Progressive troponin I loss impairs cardiac relaxation and causes heart failure in mice

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Liu J, Du J, Zhang C, Walker JW, Huang XP. Progressive troponin I loss impairs cardiac relaxation and causes heart failure in mice. Am J Physiol Heart Circ Physiol 293: H1273–H1281, 2007. First published May 25, 2007; doi:10.1152/ajpheart.01379.2006.—Cardiac troponin I (TnI) knockout mice exhibit a phenotype of sudden death at 17–18 days after birth due to a progressive loss of TnI. The objective of this study was to gain insight into the physiological consequences of TnI depletion and the cause of death in these mice. Cardiac function was monitored serially between 12 and 17 days of age by using high-resolution ultrasonic imaging and Doppler echocardiography. Two-dimensional B-mode and anatomical M-mode imaging and Doppler echocardiography were performed using a high-frequency (~20–45 MHz) ultrasound imaging system on homozygous cardiac TnI mutant mice (cTnI−/−) and wild-type littermates. On day 12, cTnI−/− mice were indistinguishable from wild-type mice in terms of heart rate, atrial and LV (LV) chamber dimensions, LV posterior wall thickness, and body weight. By days 16 through 17, wild-type mice showed up to a 40% increase in chamber dimensions due to normal growth, whereas cTnI−/− mice showed increases in atrial dimensions of up to 97% but decreases in ventricular dimensions of up to 70%. Mitral Doppler analysis revealed prolonged isovolumic relaxation time and pronounced inversion of the mitral E/A ratio (early ventricular filling wave-to-late atrial contraction filling wave) only in cTnI−/− mice indicative of impaired LV relaxation. cTnI−/− mouse hearts showed clear signs of failure on day 17, characterized by >50% declines in cardiac output, ejection fraction, and fractional shortening. B-mode echocardiography showed a profoundly narrowed tube-like LV and enlarged atria at this time. Our data are consistent with TnI deficiency causing impaired LV relaxation, which leads to diastolic heart failure in this model.

THE CONTRACTILE SARCOMERIC PROTEINS consist of a highly ordered arrangement of myosin thick filaments, actin thin filaments, and associated proteins, such as the troponin-tropomyosin complex. Contractile sarcomeric protein mutations, truncations, and deletions have been identified for various cardiac disorders in humans and experimental animals (8, 16, 18, 23, 25, 28). Troponin, a contractile protein of the thin filament of striated muscle, consists of three subunits: troponin C (TnC), troponin T (TnT), and troponin I (TnI). TnI is the inhibitory subunit that can bind to actin-tropomyosin and prevent muscle contraction by inhibition of actin-activated myosin (actomyosin) ATPase activity (33). TnI has received considerable attention as a serum biomarker of myocardial tissue damages (2, 31) and as a key phosphoprotein that regulates cardiac contractile function (13, 40). Its role in mediating cardiac dysfunction in humans and experimental animals is controversial and continues to be actively investigated. Linkage studies and animal experiments have confirmed that point mutations in cTnI are linked to hypertrophic cardiomyopathy (1, 11, 14, 27) or restrictive cardiomyopathy (4, 20). TnI degradation has been reported in myocardial cells after ischemia and cardiac stunning (7, 15, 21, 39). TnI can dramatically decrease in postinfarction left ventricular (LV) remodeled myocardium remote from the infarct zone (24), and the content of TnI in LV myocardium may significantly decrease in older men with or without cardiac disease (37). Other studies have failed to confirm widespread TnI loss in diseased myocardium (36, 38). Presently, the overall prevalence of loss and/or truncation of cTnI in ischemia-reperfusion injury, stunning, hypertrophy, and failure in human populations is far from clear (30). Nevertheless, the growing number of cardiac disorders associated with changes in TnI led us to develop an experimental system to better understand TnI function in vivo (10).

