Titin Isoforms, Extracellular Matrix, and Global Chamber Remodeling in Experimental Dilated Cardiomyopathy
Functional Implications and Mechanistic Insight

Wissam A. Jaber, MD; Calin Maniu, MD; Judith Krysiak, MSc; Brian P. Shapiro, MD; Donna M. Meyer; Wolfgang A. Linke, PhD; Margaret M. Redfield, MD

Background—Altered titin isoforms may modify cardiac function in heart failure (HF), but the nature of isoform switches and associated functional implications are not well defined. Limited studies have reported an increased compliant isoform (N2BA) expression in human systolic HF. Titin may also modulate stretch-regulated responses such as myocardial natriuretic peptide production.

Methods and Results—We characterized titin isoform expression and extracellular matrix in all 4 cardiac chambers and the left ventricular (LV) epicardium and endocardium in normal dogs and those with HF due to tachypacing and characterized functional implications at the LV myofiber and chamber level. Recognizing the potential for uncoupling of the extracellular matrix and cardiomyocyte in tachypacing, myocardial natriuretic peptide production, a molecular marker of stretch-regulated responses, was also assessed. All chambers were dilated in HF, but the extracellular matrix was not increased. HF dogs had markedly lower N2BA in the atria and right ventricle. In failing LVs, N2BA was decreased only in the epicardium, where myofiber passive stiffness was increased. However, LV chamber mechanics were driven by the marked LV dilatation, with no increase in LV diastolic stiffness. Natriuretic peptide concentrations increased markedly in the endocardium in relation to increases in LV wall stress.

Conclusions—Tachypacing HF is characterized by decreases in compliant titin isoform expression in the atria, right ventricle, and LV epicardium. However, LV chamber mechanics are principally determined by geometric and extracellular matrix changes rather than titin-based myofiber stiffness in this model. Stretch-regulated myocardial responses (natriuretic peptide production) appeared intact, suggesting that the mechanotransduction role of titin was not impaired in HF. (Circ Heart Fail. 2008;1:192-199.)

Key Words: diastole ■ heart failure ■ mechanics ■ remodeling

Experimental tachycardia-related cardiomyopathy (TRCM) is a unique model of dilated cardiomyopathy (DCM) and heart failure (HF). TRCM is associated with marked left ventricular (LV) dilatation, systolic dysfunction, and disruption of the fibrillar collagen network.1-7 Further, reduced myocyte adhesion to constituents of the basement membrane4,8 has been reported in TRCM and may contribute to myocyte elongation, chamber remodeling, dysfunction, and altered mechanotransduction because of the decreased coupling of the myocyte sarcomeroma to the extracellular matrix (ECM).

Clinical Perspective see p 199

Titin is a giant sarcomeric protein that exists in the heart in 2 isoforms coexpressed at the level of the sarcomere: the shorter, stiffer N2B and various longer, more compliant N2BA isoforms. Differential expression of these isoforms is related to alternate gene splicing, influences passive stiffness of the sarcomere, and, along with geometric changes, hypertrophy, and the ECM, may contribute to myocardial and chamber mechanics in all cardiac chambers.9,10 Titin and titin-associated proteins may play a pivotal role in mechanotransduction in the heart by regulating the bidirectional interaction among the wall stress, the ECM, and the sarcomere.9 Indeed, defects in Z-disk–based mechanotransduction involving Z-disk–based binding partners of titin are associated with an inability to upregulate natriuretic peptide (NP) production in response to stretch in vitro.11

Mechanisms regulating differential titin isoform expression and the role of these isoform changes in influencing cardiac function in disease states are not well defined. To date, studies of titin isoform changes in chronic human
cardiomyopathy, including DCM\textsuperscript{12-14} and experimental TRCM,\textsuperscript{15,16} are few and have yielded somewhat counterintuitive findings. Further, understanding of the influence of titin isoform-mediated changes in sarcomeric stiffness on myofiber and chamber mechanics is complicated by interaction with changes in the quantity or character of the ECM, hypertrophy, and altered chamber geometry, variables that may be coregulated by factors influencing titin isoform expression.

