Heart Failure

Myocardial Structure and Function Differ in Systolic and Diastolic Heart Failure

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Background—To support the clinical distinction between systolic heart failure (SHF) and diastolic heart failure (DHF), left ventricular (LV) myocardial structure and function were compared in LV endomyocardial biopsy samples of patients with systolic and diastolic heart failure.

Methods and Results—Patients hospitalized for worsening heart failure were classified as having SHF (n=22; LV ejection fraction [EF] 34±2%) or DHF (n=22; LVEF 62±2%). No patient had coronary artery disease or biopsy evidence of infiltrative or inflammatory myocardial disease. More DHF patients had a history of arterial hypertension and were obese. Biopsy samples were analyzed with histomorphometry and electron microscopy. Single cardiomyocytes were isolated from the samples, stretched to a sarcomere length of 2.2 μm to measure passive force (Fpassive), and activated with calcium-containing solutions to measure total force. Cardiomyocyte diameter was higher in DHF (20.3±0.6 versus 15.1±0.4 μm, P<0.001), but collagen volume fraction was equally elevated. Myofibrillar density was lower in SHF (36±2% versus 46±2%, P<0.001). Cardiomyocytes of DHF patients had higher Fpassive (7.1±0.6 versus 5.3±0.3 kN/m²; P<0.01), but their total force was comparable. After administration of protein kinase A to the cardiomyocytes, the drop in Fpassive was larger (P<0.01) in DHF than in SHF.

Conclusions—LV myocardial structure and function differ in SHF and DHF because of distinct cardiomyocyte abnormalities. These findings support the clinical separation of heart failure patients into SHF and DHF phenotypes.

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Key Words: myocardium ▪ heart failure ▪ diastole ▪ hypertrophy ▪ collagen

Despite an increasing awareness that heart failure (HF) can occur in the presence of a normal left ventricular (LV) ejection fraction (EF),1-2 it remains uncertain whether HF with normal LVEF and with reduced LVEF are indeed distinct HF phenotypes.3,4 Although global LV systolic performance is preserved,5 HF patients with normal LVEF have reduced LV long-axis shortening,6 depressed tissue Doppler myocardial systolic velocity,7 and deranged ventriculoarterial coupling.8 Furthermore, HF with normal LVEF is frequently referred to as diastolic heart failure (DHF) because of the presence of diastolic LV dysfunction evident from slow LV relaxation and high LV stiffness.9 Diastolic LV dysfunction, however, is not unique to patients with DHF but also occurs in HF patients with reduced LVEF, or systolic heart failure (SHF), in whom it correlates better with symptoms than LVEF.10

Because hemodynamic features apparently fail to identify SHF and DHF as distinct HF phenotypes, a comparative analysis of the structure and function of LV myocardium procured from both types of HF was proposed recently.11,12 The present study performed this analysis by investigating LV endomyocardial biopsy samples from SHF and DHF patients. Histological structure and contractile function of the biopsy samples were compared with histomorphometry, transmission electron microscopy, and force measurements of single cardiomyocytes.

Methods

Patients

Fifty-eight patients hospitalized for worsening HF were referred for cardiac catheterization and LV endomyocardial biopsy procurement because of suspicion of infiltrative or inflammatory myocardial disease. Coronary angiography showed significant (>50%) coronary stenoses in 10 patients, and histological analysis of the biopsy...
samples revealed myocardial infiltration or inflammation in 4 patients. These 14 patients were excluded from the study.

The remaining 44 patients were classified as SHF (n=22) if LVEF was <45% and as DHF (n=22) if they satisfied the “European Study Group on DHF” criteria, ie, signs and symptoms of congestive HF, LVEF >45%, and an LV end-diastolic pressure (LVEDP) >16 mm Hg.13 All DHF patients also satisfied criteria for definite DHF.14 Hemodynamic data were derived from LV angiograms, high-fidelity LV pressure catheters, and thermodilution cardiac outputs. They included LV peak systolic pressure (LVPSP), LVEDP, LV end-diastolic volume index (LVEDVIangio), LVEF, cardiac index (CI), LV dP/dt max, LV dP/dt min, and the time constant of LV relaxation (τ, Table). Doppler echocardiographic data (Table) included LVEDVIecho, mitral early velocity (E), mitral atrial velocity (A), E/A ratio, deceleration time, and LV wall thickness. LVEDVIecho correlated with LVEDVIangio (r=0.44; P=0.009). Hemodynamic and echocardiographic data were combined for LV mass index (LVMI), LVM/LVEDVI ratio, and myocardial stiffness modulus (Table). The Table compares hemodynamic, echocardiographic, and combined hemodynamic-echocardiographic data of SHF patients with DHF patients and of SHF or DHF patients with normal values derived from an age- and gender-matched control population. Data

