Diastolic Stiffness of the Failing Diabetic Heart

Importance of Fibrosis, Advanced Glycation End Products, and Myocyte Resting Tension

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Background—Excessive diastolic left ventricular stiffness is an important contributor to heart failure in patients with diabetes mellitus. Diabetes is presumed to increase stiffness through myocardial deposition of collagen and advanced glycation end products (AGEs). Cardiomyocyte resting tension also elevates stiffness, especially in heart failure with normal left ventricular ejection fraction (LVEF). The contribution to diastolic stiffness of fibrosis, AGEs, and cardiomyocyte resting tension was assessed in diabetic heart failure patients with normal or reduced LVEF.

Methods and Results—Left ventricular endomyocardial biopsy samples were procured in 28 patients with normal LVEF and 36 patients with reduced LVEF, all without coronary artery disease. Sixteen patients with normal LVEF and 10 with reduced LVEF had diabetes mellitus. Biopsy samples were used for quantification of collagen and AGEs and for isolation of cardiomyocytes to measure resting tension. Diabetic heart failure patients had higher diastolic left ventricular stiffness irrespective of LVEF. Diabetes mellitus increased the myocardial collagen volume fraction only in patients with reduced LVEF (from 14.6±1.0% to 22.4±2.2%, P<0.001) and increased cardiomyocyte resting tension only in patients with normal LVEF (from 5.1±0.7 to 8.5±0.9 kN/m², P=0.006). Diabetes increased myocardial AGE deposition in patients with reduced LVEF (from 8.8±2.5 to 24.1±3.8 score/mm²; P=0.005) and less so in patients with normal LVEF (from 8.2±2.5 to 15.7±2.7 score/mm², P=NS).

Conclusions—Mechanisms responsible for the increased diastolic stiffness of the diabetic heart differ in heart failure with reduced and normal LVEF: Fibrosis and AGEs are more important when LVEF is reduced, whereas cardiomyocyte resting tension is more important when LVEF is normal. (Circulation. 2008;117:43-51.)

Key Words: diastole diabetes mellitus heart failure collagen myocytes

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In the absence of coronary artery disease, excessive diastolic LV stiffness of the diabetic heart has been related to myocardial fibrosis and to circulating advanced glycation end products (AGEs), although associations of histological and biochemical data with in vivo LV function are largely lacking. Furthermore, the contribution of an elevated cardiomyocyte resting tension (F\text{passive}) to this increased diastolic LV stiffness has not been assessed. In patients with HF and normal LVEF (HFNEF), F\text{passive} of cardiomyocytes isolated from LV endomyocardial biopsy samples was recently demonstrated to be elevated and to be an important determinant of LV stiffness. In patients with HF and reduced LVEF (HFREF), F\text{passive} was lower and failed to correlate with LV stiffness. The high F\text{passive} in HFNEF was paralleled by cardiomyocyte hypertrophy and concentric LV remodeling. Because myocardial fibrosis...
hypertrophy is associated with insulin resistance,14 and because HFNEF is common in patients with type 2 DM,7 high Fpassive could be an important contributor to the excessive diastolic LV stiffness of the diabetic heart.

Using LV endomyocardial biopsy samples, we therefore compared myocardial fibrosis, AGE deposition, and Fpassive of isolated cardiomyocytes between diabetic (DM∗) and nondiabetic (DM−) HFNEF patients (DMHFNEF and DM−HFNEF) and between DM∗ and DM− HFREF patients (DMHFREF and DM−HFREF). All patients had been hospitalized for worsening HF and had no evidence of coronary artery disease on their coronary angiogram or of myocardial infarction or active inflammation in their LV endomyocardial biopsy samples.

Methods

Patients

The study population consisted of 90 patients hospitalized for worsening HF between October 2003 and December 2006. Patients were referred for cardiac catheterization and LV endomyocardial biopsy procurement because of suspicion of infiltrative or inflammatory myocardial disease. Fifty-eight patients had new-onset HF, and 32 had acute decompensation superimposed on chronic HF. Modes of presentation were similar in HFNEF versus HFREF patients and in DM∗ versus DM− patients. Patients were studied after medical compensation. No patient had undergone cardiac transplantation. Coronary angiography showed epicardial coronary artery stenoses in 20 patients; these patients were excluded from the present study. Histological analysis of the biopsy samples revealed active inflammatory infiltration or myocyte necrosis in 6 patients. The histologically positive biopsy sample rate of 8.6% of the study population is comparable to the rate found in previous studies, which reported active lymphocytic infiltration in 8.3% of patients with dilated cardiomyopathy15 and amyloid deposits in 6.3% of patients with LV restrictive physiology16 of hemodynamic severity comparable to that of the present study population. Patients with a positive biopsy sample were also excluded from the present study. The final study cohort, therefore, consisted of 64 patients. For 44 of these 64 patients, data on myocardial collagen volume fraction (CVF) and cardiomyocyte Fpassive were included in previous studies.12,13

