Principal strain changes precede ventricular wall thinning during transition to heart failure in a mouse model of dilated cardiomyopathy

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DILATED CARDIOMYOPATHY (DCM) is characterized by anatomical changes of the heart, including myocardial wall thinning (7). Functional abnormalities include impaired contraction with reduced stroke volume and ejection fraction (EF). This study focuses on the potential differences in principal strain between normal and thinned left ventricular (LV) walls. Few studies have explored the relationship between LV wall thickness and principal E1 and E2 strains that are important indexes of radial wall thickening and wall circumferential shortening. The few published myocardial strain measurements by MRI tagging in humans with DCM show consistent patterns of regional heterogeneity of myocardial strain (34) and severe reduction in fiber shortening (19). Variation of end-systolic wall stress in normal subjects and patients with DCM was also found using Cine MRI (10). In videofluoroscopy studies of motion of radiopaque markers implanted in LV of sheep, DCM was associated with circularization of the normally oval LV epicardium and with decreased magnitude of principal strains (27). Thus our aim was to monitor temporal changes in wall thickness in the protein kinase C-ε (PKC-ε) transgenic (TG) mouse for comparison of strain to that of normal nontransgenic (NTG) hearts.

We hypothesize that the progressive ventricular thinning over the course of the development of DCM affects principal strains compared with the normal heart and that this will provide insight into the hierarchical nature of myocardial (mal)adaptation. The hypothesis was tested with high-resolution cardiac tagged MRI by examining changes in LV wall thickness and two-dimensional (2D) principal E1 and E2 strains in the midventricular wall of the PKC-ε overexpressing mouse during progression of cardiac dilation.

METHODS

Animal preparation. Six male PKC-ε overexpressing TG mice (FVB/N strain, mean weight = 31.6 ± 2.0 g) and five age-matched NTG control mice (mean weight = 31.7 ± 1.8 g) were studied at 1-mo intervals, from 6 to 13 mo of age. The 6-mo start time was selected to correspond to the time of known onset of previously reported changes in myofilament biochemistry (11). A detailed description of the creation of the PKC-ε TG mice has been previously published (11). Animals were housed under controlled temperature, humidity, and light, with chow and water available ad libitum. All experimental protocols were approved by the Animal Care Policies and Procedures Committee at the University of Illinois at Chicago (IACUC accredited). The investigation conforms with the Guide for the Use of Experimental Animals. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
For imaging, anesthesia was induced in mice with 5% isoflurane and 100% medical grade O₂ in a closed induction chamber (E-Z System, Palmer, PA). Thereafter, anesthesia was maintained with 1.0–1.2% isoflurane in 2 l/min flow of 100% O₂ administered via vaporizer (SurgiVet, Waukesha, WI) and nose cone. Body temperature was measured rectally and maintained at 37 ± 0.5°C by continuous flow of warm water through surrounding Tygon tubing. Subcutaneous ECG electrodes were connected to a physiological monitoring system (SA Instruments, Stony Brook, NY) to provide cardiac triggering. Triggering pulses were blanked during inspiration to limit motion (4). Accidental scanner triggering from rising gradient-in-gradients and were warmed under an infrared heating lamp and, after full recovery, returned to cages.

**MRI.** ¹H anatomical and tagged cardiac imaging were performed on a pulsed nuclear magnetic resonance (NMR) spectrometer/imager (Bruker Medical, Ettlingen, Germany), consisting of 14.1-T, 89-mm vertical bore magnet, with a multineclear Avance console equipped with a microimaging gradient system capable of 1,000 mT/m maximum gradient strength and 110-μs rise time. Crops of typical end-diastole and end-systole tagged cardiac images of a 10-mo-old PKC-ε mouse are presented in Fig. 1, A and B.

Anesthetized animals were restrained in a 24-mm inner diameter cradle designated to fit linearly polarized 600-MHz whole body, bird-cage NMR resonator (26 mm inner diameter and 52 mm length) and were situated vertically, head up, in the magnet.

