Quantitative Assessment of Regional Myocardial Function in Mice by Tissue Doppler Imaging

Comparison With Hemodynamics and Sonomicrometry

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Background—Tissue Doppler imaging (TDI) is a novel echocardiographic method to quantify regional myocardial function. The objective of this study was to assess whether myocardial velocities and strain rate (SR) could be obtained by TDI in mice and whether these indices accurately quantified alterations in left ventricular (LV) systolic function.

Methods and Results—TDI was performed in 10 healthy mice to measure endocardial (v_{endo}) and epicardial systolic velocities and SR. In further experiments, TDI indices were compared with dP/dt_{max} and with sonomicrometer-derived regional velocities, at rest and after administration of dobutamine or esmolol. TDI indices were also studied serially in 8 mice before and 4 and 7 hours after endotoxin challenge. Myocardial velocities and SR were obtained in all mice with low measurement variability. TDI indices increased with administration of dobutamine (v_{endo} from 2.2 ± 0.3 to 3.8 ± 0.2 cm/s [$P<0.01$]; SR from 12 ± 2 to 20 ± 2 s^{-1} [$P<0.05$]) and decreased with administration of esmolol (v_{endo} 1.4 ± 0.2 cm/s [$P<0.05$]; SR 6 ± 1 s^{-1} [$P<0.01$]). Both indices correlated strongly with dP/dt_{max} ($r^2=0.79$ for SR and $r^2=0.69$ for v_{endo}; both $P<0.0001$). SR and shortening fraction were predictors of dP/dt_{max} even after adjustment for the confounding effect of the other variables. V_{endo} correlated closely with sonomicrometer-measured velocity ($r^2=0.71$, $P<0.0005$). After endotoxin challenge, decreases in both v_{endo} and SR were detected before decreases in shortening fraction became manifest.

Conclusions—Myocardial velocities and SR can be measured noninvasively in mice with the use of TDI. Both indices are sensitive markers for quantifying LV global and regional function in mice. **(*Circulation*. 2005;111:2611-2616.)**

Key Words: echocardiography ■ systole ■ inotropic agents

Genetically modified mice offer unique opportunities to explore molecular pathways involved in left ventricular (LV) dysfunction.[1–4] Murine cardiac function can be evaluated in vivo with the use of noninvasive methods, such as echocardiography, or with the use of invasive hemodynamic measurements. Echocardiography may be performed serially in mice with little or no anesthesia, but to date the indices of global LV systolic function obtained with M-mode and 2-dimensional (2D) echocardiography have been limited to load-dependent parameters, such as shortening fraction (SF) and ejection fraction.[1–7] Assessment of regional function by 2D echocardiography is typically determined visually, a subjective measure that is at best semiquantitative. Sensitive indices of LV function such as dP/dt_{max} can be obtained with the use of invasive hemodynamic measurements. However, in mice, acquisition of these measures typically involves a nonsurvival procedure. These measures also do not allow for the assessment of regional contractile function.

Tissue Doppler imaging (TDI) is a novel echocardiographic technique that permits quantification of systolic and diastolic regional myocardial velocities and strain rate (SR), the rate of fractional tissue deformation in response to applied force. Both systolic velocities and SR have been shown to be sensitive indices of LV regional systolic function.[8–12] Endocardial to epicardial velocity gradients and maximal SR may identify LV systolic dysfunction before ejection fraction is altered[11,13] and are less dependent on loading conditions than is ejection fraction.[8–10]

Because of their small size and rapid heart rate, the feasibility of TDI assessment of regional myocardial systolic

