Heart Failure

Cardiomyocyte Stiffness in Diastolic Heart Failure

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Background—Heart failure with preserved left ventricular (LV) ejection fraction (EF) is increasingly recognized and usually referred to as diastolic heart failure (DHF). Its pathogenetic mechanism remains unclear, partly because of a lack of myocardial biopsy material. Endomyocardial biopsy samples obtained from DHF patients were therefore analyzed for collagen volume fraction (CVF) and sarcomeric protein composition and compared with control samples. Single cardiomyocytes were isolated from these biopsy samples to assess cellular contractile performance.

Methods and Results—DHF patients (n=12) had an LVEF of 71±11%, an LV end-diastolic pressure (LVEDP) of 28±4 mm Hg, and no significant coronary artery stenoses. DHF patients had higher CVFs (7.5±4.0%, P<0.05) than did controls (n=8, 3.8±2.0%), and no conspicuous changes in sarcomeric protein composition were detected. Cardiomyocytes, mechanically isolated and treated with Triton X-100 to remove all membranes, were stretched to a sarcomere length of 2.2 μm and activated with solutions containing varying [Ca2+]i. Compared with cardiomyocytes of controls, cardiomyocytes of DHF patients developed a similar total isometric force at maximal [Ca2+]i, but their resting tension (Fpassive) in the absence of Ca2+ was almost twice as high (6.6±3.0 versus 3.5±1.7 kN/m2, P<0.001). Fpassive and CVF combined yielded stronger correlations with LVEDP than did either alone. Administration of protein kinase A (PKA) to DHF cardiomyocytes lowered Fpassive to control values.

Conclusions—DHF patients had stiffer cardiomyocytes, as evident from a higher Fpassive at the same sarcomere length. Together with CVF, Fpassive, determined in vivo diastolic LV dysfunction. Correction of this high Fpassive by PKA suggests that reduced phosphorylation of sarcomeric proteins is involved in DHF. (Circulation. 2005;111:774-781.)

Key Words: diastole ■ heart failure ■ myocytes ■ contractility ■ collagen

Heart failure with preserved left ventricular (LV) ejection fraction (EF) is frequently referred to as diastolic heart failure (DHF) in contrast to systolic heart failure, which is characterized by HF with reduced LVEF.1–4 DHF is currently diagnosed in as much as 49% of HF patients.5 Diastolic LV dysfunction is also increasingly recognized,6 as evident from a recent population-based survey in which diastolic LV dysfunction was observed 5 times more often than systolic LV dysfunction.7

Despite the increased recognition of both DHF and diastolic LV dysfunction, their pathophysiology remains incompletely understood. Whether HF with preserved LVEF results from diastolic LV dysfunction8 or from subtle systolic LV dysfunction, unappreciated by a routine LVEF measurement9 and possibly exacerbated by high arterial impedance,10 is still a matter of debate. Furthermore, explanations proposed for diastolic LV dysfunction are divergent, ranging from high LV myocardial stiffness1,8 to pericardial or right ventricular constraint.11,12 Moreover, the relative importance of myocardial fibrosis and of high cardiomyocyte resting tension for LV myocardial stiffness remains undefined.13

Failure to resolve these controversies about DHF and diastolic LV dysfunction could arise from a lack of myocardial biopsy or necropsy material,8,13 which would allow clinical and hemodynamic features to be matched with cellular and molecular myocardial properties. We therefore studied endomyocardial biopsy samples from patients with DHF in whom sample procurement was requested because of clinical suspicion of restrictive cardiomyopathy or, in the case of transplant recipients, because of clinical suspicion of rejection and in whom subsequent histological examination revealed no signs of infiltrative myocardial disease or rejection. Apart from routine histological examination, biopsies were used for determination of collagen volume fraction (CVF), sarcomeric protein expression, and isolation of single cardiomyocytes, whose developed and resting tensions were measured to detect systolic and/or diastolic dysfunction at the cellular level.14,15 To evaluate the relative contributions to

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TABLE 1. Clinical Characteristics of DHF Patients