Several factors influenced our decision to evaluate only a midventricular short-axis slice. First, cardiac MRI experiments at very high magnetic fields are prone to loss of the signal due to the susceptibility effect that causes local inhomogeneity of magnetic field (14). While this inhomogeneity can be reduced by elaborate and time-consuming manual shimming in the midventricular slice, our experience has been that it is very often not possible in the apical and basal regions. Furthermore, although complete imaging studies were performed at apex, midventricular, and basal levels, loss of the NMR signal on tagged images from the apex and base made these experiments only partially complete and impossible to process. Only the short-axis, midventricular slice provided consistent nondisturbed, high-resolution tagging patterns of 0.45 × 0.45 mm, allowing strain computation as well as unambiguous measurements of wall thickness.

A gradient echo fast imaging (GEFI) sequence provided long-axis plane and transverse plane scout images to localize the heart and to position the short-axis plane (Fig. 1A). Then the midventricular short-axis slice was located halfway between the apex and base, and corresponding anatomical and tagged images were acquired. To monitor the position of the heart over the entire experimental period, control horizontal long-axis images of the heart were acquired every 20 min.

Volumetric analysis was performed using seven to nine anatomical true short-axis contiguous slices (no interslice gap) that were taken at end diastole and end systole to cover the entire LV. Images were acquired using GEFI sequence with 1,000-μs sync excitation pulse, field of view 20 mm, slice thickness 1 mm, echo time 1.5 ms, flip angle 30°, acquisition matrix 128 × 128, and number of excitation 4. Repetition time was set to 100 ms and, due to cardiac gating, was equal to one RR interval and additionally was prolonged by blanking during inspiration. A GEFI sequence delivered rapid imaging in 2.37 ms with excellent myocardium-blood contrast and was not disturbed by the tagging grid, which makes delineation of ventricular structure less accurate.

Tagged images were obtained using a modified DANTE/GEFI sequence proved to be capable of producing very narrow tag lines in a relatively short time, thus making it particularly useful in a study of small-animal heart (8), where fast heart rate (HR) and small heart size require high spatial and temporal resolution of tagging (29). The tagging grid was generated by two composite pulses consisting of 12 hard pulses, 15 μs each, separated by 600 μs, with corresponding gradients in two orthogonal directions. Saturation of protons within tagging planes with very short radio-frequency pulses required maximum power available from 100 W/600 MHz transmitter. With tagging gradients of 88 mT/m, which is less than 10% of the available gradient strength, the sequence yielded a tag grid spacing of 0.45 mm and grid line thickness of 0.1 mm (covering 5.8 and 1.3 of 256 × 256 pixels, respectively). Immediately following the tagging interval, short-axis Cine frames were acquired with 8-ms temporal resolution and imaging parameters of repetition time 200 ms (effectively two RR intervals), echo time 1.9 ms, flip angle 90°, acquisition matrix 256 × 256, and number of excitation 4.

**Image analysis.** Anatomical and tagged images were processed with a Matlab-based program (MathWorks, Natick, MA) to calculate wall thickness, cardiac volumes, and principal strains (17). To obtain regional values of wall thickness and strain, the myocardium was divided in four segments: septal, anterior, lateral, and inferior (5).

LV end-diastolic volume (LVEDV) and end-systolic volume (LVESV) were calculated from epi- and endocardial contours traced on short-axis gradient echo images taken at end-diastolic and end-systolic phases and described in details elsewhere (21, 26). LV mass was computed using the following equation: \( LV\ mass = \frac{p \Delta \Sigma \text{area}}{\text{density of myocardium}, 1.055 \text{ g/cm}^3} \) (28). LV mass index (LVMI) was determined in each animal [LVMI (mg/g) = LV mass/body mass].

