9 \pm 0.1$</td>
<td>NS</td>
<td>$-0.002$</td>
<td>$-0.02$ &lt; 0.0001</td>
</tr>
<tr>
<td>IL WT (mm)</td>
<td>$1.7 \pm 0.2$</td>
<td>$1.8 \pm 0.2$</td>
<td>NS</td>
<td>$-0.01$</td>
<td>$-0.03$ &lt; 0.0001</td>
</tr>
<tr>
<td>LV EDD (mm/g)</td>
<td>$8.5 \pm 1.0$</td>
<td>$7.9 \pm 0.6$</td>
<td>NS</td>
<td>$-0.14$</td>
<td>$+0.29$ &lt; 0.001</td>
</tr>
<tr>
<td>LV ESD (mm/g)</td>
<td>$4.9 \pm 0.6$</td>
<td>$4.5 \pm 0.6$</td>
<td>NS</td>
<td>$-0.10$</td>
<td>$+0.15$ &lt; 0.001</td>
</tr>
<tr>
<td>FS (%)</td>
<td>$43 \pm 4$</td>
<td>$43 \pm 7$</td>
<td>NS</td>
<td>$0.18$</td>
<td>$0.22$ 0.9</td>
</tr>
<tr>
<td>LV EF (%)</td>
<td>$81 \pm 4$</td>
<td>$80 \pm 8$</td>
<td>NS</td>
<td>$0.17$</td>
<td>$0.14$ 0.9</td>
</tr>
</tbody>
</table>

AS, anteroseptal; IL, inferolateral; WT, wall thickness; ED(S)D, end diastolic (systolic) diameter; FS, fractional shortening; EF, ejection fraction. Baseline values and slopes of the regression lines (rate of change/week).

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### Doppler myocardial imaging for left ventricular radial function

Adequate velocity and deformation traces could be obtained in 97% of the segments analysed. Figure 2 shows an example of an averaged trace for radial velocity, strain and strain rate in a normal and a diabetic rat after 12 weeks of follow-up.

The baseline values (before STZ administration) and rate of changes per week are demonstrated in Table 3.

Figures 3, 4, and 5 show the mean values, respectively, for peak velocity, strain rate, and strain at baseline and during follow-up in the anteroseptal (AS) and inferolateral (IL) wall.

At baseline peak myocardial velocity, strain and strain rate measurements were systematically lower in the AS wall when compared with the IL wall, as previously described. In the AS wall, there was a decrease in myocardial velocity and a trend to decrease strain rate over time in the diabetic rats (Table 3). In the IL wall comparable changes could be observed, with lower values in the diabetic rats at each time event, although the difference between both groups was not statistically significant (Figures 3 and 4).

The total amount of deformation (peak systolic strain) remained unchanged in both groups and both wall segments during all stages of follow-up (Figure 5).

### Doppler myocardial imaging for systolic longitudinal function

Figure 6 shows the evolution of the longitudinal velocities over time in both groups. There was a slight increase of the longitudinal velocities in the controls with almost no change of the velocities in the diabetic rat (rate of change/week, respectively, 0.034 and 0.002; $P = 0.17$).

### Histology

Figure 7 represents the myocardium in control and diabetic rats at 20 weeks of follow-up. No difference in extracellular collagen deposits (trichrome staining) or endomyocardial necrosis (haematoxilin–eosin staining) could be detected in the diabetic samples. The muscular fibres were thinner in the diabetic rats ($0.44 \pm 0.07$ vs. $0.32 \pm 0.06$ AU; $P = 0.01$).

### Discussion

Our study demonstrates that (1) a decrease in myocardial velocity and a trend in decrease of myocardial strain rate can be detected in rats with moderate dose STZ-induced diabetes, dilated cardiomyopathy and preserved ejection fraction (2) there are no histopathological signs of fibrosis suggesting that other mechanisms are responsible for these changes.

