Echocardiographic Speckle-Tracking Based Strain Imaging for Rapid Cardiovascular Phenotyping in Mice

Michael Bauer,* Susan Cheng,† Mohit Jain, Soeun Ngoy, Catherine Theodoropoulos, Anna Trujillo, Fen-Chiung Lin, Ronglih Liao

Rationale: High-sensitivity in vivo phenotyping of cardiac function is essential for evaluating genes of interest and novel therapies in small animal models of cardiovascular disease. Transthoracic echocardiography is the principal method currently used for assessing cardiac structure and function; however, standard echocardiographic techniques are relatively insensitive to early or subtle changes in cardiac performance, particularly in mice.

Objective: To develop and validate an echocardiographic strain imaging methodology for sensitive and rapid cardiac phenotyping in small animal models.

Methods and Results: Herein, we describe a modified echocardiographic technique that uses speckle-tracking based strain analysis for the noninvasive evaluation of cardiac performance in adult mice. This method is found to be rapid, reproducible, and highly sensitive in assessing both regional and global left ventricular (LV) function. Compared with conventional echocardiographic measures of LV structure and function, peak longitudinal strain and strain rate were able to detect changes in adult mouse hearts at an earlier time point following myocardial infarction and predicted the later development of adverse LV remodeling. Moreover, speckle-tracking based strain analysis was able to clearly identify subtle improvement in LV function that occurred early in response to standard post–myocardial infarction cardiac therapy.

Conclusions: Our results highlight the utility of speckle-tracking based strain imaging for detecting discrete functional alterations in mouse models of cardiovascular disease in an efficient and comprehensive manner. Echocardiography speckle-tracking based strain analysis represents a method for relatively high-throughput and sensitive cardiac phenotyping, particularly in evaluating emerging cardiac agents and therapies in mice. (Circ Res. 2011;108:908-916.)

Key Words: cardiac phenotyping ■ echocardiography ■ cardiac function ■ cardiovascular physiology ■ strain imaging

The ability to assess cardiac performance in small animals is critically important for phenotyping genetic mouse models, evaluating the efficacy of novel cardiovascular disease therapies, and investigating the safety of agents with potential cardiotoxic side effects.1,2 Existing approaches for assessing cardiac performance, however, are limited. In vitro assessment of cardiac cell function, although highly sensitive, is inadequate given the functional complexity of the cardiovascular system, which integrates global pump function, neurohormonal status, vascular properties, and systemic hemodynamics.3 Conventional noninvasive in vivo methods of assessing cardiac performance allow for physiological investigations but have limited resolution, which prevents capturing subtle alterations in cardiac function that can occur early in the course of myocardial injury or healing.3,4

Despite the availability of multiple cardiac imaging modalities, a robust method for highly sensitive and rapid phenotyping of cardiac performance has yet to be established. Nuclear imaging techniques lack spatial resolution for performing detailed regional assessments, whereas cardiac magnetic resonance (CMR) and computed tomography techniques lack temporal resolution for characterizing discrete functional changes.3,5 Furthermore, application of these imaging modalities in smaller animal models, especially mice, involves prohibitively expensive equipment, is time intensive, and may require complete procedural sedation that markedly alters in vivo hemodynamics. As a result, echocardiography has emerged in recent years as the standard cardiac imaging technique in experimental small animal models of cardiovascular disease.6

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908
Echocardiography is noninvasive, widely available, cost-effective, and involves relatively short image acquisition and postprocessing times.\textsuperscript{3–6} Despite these advantages, conventional echocardiographic measures lack sensitivity for capturing subtle variations in left ventricular (LV) performance.\textsuperscript{3,4} Changes in LV structure and global function, when detected by conventional echocardiographic parameters, are typically considered late manifestations of disease.\textsuperscript{7} Recently, a novel echocardiographic imaging technique, based on myocardial strain analysis, has been found to dramatically improve assessment of LV performance in humans.\textsuperscript{8,9} By capturing segmental tissue motion in multiple planes and axes serially over the cardiac cycle, strain analysis provides integrated and detailed information regarding both regional and global LV function, with much greater sensitivity and specificity than conventional echocardiographic measures, including fractional shortening (FS) or ejection fraction (EF).\textsuperscript{8} To date, the adoption of strain imaging in small animal models has been limited, primarily because of technical differences in image acquisition and analysis in mice versus humans. However, recently developed speckle-tracking based techniques now allow for angle-independent, reproducible, and accurate strain measurements that may be applied to mice.