**LV midventricular short-axis.** A: horizontal long-axis scout and location of midventricular short-axis images. Samples are shown of typical short-axis tagged images of a 10-mo-old protein kinase C (PKC)-ε transgenic (TG) mouse taken at end diastole (B) and end systole (C). Tagged images are 10 × 10 mm crops from 20 × 20 mm originals. R, right; L, left.

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LVEF was defined as \( \text{EF} = \frac{(\text{LVEDV} - \text{LVESV})}{\text{LVEDV}} \times 100\% \).

Figure 2 depicts centroids of the triangles used for strain analysis in four segments of the LV. Traces correspond to the complex motion (rigid body motion and deformation) of the myocardium tissue during the transition from end diastole to end systole. For calculating strains at end systole, a reference image was acquired at end diastole, and the image corresponding to minimal LV volume was assigned as end systole. Tagged images of 256 × 256 original resolution were zero-filled and reconstructed to a 512 × 512 matrix. Relative displacement of tagging grid centroids was quantified. Finite-element, homogeneous strain analysis was used for calculation of 2D regional and average principal \( E_1 \) and \( E_2 \) Lagrangian strains (17). \( E_1 \) corresponds to maximum elongation strain, and \( E_2 \) corresponds to maximum compression strain.

Statistical analysis. Comparison of mean values between PKC-ε TG mice and age-matched, wild-type NTG mice were performed using the two-way ANOVA with Bonferroni posttest. Intragroup comparisons were performed using repeated-measures parametric one-way ANOVA with Tukey’s multiple-comparison posttest (GraphPad Software, San Diego, CA). All data are presented as means ± SD, with statistical significance at <5% probability.

RESULTS

Cardiac function. No animal mortality occurred over the course of the 8-mo study. HR was unchanged in both groups of anesthetized mice over 8 mo, ranging from 424 ± 45 to 497 ± 37 beats/min in NTG and 441 ± 39 to 476 ± 48 beats/min in PKC-ε TG hearts.

Analysis of LV mass (Fig. 3A, top) shows significant changes in mass within the NTG group during the first 3 mo of the study (6–8 mo of age) and no changes within the TG group. Comparison of LV mass between both groups disclosed significant differences only at 6 mo of age. Normalization of LV mass by body weight (LVMI) amplifies the differences between groups at 12 mo (Fig. 3A, bottom).

Although LVEDV in the NTG group increased gradually between 6 and 9 mo, it remained the same within the PKC-ε TG group during the entire study (Fig. 3B). Relative to the NTG, LVEDV in PKC-ε TG was significantly higher at 6–8 mo of age, as was LVESV from 6–13 mo. In PKC-ε TG, this high LVEDV remained unchanged, while LVEDV in NTG mice gradually increased over 6–9 mo.

While EF of NTG hearts was comparable to previously reported values for normal mice (13, 18, 22, 30, 32), monthly comparison (Fig. 3C) revealed consistently lower EF in PKC-ε TG than NTG (\( P < 0.001 \)), as previously reported for mouse hearts with DCM (16).
mice shows comparable values of average diastolic wall thickness from 6 through 8 mo of age (Fig. 4A). At this time, both groups present similar heterogeneity of regional wall thickness with anterior segment dominant ($P < 0.05$). Figure 4B illustrates regional wall thickness measured at the beginning of the study (6 mo) before wall thinning in PKC-ε TG.

At 9 mo of age, a thinning of the LV wall of PKC-ε TG mice was abruptly evident compared with that of the previous 6- to 8-mo period (Fig. 4A). Average wall thickness was reduced in PKC-ε TG ($P < 0.003$), with no difference occurring among NTG hearts ($P > 0.1$). Average wall thickness in PKC-ε TG was 21% below that of NTG values (PKC-ε TG = 0.90 ± 0.12 mm; NTG = 1.14 ± 0.14 mm, $P < 0.001$) and marked the onset of changes in the morphology of PKC-ε TG hearts. Additionally, at the 9-mo time point, regional wall thickness of the PKC-ε TG developed a near uniform distribution in wall thickness, which was in contrast to the shape maintained in the NTG hearts, with the thickest segment being anterior. Beyond
this 9-mo time point, average and regional wall thickness in both groups of hearts did not change. Figure 4C displays regional wall thickness for both groups of mice at the end of the study (13 mo) following wall thinning in PKC-ε TG.