The objectives of the current study were to characterize titin isoform expression, hypertrophy, and ECM changes in all cardiac chambers and in the LV epicardium and endocardium in normal and TRCM canine hearts to characterize the functional implications of these changes at the LV myofiber and chamber level and to examine the relationship among wall stress, titin isoform changes, and ECM remodeling. Recognizing the putative roles of titin and titin isoform changes to sense and compensate for increases in ECM-based stiffness\textsuperscript{9,12-14} and the potential for uncoupling of the ECM and cardiomyocyte in TRCM, we also determined whether increases in wall stress were associated with increases in NP production, a molecular marker of stretch-regulated responses.

Methods

Study Design

The study included 6 young normal (NL) mongrel dogs and 6 dogs with HF due to TRCM. Conscious echocardiography, followed by an open-chest hemodynamic study under anesthesia and tissue harvest, was performed. All experimental procedures were designed in accordance with National Institutes of Health guidelines and approved by the Mayo Institutional Animal Care and Use Committee. Dogs were euthanized by intravenous potassium chloride, consistent with guidelines of the Panel on Euthanasia of the American Veterinary Medical Association. The authors have full access to and take responsibility for the integrity of the data. All authors have read and agreed to the manuscript as written.

HF Model

Under general anesthesia (ketamine, 10 mg/kg; diazepam, 0.5 mg/kg; and isoflurane, 0.5 to 2.5%), a modified, programmable cardiac pacemaker and a permanent screw-in right ventricular epicardial lead were placed through a left thoracotomy as previously described.\textsuperscript{6} After a 2-week recovery period, the pacemaker was programmed to 180 bpm for 10 days, after which the pacing rate was decreased to 80 bpm as previously described.\textsuperscript{6,7}

Echocardiography

All dogs underwent 2D guided M-mode echocardiography in the conscious state and with pacing suspended briefly (HF dogs) for measurement of LV internal dimension and wall thickness in systole and diastole and left atrial (LA) area and volume as previously described.\textsuperscript{17} Venous blood was drawn for hormone analysis.

Hemodynamic Study

In HF dogs, the pacemaker was turned off immediately before the hemodynamic study. Animals were anesthetized with fentanyl (0.25 mg/kg bolus, then 0.18 mg/kg per hour) and midazolam (0.75 mg/kg bolus, then 0.59 mg/kg per hour) and ventilated with supplemental oxygen. The heart was exposed via a sternotomy and stabilized in a pericardial cradle. Animals were instrumented with a high-fidelity micromanometer catheter in the LV, short- and long-axis endocardial piezoelectric crystals, pulmonary inferior and superior vena cava occluders, and an atrial pacing wire. Animals were atrial paced at 10 to 20 bpm above sinus rate. Steady-state data were obtained. A 500-mL bolus of normal saline was then given to ensure that the end-diastolic pressure (EDP) and end-diastolic volume (EDV) data during vena cava occlusion would span a range reflecting the entire curvilinear end-diastolic pressure-volume relationship (EDPVR). Steady-state data before and after volume expansion were collected. Data during vena cava occlusion were collected immediately after the volume expansion to define the end-systolic pressure-volume relationship (ESPVR) and the EDPVR. Animals were euthanized, and the heart was harvested. The LV, LA, right ventricle (RV), and right atrium were separated and weighed, adding the ventricular and atrial septum to the LV and LA, respectively. The LV samples were bisected to provide equal endocardial and epicardial sections. All tissues were flash frozen in liquid nitrogen and stored at −80°C, whereas other sections were immediately placed in formalin and paraffin embedded for staining and histological analysis.

Hemodynamic Analysis

Digital data were acquired at 4-ms intervals and analyzed using customized software (Sonometrics, London, Canada). To characterize the EDPVR, EDP, and EDV points obtained from multiple beats during vena cava, occlusion were fit to the monoexponential equation $E_D P = e_{a e d} E_D P V$, where $a$ is the curve-fitting constant, and $b$ is the stiffness coefficient using least-squares nonlinear regression.\textsuperscript{18}

To reflect the combined effects of diastolic properties and LV remodeling and to account for the covariance between $a$ and $b$, a measure of LV capacitance was calculated, $E D V_{s y s t} / (\log[20]/a/b)$, or $L V E D V$ at a theoretical pressure of 20 mmHg, $b$ was normalized for wall volume ($V_{w}$, $b_{\text{normalized}} = b / V_{w}$) as previously described.\textsuperscript{18}