Herein, we describe a modified echocardiographic imaging methodology that uses speckle-tracking based strain analysis to conduct highly sensitive, noninvasive cardiac phenotyping in a mouse model of cardiovascular disease. Compared with conventional echocardiographic imaging, speckle-tracking based strain echocardiography was found to efficiently detect subtle changes in cardiac performance following myocardial infarction (MI) and also identify early differences in response to treatment with standard cardiac therapy. Thus, speckle-tracking based strain echocardiography represents a novel method for relatively high-throughput and sensitive cardiac phenotyping, including in the evaluation of emerging cardiac therapies in mouse models of cardiovascular disease.

Methods

An expanded Methods section is available in the Online Data Supplement at http://circres.ahajournals.org and includes detailed information regarding the experimental MI procedure, conventional echocardiographic measurements, in vivo speckle-tracking based strain echocardiography, pathological cardiac assessment, and statistical analyses.

Experimental Protocol

Adult C57BL/6J mice were obtained from The Jackson Laboratory at 8 weeks of age, placed on a standard mouse chow diet and water ad libitum, and housed in a temperature-controlled environment under an alternating 12-hour light/dark cycle. All animal handling procedures adhered strictly to the approved guidelines of the Institutional Animal Care and Use Committee. A total of 16 mice (mean weight, 26.3 ± 1.7 g) were randomly assigned to 1 of the following 3 groups: a group that underwent open thoracotomy without coronary ligation (sham group, N = 5); a group that underwent permanent left anterior descending artery (LAD) ligation without subsequent treatment (MI group, N = 6); and a group that underwent permanent LAD ligation followed by oral administration of an angiotensin-converting enzyme inhibitor (ACEi) (captopril, 20 mg/kg per day) in the drinking water starting on day 7 following surgery (MI + ACEi group, N = 5). Coronary ligation was performed as previously described.\textsuperscript{10} Echocardiography was performed on all mice under light sedation (1% isoflurane in oxygen) before surgery (baseline) and at 1 week following surgery (before starting any treatment with ACEi), at 3 weeks, and at 7 weeks following surgery. Echocardiography was performed using a 18 to 38 MHz linear-array transducer with a digital ultrasound system (Vevo 2100 Imaging System, VisualSonics, Toronto, Canada). Standard parasternal long- and short-axis views were obtained during each echocardiographic examination. Conventional and novel echocardiographic image measurements were performed offline. All image acquisitions and offline measurements included in the present analysis were conducted by a single investigator who was blinded to animal groups. At 7 weeks, mice were euthanized for pathological assessment of cardiac remodeling and infarct size.

Conventional Echocardiographic Measurements

Echocardiographic measurements were obtained from gray scale M-mode images, at the midpapillary level in the parasternal short-axis view, and also from B-mode images acquired in the parasternal long- and short-axis views. Conventional measurements of the LV included: end-diastolic diameter (LVEDD), end-systolic diameter, anterior and posterior wall thicknesses, FS, wall thickening, fractional area change (FAC), end-systolic and end-diastolic volumes, EF, and LV mass.

Novel Echocardiographic Speckle-Tracking Based Strain Measures of Myocardial Deformation

Based on Lagrangian and Eulerian strain tensors of finite deformation theory, extensional strain of soft tissue in a prespecified direction can be defined as the change in length of a segment divided by its original length ($\frac{(L_f - L_0)}{L_0}$), where strain rate (SR) is the rate of change of this deformation over time ($\frac{(L_f - L_0)}{L_0} \times \text{sec}^{-1}$).\textsuperscript{11} Using speckle-tracking based strain analysis of 2D gray scale echocardiographic images acquired from the parasternal long- and short-axis views, strain and SR were quantified in the longitudinal, radial, and circumferential axes; in accordance with myocardial fiber orientation at varying levels of the LV wall, longitudinal strain is most representative of myocardial shortening at the level of the endocardium, whereas radial and circumferential strains are more reflective of shortening at the level of the mesocardium (Figure 1).