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age, y</th>
<th>Sex</th>
<th>Diagnoses</th>
<th>Medications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>77</td>
<td>F</td>
<td>HT</td>
<td>ACEI, Amio, CCB, Diu, Stat</td>
</tr>
<tr>
<td>2</td>
<td>66</td>
<td>F</td>
<td>HT</td>
<td>Amio, ARB, β, Dig, Diu</td>
</tr>
<tr>
<td>3</td>
<td>67</td>
<td>M</td>
<td>HT, DM, Ob</td>
<td>ACEI, Dig, Diu, Nif</td>
</tr>
<tr>
<td>4</td>
<td>82</td>
<td>F</td>
<td>HT</td>
<td>ACEI, β, Diu, Diu, Stat</td>
</tr>
<tr>
<td>5</td>
<td>71</td>
<td>F</td>
<td>HT, DM</td>
<td>ACEI, Amio, CCB, Diu</td>
</tr>
<tr>
<td>6</td>
<td>71</td>
<td>M</td>
<td>HT, DM, Ob</td>
<td>ACEI, CCB, Nif, Diu</td>
</tr>
<tr>
<td>7</td>
<td>74</td>
<td>F</td>
<td>HT</td>
<td>ACEI, ARB, CCB, β, Diu</td>
</tr>
</tbody>
</table>

HT indicates arterial hypertension; DM, diabetes mellitus; Ob, obesity; TX, transplant recipients; ACEI, angiotensin-converting-enzyme inhibitors; Amio, amiodarone; CCB, calcium channel blockers; Diu, diuretics; Stat, statins; ARB, angiotensin II receptor blockers; β, β-blockers; Dig, digitalis; Nif, nitrates; Imm, immunosuppressants; and Fib, fibrates.

TABLE 2. LV Systolic and Diastolic Function of Control and DHF Groups (Mean±SD)

<table>
<thead>
<tr>
<th></th>
<th>HR, bpm</th>
<th>LVSP, mm Hg</th>
<th>LVEDP, mm Hg</th>
<th>LVEDVI, ml/m²</th>
<th>LVEF, %</th>
<th>CI, L/min per m²</th>
<th>σ, kN/m²</th>
<th>E, kN/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>80±16</td>
<td>135±15</td>
<td>13±4</td>
<td>78±23</td>
<td>71±12</td>
<td>2.7±0.6</td>
<td>1.6±0.3</td>
<td>2.2±0.7</td>
</tr>
<tr>
<td>DHF</td>
<td>84±11</td>
<td>186±35*</td>
<td>28±4*</td>
<td>74±16</td>
<td>71±11</td>
<td>2.8±0.6</td>
<td>5.1±1.1*</td>
<td>4.6±1.2*</td>
</tr>
</tbody>
</table>

HR indicates heart rate; LVSP, left ventricular peak systolic pressure; LVEDVI, left ventricular end-diastolic volume index; and CI, cardiac index. *P<0.05, DHF vs controls.
Data Analysis
Circumferential LV end-diastolic wall stress (σ) was computed by using a thick-wall ellipsoid model of the LV: 

\[ \sigma = \frac{P(D/2)h(1 - (h/D) - (D^2/2L^2))}{12} \]

where \( P \) is LV end-diastolic pressure, \( h \) is LV end-diastolic volume, and \( D, L \) are LV short-axis diameter and long-axis length at the midwall, respectively.\(^{20} \)

The radial myocardial stiffness modulus (\( E \)) was calculated to assess myocardial material properties from the formula: 

\[ E = \frac{\Delta \sigma / \Delta h}{\Delta P / \Delta h} = \frac{-\Delta \sigma / \Delta h}{\Delta P / \Delta h} \]

Assuming the increment in radial stress (\( \Delta \sigma \)) to be equal but opposite in sign to the increment in wall thickness (\( \Delta h \)), \( E \) equals the slope of a \( P \) versus \( h \) plot.\(^{18,21} \) Agreement between \( E \) and diastolic LV stiffness indices derived from multiple beat analyses during caval occlusion has previously been reported in patients with dilated cardiomyopathy.\(^{22} \)

The Hill constant (\( n_{\text{Hill}} \)), a measure of the steepness of the sigmoidal force-pCa relation, was derived from a modified Hill equation fitted to the individual force-pCa data points of each myocyte: 

\[ F_{\text{total}} = F_{\text{max}} \times (\text{Ca}^{2+})^{pC_{50}} (\text{Ca}_{\text{pCa}50}^{\text{initial}} + [\text{Ca}^{2+}]^{pC_{50}}) \]

where \( F_{\text{total}} \) is force, \( F_{\text{max}} \) is maximum force, \( pC_{50} \) is the pCa at which half-maximum force is reached, \( [\text{Ca}^{2+}] \) is extracellular calcium concentration, and \( \text{Ca}_{\text{pCa}50}^{\text{initial}} \) and \( [\text{Ca}^{2+}]^{pC_{50}} \) are initial and final calcium concentrations, respectively.