In accordance with a recent consensus document on the diagnosis of HFNEF,17 patients had HFNEF (n=28) if LVEF was >50%, LV end-diastolic volume index was <97 mL/m², and LV end-diastolic pressure was >16 mm Hg.17 Patients had HFREF (n=36) if LVEF was <45%. A patient had DM if a history of DM was evident from use of glucose-lowering medications and/or insulin or if fasting plasma glucose was ≥7.0 mmol/L.18 No patient was using thiazolidinediones. Three HFREF patients had type 1 DM, and 7 HFREF and all 16 HFNEF patients had type 2 DM. In DMHFNEF and DMHFREF patients who were not undergoing insulin therapy, fasting insulin plasma levels were elevated (20.1±4.3 and 22.4±3.0 μU/mL, respectively). The local ethics committee approved the study protocol. Written informed consent was obtained from all patients.

Quantitative Histomorphometry

Light and Electron Microscopy

Light microscopic quantification of cardiomyocyte diameter and CVF has been described and validated previously.12,13 Biopsy samples used for CVF averaged 2.8±0.2 samples per patient. The same automated image analyzer was also used for electron microscopic quantification13 of the sarcomeric Z-line thickness of cardiomyocytes. For each patient, 30 Z-line–thickness measurements were averaged.

Immunohistochemistry

Deposition of AGEs was inferred from measurement of the AGE N-(carboxymethyl)lysine (CML). Development of the anti-CML monoclonal antibody and immunohistochemical staining techniques for CML and E-selectin used in the present study have been described previously.19,20 AGE and E-selectin positivity was scored for intensity. The sum of all positivities times their score was subsequently divided by the area of the slide to yield an immunohistochemical score per square millimeter (score/mm²).

Immunofluorescence Light Microscopy

For analysis of Z-line thickness, fixed slides with isolated cardiomyocytes were stained with anti-α-actinin for 60 minutes at room temperature (1:50, mAb, Monosan, Uden, the Netherlands). After they were washed in PBS-Tween, the cells were incubated for 30 minutes with rabbit anti-mouse AlexaFluor 488 secondary antibody (1:40, Molecular Probes, Invitrogen, Carlsbad, Calif) and again washed in PBS-Tween. Slides were covered and sealed by mounting medium and ultrathin glass cover slips. Z-line–thickness analysis was performed under a 3D Marianas wide-field deconvolution microscopy workstation (Intelligent Imaging Innovations, Denver, Colo). For each patient, 3-dimensional stacks (step size in z=0.2 μm) of isolated cardiomyocytes were made, and 30 Z-line–thickness measurements were averaged.

Force Measurements in Isolated Cardiomyocytes

Force measurements were performed in single, mechanically isolated cardiomyocytes as described previously.12,13 Biopsy samples (5 mg wet weight) were defrosted in relaxing solution, mechanically disrupted, and incubated for 5 minutes in relaxing solution supplemented with 0.2% Triton X-100 to remove all membrane structures. Single cardiomyocytes were subsequently attached with silicone adhesive between a force transducer and a piezoelectric motor (2.7±0.4 cardiomyocytes per patient). Sarcomere length of isolated cardiomyocytes was adjusted to 2.2 μm. To assess reversibility of elevated Fpassive, myocytes were also incubated in relaxing solution supplemented with the catalytic subunit of protein kinase A (100 U/mL; Sigma, St Louis, Mo; batch 12K7495). After 40 minutes of incubation with protein kinase A, Fpassive measurements were repeated. Force values were normalized for myocyte cross-sectional area.