### Clinical, Hemodynamic, Echocardiographic, and Combined Hemodynamic-Echocardiographic Characteristics of SHF and DHF Patients

<table>
<thead>
<tr>
<th></th>
<th>SHF (n=22)</th>
<th>DHF (n=22)</th>
<th>P, SHF vs DHF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, y</strong></td>
<td>59.1±3.9</td>
<td>67.8±2.2</td>
<td>0.058</td>
</tr>
<tr>
<td><strong>Sex (M/F)</strong></td>
<td>15/7</td>
<td>13/9</td>
<td>0.536</td>
</tr>
<tr>
<td><strong>Hypertension</strong></td>
<td>3/22</td>
<td>16/22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Diabetes mellitus</strong></td>
<td>5/22</td>
<td>11/22</td>
<td>0.063</td>
</tr>
<tr>
<td><strong>Obesity</strong></td>
<td>0/22</td>
<td>10/22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Medication</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACEI</td>
<td>17/22</td>
<td>15/22</td>
<td>0.503</td>
</tr>
<tr>
<td>β-Blocker</td>
<td>12/22</td>
<td>10/22</td>
<td>0.551</td>
</tr>
<tr>
<td>Diuretic</td>
<td>19/22</td>
<td>18/22</td>
<td>0.684</td>
</tr>
<tr>
<td>CCB</td>
<td>2/22</td>
<td>7/22</td>
<td>0.065</td>
</tr>
<tr>
<td>ARB</td>
<td>1/22</td>
<td>4/22</td>
<td>0.159</td>
</tr>
<tr>
<td>Digoxin</td>
<td>7/22</td>
<td>4/22</td>
<td>0.302</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>2/22</td>
<td>5/22</td>
<td>0.222</td>
</tr>
<tr>
<td>Statin</td>
<td>4/22</td>
<td>10/22</td>
<td>0.055</td>
</tr>
<tr>
<td>Nitrate</td>
<td>1/22</td>
<td>2/22</td>
<td>0.554</td>
</tr>
<tr>
<td><strong>Hemodynamics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>82±4</td>
<td>74±2</td>
<td>0.095</td>
</tr>
<tr>
<td>LVPSP, mm Hg (Con: 120±6)</td>
<td>122±5</td>
<td>172±7*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVEDP, mm Hg (Con: 10±1)</td>
<td>22±2*</td>
<td>26±2*</td>
<td>0.103</td>
</tr>
<tr>
<td>LVEDVIangio, mL/m² (Con: 72±4)</td>
<td>120±7*</td>
<td>83±4</td>
<td>&lt;0.001</td>
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<tr>
<td>LVEF, % (Con: 70±3%)</td>
<td>34±2*</td>
<td>62±2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CI, L·min⁻¹·m⁻² (Con: 3.1±0.2)</td>
<td>2.2±0.1*</td>
<td>2.7±0.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LV dP/dtmax, mm Hg/s (Con: 1993±88)</td>
<td>954±109*</td>
<td>1517±75*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LV dP/dtmin, mm Hg/s (Con: 2320±149)</td>
<td>1076±124*</td>
<td>1698±96*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>τ, ms (Con: 38±2)</td>
<td>65±7*</td>
<td>68±5*</td>
<td>0.756</td>
</tr>
<tr>
<td><strong>Echocardiography</strong></td>
<td></td>
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<tr>
<td>LVEDVIecho, mL/m² (Con: 65±4)</td>
<td>127±9*</td>
<td>82±6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E, m/s (Con: 0.65±0.03)</td>
<td>0.94±0.1*</td>
<td>0.76±0.07</td>
<td>0.177</td>
</tr>
<tr>
<td>A, m/s (Con: 0.61±0.04)</td>
<td>0.78±0.09</td>
<td>0.86±0.08*</td>
<td>0.515</td>
</tr>
<tr>
<td>E/A ratio (Con: 1.13±0.05)</td>
<td>1.43±0.21*</td>
<td>1.02±0.14</td>
<td>0.110</td>
</tr>
<tr>
<td>Deceleration time, ms (Con: 219±7)</td>
<td>161±9*</td>
<td>226±12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVWT, mm (Con: 8.0±0.5)</td>
<td>9.0±0.3</td>
<td>11.4±0.3*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Hemodynamics and echocardiography</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVMI, g/m² (Con: 92±3)</td>
<td>124±7*</td>
<td>141±5*</td>
<td>0.059</td>
</tr>
<tr>
<td>LVMI/LVEDVI ratio (Con: 1.27±0.04)</td>
<td>1.09±0.1</td>
<td>1.74±0.1*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Myocardial stiffness modulus, kN/m² (Con: 2.2±0.3)</td>
<td>3.09±0.36*</td>
<td>5.95±0.48*</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