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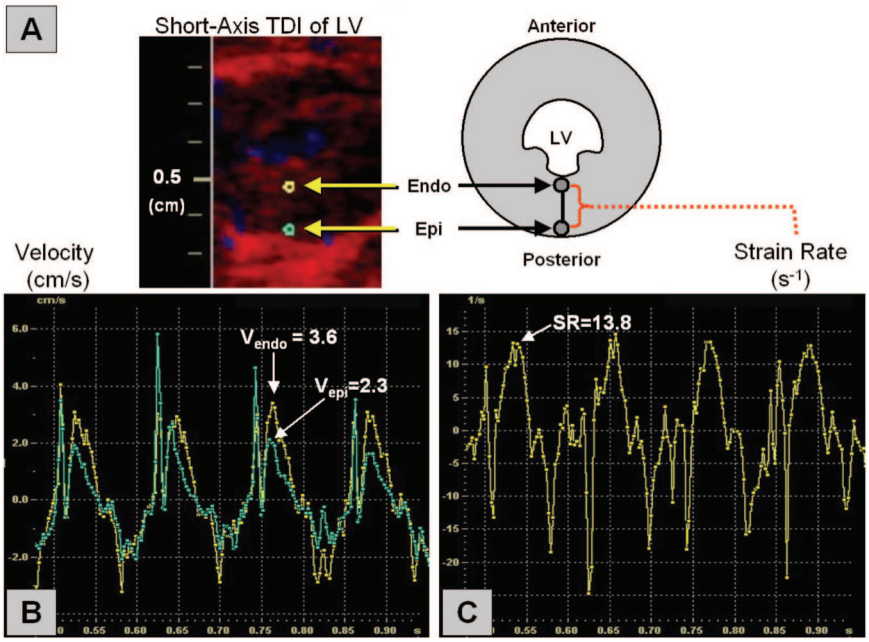

Figure 1. A, TDI of a parasternal short-axis echocardiographic view at midventricular level in a mouse. The region of interest was manually positioned along the posterior wall within the endocardium and epicardium to measure the endocardial and epicardial velocities. Endo indicates endocardial region of interest; Epi, epicardial region of interest; Anterior, anterior wall; and Posterior, posterior wall. B, Endocardial and epicardial time-velocity curves, showing an early systolic peak, short in duration and variable in amplitude, followed by a second peak, longer in duration. v_{endo} and v_{epi} are indicated. C, Time-SR curve. The plot shows little variability from 1 cycle to the next. Peak radial SR is indicated.

function in mice is uncertain. Successful advances in high-frequency and high-frame-rate TDI may overcome these obstacles. Recently, pulsed Doppler acquired at the mitral annulus has been applied to assess diastolic function in mice,[14] and a novel speckle method appears promising.[15] To validate TDI measurements in mice, the variability of murine myocardial velocities and SR was determined, and TDI-derived velocities were compared with sonomicrometer-derived velocities. TDI was performed in various inotropic states, and SF, myocardial velocities, and SR were compared with an index of LV contractility, dP/dt_{max}. TDI indices were applied in a model of endotoxin-induced LV dysfunction.

Regional myocardial velocities and SR were obtained noninvasively in mice with low variability. Changes in endocardial velocity (v_{endo}) and SR in response to agents that alter inotropy correlated with invasive hemodynamic and sonomicrometric assessments. In a model of acute LV dysfunction by endotoxin challenge, v_{endo} and SR decreased before SF was altered.

Methods

TDI in Mice

Echocardiographic images were acquired with the use of a 13-MHz linear-array transducer with a digital ultrasound system (Vivid 7, GE Medical Systems). Tissue Doppler images were collected with the use of parasternal short-axis views at the midventricular level, at a frame rate of 483 frames per second and a depth of 1 cm. The Nyquist limit of the velocity was 15 cm/s, with a pulse repetition frequency of 2.5 kHz.

