Diastolic Dysfunction

Tachycardia-Induced Diastolic Dysfunction and Resting Tone in Myocardium From Patients With a Normal Ejection Fraction

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Objectives
The purpose of this study is to evaluate tachycardia-induced relaxation abnormalities in myocardium from patients with a normal ejection fraction.

Background
Diastolic dysfunction and left ventricular (LV) hypertrophy are closely linked. Tachycardia can induce heart failure symptoms in otherwise asymptomatic patients. To study the effects of tachycardia on myocardial contractility and relaxation, we evaluated the effects of increasing pacing rates in myocardial biopsy samples obtained from patients with a normal ejection fraction.

Methods
LV biopsy samples were obtained during coronary bypass surgery. Myocardial strip preparations were electrically paced at rates from 60 to 180 beats/min. Diastolic resting tone was assessed by cross-bridge deactivation. Calcium transporting systems were functionally examined, and myofilament calcium sensitivity was studied.

Results
Incomplete relaxation developed in 7 preparations, with increased diastolic tension development at increasing pacing rates. This was absent in the remaining 7 preparations. Incomplete relaxation was found to be associated with increased LV mass and left atrial volume. Cross-bridge deactivation showed that these preparations also had a significant resting tone. Additional functional analyses suggest that incomplete relaxation is associated with disproportionately elevated cellular calcium loads due to a reduced sarcolemmal calcium extrusion reserve.

Conclusions
Tachycardia-induced incomplete relaxation was associated with increased LV mass and left atrial volumes. We also found a disproportionately increased calcium load at high rates and a substantial resting tone due to diastolic cross-bridge cycling. These observations may play a role in reduced exercise tolerance and tachycardia-induced diastolic dysfunction. (J Am Coll Cardiol 2011;58:147–54) © 2011 by the American College of Cardiology Foundation

Increased left ventricular (LV) wall thickness resulting in cardiac hypertrophy is a frequent cause of diastolic dysfunction in patients with a normal ejection fraction (1,2). Although the majority of patients are asymptomatic at rest, symptoms often develop with exertion and tachycardia. Moreover, these patients are at risk of the development of acute-onset heart failure symptoms frequently associated with tachycardia and hypertension. These clinical observations suggest an important dynamic component of diastolic dysfunction that may be different from abnormalities present at rest.

Previous studies demonstrated increased LV end-diastolic chamber stiffness as well as increased resting tension in demembranated cardiomyocytes from symptomatic patients with diastolic dysfunction (3,4). These findings have generally been ascribed to structural changes in the myocardium such as altered extracellular matrix collagen content and changes in the expression or post-translational modification of structural cardiomyocyte proteins such as titin (5).

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Abbreviations and Acronyms

- BDM = butanedione monoxime
- LV = left ventricular
- PRC = post-rest contraction
- RCC = rapid cooling contraction
- SR = sarcoplasmic reticulum

Typical for tachycardia. These abnormalities should manifest as incomplete relaxation with continued active force generation in diastole. To test this hypothesis, we studied myocardial strip preparations from patients with normal ejection fractions. After the preparations underwent a pacing protocol that encompassed rates from 60 to 180 beats/min, they were categorized into 2 groups. One group demonstrated normal relaxation behavior up to the highest rates, whereas in the other group, incomplete relaxation developed as the rate was increased. Thereafter, echocardiographic and other clinical data were disclosed to determine whether LV mass and other parameters of diastolic dysfunction are associated with incomplete relaxation. To further investigate the underlying cellular mechanisms, we also performed a series of physiological and pharmacological experiments in the same preparations. This allowed us to test for the presence of active force generation in resting preparations and assess calcium handling and myofilament calcium sensitivity.