M indicates male; F, female; ACEI, ACE inhibitors; CCB, calcium channel blockers; ARB, angiotensin II receptor blockers; HR, heart rate; and Con, control population.

*P<0.05 vs control population.
Quantitative Histomorphometry: Light Microscopy
Histomorphometric analysis of biopsy samples was performed on elastica van Gieson and hematoxylin-and-eosin-stained, 4-μm-thick sections of tissue placed in 5% formalin. Images of these sections were acquired with a projection microscope (×50). Subsequent image analysis with Slidebook 4.0 software (3I, Denver, Colo) was performed to determine cardiomyocyte diameter (MyD; μm) and extent of reactive interstitial fibrosis, which was expressed as collagen volume fraction (CVF; %). Areas of reparative and perivascular fibrosis were excluded. As previously validated,16,17 MyD was determined perpendicularly to the outer contour of the cell membrane at the nucleus level in 15 representative myocytes of the section, and CVF was calculated as the sum of all connective tissue areas divided by the sum of all connective tissue and muscle areas averaged over 4 to 6 representative fields of the section. In our laboratory, normal values of MyD and CVF for LV endomyocardial biopsy material are 13.1±0.3 μm and 5.4±2.2%, respectively.

Quantitative Histomorphometry: Electron Microscopy
Endomyocardial biopsy samples were fixed in 2% (vol/vol) glutaraldehyde for 30 minutes and 1.5% (wt/vol) osmium tetroxide for 10 minutes, dehydrated with acetone, and embedded in Epon812. Ultrathin sections were collected on 300-mesh Formvar-coated nickel grids. The sections were contrasted with uranyl acetate and lead citrate and were examined in a Jeol-1200EX electron microscope. Quantitative analysis was performed with the aforementioned automated image analyzer. Cardiomyocyte myofibrillar density, number of mitochondria, and capillary basement membrane thickness were determined. Myofibrillar density was calculated as the sum of myofibrillar areas related to total cellular area in 4 to 6 representative myocytes, and capillary basement membrane thickness was averaged over 6 measurements.

Force Measurements in Isolated Cardiomyocytes
Force measurements were performed in single, mechanically isolated cardiomyocytes as described previously.17,18 Biopsy samples (5 mg wet weight) were defrosted in relaxing solution (in mmol/L: free Mg 0.3, KCl 100, EGTA 2, Mg-ATP 4, and imidazole 10; pH 7.0), wet weight) were defrosted in relaxing solution, after which single cardiomyocytes were attached