TDI analysis was performed offline with the use of a customized version of the EchoPac Software (GE Medical). Myocardial velocities were computed from a region of interest (0.2×0.2 mm) that was manually positioned along the posterior wall on the endocardial and epicardial surfaces to measure the peak systolic v_{endo} and epicardial velocity (v_{epi}), respectively (Figure 1A). Radial SR was then measured over an axial distance of distance L of 1 mm (width 0.6 mm). In the present study a tissue Doppler frequency of 6.4 MHz was used, corresponding to a wavelength of 0.24 mm. The transmitted pulse is longer than 1 wavelength, and the received signal is eventually sampled with a sample distance of 0.35 mm. The smallest distance L, and thus the smallest possible spatial resolution, is therefore 0.35 mm. As with all gradient estimators, the quality of the strain rate estimate also improves with increasing L. A strain length of 1 mm was used in the present study to balance estimator quality versus spatial resolution. The temporal smoothing filters were turned off for all measurements. Time-velocity and time-SR plots were obtained, and peak systolic velocities and SR were measured. The values obtained in 5 consecutive cardiac cycles were averaged.

The normal patterns and intraobserver and interobserver variabilities of v_{endo}, v_{epi}, and SR were measured in 10 lightly sedated mice (ketamine 80 mg/kg administered intraperitoneally) by 2 independent observers (J.G.M. and M.S.-C.). From the color-coded images, each observer measured velocities and SR. A single observer (M.S.-C.) repeated the measurements several weeks later. Interobserver and intraobserver variabilities were calculated as the difference between the 2 observations divided by the means of the observations and were expressed as both absolute numbers and percentages.

Comparison of TDI Parameters With Hemodynamic Indices of LV Systolic Function

Hemodynamic parameters were obtained in 17 mice (weight, 26±1 g) with the use of a 1.4F high-fidelity micromanometer catheter (Millar Instruments), as described previously.[2,16] Echocardiographic parasternal short-axis 2D and M-mode images were acquired simultaneously with invasive hemodynamics. In 9 mice, after baseline measurements were obtained, dobutamine (30 μg/kg per minute) and esmolol (10 mg/kg per minute) were infused through the right jugular vein in a random order. The drugs were infused until the heart rate increased (or decreased) 10% or the dP/dt_{max} increased (or decreased) 20%. When either of these criteria was met, a second set of echocardiographic and hemodynamic measurements was recorded. The drug was then discontinued until the heart rate and dP/dt_{max} returned to baseline, and the second drug was infused. Graded inotropic modulation with lower doses of esmolol (1 and 3 mg/kg per minute) and dobutamine (10 μg/kg per minute) was similarly studied in 8 subsequent animals (1 mouse died before reliable image acquisition with the highest β-blocker dose) to provide intermediate data points in the relationship of TDI parameters with hemodynamic indices.

Comparison of TDI Parameters With Sonomicrometer-Derived Indices

Five mice (weight, 30±2 g) were anesthetized with the use of isoflurane and underwent thoracotomy. Piezoelectric crystals (0.7 mm; Sonometrics) were implanted in the midventricular portion of the LV. Two crystals were attached to the epicardium with a cyanoacrylate adhesive (1 on the anterior wall, 1 on the posterior wall), and the third crystal was implanted within the endocardial layer of the posterior wall.[17] The sonomicrometry system was set for a 960-Hz sample rate, and the individual crystal gain/reject controls were adjusted for optimal signals. The ultrasound transducer was positioned to avoid electrical or mechanical interference with the crystals. Echocardiographic epicardial short-axis views and sonomicrometer data sets were acquired simultaneously, at baseline and after administration of esmolol (5 mg/kg) or dobutamine (625 μg/kg).

Velocity values from the sonomicrometry data were calculated by taking the temporal derivative of the intercrystal distances with the use of a sliding window of 8 ms (9 samples) followed by a sliding, 5-point median filter. The velocities were then plotted versus time, and the peak systolic velocities from 5 consecutive cardiac cycles were identified and averaged. Peak systolic TDI velocities sampled from the posterior endocardial wall were compared with the peak sonomicrometry velocities computed from the anterior epicardial and posterior endocardial crystal pair.

Acute LV Dysfunction Model

Eight mice (weight, 26±2 g) were intraperitoneally injected with 50 mg/kg of *Escherichia coli* lipopolysaccharide (LPS). Echocardiography was performed before and 4 and 7 hours after injection with the use of light sedation (ketamine 80 mg/kg administered intraperitoneally).