In the present study, we exploited a predictable time-dependent loss of TnI expression in hearts of cardiac TnI knockout mice (10) to examine mechanisms underlying cardiac dysfunction associated with myocardial TnI depletion. This germ-line cardiac TnI knockout mouse (cTnI−/−) lacks cardiac TnI in the heart, but pups are born viable and healthy due to the presence of a fetal isoform of TnI (identical to ssTnI), which compensates for the absence of cTnI. However, this compensatory effect is temporary such that when ssTnI gene expression eventually switches off approximately 2 wk after birth (9), all cTnI−/− mice die on day 17 or 18 after birth (10). A systematic analysis of mRNA and protein levels revealed a progressive drop in TnI mRNA ~5 days before a drop in TnI protein (9). The remarkable predictability of these gene and protein expression changes was further demonstrated by manipulating the thyroid status in cTnI−/− mice. Hyperthyroidism accelerated by 3–4 days, whereas hypothyroidism delayed the decline in mRNA and protein by 3–4 days; in all cases mortality correlated with ~50% depletion of TnI protein (9). Data from isolated cell physiology experiments showed that as TnI loss proceeded, myofibrillar resting tension became substantially elevated and sarcomere spacing was reduced under relaxing conditions (10). Here we used high-resolution ultrasonic imaging and Doppler echocardiography to monitor in vivo cardiac function noninvasively and serially over the 6-day period immediately preceding death. The data clearly demonstrate that TnI deficiency in...
the heart impairs LV relaxation resulting ultimately in decreased cardiac output (CO) and heart failure.

METHODS

This investigation conforms to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, Revised 1996) and was in accordance with the institutional guidelines for animal care and use approved by Florida Atlantic University Institutional Animal Care and Use Committee.

cTnI−/− mice. The heterozygous cTnI mutant mice were maintained in our colony at Florida Atlantic University at Boca Raton, FL. By crosses of heterozygous mice, we obtained three genotypes of

Fig. 1. Two-dimensional B-mode imaging from wild-type and homozygous cardiac troponin I (TnI) mutation (cTnI) knockout mice. Ultrasound imaging obtained from left parasternal long-axis view on wild-type (A, C, E, G) or cTnI knockout (B, D, F, H) mice on days 12, 14, 16, and 17. The B-mode echocardiography images at the end-diastolic stage show the structure from the left ventricle (LV) to the ascending aorta (AA), innominate artery (IA), and the right ventricular (RV) outflow tract (RVOT). Note the restricted LV and enlarged left atrium (LA) in cTnI knockout on day 16 and day 17.
offspring: wild-type cTnI+/+, heterozygous cTnI+/−, and homozygous cTnI−/−. Genotyping was determined by PCR as previously reported (9). Briefly, genomic DNA was isolated from tail biopsies using the Puregene DNA isolation kit (Genta Systems, Minneapolis, MN). The specific primers used in the experiments were the following: sense primer 5′-TAGGTGTGAGGACAGAAGGCCG and antisense 5′-CCGTGAAGAGAAATCAGTGTTGCTCC were designed to produce a 630-bp fragment for the wild type; sense primer 5′-TAGGTGTGAGGACAGAAGGCCG and antisense 5′-GTGGAGATGTGCGAGGCCA were designed to produce a 390-bp fragment for the targeted alleles. Amplified DNA fragments were separated on a 1.5% agarose gel and visualized under UV light after being stained with ethidium bromide.