Data Analysis

LV end-diastolic volume, LV end-diastolic volume index, LV stroke volume, and LVEF were derived from biplane LV angiograms. Effective arterial elastance was equal to LV end-systolic pressure divided by angiographic LV stroke volume. Total arterial compliance equaled angiographic LV stroke volume divided by aortic pulse pressure. LV diastolic internal diameter (LVIDd), diastolic septal and posterior wall thicknesses (SWTd and PWTd, respectively), relative wall thickness (RWT), LV mass (LVM), and LV mass index were derived from 2-dimensional echocardiograms. LV mass and relative wall thickness were calculated in accordance with the recent recommendations for cardiac chamber quantification,21 as follows:

\[ \text{LVM} = 0.8 \times \left[ 1.04 \frac{(\text{LVIDd} + \text{PWtd} + \text{SWTd})^3}{\text{LVIDd}^2} \right] + 0.6 \text{ g} \]

and

\[ \text{RWT} = 2 \times \frac{\text{PWtd} + \text{LVIDd}}{\text{LVIDd}} \]

To calculate LV peak systolic wall stress (LVPSS) and LV myocardial stiffness modulus (SM), hemodynamic, angiographic, and 2D echocardiographic data were combined. Circumferential LVPSS was computed with a thick-wall ellipsoid model of the LV as follows:22

\[ \text{LVPSS} = \frac{\text{LVSP} \times D / (2\text{PWts})}{1 - \left( \frac{\text{PWts}}{D} \right) - \left( \frac{D^2 / 2L^2}{1.332 \text{ dyn/cm}^2} \right)} \]

where LVSP is LV peak systolic pressure, PWts is the corresponding systolic echocardiographic posterior wall thickness, and D and L...
are the corresponding angiographic LV systolic diameter and length, respectively.

To assess diastolic LV material properties, a radial LV SM was derived.\textsuperscript{23} SM was defined as the increment of radial stress (Δσ) divided by the increment of radial strain (Δε) (SM=Δσ/Δε). Δσ is equal but opposite in sign to the increment of LV pressure (LVP) at the endocardium (−ΔLVP), and Δε equals the increment in 2-dimensional echocardiographic posterior wall thickness (PWT) relative to the instantaneous PWT (ΔPWT/PWT). Early diastolic LV relaxation pressure was extrapolated from the exponential curve fit to isovolumic LV pressure decay, which was used to calculate τ, the time constant of isovolumic LV pressure decay. Early diastolic LV relaxation pressure was subtracted from measured LVP to yield residual LV diastolic pressure (LVP\textsubscript{res}). Substitution of LVP by LVP\textsubscript{res} allowed the SM to also be calculated in early diastole, when LVP is still declining.\textsuperscript{23} Because ΔPWT/PWT=ΔlnPWT, SM equals the slope of a plot of LVP\textsubscript{res} against corresponding ΔlnPWT data points.

Values are given as mean±SEM. Data of the DM\textsuperscript{−}HFREF, DM\textsuperscript{−}HFREF, DM\textsuperscript{+}HFREF, DM\textsuperscript{+}HFREF groups were analyzed by 2-factor ANOVA testing for DM status, HFREF/HFREF status, and their interaction. Subsequent comparisons (DM\textsuperscript{−}HFREF versus DM\textsuperscript{+}HFREF and DM\textsuperscript{−}HFREF versus DM\textsuperscript{+}HFREF) were performed with a Bonferroni adjusted t test. Single comparisons were assessed by an unpaired Student t test. Relations between 2 continuous variables were assessed with linear regression analysis. Statistical analysis was performed with SPSS version 9.0 (SPSS Inc, Chicago, Ill).

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

DM and LV Function

As evident from the higher LV end-diastolic pressure at similar LV end-diastolic volume index, LV end-diastolic distensibility was reduced in DM\textsuperscript{−}HFREF and DM\textsuperscript{−}HFREF patients (Figure 1A). SM was also higher in DM\textsuperscript{−}HFREF and DM\textsuperscript{+}HFREF patients than in DM\textsuperscript{−}HFREF and DM\textsuperscript{−}HFREF patients, respectively (Table; Figure 1B).