Parasternal long-axis views were found to provide the most reproducible myocardial views for longitudinal strain analyses in mice, whereas parasternal short-axis views (at the mid-papillary level) were obtained for circumferential and radial (short-axis) strain analyses (Figure 1A). All images were acquired at a frame rate of >200 frames per second (average 230 frames per second) and at an average depth of 11 mm.

Strain analyses were conducted by the same trained investigator on all animals according to the protocol detailed in the Online Data Supplement and using a speckle-tracking algorithm provided by VisualSonics (VevoStrain, VisualSonics). In brief, suitable B-mode loops were selected from digitally acquired echocardiographic im-
ages based on adequate visualization of the endocardial border and absence of image artifacts. Three consecutive cardiac cycles were selected for analysis based on image quality. Semiautomated tracing of the endocardial and epicardial borders were performed and verified over all 3 cardiac cycles and then corrected as needed to achieve good quality tracking throughout each cine loop. Tracked images were then processed in a frame-by-frame manner for strain measurements (Figure 1B). Strain measures were averaged over the obtained cardiac cycles (with temporal smoothing filters turned off for all measurements), resulting in curvilinear strain and SR data (Figure 1C). Each long- and short-axis view of the LV myocardium was divided into 6 standard anatomic segments\(^12\) for regional speckle-tracking based strain analysis throughout the cardiac cycle. In MI animals, the mid-anterior, apical-anterior, and apical-inferior wall segments in the long-axis view were designated as the infarct region and the basal-inferior and mid-inferior wall segments were designated as the remote (noninfarct) region. Peak strain and SR measurements were recorded from each of the 6 standard segments in each view, providing regional strain values. For global strain values, peak strain and SR measurements were averaged across all 6 segments. Regional strain values were obtained by averaging these same measurements across infarct and remote segments, respectively.

**Results**

**Echocardiographic Speckle-Tracking Based Strain Imaging**

During each cardiac cycle, the LV undergoes a typical pattern of tissue deformation in multiple planes. This complex functional pattern includes myocardial shortening in the longitudinal axis, thickening in the radial axis, and shortening in the circumferential axis during systole, followed by reverse changes during diastole (Figure 1A). Myocardial tissue deformation in these axes can be assessed, both regionally and globally, as measures of tissue strain and SR. Speckle-tracking–based strain analyses of myocardial motion (in the long- and short-axis images) integrates frame-to-frame data from cine loops (Figure 1B), allowing for measurements of segmental myocardial strain and calculation of SR in the longitudinal, radial, and circumferential axes. These measures are plotted as curvilinear data for each region tracked (Figure 1C).

Echocardiographic images were obtained in adult mice, as described in the Methods. Total time for image acquisition and analyses were similar for conventional and speckle-tracking based strain measures and, together, totaled less than 25 minutes per animal, typically allowing for imaging and analysis of 20 mice per day by a single operator. Acquisition of B-mode images in the parasternal long-axis view, followed by acquisition of M-mode and B-mode images in the parasternal short-axis view, involved 2 to 5 minutes of image acquisition time per view. During image acquisition, mice were lightly anesthetized (1% isoflurane in oxygen). As demonstrated by average heart rates of 560±79 bpm at baseline (Table 1), echocardiography allowed for in vivo cardiac assessment at physiological heart rates without significantly altering resting hemodynamics. All acquired images were deemed appropriate for both conventional and speckle-tracking based strain measurements.