In vivo transthoracic cardiac imaging with echocardiography. A Vevo 770 High-Resolution In Vivo Imaging System (VisualSonics, Toronto, ON, Canada) was used to perform echocardiographic studies. It has a RMVTM 707B “high frame” scan head designed for high frame rate and real-time small animal imaging applications with a center frequency of 30 MHz and a frequency band 15–45 MHz. All measurements were performed according to the standards established in human echocardiography (5, 22, 26). To decrease experimental bias, all of the echoelectrocardiogram measurements were performed by an examiner blinded to the genotype. For M-mode and mitral Doppler measurements, the imaging for each tested animal was recorded for at least 5 s (30–40 cardiac cycles) from which 3–5 representative cycles with highest quality imaging were selected to measure the dimension and attitude. Experimental mice were anesthetized with isoflurane at a concentration of 5% and then maintained at 1.5% isoflurane by a facemask during the whole procedure. Mouse body temperature was monitored with a rectal thermometer.
thermometer. Hair on the precordial region was cleanly removed with a Nair lotion hair remover (Church & Dwight Canada, Mississauga, ON, Canada), and the region was covered with prewarmed ultrasound transmission gel (Aquasonic, Parker Laboratory, Fairfield, NJ). The long-axis imaging was taken to mainly visualize left ventricle (LV), right ventricle (RV), ascending aorta (AA), and right ventricular outflow tract (RVOT) by placing the ultrasound scan head on the left parasternal position. The short-axis imaging was taken to view the LV and RV movement during diastole and systole stages by placing the scan head horizontally on the heart area. Four-chamber imaging was taken to view all chambers (LA, LV, RA, and RV) simultaneously by placing the scan head on the apical area. Anatomical M-mode (AM-Mode) provides the ability to obtain anatomically correct LV measurements. This is achieved in AM-Mode by generating the M-mode spectrum from the acquired B-mode imaging. The whole examination for each mouse took roughly 30 min. All data and images were saved and analyzed by a Advanced Cardiovascular Package Software with an automated analysis for semiautomated analysis and quantification of cardiac function (VS-11560, VisualSonics, Toronto, ON, Canada). cTnI−/− mice and wild-type littermates were measured on days 12, 14, 16, and 17 after birth. Data analysis was performed offline with the use of a customized version of Vevo 770 Analytic Software.

Doppler echocardiography analysis in mice. Animal treatment was the same as previously described. Echocardiographic images were acquired with the use of a high-resolution (40 MHz) transducer with a digital ultrasonic system. Pulse Doppler images were collected with the apical four-chamber view to record the mitral Doppler flow spectra. The Doppler sample volume was placed at the center of the orifice and at the tip level of the valves for the highest velocities. However, for the measurement of the LV systolic and diastolic time intervals, the Doppler sample volume was moved slightly toward the LV outflow tract to intersect with both the mitral inflow and the LV outflow in the same recording. cTnI−/− mice and wild-type littermates were measured on days 12, 14, 15, 16, and 17 after birth. Data analysis was performed offline with the use of a customized version of Vevo 770 Analytic Software.

Western blot assays. After echocardiographic examination, the Tnl concentration in the heart was further confirmed with Western blot analysis. The Western blot assays were carried out as previously described (12). Briefly, myofibril proteins were extracted from mouse hearts with known genotypes. Equal amount of cardiac myofibril proteins (20–30 μg) was loaded and separated on 12% SDS gels before being transferred onto the nitrocellulose membranes. Protein loads were standardized by protein concentration measurement before electrophoresis and by quantitative densitometry of Coomassie blue-stained gels.

Blots were then stained by 0.1% Ponceau S solution to visualize protein bands and confirm both consistent protein loading among wells and complete transfer of proteins to blots. An anti-Tnl monoclonal antibody (clone 6F9, Advanced ImmunoChemical, Long Beach, CA), which recognizes both mouse cTnl and ssTnl, was used at a dilution of 1:10,000. Purified cTnl and ssTnl proteins were used as standard markers. Bound antibody on immunoblots was visualized by enhanced chemiluminescence (ECL), and relative protein quantities were determined by densitometry.

Statistics. All results are presented as means ± SE. ANOVA and Student’s t-test were used to determine statistical significance. Statistical significance was set at P < 0.05.