Methods

Patient population. LV myocardial biopsy samples were obtained from 17 patients at the time of aortocoronary bypass grafting. On the basis of pre-operative echocardiography, all patients had an LV ejection fraction ≥50% and normal regional wall motion. Patients with objective evidence of myocardial ischemia in the week before aortocoronary bypass grafting were excluded. Three patients underwent aortic valve replacement for aortic stenosis in addition to aortocoronary bypass grafting. Patients with a history of myocardial infarction, diabetes mellitus, chronic renal dysfunction (creatinine >2.0 mg/dl), and any other significant valve disorders were excluded. Consent forms, tissue biopsy techniques, and experimental protocols were approved by the institutional review board. Three biopsy samples could not be sculpted into excitable preparations, leaving a total of 14 experiments.

Clinical data. Age, sex, preoperative blood pressures, and pertinent details of the medical history were tabulated. LV ejection fraction was calculated by volumetric assessment using the apical 4- and 2-chamber views. LV chamber dimensions, wall thickness, mass, and regional wall motion were assessed in accordance with published guidelines (6). Pulsed Doppler mitral inflow and lateral mitral annular tissue Doppler signals were analyzed for the following parameters of diastolic function: E-wave peak velocities and lateral E’-wave tissue velocity to calculate E/E’ (early peak mitral inflow/early peak lateral mitral annulus tissue Doppler ratio). The left atrial volume was calculated using the area-length method. The LV mass and left atrial volume were normalized to body surface area. Concentric LV hypertrophy criteria were >115 g/m² in men and >95 g/m² in women with a normal LV end-diastolic diameter (<59 mm in men, <53 mm in women). LV end-diastolic pressure at pre-operative catheterization was also tabulated.

Muscle preparation and mechanical parameters. The biopsy samples were obtained from the anterior LV wall and microdissected into strip preparations (cross-sectional area: 0.7 ± 0.3 mm²), as previously described (7,8). The preparations were then transferred to the muscle chamber containing oxygenated Tyrode solution at 37°C and attached to a force transducer and stimulating electrodes. Isometric force was evoked at a stimulation rate of 60 beats/min. Pre-load was applied until the developed tension reached a maximum. Core hypoxia was excluded by perfusion arrest (8). Force parameters were recorded using an IonOptix recording system (IonOptix, Milton, Massachusetts). A reservoir above the muscle chamber allowed precise delivery of a rapid cooling solution. Contraction and relaxation parameters were defined, as previously described (7,8). An exponential time constant, τ, was calculated from nonlinear least-squares fitting of a single exponential equation to the force data after half-maximal relaxation.

Experimental protocol. The experimental sequence for each preparation was as follows.

PACING PROTOCOL. Relaxation was evaluated after increasing the stimulation rate in 30 beats/min increments over a range from 60 to 180 beats/min to encompass normal human resting and tachycardia rates. Measurements were taken after steady-state conditions were reached. The onset rate of incomplete relaxation was defined as the stimulation rate at which diastolic tension started to increase. If diastolic tension did not increase, the preparations were assigned to the complete (normal) relaxation group. The magnitude of the increase in diastolic tension due to incomplete relaxation was defined as the percentage of increase in diastolic tension from 60 to 180 beats/min. For group statistical comparisons, the onset rate was considered to be 180 beats/min if incomplete relaxation was not observed.

POST-REST CONTRACTIONS AND RAPID COOLING CONTRACTURE. To evaluate sarcoplasmic reticulum (SR) calcium retention and release, we used a post-rest contraction (PRC) protocol. After steady-state conditions were reached at 60 beats/min, resting intervals of 5, 20, 40, 80, and 120 s were introduced. The resting periods were followed by a single applied stimulus before resuming continuous stimulation. This protocol was then repeated at 180 beats/min. The varying developed tension of the PRC is a marker of SR calcium release. The time-dependent decay of the PRCs reflects calcium leakage from the SR (9,10).

The SR calcium content was assessed by rapid cooling contractures (RCCs), a semiquantitative physiological measurement of total SR calcium content (10). A rapid switch to an
RCC solution at 1°C induces a complete SR calcium release leading to a contracture. The contracture amplitude is a measure of SR calcium content. RCCs were induced after 5 and 120 s of rest at 2 preceding pacing rates of 60 and 180 beats/min (11).