Force Measurements in Single Cardiomyocytes
The average force-pCa relations obtained for 6 control and 9 DHF patients are shown in Figure 2A. \( F_{\text{total}} \) at pCa 4.5 did not significantly differ between the DHF (20.3±7.5 kN/m^2; number of myocytes, \( n=23) \) and the control (24.2±12.4 kN/m^2; \( n=15) \) groups. However, \( F_{\text{passive}} \) was significantly higher in the DHF group. The higher LVEDP and \( \sigma \) at comparable LVEDVI implied a reduced LV diastolic distensibility and the higher \( E \), increased myocardial stiffness.\(^{16} \)

Results
Hemodynamic Characteristics of the DHF Patients
The mean hemodynamic data of the DHF group are compared with that of the control group in Table 2. Heart rate, LVEF, LV end-diastolic volume index, and cardiac index in the DHF group were similar to the values measured in the control group. LV peak systolic pressure, LV end-diastolic volume index, heart rate, LVEF, and diastolic LV stiffness indices derived from multiple beat analyses during caval occlusion has previously been reported in patients with dilated cardiomyopathy.\(^{22} \)

Figure 1. A, Single cardiomyocyte, isolated from endomyocardial biopsy sample from DHF patient, mounted between force transducer and piezoelectric motor. B, Contraction-relaxation sequence recorded in single cardiomyocyte before and after PKA treatment during maximal (pCa 4.5) and submaximal (pCa 5.8) activation. *Slack test (see text).
donor hearts. No significant differences were found between the 2 groups in terms of Ca$^{2+}$ sensitivity of the contractile apparatus (pCa50) and the steepness of the force-pCa curves (ie, nHill; Table 3).

**Myocardial Tissue Properties**

DHF patients had a higher CVF than did controls (7.5±4.0% versus 3.8±2.0%, P<0.05). CVF of the DHF patients who were transplant recipients (7.5±3.0%) was similar to the CVF of the other DHF patients (7.5±3.0%). DHF patients were equally distributed over the 3 classes of CVF (Figure 3), and one third of patients therefore had low interstitial fibrosis. The higher values of LVEDP, $\sigma$, and $E$ in these patients compared with those of controls with low interstitial fibrosis indicates that CVF is not the sole contributor to diastolic LV dysfunction. No conspicuous differences in expression of myosin heavy chain, desmin, actin, TnT, tropomyosin, TnI, or MLC-1 and MLC-2 were found between DHF and control patients.
myocardium. Western immunoblot analysis did not reveal any degradation product for desmin, TnT, TnI, MLC-1, and MLC-2 in either group. Moreover, the MLC-1–MLC-2 ratio did not differ between DHF patients (0.39±0.15) and controls (0.44±0.11). Phosphorylation status of TnI was determined in endomyocardial biopsy samples retrieved from 7 DHF patients and 7 controls. The ratio of dephosphorylated to total TnI was comparable in both groups (0.58±0.17 versus 0.53±0.17). Furthermore, there was no correlation between this ratio and $F_{\text{passive}}$.

**Correlation Between In Vivo Hemodynamics and In Vitro Force**

When the DHF and control groups were combined, a monovariate linear regression analysis revealed significant correlations between the average $F_{\text{passive}}$ of all cardiomyocytes of each individual and the LVEDP, $\sigma$, or $E$ measured in the same individual at the time of cardiac catheterization and biopsy sample retrieval (Figure 4). Note the quantitative agreement between the individual values of in vivo circumferential $\sigma$ and $F_{\text{passive}}$ obtained in the isolated cardiomyocytes. These correlations were especially evident for values of $F_{\text{passive}}$ up to 5.0 kN/m² and seemed to level off at higher values. A monovariate linear regression analysis also revealed significant correlations between CVF and LVEDP ($R=0.63$, $P=0.009$) or $\sigma$ ($R=0.68$, $P=0.004$). In a bivariate linear regression analysis, the combination of $F_{\text{passive}}$ and CVF yielded stronger correlations with LVEDP ($R=0.80$, $P=0.001$) or $\sigma$ ($R=0.78$, $P=0.002$) than $F_{\text{passive}}$ and CVF alone in monovariate analysis. $F_{\text{passive}}$ and CVF were unrelated ($P=0.26$).