Similar to conventional echocardiographic measures, speckle-tracking based strain analyses were performed offline after imaging acquisition was completed. Conventional and speckle-tracking based strain measurements involved a total of 7 to 15 minutes of analysis time per study (including image selection, image tracing/measuring, and data processing), where up to 15 minutes was needed for a minority of studies
that required repeat image tracing and processing to avoid or minimize the effects of artifact on speckle-tracking. Speckle-tracking based strain measures exhibited high intraobserver reproducibility (r=0.87 and r=0.89, P<0.001 for both), comparable to recent human studies using similar methods. Safety of the experimental procedure was demonstrated by lack of any adverse reactions or deaths observed for the animals studied over the duration of the study period.

### Table 1. Baseline Echocardiographic Characteristics

<table>
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<th>Characteristic</th>
<th>Sham</th>
<th>MI</th>
<th>MI+ACEi</th>
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<tbody>
<tr>
<td>Conventional measures</td>
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<td>Heart rate (bpm)</td>
<td>513±32</td>
<td>584±45</td>
<td>520±26</td>
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<td>LVWT (mm)</td>
<td>0.8±0.0</td>
<td>0.8±0.1</td>
<td>0.8±0.1</td>
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<td>LVEDD (mm)</td>
<td>3.2±0.2</td>
<td>3.4±0.1</td>
<td>3.4±0.2</td>
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<td>FS (%)</td>
<td>38.6±3.8</td>
<td>40.8±3.5</td>
<td>40.3±4.6</td>
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<td>LV area long axis (mm²)</td>
<td>20.5±1.1</td>
<td>16.0±1.4</td>
<td>16.4±0.8</td>
</tr>
<tr>
<td>FAC long axis (%)</td>
<td>30.6±2.1</td>
<td>42.3±3.8</td>
<td>41.6±6.9</td>
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<td>LV area short axis (mm²)</td>
<td>10.0±0.7</td>
<td>9.4±0.8</td>
<td>7.4±0.1</td>
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<td>FAC short axis (%)</td>
<td>48.7±3.3</td>
<td>56.6±5.4</td>
<td>61.4±4.8</td>
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<td>EF (%)</td>
<td>45.2±3.2</td>
<td>59.2±4.7</td>
<td>56.5±8.1</td>
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<td>LV mass (m)</td>
<td>169.4±14.8</td>
<td>140.4±6.3</td>
<td>127.3±11.4</td>
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<tr>
<td>Strain measures</td>
<td></td>
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<td>Short axis (%)</td>
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<td></td>
</tr>
<tr>
<td>Circumferential strain</td>
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<td>−30.1±3.8</td>
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<td>Circumferential SR</td>
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<td>−13.4±2.4</td>
<td>−13.0±1.9</td>
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<tr>
<td>Radial strain</td>
<td>31.6±3.8</td>
<td>39.0±2.4</td>
<td>34.9±4.8</td>
</tr>
<tr>
<td>Radial SR</td>
<td>8.9±1.2</td>
<td>11.3±0.9</td>
<td>10.8±1.1</td>
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<tr>
<td>Long axis (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longitudinal strain</td>
<td>−17.9±1.5</td>
<td>−19.1±2.7</td>
<td>−16.6±1.5</td>
</tr>
<tr>
<td>Longitudinal SR</td>
<td>−10.6±1.4</td>
<td>−12.2±2.8</td>
<td>−10.7±1.7</td>
</tr>
<tr>
<td>Radial strain</td>
<td>22.6±4.3</td>
<td>24.8±2.0</td>
<td>23.0±2.9</td>
</tr>
<tr>
<td>Radial SR</td>
<td>8.0±1.4</td>
<td>8.5±0.5</td>
<td>7.4±0.6</td>
</tr>
</tbody>
</table>

Values are shown as mean±SE. *Measured at end diastole.