PHARMACOLOGICAL CROSS-BRIDGE INHIBITION. To estimate resting tone, the nonstimulated muscle strip was exposed to a circulating solution of Tyrode solution containing 30 mmol/l 2,3-butanedione monoxime (BDM). BDM is a potent, fully reversible inhibitor of cross-bridge cycling (7). Resting tone was thus considered to be the difference between baseline resting diastolic tension and resting diastolic tension after the addition of BDM.

SARCOLEMMAL CONTRIBUTION TO CONTRACTION AND RELAXATION. To test the contribution of sarcolemmal calcium transport, we chemically inhibited SR with 20 μmol/l cyclopiazonic acid, an inhibitor of the SR calcium ATPase, and 1 μmol/l ryanodine, an inhibitor of the SR calcium release channels. These were added to the perfusate of the strip while it was paced at 60 beats/min. The combination of both compounds has been demonstrated to abolish SR function in human myocardium (12). This was confirmed by the absence of RCCs (data not shown).

MYOFILAMENT CALCIUM SENSITIVITY. To determine the potential role of myofilament calcium sensitivity, the myofilament response to varying calcium concentration was studied, as previously described (13). The negative logarithm of the calcium concentration at half-maximal force generation (EC50) was used to compare the groups. Statistical analysis. After the initial group assignment, a repeated-measure analysis of variance followed by a Student t test without corrections for multiplicity was used to assess the increase in diastolic tension. For between- and within-group comparisons, unpaired and paired Student t tests were used. Values in the graphs in the figures are plotted as mean ± SE. Values in the text and tables are presented as mean ± SD, if not otherwise indicated. p values <0.05 were considered statistically significant. The alpha value was set at 0.05 to calculate confidence intervals.

Results

Incomplete relaxation. The experimental data were grouped on the basis of the presence or absence of incomplete relaxation, as shown in Figure 1A. This resulted in 2 groups of 7 experiments each. The developed tension ± SE at 60 beats/min was 6.0 ± 1.1 mN/mm² in the incomplete relaxation group and 4.5 ± 1.1 mN/mm² in the normal relaxation group (p = 0.2). In the incomplete relaxation group, diastolic tension increased from 5.4 ± 1.3 mN/mm² at 60 beats/min to 7.8 ± 1.8 mN/mm² at 180 beats/min (p < 0.05), whereas no significant change in diastolic tension was found in the normal relaxation group. Average diastolic tensions and peak systolic tensions are shown in Figure 1B. This figure demonstrates that incomplete relax-
Incomplete relaxation is associated with a progressive increase in diastolic tension as the rate is increased. Although we observed a directional trend toward prolonged relaxation indexes at 60 beats/min in the incomplete relaxation group, none of the indexes reached statistical significance (Table 1). This result suggests that the relaxation characteristics at low resting rates cannot predict the development of incomplete relaxation as the rate is increased.

**Clinical data correlations.** The baseline clinical data and relevant clinical correlations categorized by the principal experimental finding of incomplete relaxation at increasing rates are shown in Table 2. All patients with aortic stenosis demonstrated incomplete relaxation, whereas clinical documentation of hypertension was not different between the groups. Most importantly, the results demonstrate that the presence of incomplete relaxation is associated with a greater LV mass and left atrial volume, a marker of chronic diastolic dysfunction (14). Trends in age, E/E’, and LVEDP toward higher values were also evident in the incomplete relaxation group. The presence of incomplete relaxation predicted or excluded LV hypertrophy in all but 1 patient, a man with a mass index of 89 g/m².

**Resting tone.** Use of the reversible cross-bridge inhibitor BDM allowed us to evaluate whether unstimulated preparations have an active resting tone due to cross-bridge formation. These experiments demonstrated that active resting tone was detectable in all the strips with incomplete relaxation but none of the strips from the normal relaxation group. The average resting tone in the preparations with incomplete relaxation was 1.3 ± 0.6 mN/mm² (p < 0.01). The presence of an active resting tone in a preparation with incomplete relaxation at 180 beats/min is shown in the lower panel of Figure 2.