Titin Isoform Gel Electrophoresis
Titin isoform gel electrophoresis was performed on myocardial samples (n=4) of DHF patients and on myocardial samples (n=5) of SHF patients. Endomyocardial biopsy samples of either DHF or SHF patients needed to be pooled to provide sufficient tissue to perform the titin isoform gel electrophoresis. Because no previous study had determined titin isoforms on LV myocardial biopsy samples, the assay was validated by the repetition of titin isoform gel electrophoresis on LV myocardium of explanted dilated cardiomyopathic hearts (n=3), which have been studied previously.19,20 The N2BA/N2B titin isoform ratio observed in the explanted myocardium (39/61) was similar to previously reported values (42/58)17 and higher than previously reported values of normal human myocardium (30/70).17 Tissue samples were homogenized in 50 to 100 μL of Tris-SDS buffer (pH 6.8) containing 8 μg/mL leupeptin (Peptide Institute, Osaka, Japan). Titin isoforms were separated on agarose-strengthened 2% SDS-polyacrylamide gels and stained with Coomassie brilliant blue. Gels were digitized, and the optical volume of protein bands was determined with Total Laboratory software (Phoretix, Nonlinear Dynamics, Newcastle upon Tyne, UK).

Data Analysis
The time constant of LV relaxation (τ) was derived from an exponential curve fit with zero-asymptote pressure to the pressure data points of isovolumic LV relaxation. To assess LV myocardial material properties, a radial myocardial stiffness modulus was calculated.16,17,22 Agreement between myocardial stiffness modulus and diastolic LV stiffness indices derived from multiple beat analyses during caval occlusion was previously established in SHF patients.22 Values are given as mean±SEM. A 2-tailed test with a probability value <0.05 was considered significant. SHF and DHF groups were compared by an unpaired t test. Effects of CVF and PKA in SHF and DHF were analyzed respectively by 2-factor ANOVA and by 2-factor repeated-measures ANOVA. Bonferroni-adjusted t tests served as subsequent multicomparison tests. The significance for categorical data were determined by the χ2 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 the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results
Clinical and Hemodynamic Characteristics
More DHF patients had a history of arterial hypertension and were obese (body mass index >30 kg/m2; Table). Use of medications was similar in both patient groups. LVPSWP, LVEF, CI, LVDp/dtmax, LVDp/dtmin, deceleration time, and myocardial stiffness modulus were higher in DHF patients. In both SHF and DHF, LVMi was higher than in the control population. In SHF patients, the elevated LVMi was compensatory to preserve LV wall thickness at the larger LVEDVI (eccentric LV hypertrophy). In DHF patients, the elevated LVMi resulted in significant increases in LV wall thickness and in LVMi/LVEDVI ratio (concentric LV hypertrophy).
Quantitative Histomorphometry of LV Myocardium

MyD was significantly larger in DHF (20.3±0.6 μm) than in SHF patients (15.1±0.4 μm; P<0.001; Figure 1A). CVF was equally elevated in SHF (14.4±1.5%) and DHF (12.2±1.4%; Figure 1B). At each level of CVF, MyD was larger in DHF (Figure 1C). As CVF rose, MyD increased linearly in both SHF (r=0.58, P=0.005) and DHF (r=0.67, P=0.001; Figure 1C).

The relation between MyD and CVF is further illustrated in Figure 2, which used mean CVF to divide SHF and DHF patients into low (<mean) or high (>mean) CVF groups. Representative histological examples of SHF with low CVF, SHF with high CVF, DHF with low CVF, and DHF with high CVF are depicted in Figure 2A. In both DHF and SHF, the largest MyD is observed at high CVF (Figure 2B). In both low and high CVF, the largest MyD is observed in DHF (Figure 2B). Myofibrillar density was significantly lower in SHF (36±2%) than in DHF (46±2%; P<0.001; Figure 3A). Myofibrillar loss in SHF is illustrated in Figure 3B. The number of mitochondria per micrometer squared and capillary basement membrane thickness were comparable in SHF and DHF.