**Myocardial strains.** Importantly, differences in 2D LV wall E1 strain between experimental groups precedes significant LV wall thinning in the PKC-ε TG hearts. As shown in Fig. 5A, values of average E1 in the two groups diverged at the 7-mo time point (2 mo before the wall thinning seen in the TG group), maintaining an ~25% difference over the remaining 6 mo of the study. Figure 5, B and C, displays segmented E1 strain values during the prethinning period (6 mo) and following thinning of dilated PKC-ε TG (13 mo) and illustrates the trend in regional E1 strain changes, with the lateral segments providing the dominant contribution to average strain from all segments, as summarized in Table 1. A significant increase of the lateral E1 in NTG hearts occurred over time, but not within the TG group.

In contrast, the magnitude of both the average and regional E2 strains did not change over the 8-mo study period in either NTG mice or PKC-ε TG mice (Figs. 6). Although magnitudes of E2 for PKC-ε TG did remain consistently below those of NTG hearts, no significant differences in E2 were established between the two groups.

**DISCUSSION**

While focusing on the relationship between potential changes in 2D principal strains in the LV wall of the heart and LV wall thinning, the present study offers a comprehensive analysis of the development of DCM in hearts of mice over-expressing PKC-ε. Lower EF at 6 mo in PKC-ε TG mice vs. NTG indicate that the compromised cardiac function that is induced by this genetic modification presents earlier than previously appreciated using more conventional echocardiographic imaging (11). This observation may be due to the relatively high resolution of the MRIs compared with previous evaluations using other methods. Differences in EF between groups did not occur as a consequence of age and, in PKC-ε TG mice, preceded changes in E1 strain and LV wall thickness at midventricular level.

Midventricular wall thickness within the PKC-ε TG group dropped significantly at 9 mo of age (Fig. 4A). However, EF, LV epi- and endocardial volumes, and LV mass remained unchanged (Fig. 3). This result highlights the importance regional, as opposed to global, factors that influence the geometric progression toward cardiac dilation.

The study indicates that E1, but not E2, strain was reduced during the development of DCM, and this change was temporally linked to subsequent thinning of the LV wall. At 9 mo, LV wall thinning in TG mice affected the septal, lateral, and primarily anterior segments. The lateral segment has the highest E1 principal strain in normal LVs. Interestingly, the regions displaying the greatest magnitude of E1 strain in normal LV also displayed the greatest difference in E1 compared with the dilated LV of PKC-ε TG hearts. In relation to NTG mice, lateral E1 strain in TG mice was smaller by 30–100% between 7 and 13 mo (Table 1), the biggest difference among all four identified segments. However, segments showing the greatest difference in E1 strain were not those displaying the greatest

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Values are means ± SD. TG, transgenic; NTG, nontransgenic. *P < 0.05 between groups.

Fig. 6. Principal strain E2 in PKC-ε TG and control NTG mice. A: age changes of average E2 strain. Regional E2 strain are shown for 6-mo-old (B) and 13-mo-old mice (C).
reduction in wall thickness. The observation that both preexisting differences in EF and the divergence of E1 between groups preceded LV wall thinning in dilated hearts suggests that strain changes are more closely linked to the development of cardiac dysfunction than later stage changes in wall thickness.

A recently published cardiac MRI tagging study of children with Duchenne muscular dystrophy showed similar differences in average and regional midventricular strains compared with controls (1). Duchenne muscular dystrophy is associated with progression, often undetected, to DCM during adolescence. The study links changes in myocardial contractility expressed in the early decrease of strains with gradual loss of sarcomeres.