**Evaluation of mechanisms.** The experimental design of this study allowed us to evaluate the mechanism of incomplete relaxation and resting tone by adding a sequence of experiments in each preparation. For clarity, the results are not reported in sequence of performance.

**MYOFILAMENT CALCIUM SENSITIVITY.** As shown in Figure 3, there was no difference in calcium sensitivity between preparations with and without incomplete relaxation (EC50 values: incomplete relaxation, 6.4 ± 0.4 and normal relaxation, 6.4 ± 0.2). Thus, incomplete relaxation is not associated with increased myofilament calcium sensitivity.

**PRCs AND RCCs.** The developed tension of PRCs after varying resting intervals were used to quantify SR calcium release and retention (Fig. 4A). PRCs after short resting intervals were more pronounced in preparations that displayed incomplete relaxation (p < 0.05). The time-dependent decay in PRCs was similar in both groups. These results suggest greater SR calcium release in preparations that display incomplete relaxation at both rates.

The RCCs provide a semiquantitative assessment of SR calcium content (10). As shown in Figure 4B, there appears to be a disproportionate increase in SR calcium content at 180 beats/min, which re-equilibrates to more normal levels after a prolonged rest interval. This slow re-equilibration is likely also responsible for the delay in diastolic tension readjustment after the abrupt cessation of tachycardia, as shown in the lower panel of Figure 2. These data suggest that tachycardia dramatically increases SR calcium content in the group with incomplete relaxation. It is important to note that this increase in SR calcium did not translate into an increase in developed tension at 180 beats/min, as shown in Figure 1B.

**COMPLETE SR INHIBITION.** In preparations without functioning SR, cytosolic calcium elimination from the cytoplasm is almost exclusively dependent on the sarcolemmal sodium–calcium exchanger (10). After SR inhibition, the developed tension of the contraction (at 60 beats/min) decreased by 47 ± 24% in the normal relaxation group versus 57 ± 24% in the group that demonstrated incomplete relaxation (p = 0.2). Due to the limited number of preparations, we were not able to determine whether the observed differences in SR calcium content were statistically significant. This may indicate that incomplete relaxation was not associated with increased calcium sensitivity.

### Table 1

<table>
<thead>
<tr>
<th>Relaxation, beats/min</th>
<th>TPT, ms</th>
<th>RT50, ms</th>
<th>RT90, ms</th>
<th>Tau, ms</th>
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<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>152 ± 21</td>
<td>280 ± 34</td>
<td>416 ± 54</td>
<td>8 ± 1</td>
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<tr>
<td>180</td>
<td>113 ± 11</td>
<td>200 ± 11</td>
<td>284 ± 9</td>
<td>7 ± 1</td>
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<tr>
<td>Incomplete</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>60</td>
<td>155 ± 24</td>
<td>287 ± 29</td>
<td>454 ± 79</td>
<td>12 ± 7</td>
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<tr>
<td>180</td>
<td>114 ± 12</td>
<td>206 ± 17</td>
<td>287 ± 2</td>
<td>7 ± 1</td>
</tr>
</tbody>
</table>

No significant changes between the groups were found.

RT50 = time to half maximal relaxation; RT90 = time to 90% relaxation; Tau = time index of relaxation after RT50; TPT = time to peak tension.

### Table 2

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Incomplete Relaxation (n = 7)</th>
<th>Normal Relaxation (n = 7)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>76 ± 14</td>
<td>62 ± 15</td>
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<td>Sex</td>
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<td></td>
</tr>
<tr>
<td>Male</td>
<td>4</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>3</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Aortic stenosis</td>
<td>3</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>History of hypertension</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td>133 ± 12/74 ± 6</td>
<td>134 ± 9/75 ± 8</td>
<td>NS</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>61 ± 8</td>
<td>62 ± 5</td>
<td>NS</td>
</tr>
<tr>
<td>LV mass, g/m²</td>
<td>132 ± 47</td>
<td>78 ± 14</td>
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</tr>
<tr>
<td>LA volume, ml/m²</td>
<td>43 ± 15</td>
<td>23 ± 7</td>
<td>&lt;0.01</td>
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<tr>
<td>E/E’</td>
<td>11 ± 5</td>
<td>6 ± 1</td>
<td>0.11</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>16 ± 5</td>
<td>13 ± 3</td>
<td>0.18</td>
</tr>
</tbody>
</table>

### Comorbidities

PVD: 2 —
PVD: 2 —
AF: 1 1
VT: — 1

Values are reported as mean ± SD or n.