**PKA and Cardiomyocyte Force Development**

After PKA treatment, a second force-pCa relation could be constructed for 11 cardiomyocytes isolated from biopsy samples of 4 control patients and for 16 cardiomyocytes isolated from biopsy samples of 7 DHF patients. Figure 1 (bottom) shows representative force recordings of a single cardiomyocyte retrieved from a DHF patient before and after PKA treatment at maximal (pCa 4.5) and intermediate (pCa 5.8) activation. At pCa 4.5, $F_{\text{total}}$ remained the same, whereas at pCa 5.8, $F_{\text{total}}$ was reduced after PKA. In addition, Figure 1 illustrates the reduced $F_{\text{passive}}$ (pCa 9.0) after PKA. For control and DHF groups, $F_{\text{total}}$ at pCa 4.5 was similar before and after PKA (Table 3; Figure 2B and 2C). At intermediate pCa (eg, pCa 5.8), $F_{\text{total}}$ was reduced after PKA because of PKA-induced myofilament desensitization (Figure 2B and 2C). The latter was also evident from the reduced pCa_{50} value observed in both DHF and control groups (Table 3). After PKA treatment, $F_{\text{passive}}$ of cardiomyocytes from DHF patients dropped to values observed in the control group both at baseline and after PKA treatment (Figures 2B, 2C, and 5A). In addition, in DHF patients, the PKA-induced fall in $F_{\text{passive}}$ was larger when baseline $F_{\text{passive}}$ was higher (Figure 5B).

**Discussion**

The present study analyzed endomyocardial biopsy samples obtained from patients with DHF and yielded the following information: (1) When cardiomyocytes isolated from these samples were stretched to a sarcomere length of 2.2 μm, $F_{\text{total}}$ at maximal [Ca^{2+}] was comparable to that of control cardiomyocytes, but their $F_{\text{passive}}$ was twice as high. (2) The increase in $F_{\text{passive}}$ was reversible because administration of PKA lowered $F_{\text{passive}}$ to a level observed in control cardiomyocytes. (3) In vivo hemodynamic measures of diastolic LV function such as LVEDP, $\sigma$, and $E$ were correlated with in vitro measurements of both $F_{\text{passive}}$ and CVF.

**High $F_{\text{passive}}$**

Because the mechanical isolation procedure removed endomysial collagen structures, the high $F_{\text{passive}}$ of cardiomyocytes retrieved from DHF patients can only result from deranged diastolic stiffness of the cardiomyocytes themselves. Because cardiomyocytes were incubated in solution supplemented with 0.2% Triton X-100 before the experiments, the integrity of sarcolemmal and sarcoplasmic membranues was disrupted, and the cardiomyocytes became dependent on externally supplied calcium for active force development. Under these conditions, disturbed calcium handling because of modified expression and/or phosphorylation of sarcoplasmic reticular Ca^{2+}-ATPase, phospholamban, sarcoplasmic calcium release channel, and sodium/calcium exchanger is effectively ruled out as a cause of the observed elevation of $F_{\text{passive}}$, which therefore needs to be attributed to alterations of myofilament or cytoskeletal proteins.

The present study revealed no difference between the DHF and control groups in the expression of cardiac sarcomeric proteins such as myosin heavy chain, actin, TnT, TnI, desmin, and tropomyosin. Protein composition may also change as a result of enhanced proteolysis. This is especially evident for TnI, whose calpain-mediated breakdown is accelerated by high LVEDP. Western immunoblot analysis ruled out degradation of several contractile proteins, including TnI, in both the control and DHF groups. Therefore, it is unlikely that a change in isoform composition or protein degradation accounts for the high $F_{\text{passive}}$ of cardiomyocytes observed in the DHF group.