The LV midwall stress ($\sigma$, at end systole and end diastole) was estimated using the LV pressure ($P_{L V}$), LV cavity volume, and $V_{w}$ in a spherical model, and the dimensionless myocardial stiffness index ($K$) was calculated from the EDPVR data as previously described.\textsuperscript{18}

Similar methods were used to calculate end-diastolic LA midwall stress using echo-derived LA volume, LA wall volume, and LV EDP. The ESPVR was characterized as end-systolic pressure=Es pressure (ESV$-V_{0}$), where Ees is end-systolic elastance, ESV is end-systolic volume, and $V_{0}$ is the x intercept of the extrapolated ESPVR.\textsuperscript{18}

Tissue Analysis

Plasma and LV atrial and canine brain NP concentrations were measured by radio-immunoassay, as previously described, with tissue concentrations normalized to protein concentration measured by the Lowry method.\textsuperscript{19,20} Total collagen content in cardiac tissue was quantified using the hydroxyproline assay\textsuperscript{21} and indexed to tissue weight. LV and LA samples were stained with picrosirus red for measurement of collagen volume fraction using quantitative histomorphometry as previously described.\textsuperscript{21} All analyses on LV were performed in endocardial and epicardial tissues, and as the tissue samples were equally bisected, endocardial and epicardial values were averaged for overall LV data.

Titin Isoform Composition and Myofiber Stiffness

Tissue samples were processed and loaded onto a 2% sodium dodecyl sulfate-polyacrylamide gel to measure relative concentrations of the titin isoforms N2B and N2BA as previously described.\textsuperscript{13} Mean titin isoform composition was obtained by averaging densitometry data for at least 10 gel lanes per tissue type. Passive tension of isolated, Triton X-100–skinned, myofiber bundles (diameter, 200 to 300 μm; length, 2.5 to 3.0 mm) was determined as previously described.\textsuperscript{13} Sarcomere length was measured by laser diffraction microscopy.\textsuperscript{13} The means of 9 individual fiber measurements on epicardial and endocardial samples from 2 dogs in each group were averaged.

Statistical Analysis

Results are presented as mean±SD. Given the small number of dogs and the large variation, groups were compared using the Wilcoxon rank-sum nonparametric test, the probability values of which were
reported in the tables. Student t test was also performed and yielded similar statistical significance as the Wilcoxon test. Statistical significance was set at a probability value of \(P < 0.05\). The relationship between myofiber tension (normalized to fiber area) and sarcomere length was modeled as a polynomial regression as previously described\(^{13}\) and differences between groups were compared by ANOVA.

**Results**

**Cardiac Chamber Geometry and Hemodynamics**

Table 1 shows the plasma hormone and hemodynamic and geometric characteristics of NL and HF dogs. Steady-state data reflect values after volume expansion, but differences between groups before volume expansion (data not shown) were similar. Plasma concentrations of atrial and canine brain NP were increased in HF. At conscious echocardiography, HF dogs had LV and LA enlargement and reduced ejection fraction. At hemodynamic study, HF dogs had a lower ejection fraction, reduced end-systolic elastance with larger \(V_0\), LV enlargement (higher EDV), and higher LV diastolic and systolic and LA diastolic wall stress. Relaxation was more impaired, diastolic pressures were higher, and the LV EDPVR curves were shifted to the right (Figure 1), with higher EDV\(_{20}\) values, consistent with the marked chamber remodeling. In the setting of this marked LV remodeling and despite higher filling pressures, the stiffness coefficient \(\beta\) was lower, \(\beta_{\text{Normalized}}\) tended to be lower, and myocardial stiffness (\(\kappa\)) was lower in HF.

![Figure 1.](image-url) Individual end-diastolic passive pressure-volume relationships (dashed lines) of all NL (normal, blue) and HF (red) dogs defined during acute caval occlusion with the average curve (solid lines) for each group. Compared with the NL group, HF dogs had the diastolic stiffness curve shifted to the right, with a modest decrease in the slope.
Myocardial Structure
There was global cardiac hypertrophy with increased LA, right atrial, and LV masses (indexed to body weight) and a trend toward increased RV mass (Table 2). Collagen content was higher in atria than in the LV in NL and HF dogs. The collagen content in the atria and LV in HF dogs was similar to or lower than observed in NL dogs (Table 2).