Force Measurements of Single Cardiomyocytes

When single cardiomyocytes were stretched to a sarcomere length of 2.2 μm, F_{passive} was higher in DHF (7.1±0.6 kN/m²) than in SHF (5.3±0.3 kN/m²; P<0.01; Figure 4A). F_{total} at
maximal activation was comparable in both patient groups (Figure 4B). pCa50 was higher in DHF (5.88 ± 0.01) than in SHF (5.84 ± 0.01; P < 0.05; Figure 4C). Average force-pCa relations of pooled cardiomyocytes of SHF and DHF patient groups are shown in the Figure of the online-only Data Supplement (panel A). As previously reported,17 Fpassive correlated with myocardial stiffness modulus (r = 0.56; P = 0.02; Figure 4D) and with LVEDP (r = 0.52, P = 0.001) in DHF patients. These correlations were absent in SHF. After PKA treatment, the drop in Fpassive was larger (P < 0.01) in DHF than in SHF because Fpassive fell to a similarly low level in SHF (3.2 ± 0.2 kN/m²) and DHF (3.7 ± 0.3 kN/m²; Figure 4A). Ftotal at maximal activation failed to change after PKA in either group (Figure 4B). pCa50 fell significantly in both groups (Figure 4C). The effect of PKA treatment on the average force-pCa relations of pooled cardiomyocytes of SHF and DHF patient groups is shown in the Figure of the online-only Data Supplement (panels B and C). To explain the higher Fpassive of DHF cardiomyocytes, titin isoform separation was performed on pooled endomyocardial biopsy material (Figure 4E). The titin N2BA/N2B ratio of DHF myocardium (17/83; SEM of the ratio 0.02) was lower (P < 0.05) than that of SHF myocardium (35/65; SEM of the ratio 0.13).

**Discussion**

A comparative analysis of LV myocardium in SHF and DHF revealed the following: (1) MyD is larger in DHF, but CVF is similar. (2) At each level of CVF, MyD is larger in DHF, but MyD increases similarly in both SHF and DHF as fibrosis.
progresses. (3) Myofibrillar density is lower in SHF. (4) \( F_{\text{passive}} \) of single cardiomyocytes is higher in DHF, and after administration of PKA to the cardiomyocytes, the drop in \( F_{\text{passive}} \) is larger in DHF. (5) Calcium sensitivity of single cardiomyocytes is higher in DHF, but \( F_{\text{total}} \) at maximal activation is similar.

**Cardiomyocyte Hypertrophy**

The present study provides histological evidence of considerable cardiomyocyte hypertrophy in DHF. LV hypertrophy had already been reported in DHF\(^{23-25} \) and was confirmed in the present study by the higher than normal LVMI. LV hypertrophy in DHF became especially evident when \( \text{LVMI/LVEDVI} \) was accounted for by expressing LV hypertrophy as an \( \text{LVMI/LVEDVI} \) ratio.\(^{23-24} \) A higher than normal LVMI was also observed in SHF, but in contrast to DHF, it only resulted in a minor increase in MyD as it was compensatory to normalize LV wall thickness in a dilated LV. The considerable increase of MyD in DHF and the minor increase of MyD in SHF resemble the previously reported cardiomyocyte remodeling in concentric and eccentric human or animal LV hypertrophy.\(^{26} \) In concentric LV hypertrophy, cardiomyocytes grow in a transverse direction while keeping cell length constant, whereas in eccentric hypertrophy, cardiomyocytes grow proportionally in longitudinal and transverse directions. This difference in cardiomyocyte remodeling correlates with distinct patterns of peptide growth factor induction in both conditions.\(^{27} \)

The increase of MyD in SHF was accompanied by collagen deposition (Figure 2B). In DHF, collagen deposition was associated with a similar additional increase in MyD (Figure 2B). Because collagen deposition and myocyte hypertrophy development were present in both SHF and DHF, use of ACE inhibitors, angiotensin II receptor blockers, and aldosterone antagonists, which oppose myocardial fibrosis and maladaptive hypertrophy, appears justified in both HF phenotypes. Indeed, the CHARM trial (Candesartan in Heart Failure—Assessment of Reduction in Mortality and Morbidity) using the angiotensin II receptor blocker candesartan reported beneficial effects not only in SHF but also in DHF.\(^{28} \) At each level of CVF, MyD of DHF patients exceeded MyD of SHF patients (Figure 1C). This excess cardiomyocyte hypertrophy in DHF probably related to a history of arterial hypertension, which was present in 73% of DHF patients and only in 13% of SHF patients. Arterial hypertension in DHF patients was also evident at cardiac catheterization from their high LVPSP (172 ± 7 mm Hg). The importance of arterial hypertension for the development of DHF was recently demonstrated in the first DHF large-animal model, in which arterial hypertension was created in old dogs by wrapping of both kidneys.\(^{29} \) Sixty percent of the DHF patients in the present study had diabetes mellitus, and 50% were obese. In experimental models of diabetes mellitus and insulin resistance, development of cardiac hypertrophy has also been reported.\(^{30,31} \)