Myocardial AGE Deposition and Fibrosis

AGE deposition was inferred from CML immunostaining and occurred mainly in the wall of small intramyocardial vessels (Figure 2A). In 2-factor ANOVA, CML deposition depended on the presence of DM (\textit{P}<0.001) but not on HFREF/HFREF status. CML deposition was especially evident when DM\textsuperscript{−}HFREF patients were compared with DM\textsuperscript{−}HFREF patients (24.1±3.8 versus 8.8±2.5 score/mm\textsuperscript{2}, \textit{P}=0.005) and was less evident when DM\textsuperscript{+}HFREF patients were compared with DM\textsuperscript{−}HFREF patients (15.7±2.7 versus 8.2±2.5 score/mm\textsuperscript{2}, \textit{P}=NS; Figure 2B). CML deposition correlated with the myocardial SM in HFREF (\textit{r}=0.48, \textit{P}=0.014). The DM-induced rise in myocardial CML deposition in HFREF was paralleled by a rise in E-selectin expression from 3.6±0.9 to 9.2±1.9 score/mm\textsuperscript{2} (\textit{P}=0.022). E-selectin is a marker of inflammatory endothelial activation (Figure 2C).

In 2-factor ANOVA, myocardial CVF depended on DM (\textit{P}=0.006) and on HFREF/HFREF status (\textit{P}<0.001). Moreover, the effect of DM on CVF depended on HFREF/HFREF status (\textit{P}=0.007). CVF was higher when DM\textsuperscript{−}HFREF patients were compared with DM\textsuperscript{−}HFREF patients (22.4±2.2% versus 14.6±1.0%, \textit{P}<0.001) and was similar when DM\textsuperscript{−}HFREF patients were compared with DM\textsuperscript{−}HFREF patients (11.6±1.1% versus 11.7±1.1%, \textit{P}=NS; Figure 2D). In HFREF patients, CVF correlated with SM (\textit{r}=0.37, \textit{P}=0.039) and with plasma glycohemoglobin (\textit{r}=0.61, \textit{P}=0.0014).

Myocyte $F_{\text{passive}}$, Myocyte Hypertrophy, and LV Remodeling

In all cardiomyocytes, $F_{\text{passive}}$ was measured at the same sarcomere length of 2.2 μm (Figure 3A). In 2-factor ANOVA, $F_{\text{passive}}$ depended on DM (\textit{P}=0.009) and on HFREF/HFREF status (\textit{P}<0.001). Similar to myocardial CVF, the effect of DM on $F_{\text{passive}}$ was dependent on HFREF/HFREF status (\textit{P}=0.021). $F_{\text{passive}}$ of DM\textsuperscript{−}HFREF cardiomyocytes was higher than $F_{\text{passive}}$ of DM\textsuperscript{−}HFREF cardiomyocytes (8.5±0.9 versus 5.1±0.7 kN/m\textsuperscript{2}, \textit{P}=0.006), whereas $F_{\text{passive}}$ of DM\textsuperscript{−}HFREF cardiomyocytes was comparable to $F_{\text{passive}}$ of DM\textsuperscript{−}HFREF cardiomyocytes (3.9±0.5 versus 3.7±0.4 kN/m\textsuperscript{2}, \textit{P}=NS; Figure 3B). Higher $F_{\text{passive}}$ of DM\textsuperscript{−}HFREF cardiomyocytes was unrelated to isolation-associated cell damage, because active force development at a saturating calcium concentration (pCa\textsubscript{4}=4.5) was comparable in DM\textsuperscript{−}HFREF (14.7±1.3 kN/m\textsuperscript{2}) and DM\textsuperscript{−}HFREF (16.9±1.9 kN/m\textsuperscript{2}) cardiomyocytes. After administration of protein kinase A, $F_{\text{passive}}$...
Table. Clinical and Hemodynamic Characteristics