Statistical Analysis

The JMP 5.1 statistical software (SAS Institute Inc) was used. Values are expressed as mean±SEM except for intraobserver and interobserver variabilities, which are expressed as mean±SD. After we tested for inequality of variances, the differences between echocardiographic and hemodynamic variables before and after inotropic alterations were tested with ANOVA for repeated measurements with a contrast analysis (each group/dose versus rest/baseline).[18] When differences in variances were found, ordinal logistic fit was applied.[18] The echocardiographic and hemodynamic variables were correlated with the use of a mixed model accounting for the mouse effect.[18] A multiple linear regression analysis was used to test the echocardiographic predictors of dP/dt_{max}. A probability value <0.05 was considered significant.

Results

TDI Parameters: Normal Patterns and Values

All mice had normal LV size and function (LV end-diastolic dimension=3.3±0.1 mm; SF=51±1%). Average heart rate was 539±15 bpm. During systole, 2 positive peaks in the time-velocity curves were noted (Figure 1B). The first peak was short (between 5 and 15 ms) and variable in amplitude. The second peak was longer (36±2 ms) and was reproducible from cycle to cycle, and its magnitude was 3.4±0.1 cm/s in the endocardial region and 2.3±0.2 cm/s in the epicardial region. Endocardial and epicardial maximal systolic velocities were measured from the second peak. The pattern in diastole was variable, with 1 or 2 negative peaks. SR curves were interpretable in all mice (Figure 1C). Peak radial SR averaged 14.2±0.7 s^{-1}. One positive peak (occasionally with an early notch) was noted in systole. The diastolic aspect was more variable, with 1 or 2 negative peaks separated by rebounds into positive values.

TABLE 1. Intraobserver and Interobserver Variabilities of TDI Parameters in Mice

	Mean±SD	Mean±SD Difference	Mean±SD Difference, %
Intraobserver variability			
v_{endo}, cm/s	3.32±0.36	0.12±0.08	3.5±2.5
v_{epi}, cm/s	2.17±0.50	0.22±0.22	10.9±10.6
SR, s^{-1}	14.09±1.70	0.92±1.36	6.2±8.4
Interobserver variability			
v_{endo}, cm/s	3.29±0.36	0.17±0.12	5.0±3.6
v_{epi}, cm/s	2.23±0.48	0.17±0.12	8.2±5.8
SR, s^{-1}	14.22±1.80	1.04±0.70	7.1±4.4

Values are mean±SD; n=10. Intraobserver and interobserver variabilities were calculated as the difference between the 2 observations divided by the means of the observations and expressed as both absolute numbers and percentage.

Intraobserver and Interobserver Variabilities

Intraobserver and interobserver variabilities were smallest for v_{endo} (the SD of the error was 2.5% for intraobserver variability and 3.6% for interobserver variability) and largest for v_{epi} (the SD of the error was 10.6% for intraobserver variability and 5.8% for interobserver variability) (Table 1). The SR had a SD of the error of 8.4% for intraobserver variability and 4.4% for interobserver variability.

Comparison of LV Systolic Function Assessment by Echocardiography and Hemodynamic Variables

Dobutamine infusion increased SF by 22% ($P<0.05$), v_{endo} by 73% ($P<0.01$), and SR by 67% ($P<0.05$) (Table 2). Esmolol infusion decreased SF by 20% ($P<0.01$), v_{endo} by 36% ($P<0.05$), and SR by 50% ($P<0.01$). The SF, v_{endo}, and SR correlated with the maximal derivative of the developed pressure dP/dt_{max} (Figure 2). Tissue Doppler parameters correlated strongly with dP/dt_{max} ($r^2=0.79$ for SR and $r^2=0.69$ for v_{endo}; $P<0.0001$ for both). SF also correlated with dP/dt_{max} ($r^2=0.46$, $P<0.0001$). When all echocardiographic parameters were analyzed by multivariate analysis, SR (<0.0001) and SF (0.03) were each significantly related to