**Higher \( F_{\text{passive}} \) in DHF**

When single cardiomyocytes of DHF patients were stretched to a sarcomere length of 2.2 μm, \( F_{\text{passive}} \) was 34% higher than in cardiomyocytes of SHF patients. Higher \( F_{\text{passive}} \) of cardiomyocytes can result from altered calcium handling or from modified myofilamentary and cytoskeletal proteins. Because the cardiomyocytes were incubated in Triton X-100 before the experiments, the integrity of sarcolemmal and sarcoplasmic membranes was disrupted. Altered calcium handling was therefore excluded as a cause of the higher \( F_{\text{passive}} \). In a previous study, no changes in myofilamentary protein expression or phosphorylation were found in endomyocardial biopsy samples of DHF patients.\(^{17} \) The higher \( F_{\text{passive}} \) is therefore also unrelated to myofilamentary proteins and must be attributed to altered expression or phosphorylation of cytoskeletal proteins. Because PKA corrected \( F_{\text{passive}} \), the cytoskeletal protein involved must have phosphorylation sites. Titin has phosphorylation sites, and its phosphorylation by PKA lowers \( F_{\text{passive}} \) in isolated cardiac muscle strips, especially when the stiff isoform of titin (N2B) is overexpressed.\(^{32,33} \) The present study observed a higher expression of the stiff N2B titin isoform in pooled endomyocardial biopsy samples of DHF patients. If future studies can confirm this titin isoform shift in myocardial samples of individual DHF patients, the higher \( F_{\text{passive}} \) observed in cardiomyocytes of DHF patients could be attributed to this titin isoform shift. Furthermore, the fall in \( F_{\text{passive}} \) after PKA was larger in DHF than in SHF (Figure 4A). This larger fall after PKA was also consistent with the higher N2B expression in the pooled endomyocardial biopsy samples of DHF patients. A similar shift in titin isoform expression from the compliant N2A to the stiff N2B isoform was previously reported in experimental hypertensive myocardium.\(^{34} \) It remains to be investigated whether lower baseline phosphorylation of titin contributed to the higher \( F_{\text{passive}} \) in DHF.

Cardiomyocyte \( F_{\text{passive}} \) of DHF patients correlated with LVEDP and with myocardial stiffness modulus in both the present and a previous study.\(^{17} \) Because PKA corrected the high \( F_{\text{passive}} \) (Figure 4A), raising myocardial PKA activity by \( \beta \)-adrenoceptor stimulation could improve LV diastolic function in DHF. Improved diastolic LV function during administration of isoprotenerol has indeed been demonstrated in patients with hypertrophic cardiomyopathy, who frequently have DHF. At present, it is unclear whether only PKA can phosphorylate titin or whether protein kinase C and protein kinase G are also effective. Protein kinase G is especially of interest, because myocardial activity of PKG can be increased by nitric oxide and by phosphodiesterase 5A-inhibitors. Nitric oxide is known to improve diastolic LV function in normal, hypertrophied, and failing hearts, and sildenafil has recently been shown to favorably modify cardiac hypertrophy.\(^{37} \) DHF patients had 20% higher myofilamentary density than SHF patients. This higher myofilamentary density could have contributed to the higher \( F_{\text{passive}} \) in DHF. Reduction by PKA of \( F_{\text{passive}} \) to a similar level in DHF and SHF, however, argues against higher myofilamentary density accounting for the higher \( F_{\text{passive}} \) in DHF. The lower myofilamentary density in SHF could have resulted from myofilamentary breakdown, as occurs in coxsackie myocarditis, or from reduced myofilamentary synthesis, as occurs in alcoholic cardiomyopathy.\(^{39} \) Although myofilamentary density was lower in SHF, cardiomyocytes had comparable \( F_{\text{total}} \) at maximal activation, possi-
bly because of enhanced actomyosin interaction resulting from altered titin isoforms or phosphorylation.32