<table>
<thead>
<tr>
<th></th>
<th>DM&lt;sub&gt;HREF&lt;/sub&gt; (n=26)</th>
<th>DM&lt;sub&gt;HREF&lt;/sub&gt; (n=10)</th>
<th>DM&lt;sub&gt;HREF&lt;/sub&gt; (n=12)</th>
<th>DM&lt;sub&gt;HREF&lt;/sub&gt; (n=16)</th>
<th>DM&lt;sub&gt;HREF&lt;/sub&gt; vs DM&lt;sup&gt;+&lt;/sup&gt;</th>
<th>HFREF vs HFREF</th>
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<td>Age, y</td>
<td>60.2 ± 3.4</td>
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<td>NS</td>
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<td>7/3</td>
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<td>9/7</td>
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<td>Fasting glucose, mmol/L</td>
<td>4.97 ± 0.23</td>
<td>7.10 ± 0.56*</td>
<td>5.78 ± 0.28</td>
<td>8.64 ± 0.94§</td>
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<td>Glycated hemoglobin, %</td>
<td>5.54 ± 0.20</td>
<td>7.04 ± 0.58*</td>
<td>5.50 ± 0.04</td>
<td>7.26 ± 0.51§</td>
<td>&lt;0.001</td>
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<td>Body mass index, kg/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>26.4 ± 0.95</td>
<td>28.9 ± 1.71</td>
<td>25.9 ± 1.86</td>
<td>32.6 ± 1.18‡</td>
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<td>DM duration, y</td>
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<td>6.9 ± 0.7</td>
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<tr>
<td>Creatinine, μmol/L</td>
<td>94.3 ± 4.0</td>
<td>101.0 ± 3.0</td>
<td>94.8 ± 4.0</td>
<td>96.2 ± 3.0</td>
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<td>GFR, mL·min&lt;sup&gt;-1&lt;/sup&gt;·1.73 m&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>75.3 ± 3.0</td>
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<td>Insulin</td>
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<td>5/16</td>
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<tr>
<td>LVPSp, mm Hg</td>
<td>117.4 ± 3.4</td>
<td>126.4 ± 8.2</td>
<td>166.0 ± 8.5</td>
<td>172.0 ± 9.7</td>
<td>NS</td>
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<td>LVEDP, mm Hg</td>
<td>19.9 ± 1.7</td>
<td>27.8 ± 2.5*</td>
<td>22.2 ± 1.9</td>
<td>28.0 ± 1.7</td>
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<td>RAP, mm Hg</td>
<td>6.7 ± 1.0</td>
<td>9.5 ± 1.9</td>
<td>8.1 ± 1.0</td>
<td>8.4 ± 1.2</td>
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<td>τ, ms</td>
<td>66.5 ± 10</td>
<td>63.0 ± 8.5</td>
<td>58.3 ± 5.3</td>
<td>72.5 ± 5.7</td>
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<td>Ea, mm Hg/mL</td>
<td>2.2 ± 0.2</td>
<td>2.5 ± 0.7</td>
<td>2.3 ± 0.2</td>
<td>2.7 ± 0.3</td>
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<tr>
<td>PP, mm Hg</td>
<td>49.1 ± 2.9</td>
<td>51.2 ± 6.2</td>
<td>82.9 ± 5.0</td>
<td>97.9 ± 7.6</td>
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<td>Total arterial compliance, mL/mm Hg</td>
<td>1.7 ± 0.2</td>
<td>1.4 ± 0.2</td>
<td>1.4 ± 0.2</td>
<td>1.0 ± 0.1</td>
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<td>SVR, dyne·sec&lt;sup&gt;-1&lt;/sup&gt;·cm&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>1832 ± 101</td>
<td>1963 ± 114</td>
<td>1781 ± 158</td>
<td>1913 ± 263</td>
<td>NS</td>
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<td>LVEDV, mL</td>
<td>236.6 ± 12.5</td>
<td>226.1 ± 17.6</td>
<td>176.3 ± 17.8</td>
<td>148.0 ± 10.9</td>
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<td>LVEDVI, mL/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>127.9 ± 6.6</td>
<td>117.8 ± 7.2</td>
<td>88.0 ± 7.2</td>
<td>77.0 ± 4.2</td>
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<td>LVEF, %</td>
<td>33.0 ± 1.8</td>
<td>26.0 ± 2.9</td>
<td>60.5 ± 2.2</td>
<td>60.2 ± 3.2</td>
<td>&lt;0.001</td>
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<tr>
<td>CO, L/min</td>
<td>3.9 ± 0.2</td>
<td>3.6 ± 0.2</td>
<td>5.3 ± 0.6</td>
<td>5.1 ± 0.1</td>
<td>&lt;0.001</td>
<td>NS</td>
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<td>Cl, L·min&lt;sup&gt;-1&lt;/sup&gt;·m&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>2.1 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>2.7 ± 0.3</td>
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<td>LVIdd, cm</td>
<td>7.1 ± 0.1</td>
<td>6.9 ± 0.2</td>
<td>5.4 ± 0.2</td>
<td>5.1 ± 0.1</td>
<td>&lt;0.001</td>
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<tr>
<td>PWId, mm</td>
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<td>10.3 ± 0.4</td>
<td>11.4 ± 0.3</td>
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<td>RWT</td>
<td>0.25 ± 0.01</td>
<td>0.30 ± 0.02*</td>
<td>0.40 ± 0.02</td>
<td>0.45 ± 0.02‡</td>
<td>0.005</td>
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<td>LVM, g</td>
<td>298.8 ± 18.1</td>
<td>338.6 ± 16.3</td>
<td>227.9 ± 14.6</td>
<td>231.0 ± 10.6</td>
<td>NS</td>
<td>&lt;0.001</td>
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<tr>
<td>LVMI, g/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>163.1 ± 10.1</td>
<td>181.2 ± 1.1</td>
<td>114.6 ± 5.5</td>
<td>121.3 ± 4.4</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVMI/LVEDVI, g/mL</td>
<td>1.3 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>LVPSs, dyne/cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>243.4 ± 8.0</td>
<td>255.0 ± 7.0</td>
<td>137.8 ± 8.4</td>
<td>130.3 ± 9.9</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>SM, kN/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>2.5 ± 0.2</td>
<td>6.3 ± 2.0†</td>
<td>4.5 ± 0.8</td>
<td>7.4 ± 0.7</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
</tbody>
</table>