RESULTS

Short-axis view and AM-Mode were used for analysis of LV function during the working cardiac cycle. AM-Mode allows for anatomically correct LV measurements. Using high-resolution short-axis imaging and AM-Mode analysis, we evaluated LV size and function during systole and diastole. Figure 1 shows the B-mode images obtained from long-axis measurements in wild-type and cTnl knockout mice on days 12, 14, 16, and 17 after birth. It clearly shows that the LV end-diastolic dimension (LVEDD) was similar in wild-type and cTnl knockout mice on days 12 and 14. However, on day 16 the LV dimension in cTnl knockout mouse was significantly decreased and became a narrowed tube-like shape in 17-day-old cTnl knockout hearts (Fig. 1, F and H). Figure 2 shows the M-mode images obtained from short-axis measurements in wild-type and cTnl knockout mice on days 12, 14, 16, and 17 after birth. Since mice and their hearts are probably growing rapidly over this time frame, we evaluated several parameters in wild-type mice to assess growth. The body weight increase was <20%, similar to the increase of LVEDD. LV end-systolic dimension (LVEDS) increased by ~30%, whereas right and left atrial end-diastolic dimension (EDD) increased by ~40%. Thus increases in cardiac dimensions by up to 40% could be accounted for by normal growth of wild-type hearts over the examined 12- to 17-day window.

Table 1. Ultrasonic imaging data from cTnl knockout mice and WT littermates

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Day 12</th>
<th>Day 14</th>
<th>Day 16</th>
<th>Day 17</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT (±SD)</td>
<td>WT (±SD)</td>
<td>WT (±SD)</td>
<td>WT (±SD)</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>9.17±0.3</td>
<td>9.10±0.1</td>
<td>9.93±0.5</td>
<td>9.47±0.4</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>324±21</td>
<td>342±18</td>
<td>365±18</td>
<td>375±15</td>
</tr>
<tr>
<td>RA EDD, mm</td>
<td>1.48±0.03</td>
<td>1.54±0.03</td>
<td>1.68±0.01</td>
<td>1.90±0.02</td>
</tr>
<tr>
<td>LA EDD, mm</td>
<td>0.87±0.04</td>
<td>0.98±0.02</td>
<td>1.04±0.03</td>
<td>1.18±0.02</td>
</tr>
<tr>
<td>LV (end diastole)</td>
<td>2.16±0.18</td>
<td>2.23±0.10</td>
<td>2.40±0.05</td>
<td>2.39±0.10</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>0.58±0.10</td>
<td>0.50±0.02</td>
<td>0.52±0.01</td>
<td>0.59±0.03</td>
</tr>
<tr>
<td>LV (end systole)</td>
<td>1.32±0.10</td>
<td>1.45±0.10</td>
<td>1.41±0.20</td>
<td>1.74±0.06</td>
</tr>
<tr>
<td>LVEDS, mm</td>
<td>0.71±0.10</td>
<td>0.65±0.09</td>
<td>0.77±0.09</td>
<td>0.71±0.10</td>
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<tr>
<td>LV FS, %</td>
<td>40.3±5.10</td>
<td>35.0±10.0</td>
<td>40.7±4.10</td>
<td>35.5±3.0</td>
</tr>
<tr>
<td>LV EF, %</td>
<td>73.7±5.60</td>
<td>66.9±10.1</td>
<td>70.0±2.50</td>
<td>58.9±3.50</td>
</tr>
<tr>
<td>CO, ml/min</td>
<td>4.64±0.08</td>
<td>3.62±0.02</td>
<td>3.95±0.10</td>
<td>3.84±0.60</td>
</tr>
</tbody>
</table>