### Table 2. Conventional Echocardiographic Measurements Over the Treatment Period

<table>
<thead>
<tr>
<th>Measurement</th>
<th>1 Week</th>
<th>3 Weeks</th>
<th>7 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>MI+ACEi</td>
<td>Sham</td>
</tr>
<tr>
<td>LVWT (mm)</td>
<td>0.8±0.0</td>
<td>1.0±0.1</td>
<td>0.8±0.1</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>3.1±0.2</td>
<td>4.3±0.2†</td>
<td>4.4±0.2†</td>
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<tr>
<td>FS (%)</td>
<td>35.1±5.2</td>
<td>18.6±2.1†</td>
<td>19.9±4.2†</td>
</tr>
<tr>
<td>LV area long axis (mm²)</td>
<td>16.0±1.6</td>
<td>30.2±3.3†</td>
<td>27.9±2.1†</td>
</tr>
<tr>
<td>FAC long axis (%)</td>
<td>43.7±2.6</td>
<td>16.3±4.5†</td>
<td>17.9±5.6†</td>
</tr>
<tr>
<td>LV area short axis (mm²)</td>
<td>8.0±0.7</td>
<td>16.4±2.1†</td>
<td>15.9±1.6†</td>
</tr>
<tr>
<td>FAC short axis (%)</td>
<td>52.3±4.9</td>
<td>25.4±3.9†</td>
<td>27.0±4.2†</td>
</tr>
<tr>
<td>EF (%)</td>
<td>60.5±3.4</td>
<td>23.4±7.0†</td>
<td>25.3±7.3†</td>
</tr>
<tr>
<td>LV mass (m)</td>
<td>129.6±4.8</td>
<td>186.5±12.2†</td>
<td>186.1±8.9†</td>
</tr>
</tbody>
</table>

Values are shown as mean±SE. *P<0.05 vs MI; †P<0.05 vs sham. ‡Measured at end diastole.

### Novel Measures of Myocardial Performance Following Regional Myocardial Injury

To determine the relative sensitivity of speckle-tracking based strain measures versus conventional echocardiographic measures in the assessment of impaired cardiac function, adult mice were serially imaged following MI or sham operation. Both conventional and speckle-tracking based strain measures of myocardial performance were similar across all animals at baseline, before either MI or sham operation (Table 1).

### Global Function

Within 1 week following MI or sham operation, conventional measures of remodeling (LVEDD and LV mass) increased and conventional measures of global LV function (FS and EF) decreased in MI compared with sham animals (Table 2; Figure 2A and 2B). Advanced measures of cardiac performance, represented by myocardial strain in all axes (longitudinal, radial, and circumferential), were also reduced early following MI (Table 3; Figure 2C and 2D), and these changes persisted over the total 7-week observation period (Table 3). These changes were most consistently significant for longitudinal compared with radial and circumferential strains, in accordance with greater vulnerability of endocardial and subendocardial tissue to ischemia from coronary obstruction. Importantly, changes in myocardial longitudinal strain and SR were more marked than changes in conventional LV measures, even FS (Figure 2E).

### Regional Function

Strain analyses further allow for quantification of regional cardiac function within the infarct zone (mid-anterior, apical-anterior, and apical-inferior segments) and the remote, non-infarct zone (basal-inferior and mid-inferior segments). Interestingly, myocardial performance decreased at 1 week not only in the infarct segments but also in regions remote from the designated infarct area (Table 3; Figure 2G), consistent with prior evaluations of regional myocardial function using CMR imaging in mice. This pattern of regional dysfunction...
performed before and after ACEi administration, which served as vehicle control (MI). Serial echocardiography was performed at 7 weeks. In the sham, MI, and MI + ACEi groups, heart-to-body weight ratios were 6.46 ± 0.27, 8.82 ± 0.57, and 7.33 ± 0.41, respectively. These weights were strongly correlated with echocardiographically assessed LV mass at 7 weeks (r = 0.85; P < 0.01). Histological examination of infarct size was also performed on all animals. Echocardiographically determined infarct size at 7 weeks for the MI and MI + ACEi groups was 47 ± 3% and 47 ± 7%, respectively, whereas histological assessment of infarct size for the MI and MI + ACEi animals was 46 ± 9% and 53 ± 9%, respectively. For the total sample of MI animals, echocardiographic infarct size was strongly correlated to histological infarct size (r = 0.93; P < 0.001).