M indicates male; F, female; DM1, type 1 DM; DM2, type 2 DM; GFR, glomerular filtration rate; ACEI, ACE inhibitors; CCB, calcium channel blockers; ARB, angiotensin II receptor blockers; LVSPs, LV peak systolic pressure; LVEDP, LV end-diastolic pressure; RAP, right atrial pressure; Ea, effective arterial elastance; PP, aortic pulse pressure; SVR, systemic vascular resistance; LVEDV, LV end-diastolic volume; LVEDVI, LV end-diastolic volume index; CO, cardiac output; CI, cardiac index; LVIdd, LV diastolic internal diameter; PWId, diastolic posterior wall thickness; RWT, relative wall thickness; LVM, LV mass; LVMI, LV mass index; and LVPSs, LV peak systolic wall stress.

Values are number of patients unless otherwise indicated.

*P<0.05 vs DM<sub>HREF</sub>; †P<0.001 vs DM<sub>HREF</sub>; ‡P<0.05 vs DM<sub>HREF</sub>; §P<0.01 vs DM<sub>HREF</sub>; ‖P<0.001 vs DM<sub>HREF</sub>. For all variables listed, 2-factor ANOVA showed no significant interaction between DM status and HFNEF-HFREF status.
fell, especially in DM+HFREF cardiomyocytes (from 8.5±0.9 to 4.0±0.3 kN/m², P<0.001), and the values of Fpassive became comparable in all patient groups (Figure 3C). The higher Fpassive in DM+HFREF than in DM−HFREF cardiomyocytes was paralleled by widening of the sarcomeric Z line, which was significantly larger in DM+HFREF than in DM−HFREF both on immunofluorescent images stained for α-actinin (+16.9%, P=0.045; Figure 3D) and on electron microscopy images (+15.2%, P<0.001; Figure 3E). In HFNEF patients, Fpassive correlated with SM (r=0.55, P=0.022) and with the duration of DM (r=0.35, P=0.04).

In control conditions, Fpassive rose progressively from HFREF to DM−HFREF and to DM+HFREF (Figure 3B). This trend was paralleled by the rise in cardiomyocyte diameter, PWTd, and relative wall thickness. Cardiomyocyte diameter rose from 16.0±1.5 μm in HFREF to 19.8±1.7 μm in DM−HFREF and 22.4±0.9 μm in DM+HFREF cardiomyocytes (P=0.98, P<0.001). PWTd rose from 9.4±0.3 mm in HFREF to 10.3±0.4 mm in DM−HFREF and 11.4±0.3 mm in DM+HFREF (P=0.98, P=0.037). Relative wall thickness rose from 0.27±0.01 in HFREF to 0.40±0.02 in DM−HFREF and 0.45±0.02 in DM+HFREF (P=0.97, P=0.03). The progressive rise in relative wall thickness indicates a shift from eccentric to concentric LV remodeling.

**Discussion**

The prevalence of DM in heart failure is increasing, and mortality and hospitalization rates in diabetic patients with heart failure remain particularly high.26–28 Although coronary artery disease is the most important contributor to the myocardial dysfunction observed in DM, DM-related disturbances such as hyperglycemia, insulin resistance, and hyperlipidemia can also act directly on the myocardium and induce myocardial dysfunction because of a shift in myocardial energy production from glucose utilization to fatty acid oxidation.30–32 In the first clinical description of DM-induced myocardial dysfunction, LV dilatation and systolic LV dysfunction were prominent features,33 and DM-induced myocardial dysfunction was therefore classified as a dilated cardiomyopathy. Subsequently, diastolic LV dysfunction was recognized as an earlier manifestation of DM-induced myocardial dysfunction.1–5 The present study confirmed diastolic LV stiffness to be greater in failing hearts of diabetic patients in the absence of significant coronary artery disease. Mechanisms responsible for this DM-induced diastolic myocardial stiffening were identified in endomyocardial biopsy samples of these patients. The main finding of the present study is that DM-induced diastolic LV stiffness mainly through higher Fpassive of hypertrophied cardiomyocytes.