Values are means ± SD from 5 wild-type (WT) and 5 homozygous cardiac troponin I (cTnl−/−) mice at each time point from days 12 to 17 of age. RA, right atrium; EDD, end-diastolic dimension; LA, left atrium; LV, left ventricle; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; LV FS, fractional shortening of LV; LV EF, ejection fraction; CO, cardiac output. *P < 0.05; †P < 0.01 represent significant differences between the measurements in cTnl−/− mice compared with that of WT littermates at the same age.
The dynamic cardiac dimensions of LVEDD, LVESD, and atrial EDD were similar in cTnI$^{-/-}$ and wild-type mice at day 12. However, two cardiac dimensions, LVEDD and atrial EDD, changed quite differently in cTnI$^{-/-}$ compared with wild-type mice by day 16. There was a statistically significant 86% increase in left atrial EDD of cTnI$^{-/-}$ mice compared with the same mice on day 12, which was also statistically different from a 40% increase in wild-type littermates on day 16. By day 17, these changes were exaggerated such that cTnI$^{-/-}$ mice showed increases of both left and right atrial EDD by up to 97%, which were highly significant compared with the same mice at day 12 or to wild-type littermates at day 16.

Fig. 3. Four-chamber B-mode imaging from wild-type and cTnI knockout mice. Typical ultrasound imaging from the apical 4-chamber view at the end-diastolic stage was obtained in 17-day-old wild-type (A) or cTnI knockout (B) mice. RA, right atrium. Note the significant decrease of LV accompanied by the dramatic enlargement of both LA and RA in 17-day-old cTnI knockout mice.

Fig. 4. Doppler echoelectrocardiogram imaging from wild-type and cTnI knockout mice. Typical Doppler flow spectra obtained from the mitral orifice in wild-type and cTnI knockout mice on day 12 to day 17 after birth. Mitral Doppler spectra in all wild-type mice show a higher early ventricular filling wave (E wave) and a lower late filling wave caused by atrial contraction (A wave). IVCT, isovolumic contraction time; ET, ejection time; IVRT, isovolumic relaxation time; DT, deceleration time. All these parameters were measured and averaged from 3 to 5 cardiac cycles from each experimental animal and 5 mice for each group. Note the relative changes of E and A wave amplitudes in cTnI knockout mice from day 14 to day 17 after birth.
17 (Fig. 2 and Table 1). LVEDD in cTnI\textsuperscript{−/−} mice was significantly decreased on day 16 compared with wild-type littermates at the same age. By day 17, LVEDD in a four-chamber view of the cTnI\textsuperscript{−/−} heart showed dramatically restricted ventricles and enlarged atria (Fig. 3). LVEDD, LVESD, and LV percent fractional shortening (%FS), and LV ejection fraction decreased dramatically in cTnI\textsuperscript{−/−} mice at day 17 (Table 1). Finally, also on day 17, i.e., less than 1 day before death, the CO in cTnI\textsuperscript{−/−} mice was significantly reduced to <20% of normal (Table 1). All of these indicators are consistent with overt heart failure on day 17 in cTnI\textsuperscript{−/−} mice.

These functional changes were not significant in cTnI\textsuperscript{−/−} mice at age 12 or 14 days. On day 14, cardiac contraction measured as the annular motion toward the apex in systole was similar in wild-type and cTnI knockout mice, indicating that cardiac contraction was not impacted at that time (Fig. 2). The CO in cTnI\textsuperscript{−/−} mice on day 14 did not show any significant decrease compared with the same animal at day 12 or to wild-type littermates at the same age (Table 1). Significant changes observed in cTnI knockout mice at the age of 16 days, which became more pronounced at 17 days, included a restricted left ventricle, enlarged atria, and reduced LVEDD.

We further evaluated systolic and diastolic function of the left ventricle using Doppler echocardiography. Figure 4 shows the Doppler flow spectra obtained from the mitral orifice in wild-type and cTnI knockout mice on days 12–17 after birth. Figure 5 summarizes the Doppler measurements in cTnI\textsuperscript{−/−} mice compared with wild-type littermates at the same age.