In NL dogs, the relative expression of the compliant titin isoform (N2BA:N2B ratio) was greater in the atria and RV than in the LV (Table 2 and Figure 2). However, in HF dogs, these chamber differences were diminished. As compared with NL, the N2BA:N2B ratio was decreased in the HF group in the atria and RV but not in the LV.

LV Endocardium Versus Epicardium
Atrial and canine brain NP tissue concentration was or tended to be higher in the LV endocardium than epicardium in both the NL and HF groups (Table 3), and this transmural gradient was greatly increased in HF dogs. As compared with NL, NP concentrations were markedly higher in the failing endocardium but were slightly, and not significantly, higher in failing versus normal epicardium. Further, LV endocardial and epicardial brain natriuretic peptide (BNP) correlated with LV end-diastolic wall stress (Figure 3). Similar relationships also existed between tissue BNP and end-systolic wall stress ($r=0.59$ and $P=0.04$ for the endocardium; $r=0.65$ and $P=0.02$ for the epicardium).

Collagen content was similar in the endocardium and epicardium in both the NL and HF groups and was not different between groups. The N2BA:N2B ratio was similar in the epicardium and endocardium in NL dogs but was lower in the epicardium than endocardium in HF dogs. In the endocardium, the N2BA:N2B ratio was similar in HF and NL dogs. In the epicardium, the N2BA:N2B ratio was lower in HF as compared with NL dogs. Consistent with this, passive myofiber stiffness was increased in the epicardium of HF dogs as compared with the HF endocardium or NL epicardium or endocardium (Figure 4).

Discussion
Similar to human DCM, HF related to TRCM in the dog was associated with marked cardiac dilatation, LV and bialtral hypertrophy, LV systolic dysfunction, impaired LV relaxation, higher LV filling pressures, and increases in diastolic LV and LA wall stress. Unlike LV samples from humans with end-stage HF and DCM, collagen content was not increased in TRCM. Although shifts to the more compliant titin isoform (N2BA) have been described in the LV in end-stage human HF, including DCM,12-14 here we observed decreases in the relative expression of the more compliant isoform

Table 2. Cardiac Chamber Hypertrophy, ECM, and Titin Isoform Profile

<table>
<thead>
<tr>
<th></th>
<th>NL (n=6), Mean±SD</th>
<th>HF (n=6), Mean±SD</th>
<th>P (HF vs NL)</th>
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</thead>
<tbody>
<tr>
<td>Cardiac chamber hypertrophy</td>
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<tr>
<td>LA weight/body weight, g/kg</td>
<td>0.40±0.11</td>
<td>1.03±0.16</td>
<td>&lt;0.0001</td>
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<tr>
<td>RA weight/body weight, g/kg</td>
<td>0.27±0.04</td>
<td>0.72±0.13</td>
<td>&lt;0.0001</td>
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<td>RV weight/body weight, g/kg</td>
<td>1.38±0.28</td>
<td>1.54±0.08</td>
<td>0.3</td>
</tr>
<tr>
<td>LV weight/body weight, g/kg</td>
<td>4.07±0.61</td>
<td>5.22±0.61</td>
<td>0.01</td>
</tr>
<tr>
<td>Cardiac chamber collagen</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LA collagen, μg/mg tissue</td>
<td>12.9±6.3*</td>
<td>10.9±6.6*</td>
<td>0.6</td>
</tr>
<tr>
<td>RA collagen, μg/mg tissue</td>
<td>18.5±4.1*</td>
<td>12.7±2.3*</td>
<td>0.02</td>
</tr>
<tr>
<td>LV collagen, μg/mg tissue</td>
<td>5.0±1.6</td>
<td>4.9±6.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Cardiac chamber N2BA:N2B expression</td>
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<tr>
<td>LA N2BA:N2B ratio</td>
<td>1.5±0.5*</td>
<td>0.58±0.12</td>
<td>0.003</td>
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<tr>
<td>RA N2BA:N2B ratio</td>
<td>0.97±0.17*</td>
<td>0.64±0.1</td>
<td>0.01</td>
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<tr>
<td>RV N2BA:N2B ratio</td>
<td>1.30±0.4*</td>
<td>0.85±0.2</td>
<td>0.04</td>
</tr>
<tr>
<td>LV N2BA:N2B ratio</td>
<td>0.68±0.10</td>
<td>0.63±0.13</td>
<td>0.4</td>
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</tbody>
</table>

RA indicates right atrial.
*P<0.05 vs LV.