Myocardial AGE Deposition and Fibrosis

DM−HFREF patients had higher diastolic LV stiffness than DM+HFREF patients. This higher diastolic LV stiffness was related to both AGE deposition and interstitial fibrosis. AGE deposition results from long-standing hyperglycemia and affects diastolic LV stiffness by direct and indirect mechanisms.29,34,35 AGE cross-linking of collagen increases its tensile strength, and this altered biophysical
property of collagen increases diastolic LV stiffness. AGE deposition can also indirectly augment diastolic LV stiffness through enhanced collagen formation and reduced nitric oxide bioavailability. Enhanced collagen formation in the presence of AGEs was observed in the present study. AGEs quench endothelially produced nitric oxide, and low myocardial nitric oxide bioavailability was previously demonstrated to increase diastolic LV stiffness in HFREF patients.36

Previous myocarditis, not DM, is the most likely cause of the dilated cardiomyopathy in the majority of the DM+/HFREF patients, because fasting glucose, glycohemoglobin, and DM duration were all similar in the DM+/HFREF and DM−/HFREF groups. Even in the absence of cellular infiltration, patients with postmyocarditis HFREF frequently have persistent myocardial microvascular inflammation. Inflammation facilitates AGE deposition, and persistent microvascular inflammation could therefore explain the preferential CML deposition in small intramyocardial vessels of DM+/HFREF patients. In rodent DM animal models, AGE deposition also occurs in the myocardial interstitium. Failure to observe interstitial CML deposition in the present study probably relates to better glycemic control in patients treated with glucose-lowering medication or insulin than in untreated rodent animal models. The clinical importance of endothelial AGE deposition was recently confirmed in hypertensive patients in whom a cross-link breaker improved endothelial function.40

In the present study, we observed a higher CVF in DM+/HFREF than in DM−/HFREF patients. Activation of fibroblasts in DM+/HFREF patients may have resulted from the aforementioned AGE deposition, protein kinase C activation, or high intracellular glucose concentrations.35,41

Cardiomyocyte Resting Tension

DM+/HFREF patients had a higher SM than DM−/HFREF patients. The higher SM related more to cardiomyocyte F passive and less to AGE deposition. Correction of high cardiomyocyte F passive by protein kinase A suggests a phosphorylation deficit of myofilamentary or cytoskeletal proteins,12,13 because the cardiomyocytes had been pretreated with Triton X-100 to remove all membranes. High F passive of DM+/HFREF cardiomyocytes was accompanied by Z-line widening. Z-line widening has been observed in transgenic mice after nebulin or muscle LIM protein knockout.42,43

The present study is the first to report Z-line widening in humans, and because of the simultaneous elevation of F passive, it suggests that Z-line widening results from altered elastic properties of cytoskeletal proteins, which pull at and open up adjacent Z lines. In a previous study comparing HFREF with HFNEF,13 a significant correlation was observed between cardiomyocyte hypertrophy and F passive.
In the present study, $F_{\text{passive}}$ rose progressively from HFREF to DM$^{+}$HFNEF and to DM$^{-}$HFNEF, and this rise was paralleled by an increase in cardiomyocyte diameter and a shift from eccentric to concentric LV remodeling. Because LV peak systolic pressure and peak systolic wall stress were similar in DM$^{+}$HFNEF and DM$^{-}$HFNEF, excess cardiomyocyte hypertrophy in DM$^{+}$HFNEF was unrelated to pressure overload and was probably induced by insulin resistance. All of the DM$^{+}$HFNEF patients had type 2 DM and elevated fasting insulin plasma levels. Furthermore, hyperinsulinemia is known to stimulate prohypertrophic signaling in insulin-responsive tissues such as the myocardium.