AF = atrial fibrillation; COPD = chronic obstructive pulmonary disease; E/E’ = early peak mitral inflow/early peak lateral mitral annulus tissue Doppler ratio; LA volume = left atrial volume index; LVEDP = left ventricular end-diastolic pressure; LVEF = left ventricular ejection fraction; LV mass = left ventricular mass index; PVD = peripheral vascular disease; VT = ventricular tachycardia.
to the general prolongation of the mechanical twitch, incomplete relaxation and the rate-dependent increase in diastolic tension was more pronounced in both groups after SR inhibition. However, the increase in diastolic tension after SR inhibition was much more pronounced in strips exhibiting incomplete relaxation, as exemplified in Figure 5. After SR inhibition, the increase in diastolic tension was 129 ± 44% compared with 36 ± 32% with an intact SR, a more than 3-fold increase (p < 0.01). In contrast, biopsy samples from patients with normal relaxation behavior showed only a 12 ± 5% increase in diastolic tension after SR inhibition, a major difference compared with the preparations that showed incomplete relaxation with an intact SR (p < 0.01). This suggests that sarcolemmal function can partially compensate for the loss of SR function in preparations with normal relaxation, whereas preparations with incomplete relaxation demonstrated a lack of sarcolemmal calcium extrusion reserve. This is very likely the primary reason for the disproportionate increase in SR calcium load at high rates.

Discussion

The present study is the first to systematically relate diastolic function in isolated, contracting myocardium from patients with a normal ejection fraction to clinical variables of diastolic function. Our data demonstrate that incomplete relaxation at rates typical for clinical tachycardia was strongly associated with an increased LV mass and an increased left atrial size. Incomplete relaxation was present in all preparations from patients with concentric left ventricular hypertrophy. With incomplete relaxation, the myocardium never completely relaxes and remains in an activated state that can be described as a partial or diastolic contracture (15). Tachycardia-induced incomplete relaxation is a physiological finding at very high rates that was first documented by Frank in 1895 (16). His pressure tracings obtained in isolated animal hearts showed incomplete relaxation with bursts of ventricular tachycardia that increased the diastolic pressure baseline.

In our samples from patients with LV hypertrophy, incomplete relaxation was observed at substantially lower rates compared with samples from patients with a normal LV mass (Fig. 6). Our experiments also indicate that myocardium from patients with incomplete relaxation has a substantial active resting tone due to cross-bridge cycling. In other words, complete relaxation can never be achieved, even with an indefinitely prolonged diastole. The finding of incomplete relaxation in addition to abnormal resting tone may play a role in the dynamic clinical presentation of patients with acute diastolic heart failure symptoms.

If our observation of incomplete relaxation is of clinical relevance, it would be manifest as a tachycardia-induced reduction in LV end-diastolic volumes. This was indeed observed by Westermann et al. (17), who measured pressure-volume loops in 70 patients after hospital admission for heart failure with a normal ejection fraction. Compared with 20 control subjects, these patients had an increased LV wall thickness, and when paced at 120
beats/min, the LV end-diastolic volume decreased by 28%. In contrast, in control subjects, they found an incremental increase in LV end-diastolic volume. Although the authors could not explain this finding, they argued that this observation may play a substantial role in the development of symptoms in these patients.