Mitrail Doppler spectra in wild-type mice showed a higher early ventricular filling wave (E wave) and a lower late filling wave caused by atrial contraction (A wave). Thus the ratio of E wave to A wave amplitudes was greater than 1.1–1.5 in all wild-type mice (Fig. 5F). These E and A wave values and their ratio did not change over the 12–17-day window in wild-type mice (Fig. 5). However, in cTnI\textsuperscript{−/−} mice, the E and A wave velocities gradually declined between 12 and 17 days of age, indicating an increase in ventricular stiffness as TnI was depleted (Figs. 4 and 5). Moreover, the E/A ratio gradually reversed in cTnI knockout mice and was <1.0 on days 16 through 17 (Figs. 4 and 5), consistent with impaired LV relaxation. The reduced E/A ratio indicated that the early (passive) ventricular filling phase was more impacted, whereas the later filling phase assisted by atrial contraction was less impacted in cTnI\textsuperscript{−/−} mice. Moreover, the deceleration time of early mitral flow was prolonged in cTnI\textsuperscript{−/−} mice on day 17, indicating impaired LV relaxation and diastolic dysfunction (Fig. 5C).

Measurements of isovolumic contraction time (IVCT) and isovolumic relaxation time (IVRT) were also performed. IVCT, defined as the interval between mitral valve closure and aortic valve opening, can provide insight into the dynamics of LV contraction. In contrast, IVRT, defined as the interval between aortic valve closure and mitral valve opening, can provide information about LV filling and LV relaxation. The data reveal that LV IVCT was not changed in either wild-type or cTnI\textsuperscript{−/−} mice from day 12 to day 17 (Fig. 5A). However,
LV IVRT was greater in cTnI⁻/⁻ mice compared with wild-type littermates at the same age (Fig. 5B). An increase in IVRT without a corresponding change in IVCT is most consistent with impaired LV relaxation associated with TnI loss. Interestingly, IVRT was also prolonged at day 14 in TnI⁻/⁻ mice, making it among the first of the parameters to become abnormal. IVRT may be one of the more sensitive Doppler indexes to detect impaired relaxation (5, 6). All of the data from B-mode echocardiography and Doppler analysis demonstrate that impaired LV relaxation is the primary and earliest characteristic defect in TnI-deficient mice. In addition, no significant abnormalities in ECG, such as arrhythmia or atrial fibrillation, were detected in cTnI⁻/⁻ mice even at end-stage failure (Fig. 4).

In each mouse subjected to echocardiography, TnI protein levels were determined by Western blot analysis after completion of the final functional measurements at day 17. This strategy confirmed a number of unique properties of the mouse model including the extent of TnI depletion in 17-day-old cTnI⁻/⁻ mice and the remarkable reproducibility of these changes; TnI Western blot analysis also served as a double check on genotyping of experimental animals initially determined by PCR. As shown in Fig. 6, 17-day-old wild-type mice expressed only cardiac TnI without any detectable fetal TnI isoform, ssTnI, whereas cTnI knockout mice expressed only ssTnI. The ssTnI level in 17-day-old cTnI knockout mice declined to about 40% of the original ssTnI level compared with 12-day-old cTnI knockout mice (Fig. 6B). The range of values for all 17-day-old cTnI⁻/⁻ mice used in this study was 30–42%. These levels of ssTnI expression at days 12 and 17 in cTnI⁻/⁻ mice recapitulate what has been measured in previous studies (9, 10) and reinforce the predictable and reproducible nature of TnI depletion in this model.

DISCUSSION

We have generated a mouse model of myocardial TnI deficiency by using gene targeting in murine embryonic stem cells. A salient phenotype of the cTnI homozygous mutants (cTnI⁻/⁻) is highly predictable mortality on days 17 through 18 after birth (10). In the present study, we quantitatively evaluated cardiac function, in particular LV function, in cTnI⁻/⁻ mice from day 12 to day 17 after birth using a high-resolution ultrasonic imaging system and Doppler echocardiography. Serial noninvasive monitoring of the heart by echocardiography (3) was used to obtain a more complete phenotypic assessment and better understanding of the functional impact of myocardial TnI depletion in vivo. The Vevo 770 High-Resolution Imaging System features a RMVTM 707B Scan head designed for high frame rate, real-time small animal imaging applications with a center frequency of 30 MHz and a frequency band 15 to 45 MHz. With this instrument, it was possible to measure cardiac dimensions and function even in neonatal mouse hearts (32, 41, 42).