TABLE 2. Echocardiographic and Hemodynamic Variables at Baseline and After Dobutamine and Esmolol in Mice

	Rest	Dobutamine	Esmolol	ANOVA
HR, bpm	557±30	603±19	424±43*	0.01
LVID, mm	2.7±0.1	2.6±0.1	3.0±0.1*	0.01
SF, %	45±2	55±2*	34±2†	0.0007
v_{endo}, cm/s	2.2±0.3	3.8±0.2†	1.4±0.2*	<0.0001
SR, s^{-1}	12±2	20±2*	6±1†	0.0024
dP/dt_{max}, mm Hg/s	8543±915	14130±741†	5060±344*	<0.0001
dP/dt_{min}, mm Hg/s	8684±881	8872±453	5636±453*	0.0286
LVSP, mm Hg	91±6	98±2	75±5*	0.008

Values are mean±SEM; n=9. HR indicates heart rate; LVID, LV internal diameter; dP/dt_{max}, maximal pressure derivative; dP/dt_{min}, minimal pressure derivative; and LVSP, LV systolic pressure. Data were analyzed as ANOVA for repeated measurements and contrast analysis and ordinal logistic fit for v_{endo}, SR, and dP/dt_{max}.

*$P<0.05$ vs baseline; †$P<0.01$ vs baseline.

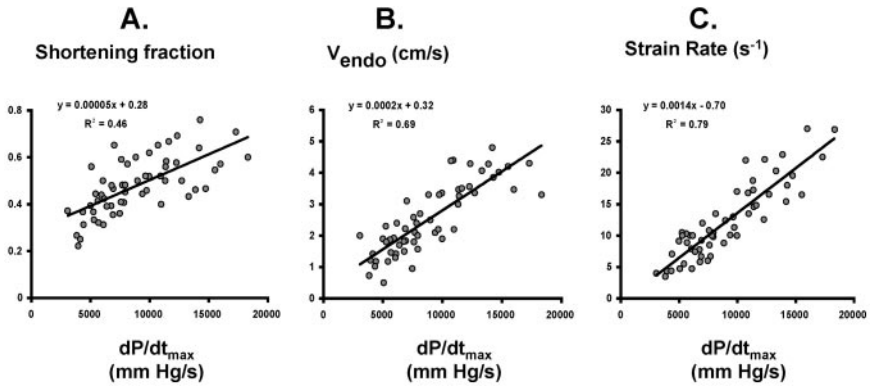

Figure 2. Correlation between dP/dt_{max} and SF (A) v_{endo} (B), and SR (C). Mixed model (doses and mouse) was applied to 58 data points.

dP/dt_{max} even after adjustment for the confounding effects of the other variables, whereas v_{endo} did not improve prediction of dP/dt_{max} based on SF and SR. When SR was omitted from the multivariate analysis, both SF and v_{endo} were predictors of dP/dt_{max} ($P<0.02$ for SF and $P<0.0001$ for v_{endo}).

Relationship of TDI Velocity With Sonomicrometry

The peak systolic velocity measured by sonomicrometry was 1.9±0.2 cm/s, and the wall thickening was 26±3%. An example of the sonomicrometer-derived posterior wall velocity curves is shown in Figure 3. Peak systolic velocity measured by TDI (v_{endo}) was 1.2±0.2 cm/s and correlated closely with sonomicrometer-measured velocity (TDI velocity=0.71×sonomicrometer velocity−0.17; $r^2=0.72$, $P<0.0005$). However, TDI-derived velocity consistently underestimated sonomicrometer-derived velocity ($P<0.0001$).