Discussion

In this report, we describe the utility of a noninvasive imaging method for highly sensitive and rapid cardiac phenotyping in

**Figure 2. Echocardiographic assessment of post-MI LV function and remodeling.** Among animals that underwent MI, changes in echocardiographic measurements are shown at baseline (BL) and at 1 week after MI (1). LV end-diastolic dimension (A) was increased after MI, whereas FS (B), global peak longitudinal strain (LS) (C), and global peak longitudinal SR (D) all decreased with cardiac injury. Percentage change in strain measures was greater than for conventional measures (E). A schematic of myocardial regions identified from the parasternal long axis view is shown in Panel (F). AA indicates apical anterior; AL, apical inferior; BA, basal anterior; BI, basal inferior; MA, mid-anterior; MI, mid-inferior. Peak longitudinal strain across these regions was normally distributed at baseline but globally reduced at 1 week after MI (G). *P < 0.05; **P < 0.01.

Ability of Myocardial Performance Measures to Differentiate Effects of MI Treatment

To determine the relative sensitivity of speckle-tracking based strain versus conventional measures in assessing the effects of potential cardiac therapies, a subset of the MI animals underwent treatment with ACEi (MI + ACEi) or served as vehicle control (MI). Serial echocardiography was performed before and after ACEi administration, which represents a mainstay of post-MI cardiac therapy and has been shown to improve both cardiac function and survival in animal models and humans.15–17

At serial time points following MI, progressive changes consistent with adverse ventricular remodeling were observed in all animals but, as expected, to a lesser degree in ACEi-treated compared with untreated mice. Significant differences between MI and MI + ACEi mice in conventional structural measures, including LV chamber size and mass, were observed at 7 weeks following MI (6 weeks following treat-
a small animal model of cardiovascular disease. Advanced speckle-tracking based strain measures of LV performance were not only more sensitive than conventional echocardiographic measures in detecting global changes in post-MI cardiac function, but they also allowed region-specific functional assessments in infarcted versus noninfarcted areas of myocardium. Furthermore, speckle-tracking based strain measures of myocardial performance differentiated response to standard cardiac therapy with an ACEi earlier than did conventional measures of either LV function or remodeling.

In clinical studies, subtle abnormalities in strain are associated with the presence of myopathic disease even in the setting of normal cardiac structure and function by conventional measures.8 Moreover, cardiovascular risk factors result in impaired strain measures before the development of decreased LV EF or ventricular dilation.8,21 In experimental studies, the adoption of strain analysis to facilitate detailed cardiac phenotyping has been limited, primarily because of technical differences in imaging mice versus humans, including limited echocardiographic views, translational motion during image acquisition, and the effect of very high heart rates on the number of frames per second that may be captured and, in turn, analyzed. However, recently developed image analysis techniques, based on speckle tracking, now allow for angle-independent and accurate strain-related measurements of mice at high frame rates.22,23 As such, recent studies have described the ability of speckle-tracking analyses to characterize serial changes in myocardial strain occurring in parallel with decrements in conventional measures of cardiac function in the setting of transverse aortic constriction23,24 and MI.25 However, prior investigations have focused on speckle-tracking analyses of short-axis images, which precludes measures of longitudinal strain and, thus, limits the ability to assess the relative sensitivity of speckle-tracking versus conventional measures in mice.

Normal LV function involves a typical pattern of myocardial deformation that includes longitudinal shortening, circumferential shortening, and radial thickening in systole, followed by reverse changes in diastole.26,27 Reflecting myocardial fiber shortening at the level of the endocardium, alterations in longitudinal myocardial deformation tend to be among the earliest signs of cardiac dysfunction.18–20 Compared with strain in the circumferential or radial axes, which predominantly reflect activity of the mesocardium (mid-myocardium), longitudinal strain is a particularly sensitive marker of the subendocardial myofiber dysfunction that tends to occur early in the setting of hypoperfusion or mechanical stress.8,28,29 Accordingly, we observed that regional and global measures of longitudinal strain worsened dramatically and soon after experimental MI, which parallels findings from prior animal25,30,31 and human32–34 studies. In contrast to conventional echocardiographic measures, speckle-tracking based strain measures provided additional region-specific assessments of LV function, demonstrating impaired performance in noninfarcted, as well as infarcted, areas of the myocardium. Functional abnormalities affecting the myocardial tissue remote to the infarct region may be attributable to global myocardial stunning and hypoperfusion or continued