**TABLE 3. Measures of Cardiomyocyte Force and Ca\textsuperscript{2+} Sensitivity Before and After PKA Treatment**

<table>
<thead>
<tr>
<th></th>
<th>Before PKA</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>After PKA</th>
<th></th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>$F_{\text{total}}$</td>
<td>$F_{\text{passive}}$</td>
<td>$nHill$</td>
<td>pCa_{50}</td>
<td>$n$</td>
<td>$F_{\text{total}}$</td>
<td>$F_{\text{passive}}$</td>
<td>$nHill$</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>24.2±12.4</td>
<td>3.5±1.7</td>
<td>2.24±0.33</td>
<td>5.8±0.08</td>
<td>11</td>
<td>24.3±14.1</td>
<td>2.6±1.4</td>
<td>2.67±0.47</td>
</tr>
<tr>
<td>DHF</td>
<td>20</td>
<td>20.3±7.5</td>
<td>6.6±3.0*</td>
<td>2.43±0.53</td>
<td>5.86±0.07</td>
<td>16</td>
<td>22.2±6.8</td>
<td>3.4±1.0†</td>
<td>2.99±0.59</td>
</tr>
</tbody>
</table>

$n$ indicates number of cardiomyocytes.

* $P<0.05$, DHF vs controls.
† $P<0.05$, after vs before PKA.

**Before PKA**

**After PKA**

**pCa_{50}**

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Moreover, the correction by PKA treatment provides evidence that the high $F_{\text{passive}}$ resulted from phosphorylation of its sarcomeric target proteins: TnI, myosin binding protein-C, and/or titin. Treatment with PKA induced a large drop in $F_{\text{passive}}$ in cardiomyocytes of DHF patients, whereas neither $F_{\text{passive}}$ in control cardiomyocytes nor $F_{\text{total}}$ at pCa 4.5 in cardiomyocytes from both groups was altered (Table 3 and Figure 2). Such an isolated drop in $F_{\text{passive}}$ unaccompanied by a fall in $F_{\text{total}}$ is more easily reconciled with an action of PKA on a myofilament rather than on a cytoskeletal phosphorylation site because the parallel alignment of the cytoskeleton with the myofilaments would predict a fall in $F_{\text{passive}}$ generated by the cytoskeleton to also lower $F_{\text{total}}$.

The present study also determined the phosphorylation status of TnI but found no difference in the ratio of dephosphorylated to total TnI between control and DHF groups. It has recently been demonstrated in animal studies that phosphorylation of myosin binding protein-C and titin modifies diastolic properties. Phosphorylation or expression of a mutant isoform releases the “braking” action of myosin binding protein-C on cross-bridge cycling, thereby decreasing $F_{\text{passive}}$ in skinned mouse myocardial strips. Similarly, PKA-mediated phosphorylation of the elastic N2B spring element of titin reduces diastolic stiffness in isolated rat cardiomyocytes. Because of limited procurement of myocardial tissue by endomyocardial biopsy techniques, phosphorylation of both proteins could not be assessed in the present study. Future studies on myocardial tissue from DHF patients should focus on the phosphorylation level of both proteins to detect the sarcomeric protein responsible for the high $F_{\text{passive}}$ of cardiomyocytes isolated from DHF patients.

**Figure 4.** Correlations between $F_{\text{passive}}$ averaged for all cardiomyocytes of each individual and LVEDP, $\sigma$, and $E$ measured at time of cardiac catheterization.

**Figure 5.** A, PKA treatment reduces $F_{\text{passive}}$ of DHF patients to values observed in control group at baseline and after PKA treatment. B, Correlation between PKA-induced fall in $F_{\text{passive}}$ and baseline value of $F_{\text{passive}}$. 

![Graphs showing correlations between various parameters and LVEDP, $\sigma$, and $E$.](http://example.com/graphs.png)
In Vitro Versus In Vivo

When $F_{\text{passive}}$ values of control and DHF cardiomyocytes were pooled, in vitro measurement of $F_{\text{passive}}$ was correlated with in vivo indices of diastolic LV dysfunction, such as LVEDP, $\sigma$, and $E$ (Figure 4). The quantitative agreement between $\sigma$ and $F_{\text{passive}}$ indicates that diastolic LV dysfunction is determined to an important extent by the rise in $F_{\text{passive}}$ of the cardiomyocytes. The relations between $F_{\text{passive}}$ and indices of diastolic LV function all leveled off at higher values of LVEDP, $\sigma$, and $E$. This could have resulted from diuretic therapy to compensate the patients before catheterization or from more intense interstitial fibrosis at the top end of diastolic LV dysfunction. In the in vitro setting, all cardiomyocytes were stretched to the same sarcomere length of 2.2 $\mu$m. In the in vivo setting, LV preload was uncontrolled, and especially the DHF patients, who underwent more intense diuretic therapy in the interval between admission for pulmonary edema and diagnostic cardiac catheterization, could have been operating at LV filling pressures lower than needed to achieve optimal sarcomere length. More intense interstitial fibrosis also provides an explanation for the relations between $F_{\text{passive}}$ and indices of diastolic LV function to level off at higher values of LVEDP, $\sigma$, and $E$. Endomyocardial biopsy samples from patients with DHF had higher CVF than did controls, and in a bivariate linear regression analysis, both $F_{\text{passive}}$ and CVF were significantly correlated with LVEDP and $\sigma$. A predominant effect of interstitial fibrosis at the top end of diastolic LV dysfunction is in line with previous experimental studies, which showed diastolic muscle stiffness to originate from structures within the sarcomere for sarcomere lengths $<2.2$ $\mu$m and from perimysial fibers once filling pressures exceeded 30 mm Hg.