Different imaging modalities have been used to assess the presence, onset and time course of diastolic and
Figure 2  Example of a velocity, strain, and strain rate curve, for the anteroseptal and inferolateral wall, in a normal and diabetic rat at 12 weeks of follow-up. Notice the lower systolic velocity and strain rate in the diabetic rat with no change in systolic strain.
systolic dysfunction in small animals with STZ-induced diabetes, especially in rats.\textsuperscript{9–13,20} Anatomical M-mode and bi-dimensional echocardiography have been used to demonstrate systolic LV dysfunction and dilatation of the left ventricle in STZ treated rats after a few weeks\textsuperscript{6–12} of diabetes.\textsuperscript{9–13}

Our study could not demonstrate a significant change in fractional shortening or LV EF in the diabetic rats after a follow-up of 16 weeks, but left ventricles significantly dilated. The absence of change in fractional shortening could be due to several factors. First, the strain we used in our study (Wistar) is different from the strain used in most of the previous studies (Sprague–Dawley), what could explain the difference in time course of onset of pronounced LV dysfunction. Secondly, we used a relatively low dose of STZ (45 mg/kg) in order not to induce severe cardiomyopathy. Thirdly, the age at which the STZ was administrated in our study was higher when compared with previous studies, which can lead to a less severe diabetic state.\textsuperscript{21}

The use of DMI in the regional quantification of LV function is a challenge in small animals as not only their hearts are very small but also the heart rate is very high. Nevertheless, a few recent publications demonstrated that it is a feasible and reproducible tool in rats and even in mice.\textsuperscript{16–18} Our present study shows that this technique can be used in a rat model of diabetic cardiomyopathy to determine early

<table>
<thead>
<tr>
<th>Anterior wall</th>
<th>Control</th>
<th>Diabetic</th>
<th>( P )</th>
<th>Rate of change/week</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak velocity (cm/s)</td>
<td>(0.67 \pm 0.14)</td>
<td>(0.85 \pm 0.18)</td>
<td>NS</td>
<td>+0.01</td>
<td>−0.003</td>
</tr>
<tr>
<td>Peak strain rate (s(^{-1}))</td>
<td>10.3 ± 3.0</td>
<td>11.4 ± 2.0</td>
<td>NS</td>
<td>+0.003</td>
<td>−0.205</td>
</tr>
<tr>
<td>Peak strain (%)</td>
<td>39 ± 12</td>
<td>44 ± 12</td>
<td>NS</td>
<td>+0.26</td>
<td>−0.27</td>
</tr>
<tr>
<td>Inferior wall</td>
<td>Peak velocity (cm/s)</td>
<td>3.5 ± 0.6</td>
<td>3.5 ± 0.7</td>
<td>NS</td>
<td>−0.013</td>
</tr>
<tr>
<td>Peak strain rate (s(^{-1}))</td>
<td>15.3 ± 2.5</td>
<td>16.4 ± 3.6</td>
<td>NS</td>
<td>−0.111</td>
<td>−0.155</td>
</tr>
<tr>
<td>Peak strain (%)</td>
<td>51 ± 18</td>
<td>55 ± 14</td>
<td>NS</td>
<td>+0.08</td>
<td>−0.05</td>
</tr>
</tbody>
</table>

\( S' \) velocity mitral annulus (mm/s) 27.9 ± 7.2 28.6 ± 4.7 NS +0.034 +0.002 0.173

Baseline values and slopes of the regression lines (rate of change/week).

Figure 3  Mean ± SD for peak systolic velocity in diabetic (closed circle) and normal (closed triangle) rats at baseline and after 2, 4, 8, 12, and 16 weeks of diabetes in the anteroseptal (AS) and inferolateral (IL) wall. Mean regression line for diabetic (straight line) and controls (dotted line); \( P = 0.007 \) (AS) and \( P = 0.09 \) (IL).

Figure 4  Mean ± SD for peak systolic strain rate in diabetic (closed circle) and normal (closed triangle) rats at baseline and after 2, 4, 8, 12, and 16 weeks of diabetes in the anteroseptal (AS) and inferolateral (IL) wall. Mean regression line for diabetic (straight line) and controls (dotted line); \( P = 0.076 \) (AL) and \( P = 0.68 \) (IL).
onset of regional systolic LV dysfunction. Although there was a significant decrease in myocardial velocity in the diabetic rat \( (P = 0.007) \), the change in myocardial strain rate was small \( (P = 0.076) \) and only prominent in the AS wall. However, in both the walls and at each time event values of velocity and strain rate were lower in the diabetic group. The dosis of STZ used and the age at which STZ was administered might explain why only subtle changes in strain rate were present.