**Study Limitations**

The clinical characteristics of the HFNEF patients in the present study differed from clinical characteristics observed in epidemiological studies. In the present study, HFNEF patients were younger (mean age 64 years) and less often female (46%) than in epidemiological studies, in which patients are typically older (mean age 76 years) and more often female (55%). Patient recruitment from tertiary referral because of suspicion of inflammatory or infiltrative myocardial disease explains this discrepancy.

In the present study, diastolic LV material properties were analyzed by a radial LV SM. Use of a radial LV SM avoids geometric assumptions of LV shape. Furthermore, substitution of measured LVP by LVP$_{\text{pres}}$ enables early diastole to be included in the LV stiffness analysis, because it corrects for the upward displacement of the early diastolic LV pressure-volume relation. In a previous study of HFREF patients, close agreement was observed between the radial LV SM and the LV chamber stiffness constant derived from a curve fit to multiple LV end-diastolic pressure-volume points during balloon caval occlusion.

Higher LV end-diastolic pressure at similar or smaller LV end-diastolic volume can result from altered myocardial material properties and from external constraints on the LV by the right ventricle or the pericardium. Right ventricular constraints because of the shared interventricular septum are prominent in patients with hypertrophic cardiomyopathy but not in patients with HFNEF, who usually have hypertensive heart disease. Right atrial pressure was determined as a measure of intrapericardial pressure or pericardial constraint and was comparable in all patient groups.

Because of microvascular CML deposition, a relative reduction of the number of microvessels in hypertrophied myocardium could have lowered the CML score in the DM$^{+}$HFNEF patients. A similar CML score in DM$^{+}$HFNEF and DM$^{-}$HFREF patients, however, argues against such an artifact. Isolation of cardiomyocytes and assessment of myocardial tissue properties were performed on a limited number of samples procured by an endomyocardial biopsy technique and potentially overlook tissue heterogeneity. The extent of tissue heterogeneity was assessed in the present and previous studies. Sampling-related variability was <5% for cardiomyocyte force measurements and <15% for histomorphometric data.

Development of HFNEF results from diastolic LV dysfunction, deficient chronotropic or vasomotor responses, and arterial stiffening. In DM, not only diastolic LV dysfunction but also arterial stiffness becomes a more important contributor to HFNEF.

**Conclusions**

In the absence of coronary artery disease, the failing diabetic heart has an elevated diastolic LV stiffness. Mechanisms responsible for this increase in diastolic LV stiffness differ between HFREF and HFNEF patients. Deposition of AGEs and deposition of collagen are important determinants of the increased LV stiffness in diabetic patients with HFREF, whereas high cardiomyocyte $F_{\text{passive}}$ is the main determinant of the increased LV stiffness in diabetic patients with HFNEF.

**Disclosures**

None.

**References**


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CLINICAL PERSPECTIVE

Mortality among diabetic patients with heart failure is high. Diabetes mellitus–related metabolic disturbances contribute importantly to their myocardial dysfunction. Increased diastolic left ventricular (LV) stiffness is an early manifestation of myocardial dysfunction and frequently becomes an important functional deficit, because many diabetic patients present with heart failure and normal LV ejection fraction. Excessive diastolic LV stiffness of the diabetic heart is usually attributed to myocardial fibrosis or to myocardial deposition of advanced glycation end products. Hypertrophied cardiomyocytes isolated from LV biopsy samples of heart failure patients with normal LV ejection fraction have a high resting tension, which correlates with greater in vivo diastolic LV stiffness. This increased resting tension could be an important contributor to the increased diastolic LV stiffness of the diabetic heart. With the use of LV endomyocardial biopsy samples, the present study assessed myocardial fibrosis, myocardial advanced glycation end product deposition, and resting tension of isolated cardiomyocytes in diabetic patients with heart failure and either normal or reduced LV ejection fraction. All patients were free of coronary artery disease and had an elevated diastolic LV stiffness. The mechanisms responsible for the elevated diastolic LV stiffness differed between heart failure patients with normal and reduced LV ejection fraction. Myocardial deposition of collagen and advanced glycation end products was more important in patients with reduced ejection fraction, whereas a high cardiomyocyte resting tension was more important in patients with normal ejection fraction. These mechanistic studies suggest that correction of high cardiomyocyte resting tension, possibly through regression of cardiomyocyte hypertrophy, may be an important therapeutic target for diabetic patients with heart failure and normal ejection fraction.
Diastolic Stiffness of the Failing Diabetic Heart: Importance of Fibrosis, Advanced Glycation End Products, and Myocyte Resting Tension


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