Endotoxin Challenge

Injection of 50 mg/kg LPS resulted in a progressive decline of LV systolic function (Figure 4). Four hours after LPS injection, SF was unchanged (50±3% at baseline and 45±11% 4 hours after injection), whereas v_{endo} decreased by 32% (from 3.1±0.4 to 2.1±0.5 cm/s; $P<0.005$) and SR by 38% (from 17±5 to 11±4 s^{-1}; $P<0.01$). Seven hours after injection, all echocardiographic parameters of LV systolic function were decreased.

Discussion

This study demonstrates that myocardial velocities and SR can be obtained noninvasively in mice with the use of TDI and describes their normal patterns and values in these animals. Low intraobserver and interobserver variabilities were observed. TDI-derived v_{endo} correlated well with sonomicrometric measurements of regional velocities. Both v_{endo} and SR detected alterations in contractility and correlated closely with dP/dt_{max}. In a model of LV dysfunction induced by endotoxin challenge, v_{endo} and SR detected LV dysfunction earlier than did SF.

Applying TDI to murine hearts presents unique challenges because of the small size and fast heart rate of the mouse. In the present study the axial and lateral resolutions of the color Doppler at the depth studied were 0.35 mm, allowing spatial differentiation of the endocardial and epicardial layers in systole (the wall thickness is 1.5 to 1.8 mm at midsystole). No attempts were made to study the diastolic phase of the cardiac cycle when the wall thickness is 0.6 to 0.8 mm. The frame rate was 483 frames per second. With an average murine heart rate of 539 bpm, 54 frames per cardiac cycle were obtained, a rate similar to that used by others (with close correlation of TDI-derived to MRI-derived measurements of strain[9]). The myocardial velocities and radial SR patterns obtained in the healthy mice were very similar to those observed in large animals, with 2 positive velocity peaks.[8,12,19] In larger animal studies, the first systolic peak has

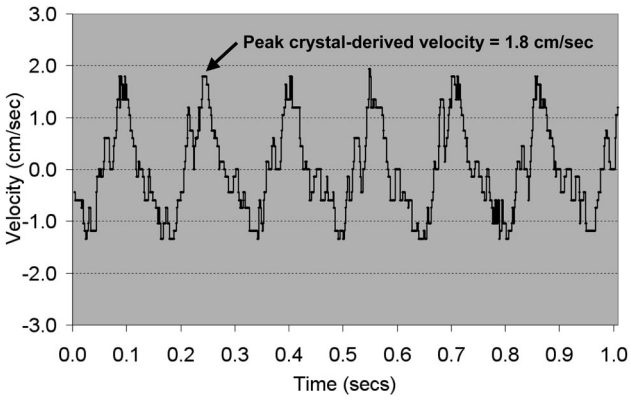

Figure 3. Sonomicrometer-derived velocity of the posterior wall endocardium over time.

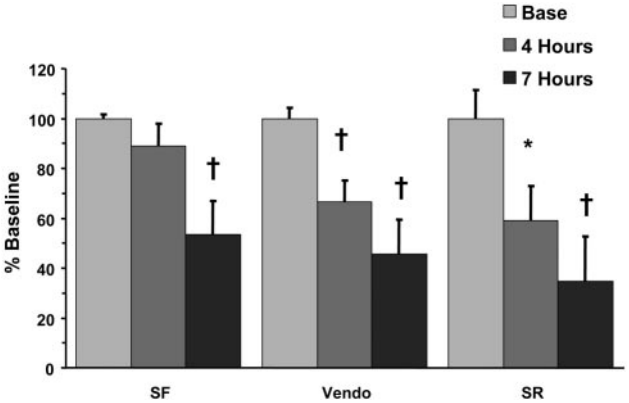

Figure 4. Echocardiographic indices of LV systolic function before and 4 and 7 hours after intraperitoneal injection of 50 mg/kg LPS. Values are mean±SEM; n=8. Vendo indicates posterior wall endocardial maximal velocity; SR, posterior wall maximal systolic strain rate. Data were analyzed as ANOVA for repeated measurements and contrast analysis. *$P<0.01$ vs baseline; †$P<0.005$ vs baseline.

been identified as concomitant to isovolumic contraction.[20] The normal value of the posterior wall v_{endo} reported in the present study was similar to that found in large animals[10,12]; however, the SR was significantly higher.