Diabetic cardiomyopathy is known to develop in humans in the absence of coronary or hypertensive disease. 22 The mechanism by which diabetic cardiomyopathy develops has been studied using \textit{in vivo} and \textit{ex vivo} experiments. 23 It has been postulated that endothelial dysfunction, endomyocardial fibrosis, direct toxic effect of hyperglycaemia on cardiomyocytes and autonomic neuropathy play an important role. In humans especially subendocardial fibres are prone to ischaemic and fibrotic changes. 24 This explains, as demonstrated in this latter study, why in the initial phase of diabetic cardiomyopathy longitudinal function can be diminished while radial function is still normal, or even compensatory increased, resulting thereby in a preserved global LV EF.5,24

It is not known if these changes can be extrapolated to small animals. In our rat model, the decrease in regional LV function was more pronounced in radial direction than in longitudinal direction. Measuring longitudinal function was performed at the level of the septal mitral annulus. Good allignment of the beam is sometimes difficult resulting in quite great standard deviation of the measurements.

Furthermore, the different results in small animals might be explained by the fact that there appears to be a higher contribution of circumferential shortening and/or torsion to EF when compared with human. 25 Therefore, it is difficult to extrapolate the results of human studies to small animals and further experience is necessary to elucidate this issue, perhaps by studying a more diseased rat model.

In our study, the changes in velocity and strain rate were accompanied by changes in heart rate what in theory might influence our findings. However, a few authors have studied the influence of heart rate on deformation parameters. 26,27 Weidemann \textit{et al.} studied the effect of heart rate in a pacing
pig model and could not find any significant change in strain rate in response to higher pacing rates. Myocardial strain on the other hand was clearly influenced by heart rate or ejection time.26 Boettler et al. nicely showed that in healthy children systolic strain significantly increases with decrease in heart rate while systolic strain rate remains constant.27

Moreover, in our study, we could not demonstrate any significant correlation between the change from baseline in heart rate and velocity \((r = 0.45; P = NS)\) or strain rate \((r = 0.16; P = NS)\).

Our model was also characterized by a significant loss in body weight but there was no significant correlation between the amount of weight loss and the decrease in velocity \((r = -0.04; P = NS)\) and strain rate parameter \((r = -0.26; P = NS)\). Ideally, a starving model would help to distinguish the effect of weight loss from the effect of diabetes on myocardial contractility. In a starving model, however, we expect a decrease in LV diameters instead of an increase as was the case in our model.

Several studies in rats have demonstrated the presence of excessive fibrosis by histological examination in diabetic cardiomyopathy.9,23 This finding was not confirmed by other investigators.12,28 Our study did not show excessive extracellular matrix due to collagen deposits, suggesting that this is not the main cause in the development of diabetic cardiomyopathy in this rat model. The direct toxic effect of hyperglycaemia on cardiomyocites might be more important in this model of acute onset of diabetes.

Limitations

Since in small animals the AS and IL walls, in short-axis view, are most easily analysed, deformation imaging has till now been restricted to radial function. Although it is possible to obtain an apical four-chamber view, good alignment with the beam is more difficult and the lateral wall is rarely visualized. The quality of the longitudinal myocardial deformation traces that we experienced is poor due to artefacts and therefore not interpretable. To look at longitudinal function, we therefore used pulsed Doppler velocity of the septal mitral annulus.

Velocity, strain, and strain rate are known to be load dependent.29 In diseased myocardium, higher preloads can lead to decrease in intrinsic contractility. No invasive evaluation of the preload was performed in this study but diastolic parameters (E/A ratio and E/E’ ratio) were analysed (results not shown) and did not suggest higher preload status in the diabetic rats.

The exact pathophysiological mechanisms in the development of diabetic cardiomyopathy remain unclear. The influence of endothelial dysfunction and ischaemia needs to be investigated. Indeed, although less likely, we cannot completely exclude the presence of CAD in this model as no stress testing or perfusion imaging was performed.

Conclusion

The diagnosis of diabetic cardiomyopathy in rats can be made non-invasively by high-resolution echocardiography. Dilatation of the LV, decrease in myocardial velocity and a trend in decrease in myocardial strain rate can be detected after a few weeks of diabetes, before the onset of global LV dysfunction. The absence of histological signs of fibrosis indicates that other mechanisms are responsible for the development of diabetic cardiomyopathy.

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Bibliography