Study Limitations

The origin of DHF is diverse, including coronary artery disease, arterial hypertension, hypertrophic cardiomyopathy, and infiltrative cardiomyopathy.13 Because patients with coronary artery disease and infiltrative cardiomyopathy were excluded, LV pressure overload induced by arterial hypertension became the most prevalent cause of DHF in the present study. Because of this patient selection and because of the limited size of the patient groups, caution is needed before the current observations are extrapolated to the HF population at large.

Because cardiomyocytes were incubated before the experiments with 0.2% Triton X-100, the integrity of sarcomlemal and sarcoplasmic membranes was disrupted, and cardiomyocytes became dependent on externally supplied calcium for force development. Under these conditions, an effect of disturbed Ca2+ handling or of cytosolic Ca2+ overload on Fpassive or Ftotal could not be studied.

Assessment of myocardial structure and function was performed on a limited number of LV biopsy samples and could have overlooked LV myocardial tissue heterogeneity. The extent of LV myocardial tissue heterogeneity was previously addressed in explanted hearts18 and in surgically procured biopsy samples.40 In those studies, the variability of force measurements in cardiomyocytes isolated from different portions of the heart was always ≤5%. Variability of the titin N2BA/N2B ratios was also ≤5% when myocardial samples procured from different locations within the LV were compared.41 The present study observed a shift from the compliant N2BA to the stiff N2B titin isoform in pooled endomyocardial biopsy samples of DHF patients. Biopsy samples needed to be pooled to provide sufficient tissue for the titin isoform gel electrophoresis. Before the higher Fpassive observed in cardiomyocytes of DHF patients is attributed to this titin isoform shift, the shift must be confirmed in myocardial samples of individual DHF patients.

Characterization of the extracellular matrix in both types of HF was limited to measurement of CVF and did not include eventual shifts in collagen isotypes and cross-linking. Because half of the DHF patients had diabetes mellitus, collagen cross-links formed by advanced-glycation end products42 could also have contributed to the high myocardial stiffness observed in this group.

Conclusions

The present study observes the LV myocardium in SHF and DHF to differ in both cellular architecture and function and suggests SHF and DHF to be associated with phenotypically distinct cardiomyocyte abnormalities. These differences support the clinical discrimination of HF patients into SHF and DHF groups.

Disclosures

None.

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**CLINICAL PERSPECTIVE**

Approximately half of the heart failure patient population has a left ventricular (LV) ejection fraction greater than 45%. These patients are frequently referred to as diastolic heart failure (DHF) patients in contrast to patients with an LV ejection fraction less than 45%, who are referred to as systolic heart failure (SHF) patients. The separation of heart failure patients into these 2 phenotypes is challenged because DHF patients can have subtle abnormalities of systolic LV function that are not appreciated from an LV ejection fraction measurement. Moreover, SHF patients can have diastolic LV dysfunction, which often predicts their exercise intolerance better than LV ejection fraction. Because hemodynamic features apparently fail to support DHF and SHF as distinct heart failure phenotypes, the structure and function of LV myocardium of DHF and SHF patients were compared. LV myocardium was procured by an endomyocardial biopsy technique in DHF and SHF patients without coronary artery disease. Myocardial collagen volume fraction was equally elevated in DHF and SHF patients. Cardiomyocytes of DHF and SHF patients were structurally different: cardiomyocyte diameter was larger and myofibrillar density higher in the DHF patients. Furthermore, isolated single cardiomyocytes were functionally different: when stretched to the same sarcomere length, passive force was higher in the DHF patients, and when activated with calcium-containing solutions, calcium sensitivity was also higher in the DHF patients. These distinct cardiomyocyte abnormalities support DHF and SHF as separate heart failure phenotypes. Regression of cardiomyocyte hypertrophy and reduction of cardiomyocyte passive force are potential targets for a specific DHF treatment strategy.
Myocardial Structure and Function Differ in Systolic and Diastolic Heart Failure
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