Figure 2. Representative gels and summary of data showing relative content of N2B and N2BA titin isoforms in LV endocardium (endo) and epicardium (epi), RV, LA and right atrial (RA) tissue from NL and HF dogs.
(N2BA:N2B ratio) in the LV epicardium. Further, for the first time, we document even more dramatic decreases in the N2BA:N2B ratio in the RV and atria in TRCM. Although decreased LV epicardial N2BA:N2B ratio in HF was associated with expected increases in epicardial myofiber passive stiffness, chamber mechanics and myocardial stiffness were driven by the marked remodeling (dilatation without fibrosis), with no increase in passive LV diastolic stiffness or myocardial stiffness calculated from the EDPVR data. Finally, using myocardial NP production as a molecular marker of stretch-regulated responses, we did not find evidence of impaired mechanotransduction, as endocardial NP production was increased in HF, in relation to increases in wall stress.

**Experimental TRCM**

Although different types of human and experimental DCM share many phenotypic characteristics, genetic, myocardial, and chamber characteristics in human or experimental DCM may vary according to etiology and duration.22-25 Previous studies in the TRCM model have reported LV dilatation and systolic dysfunction similar to that reported here.1-7 The particular pacing protocol used in the current study is unique in the progressive nature of the tachypacing insult, a variation that seems to result in mild hypertrophy not seen in other studies that use a constant high pacing rate.6,7

Assessment of myocardial and LV chamber passive diastolic properties in TRCM have been more variably described. Neumann et al26 reported marked increases in LV diastolic stiffness in conscious dogs with TRCM. However, several other studies in TRCM consistently describe a rightward shift without an increase (or with an actual decrease) in the steepness of the EDPVR or in myocardial or chamber diastolic stiffness parameters, findings consistent with those of the current study.3,5,27,28

In terms of ECM changes, consistent with the current findings, several studies have documented no change5,26,29 or slight decreases3,4,28 in collagen, depending on the method used to assess collagen and the sampling site. Spinale et al also reported that collagen cross-linking is decreased in TRCM and that types I and III collagen mRNA expression were unchanged.4 Importantly, although not assessed here, several groups have reported loss of the collagen weave connecting myocytes to each other,3,30 with disruption of the lateral sarcomere alignment and Z-line expansion 4,8,30 in TRCM. Further, myocytes isolated from TRCM hearts display marked reduction in attachment to constituents of the ECM basement membrane.4,8 These findings suggest that TRCM may be characterized by fundamental defects in the coupling of the ECM to the sarcomere. Although this defect

<table>
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<th>Table 3. Natriuretic Peptide Concentration, Collagen Content, and Titin Isoform Expression in the LV Epicardium Versus Endocardium</th>
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<tr>
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<tr>
<td><strong>ANP, pg/mg protein</strong></td>
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<tr>
<td>Epicardial</td>
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<tr>
<td>Endocardial</td>
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<tr>
<td><strong>cBNP, pg/mg protein</strong></td>
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<tr>
<td>Epicardial</td>
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<tr>
<td>Endocardial</td>
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<tr>
<td><strong>Collagen, μg/mg tissue</strong></td>
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<tr>
<td>Epicardial</td>
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<tr>
<td>Endocardial</td>
</tr>
<tr>
<td><strong>Collagen volume fraction, %</strong></td>
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<tr>
<td>Epicardial</td>
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<tr>
<td>Endocardial</td>
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<tr>
<td><strong>Titin N2BA:N2B ratio</strong></td>
</tr>
<tr>
<td>Epicardial</td>
</tr>
<tr>
<td>Endocardial</td>
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</tbody>
</table>

ANP indicates atrial natriuretic peptide; cBNP, canine brain natriuretic peptide.

*P<0.05 vs epicardial.
is thought to contribute to the dilatation and systolic dysfunction in the model, it could also have implications for titin-based mechanosensing and even for the stimulus for titin isoform changes. Indeed, altered mechanical feedback between the matrix and the myocyte likely affects titin isoform composition.