Interestingly, average and regional principal E2 strains were similar in both NTG and PKC-ε TG mice and did not change with age. This suggests that the strain associated with compression is not compromised in the myocardium during the onset and progression of this DCM. Indeed, impairment in E2 may well be more likely associated with and predictive of a hypertrophic than a dilated phenotype.

Regional E2 strain in NTG control mice was more homogeneous than E1 strain, with no dominant contribution from any one segment. This finding is consistent with previously published studies of normal C57 mice (35). Figures 5, B and C, and 6, B and C, show representative regional E1 and E2 strains before and following LV wall thinning in PKC-ε TG hearts.

We have studied many aspects of the molecular phenotype of this PKC-ε overexpressing mouse, and increases in β-myosin heavy chain (MHC) proved to be one reliable indicator of the underlying contractile dysfunction (11, 20, 24). It is now well appreciated that, in normal myocardium (both rat and human), there is a gradient of expression from endocardium to epicardium of several biochemical components; critical among these are the MHC isoforms and myosin light chain kinase (2–4, 25). As regards MHC, Stelzer et al. (25) have argued persuasively that small gradients of expression seen across species from rat (3) to human (2) are likely responsible for the timing of force generation and for maintenance of coordinate and forceful contractility. Conversely, loss of this gradient, which is seen in almost all models of DCM, would result in a slowing of the stretch activation response and would significantly slow the rate of force development during systole and diminish both stroke volume and work production during ventricular ejection. This would be reflected by precisely the strain changes seen in our present study.

There are few in vivo murine cardiac magnetic resonance studies conducted in vertical bore magnets. Work by Schneider et al. (23) at 11 T concluded that vertical bore magnet systems can be used for accurate measurement of cardiac functions in normal and failing mice. Wiesmann et al. (31) reported no significant differences in hemodynamic values obtained from either horizontal or vertical positioning of mice. However, there are no reports of principal strain measurements in murine hearts conducted in vertical bore magnets. Although the first 3D motion mapping of murine myocardium with phase-contrast MRI in 17.6-T vertical bore magnet has been published (14), it does not offer any strain-related data. To date, the consequences of prolonged upright body position in anesthetized animals on cardiac strain measurements remain purely speculative.

Systolic E1 and E2 strains obtained with the DANTE tagging method in this study for the NTG group are slightly smaller than previously reported results in normal mice held in a horizontal position with HR maintained around 500 beats/min (35). The differences between previous work and this present study, although within experimental error, are due to the use of spatial modulation of magnetization spatial coding method that inherently produce tagging grids faster than DANTE but at the cost of tagging resolution.

Of considerable clinical interest is the possibility that localized changes in E1 strain in the TG hearts (Table 1), which result in a more homogeneous distribution of strain across the heart, would lead to changes in LV geometry. This measurement, generally determined echocardiographically, has long been felt to represent a marker of progressive systolic dysfunction and to predict the onset of LV decompensation and functional mitral insufficiency (15, 33).

In summary, we investigated the fundamental role of myocardial stretch in active cardiac contraction (9), from temporal analysis of regional, 2D strain changes in the thinning LV myocardium (<1 mm) of a murine mouse model of DCM. High-resolution magnetic resonance tagging revealed a divergence of E1 principal strain between DCM and normal control hearts that preceded detectable wall thinning in the midventricle of the PKC-ε TG mouse heart, presaging the eventual transition to the dilated phenotype and, eventually, heart failure. The observation that changes in strain predate the geometric remodeling associated with thinning of the ventricle implies that dysfunction at the level of the myofibril is causative. Our findings suggest that monitoring of E1 can provide quantitative assessments of LV function during the progression of DCM before significant changes in LV wall thickness. This approach may potentially provide an imaging strategy to direct the timing and anatomical location of therapeutic interventions.

REFERENCES