<table>
<thead>
<tr>
<th>Measurement</th>
<th>1 Week</th>
<th>3 Weeks</th>
<th>7 Weeks</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>MI</td>
<td>MI+ACEi</td>
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<tr>
<td><strong>Global (%)</strong></td>
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<td>Short axis</td>
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<tr>
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<td>-25.9±2.7</td>
<td>-13.9±0.8†</td>
<td>-10.7±1.6†</td>
</tr>
<tr>
<td>Circumferential SR</td>
<td>-13.2±1.4</td>
<td>-5.5±0.5†</td>
<td>-5.2±0.8‡</td>
</tr>
<tr>
<td>Radial strain</td>
<td>32.2±2.4</td>
<td>15.6±2.9‡</td>
<td>13.0±2.7†</td>
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<td>Radial SR</td>
<td>10.7±0.8</td>
<td>6.0±0.4‡</td>
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<td><strong>Infarct region (%)</strong></td>
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<td>Long axis</td>
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<tr>
<td>Longitudinal strain</td>
<td>-15.7±1.5</td>
<td>-4.9±0.6‡</td>
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<td>-7.4±0.6</td>
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<td>Long axis</td>
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<td>Longitudinal strain</td>
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<td>Radial SR</td>
<td>21.7±2.1</td>
<td>10.4±3.5†</td>
<td>9.6±2.1†</td>
</tr>
</tbody>
</table>

Values are shown as means±SE. *P<0.05 vs Sham; †P<0.05 vs MI. |
Our results also demonstrate that the serial application of speckle-tracking based strain measures is highly sensitive for identifying the beneficial effects of a pharmacological therapy following MI. Whereas conventional LV measures such as FS and LVEDD did not significantly differ between vehicle versus ACEi-treated mice until 7 weeks following MI, measures of longitudinal strain exhibited the benefit of standard therapy as early as 3 weeks following MI. Prior studies have shown that alterations in strain coincide with alterations in conventional measures of LV structure and function following MI, likely because of the strong correlation between abnormal strain values and the extent of infarct. Our data indicate that alterations in myocardial strain can both directly reflect evolving effects of a rescue therapy and also predict subsequent differences in LV remodeling. Although the long-term benefits of ACEi therapy on preventing LV remodeling and prolonging survival following MI have been long recognized, our results highlight how the early ameliorative effects of ACEi therapy on myocardial performance can be observed even before differences in gross LV function or structure are detected. Furthermore, we demonstrate that strain abnormalities in both the infarcted and noninfarcted regions measurably improved in the setting of ACEi therapy. As with global strain, regional strain distinguished MI+ACEi from MI animals at an earlier time point than conventional measures of LV structure or function. The differential trajectories of myocardial response to MI, reflected by serial speckle-tracking based strain measures, may reflect the specific actions of ACEi on attenuating subendocardial hypoperfusion, hemodynamic load, and/or remodeling.

**Implications**

We demonstrate the utility of a novel noninvasive imaging method for assessing cardiac function. Importantly, given the growing availability of genetically altered mice, this methodology may be used for rapid cardiovascular phenotyping in a relatively large number of animals. Furthermore, speckle-tracking–based strain measures allow for rapid and sensitive assessment of cardiac function in response to either emerging therapies following injury or in response to potential cardiotoxic agents. This technique may be particularly useful when studying therapies such as cardiac regeneration, which may have specific regional, rather than global, effects.

**Study Limitations**

Several limitations of this study merit consideration. Sources of variation exist in speckle-tracking based strain analysis, including the echocardiographic views obtained for image acquisition. Changes in the imaging angle of incidence can result in capturing different fiber layers at different levels and, thus, yield variable results. This is especially important for short-axis views, where even small differences in image angle can lead to highly variable rotation and, in turn, circumferential strain measurements. Due to the presence of multiple orientation points (eg, LV outflow tract, mitral valve leaflet plane, and LV apex), parasternal long-axis views are less prone to angular variation in mice. Unlike in humans, apical 4-chamber views are difficult to obtain in mice and, therefore, parasternal long axis images are preferred for obtaining longitudinal strain measurements. Another source of variability is the tracing performed by the investigator, because strain values can depend on the quality and location of the tracing. As demonstrated by data from our laboratory, interobserver variability can be minimized through use of a standardized detailed image analysis protocol.