**Titin Isoforms in Experimental and Human DCM**

Insight into the significance of titin isoform changes in cardiac disease is complicated by developmental and possible age-related changes, variability among species, chamber and transmural heterogeneity, and marked functional alterations with titin phosphorylation status. It is also impossible to determine whether the titin isoform changes observed in this experiment are solely related to HF with increased wall stress or whether tachycardia itself is contributory. In fact, smaller species with a higher heart rate have been shown to have a much lower N2BA:N2B ratio. To date, all factors regulating alternative splicing and differential isoform expression remain unclear, but a recent study suggests that hormonal (thyroid [T3], angiotensin) as well as mechanical factors may influence isoform changes from the fetal (more N2BA) to the adult (more N2B) phenotype through PI3K/Akt-dependent signaling. In humans with advanced HF due to ischemic or nonischemic DCM, LV samples were shown to have an increased cardiac N2BA:N2B ratio, which suggests reversion to a fetal phenotype as well as increases in total collagen and increases in the ratio of type I to type II collagen. Experimental evidence suggested an inverse relationship between collagen stiffness and titin stiffness in chronic human end-stage cardiomyopathy, including DCM, as well as in myocardium of a hypothyroid rat model. In the study of Nagueh et al in human DCM, decreases in relative N2BA expression correlated with worse LV diastolic function as assessed by echocardiography.

In dogs with TRCM (2 weeks of tachypacing), Bell et al demonstrated a lower-than-normal N2BA:N2B ratio in the epicardium but unchanged titin isoform composition in midwall segments and an increased N2BA:N2B ratio in the endocardium; this, the transmural gradient tended to be more prominent in TRCM dogs. Wu et al observed a lower N2BA:N2B ratio in the midmyocardial segment in dogs with TRCM (4 weeks of tachypacing), a finding associated with increases in ex vivo, midmyocardial myofiber passive stiffness. These findings are not disparate with our own. However, Wu et al also reported that collagen-based muscle stiffness determined in KCl/KI-treated myofibers was increased and speculated that increased collagen-based and titin-based stiffness contribute to increases in LV diastolic stiffness in TRCM. However, neither LV diastolic properties nor ECM changes were measured.

We confirm the findings of Wu and Bell, as we found a decrease in the N2BA:N2B ratio in the epicardium in TRCM but extend their findings by demonstrating that this change occurred in all 4 cardiac chambers and, more significantly, in the thinner-walled chambers. More importantly, by assessing the ECM and LV diastolic properties concomitantly with titin isoform changes, we draw different conclusions regarding the functional implications of the titin isoform changes observed in these 3 studies. First, our findings and those of several other groups suggest that LV chamber and myocardial diastolic stiffness is not increased and is indeed decreased in TRCM in the setting of marked dilatation as noted earlier. Thus, as acknowledged by Wu et al, other remodeling changes may dissociate changes in sarcomeric passive diastolic stiffness from chamber and even global myocardial stiffness. Second, we and others (as noted earlier) have found no increase but, if anything, a trend toward decreases in ECM content and no evidence of changes in collagen character, which could explain increases in collagen-based stiffness in TRCM, consistent with the findings with regard to chamber and myocardial stiffness seen here and by others as noted earlier.

With regard to a potential role for uncoupling of the ECM and the sarcomere in TRCM in contributing to differences in titin-based mechanical signaling in human DCM and experimental TRCM, we did not find evidence of a loss of mechanotransduction (at least in the endocardial layers), as increases in NP production in the endocardium were intact and were related to LV diastolic wall stress. Increases in NP production by the LV myocardium occur in response to wall stress, and a transmural difference with greater NP in the endocardium has been reported in humans with end-stage HF due to ischemic or nonischemic DCM where total NP content, as well as the endocardial to epicardial gradient, decreased with unloading of the LV with a ventricular assist device. The Z-disk mechanosensor involves titin, telethonin, and muscle-specific LIM protein. Disruption of this complex in a muscle-specific MLP protein–null mouse model resulted in DCM. In neonatal cardiomyocytes cultured from muscle-specific LIM protein–null mice, loss of upregulation of BNP in response to stretch, but not pharmacological stimulation, was observed. Thus, the preservation of NP activation in the endocardium in TRCM suggests that the stress-sensing function of the cardiomyocyte is intact. Although NP production can also be upregulated because of biochemical pathways, NP production correlated with wall stress and was not increased significantly in the epicardium, suggesting that the upregulation was not primarily hormonally mediated. Although stress is expected to increase in both epicardial and endocardial layers in HF, there is probably an exaggerated increase in stress in the endocardium compared with that in the epicardium, leading to more prominent changes in endocardial versus epicardial NP (especially that the relationship between stress and NP production may be exponential rather than linear, as suggested by Figure 3). The NP increase in the epicardium may have been too small in this small sample size (n=6) to be statistically significant. In fact, there was a positive correlation between wall stress and both endocardial and epicardial log (BNP).