The mechanisms underlying diastolic dysfunction in patients with LV hypertrophy are unclear. There are several possible explanations, including an increased mass-to-volume ratio, increased passive stiffness due to changes in collagen and titin, altered calcium sensitivity of the myofilaments, and abnormal calcium handling. Before our study, the only pertinent data obtained in human myocardium were those of Borbély et al. (4,5) and van Heerebeek et al. (18). They studied the passive mechanical characteristics of demembranated cardiomyocytes obtained from patients with heart failure and a normal ejection fraction of various etiologies and found increased myofilament tension and stiffness. These findings were linked to changes in structural proteins such as titin.

Our results suggest a significant active resting tone in preparations that displayed incomplete relaxation, as shown by the force reduction in unstimulated preparations after cross-bridge inhibition. No active resting tone was found in the group with normal relaxation behavior. This observation may be explained by an inability to restore diastolic calcium to levels that prevent force generation or an increase in myofilament calcium sensitivity. However, calcium sensitivity was not found to be altered in these preparations. This suggests that an abnormality of calcium handling is the predominant mechanism. The single most important calcium-extruding mechanism in cardiomyocytes is the sarcolemmal sodium calcium exchanger. In concert with the SR, the exchanger restores cytoplasmic calcium to diastolic levels, thus inducing relaxation. Our data demonstrate a tachycardia-induced disproportionate increase in SR calcium content that did not translate into a stronger contraction. This observation strongly suggests that calcium is released into a high calcium environment, which manifests as diastolic contracture. These data also suggest

![Figure 4 Calcium Handling](image_url)

(A) Post-rest contractions (PRCs) and the time-dependent decay of PRCs at 2 preceding pacing rates (60 and 180 beats/min). The rest intervals were incrementally increased as indicated. The developed tension of the PRC is a measure of sarcoplasmic reticulum (SR) calcium release. These data demonstrate an increase in SR-mediated contractility in the group that demonstrates incomplete relaxation. (B) Rapid cooling contractures (RCCs) as a measure of the total SR calcium content at 60 and 180 beats/min in the normal and incomplete relaxation groups. Rapid switching to a solution at 1°C releases all calcium from the SR, which then activates the myofilaments. The group with incomplete relaxation demonstrates a disproportional increase in SR calcium content at 180 beats/min that recovers to more normal levels after a prolonged resting interval. *p < 0.05 for between-group comparisons at the respective rates.
that the SR calcium ATPase–mediated calcium sequestration was increased in these biopsy samples.

Elevated diastolic calcium levels at low rates may be beneficial and increase contractility by enhancing SR-dependent calcium cycling. This was evident in stronger PRCs, a trend toward increased developed tension, and a trend toward a more substantial loss in contractility after complete SR inhibition. However, the removal of the SR revealed a more important difference that became apparent as the preparations were challenged by tachycardia. In the group with normal relaxation at baseline, a minor increase in diastolic tension developed, suggesting that the exchanger-mediated calcium extrusion can largely compensate for the tachycardia-mediated increase in cellular calcium influx. In contrast, diastolic tension increased by more than 3-fold in the group with incomplete relaxation, suggesting a substantially reduced cellular calcium extrusion reserve. Hence, the principal defect causing incomplete relaxation appears to be at the level of the sarcolemma (i.e., insufficient sodium calcium exchanger–mediated calcium extrusion that overwhelms the compensatory calcium sequestration by the SR).

The energetic implications of tachycardia-induced diastolic contractures in addition to resting tone are likely profound. On the basis of the well-established linear relationship between force-time integral and myocardial oxygen consumption, our calculations indicate that at 180 beats/min, more energy is consumed for maintaining contracture and resting tone than for contraction and relaxation (8,19). Such a wasteful energy expense may play a substantial role in the development of ischemia, as described in canine hearts with concentric LV hypertrophy (20).

**Study limitations.** Despite its prevalence in this age group, the presence of coronary artery disease may present a confounding factor in our results. It is also important to reiterate that our patients did not carry a clinical diagnosis of heart failure.
Conclusions

Our data point to an intrinsic difference in tachycardia-induced relaxation characteristics in LV myocardium from patients with a normal ejection fraction and increased LV mass. This finding is associated with a substantial active resting tone. These observations may have a role in the pathophysiology of exercise and tachycardia-induced symptoms.

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