Echocardiography including Doppler imaging of cTnI⁻/⁻ mice permitted a detailed evaluation of the nature of morphological and functional changes that preceded death. The observation interval began at 12 days after birth, at which time few detectable differences were observed between cTnI⁻/⁻ and wild-type littermates in the functional or morphological parameter measured. Importantly, even LV IVRT was not significantly different in 12-day-old cTnI⁻/⁻ and wild-type littermates despite expressing different TnI isoforms. This observation is consistent with studies of adult mice overexpressing ssTnI in the heart, which also showed few relaxation abnormalities by echocardiography (35).

As littermates aged in parallel, several differences developed. The earliest change in cTnI⁻/⁻ mice not observed in wild-type mice was a prolongation of IVRT at day 14. IVRT is a measure of the duration of isovolumetric relaxation (while both cardiac valves are closed), and this change suggests that load-dependent relaxation may be particularly sensitive to TnI depletion. Virtually all other measures of cardiac function were indistinguishable in cTnI⁻/⁻ and wild-type littermates at day 14 after birth, including atrial EDD, LVEDD, LVEDD, IVCT, %FS, E and A wave velocity, annular motion in systole, CO, and cardiac mass.

By 16 days after birth, structural and functional differences between cTnI⁻/⁻ and wild-type hearts became widespread. In general, these differences reflected diastolic rather than systolic dysfunction in cTnI⁻/⁻ mice. Atrial EDD increased by 86% compared with day 12 (approximately double the increase observed with normal growth). Ventricular EDD (LVEDD) tended to decline compared with day 12 (instead of the ex-
expected 30% increase due to normal growth). The ratio of E to A wave velocities declined, and IVRT was further prolonged. By day 17, the deceleration time of early mitral flow became prolonged consistent with diastolic dysfunction, and the other defects in LV relaxation were more pronounced. The reduced E/A ratio in cTnI−/− mice indicated that the early (passive) ventricular filling phase was more impacted than the later filling phase, which was assisted by atrial contraction. A four-chamber view of the 17-day-old cTnI−/− heart revealed dramatically restricted ventricles and enlarged atri. By day 17, cTnI−/− mice were also experiencing rather profound overt heart failure as indicated by large decreases in LV %FS, %EF, and CO.

IVRT was remarkably elevated as Tn levels declined and was among the first indexes of cardiac function to become abnormal. This stands in contrast to LV IVCT, which showed no significant differences in cTnI−/− or wild-type mice over the entire 12- to 17-day age range. The data demonstrate that impaired relaxation was the earliest primary defect in cTnI-deficient mice detectable by Doppler echocardiography. This is consistent with studies in isolated myocyte force measurements, which revealed a higher resting tension in TnI-deficient myofibrils (10). Increased resting tension would translate directly to an increase in LV stiffness, which would create resistance to LV expansion during ventricular filling in diastole. This was observed here by echocardiography as a decrease in LV end-diastolic volume and enlargement of atri resulting from working against substantial resistance to achieve ventricular filling. Eventually, difficulties with ventricular filling progress and become apparent as decreased LVEDD and ventricular filling. Eventually, difficulties with ventricular filling are likely to be the underlying cause of heart failure and death.

Our present echocardiography data indicate that no significant changes of cardiac wall thickness are observed in cTnI−/− mice even at the end stages. Most cTnI−/− mice died during the night of day 17 or during the early morning of day 18. Although we cannot exclude the possibility of arrhythmia or atrial fibrillation happening immediately before death, the profoundly reduced CO due to the narrowed tube-like LV is likely to be the underlying cause of heart failure and death.

References