In the absence of unique disruption of stress sensing, the mechanism for differential changes in titin isoform expression in human DCM and experimental TRCM remains unclear, as both human HF and this model are characterized by increases in wall stress, activation of the renin-angiotensin-aldosterone system, and alterations in thyroid function consistent with a “euthyroid sick” state. As titin composition is altered via the induction of the PI3K/Akt pathway...
during heart development,31 there is a possibility that one or more of the many triggers of this pathway activate the transition toward increased N2B proportions in TRCM.

It is of note that the atria and RV showed dramatic reduction in the more compliant isoform expression. Although both ventricles are subjected to tachycardia stress, ventricular-atrial conduction may not be 1:1, and the higher ventricular filling pressures may expose the atria to a more conventional pressure overload stress. Although not assessed, atrial chamber properties may be more sensitive to increases in titin-based stiffness, and these changes may be important in the pathophysiology of HF, as all cardiac chambers contribute to the final homeostasis.

Limitations

Atrial and RV myofiber and chamber mechanics were not assessed nor was collagen-based myofiber stiffness. Study of other canines with other etiologies of HF may provide insight into regulation of titin isoform changes. Tachycardia by itself, besides HF, may contribute to the titin isoform expression changes. As RV is paced first in this model, some changes observed in the atria may also be related to retrograde activation or atrioventricular dissociation with contraction against closed valves and may not fully represent what happens in other HF models. However, increases in atrial afterload because of increased ventricular diastolic pressures or contracting against a closed valve still present a similar type of stress (pressure overload) to the atria. Moreover, as heart rate may alter myocardial stiffness, it would have been interesting to define myocardial properties during tachycardia, which was not done here. Studying diastolic properties during extreme tachycardia would be challenging given the very short duration of diastole and the effect of incomplete relaxation.

Conclusions

The current study demonstrates that in contrast to human HF, dogs with TRCM develop decreases in the relative expression of the N2BA titin isoform in all cardiac chambers, that LV mechanics in this model are more driven by geometric and ECM changes than by changes in titin-based myofiber stiffness, and that the difference in human DCM and canine TRCM does not appear driven by disruption of stretch-regulated responses. Further understanding of the role of titin and titin-associated proteins in regulating global cardiac structure and function will require more studies in human tissue and in alternative animal models that mimic the human condition. Nonetheless, the unique findings in TRCM may provide an opportunity to further dissect the factors that regulate titin isoform changes and their impact on cardiac function in disease.

Disclosures

None.

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References


**CLINICAL PERSPECTIVE**

In this study, we characterized titin isoform expression and extracellular matrix in all 4 cardiac chambers and the left ventricular (LV) epicardium and endocardium in normal dogs and those with heart failure due to tachypacing, in addition to the functional implications at the LV myofiber and chamber level. We found that all chambers were dilated in heart failure, but the extracellular matrix was not increased. Dogs with heart failure had a markedly lower ratio of the compliant titin isoform in the atria and right ventricle. In failing LVs, the compliant titin isoform was decreased only in the epicardium, where myofiber passive stiffness was increased. However, LV chamber mechanics were driven by the marked LV dilatation, with no increase in LV diastolic stiffness. Tissue natriuretic peptide levels increased dramatically in the endocardium in relation to increases in LV wall stress, suggesting that the mechanotransduction role of titin was not impaired in heart failure. Further understanding of the role of titin and titin-associated proteins in regulating global cardiac structure and function will require more studies in human tissue and in alternative animal models that mimic the human condition. Nonetheless, the unique findings in tachypacing canine cardiomyopathy may provide an opportunity to further dissect the factors that regulate titin-isoform changes and their impact on cardiac function in disease.
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