The intraobserver and interobserver reproducibility values of TDI measurements in mice were low and comparable to those reported for M-mode measurements routinely described in the literature.[5,21] Of note, the variability of the SR measurement is slightly higher than that of v_{endo}, likely because of the fact that SR is the spatial derivative of the velocities, increasing the level of noise detected. The variability of v_{epi} was the highest, and it was not used in subsequent experiments.

In the present study both v_{endo} and SR correlated closely with dP/dt_{max}, a sensitive measure of LV systolic function.[22,23] SR was predictive of dP/dt_{max} even after adjustment for the confounding effects of SF and v_{endo}. Peak systolic myocardial velocity has been reported to correlate with dP/dt_{max} in patients,[24] and peak systolic SR closely correlated with dP/dt_{max} in large animals.[10,12] Both findings were confirmed in mice in the present study.

Sonomicrometry remains the gold standard for measuring wall thickening in animals. This technique has previously been applied to mice to measure wall thickening and global LV function.[17,25] We used an endocardial crystal to obtain endocardial thickening measurements and derived the endocardial velocities. V_{endo} correlated well with the sonomicrometer-derived peak systolic velocity, used as a gold standard of regional myocardial velocity. There was, however, a significant underestimation of the sonomicrometer-derived velocity by v_{endo} (0.71 ± 0.11 cm/s). A possible explanation for this discrepancy is that TDI velocities were sampled at a point in the posterior myocardium relative to the tip of the transducer, whereas, in the sonomicrometry analysis, the velocity was computed from the distance between a crystal moving with the posterior endocardium relative to a crystal moving with the anterior epicardium wall. In the sonomicrometry-derived velocity measurements, the velocity of the anterior epicardium is added to the velocity of the posterior endocardium and should be taken into account in mice models. In a murine model of endotoxin-induced LV failure, v_{endo} and SR detected LV systolic dysfunction earlier than conventional echocardiographic indices such as SF. The sensitivity of TDI parameters in detecting subtle LV dysfunction had been reported in human asymptomatic carriers of muscular dystrophy.[13] In rats, the gradient between endocardial and epicardial velocity, a measure of the rate of deformation, was more sensitive than SF in the detection of early LV systolic dysfunction after aortic banding.[11] In mice, TDI appears to have the potential to detect subtle differences in LV function between murine strains or genetically modified mice.

There were several limitations in the present study. First, apical views are difficult to obtain and poorly reproducible in small rodents, precluding the reliable analysis of longitudinal velocities and SR.[26] Thus, we relied on the parasternal images. Great care must be taken to avoid motion and rib artifacts and poor alignment of the posterior wall perpendicular to the beam. Second, the posterior wall myocardial velocities were easier to analyze than those of the anterior/anteroseptal wall. Anterior wall velocities were much lower than posterior wall velocities, and their variability was large, precluding reliable analysis. This has been reported previously in humans[27,28] and, very recently, in mice.[15] The reason for the discrepancy between the velocities of the posterior wall and the anterior wall remains unclear. Third, the measurement of sonomicrometer-derived wall thickening required the subtraction of the distance of 2 pairs of crystals, the epicardium to epicardium and the epicardium to endocardium pairs, which may double the possible error due to positioning of the crystals and to the level of noise.

In conclusion, TDI is feasible in mice, and indices of LV regional systolic function can be obtained reproducibly. In addition to simple parameters such as peak velocities and strain rate, the temporal pattern of the curves may provide additional information on LV function. Peak systolic endocardial velocity and myocardial SR correlate closely with dP/dt_{max} and detect LV dysfunction earlier than SF, thereby potentially providing more information and detecting more subtle differences in LV systolic function compared with conventional echocardiographic measurements.

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