Degradation of collagen in pressure-overloaded, hypertrophied papillary muscles with plasmin did not reduce muscle stiffness to levels observed in normal muscles. Similarly, in the present study, patients with DHF and low CVFs still had higher LVEDP, $\sigma$, and $E$ than did controls (Figure 3). Therefore, our data support the concept that diastolic LV dysfunction in the presence of a low CVF is explained by the higher $F_{\text{passive}}$ of the cardiomyocytes. However, because half of the DHF patients suffered from diabetes mellitus, collagen cross-links formed by advanced glycation end products could also explain the impairment of diastolic LV function at low CVF.

The present study observed low CVF and high $F_{\text{passive}}$ values in some patients with DHF but failed to detect DHF patients with high CVF and low $F_{\text{passive}}$ values. This suggests diastolic LV dysfunction to result from a sequence of events, which start with a rise in cardiomyocyte $F_{\text{passive}}$ followed by the development of interstitial fibrosis. A similar sequence of events has also been reported in experimental tachypacing-induced HF models. In those models, elevation of diastolic LV muscle stiffness was paralleled by expression of the shorter and stiffer N2B isoform of titin and not by interstitial fibrosis, which only developed when an angiotensin II infusion was superimposed on the pacing stress. Similar coordination between titin isoform shift and extracellular matrix deposition has also been reported in other experimental models. The evidence provided by the present study that DHF patients can have low CVF also explains why angiotensin II receptor blockers and angiotensin-converting enzyme inhibitors, which reduce interstitial fibrosis, have not been uniformly successful in large clinical trials in DHF patients.

Study Limitations

Five of the 12 DHF patients and 6 of the 8 controls had undergone cardiac transplantation. Transplant recipients were included in the study because they frequently suffer DHF and because their myocardial biopsy material is readily available. The pathogenetic mechanisms responsible for their DHF could differ from that of other DHF patients because of ongoing rejection and use of immunosuppressant drugs. Force recordings of isolated cardiomyocytes and the extent of interstitial fibrosis of the transplanted subgroup of DHF patients were, however, similar to the measurements obtained in the other DHF patients, and both data sets were therefore merged into a single DHF group. The same also applied to the control group.

Isolation of cardiomyocytes and assessment of myocardial tissue properties was performed on a limited number of RV or LV samples and could potentially have overlooked tissue heterogeneity. The extent of tissue heterogeneity was addressed in previous studies on explanted hearts or surgically procured biopsy samples. In those studies, the variability of force measurements in cardiomyocytes isolated from different portions of the heart was always <5%. To validate the use of defrosted biopsy samples, force recordings of cardiomyocytes isolated from a biopsy sample immediately after procurement were compared with force recordings of cardiomyocytes isolated from a defrosted biopsy sample from the same patient. These force recordings yielded identical results.

Conclusions

Cardiomyocytes isolated from endomyocardial biopsy samples of DHF patients had elevated $F_{\text{passive}}$, which together with CVF, determined in vivo diastolic LV dysfunction. Administration of PKA to these cardiomyocytes normalized $F_{\text{passive}}$. Because the integrity of sarcomemal and sarcoplasmic membranes was disrupted by prior Triton incubation and because expression of sarcomeric proteins and the phosphorylation level of TnI were unaltered, the PKA-induced fall in $F_{\text{passive}}$ probably resulted from correction of a phosphorylation deficit of myosin binding protein-C or titin. This hypophosphorylated sarcomeric protein could, together with extracellular matrix modification, be a specific myocardial target for drug therapy of DHF.

Acknowledgments

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References


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