Although segment-to-segment comparisons of LV strain measures are theoretically possible using speckle-tracking
based strain analysis, discrimination of measurements at the single-segment level is technically limited for serial evaluations in a post-MI model, in part because of the anatomic changes that result from remodeling. Notwithstanding this limitation, measures that are averaged across multiple segments, as used in regional and global analyses, are both discriminating and reproducible. Speckle-tracking based analyses have also been used to evaluate post-MI dyssynchrony in humans\(^4\); however, these methods are not easily translated to mouse models, largely because of the much faster heart rates in mice. The applicability of the methods and generalizability of the findings reported herein may be limited to the use of similar equipment and software. However, continued advancements in the field of cardiac ultrasound are likely to broaden the availability of speckle-tracking based strain analyses, which rely on imaging systems that can accommodate high frame rates while preserving spatial, as well as temporal, resolution.

**Conclusion**

In a small animal model, noninvasive echocardiographic assessment of myocardial deformation using speckle-tracking based strain analyses provided a highly sensitive in vivo characterization of distinct trajectories of LV dysfunction and remodeling following acute MI. Speckle-tracking based strain measures allowed for both detailed and efficient assessments of global and regional dysfunction, which, in turn, predicted adverse remodeling. Thus, the use of speckle-tracking based strain measures to serially track regional and global myocardial performance parameters can provide insight into patterns of structural, as well as functional, myocardial recovery in response to cardiac therapies. This technique represents a novel approach to rapidly assessing phenotypic variation in the cardiac response to experimental myocardial injury and in the evaluation of therapies designed to attenuate the sequela of such injury.

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**Disclosures**

Catherine Theodoropoulos and Anna Trujillo are employees of VisualSonics. The remaining authors have no disclosures.

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**Novelty and Significance**

**What Is Known?**

- Echocardiography (cardiac ultrasound) is frequently used to assess in vivo cardiac structure and function; however, conventional echocardiographic measures lack sensitivity for detecting changes early in the course of disease progression, particularly in common small animal models of cardiovascular disease.
- A new echocardiographic method for high-throughput and high-sensitivity in vivo phenotyping of cardiac function is needed for evaluating genes of interest and novel therapies in small animal models of cardiovascular disease.

**What New Information Does This Article Contribute?**

- This article describes the novel application of echocardiographic strain imaging to mouse models of cardiovascular disease.
- In a mouse model of myocardial infarction (MI), echocardiographic strain-based measures allowed for assessment of global (whole heart) and regional (specific heart areas) cardiac function.
- Echocardiographic strain-based measures provided more sensitive and rapid assessment of cardiac structure and function in mice following MI and in response to cardiac therapy, relative to conventional echocardiographic measures.

Although transthoracic echocardiography is the principal method used for assessing cardiac structure and function, standard echocardiographic techniques are relatively insensitive to early or subtle changes in cardiac performance, particularly in mice. In this study, we describe a modified echocardiographic technique that uses speckle-tracking based strain analysis for the noninvasive evaluation of cardiac performance in adult mice. This method is rapid, reproducible, and highly sensitive in analyzing regional and global LV function. Compared with conventional echocardiographic measures, peak longitudinal strain and strain rate were able to detect changes in adult mouse hearts at an earlier time point following MI and predicted the later development of adverse LV remodeling. Moreover, speckle-tracking based strain analysis was able to identify subtle improvement in LV function that occurred early in response to standard post-MI cardiac therapy. These results highlight the utility of speckle-tracking based strain imaging for detecting discrete functional alterations in mouse models of cardiovascular disease in an efficient and comprehensive manner. Thus, echocardiographic speckle-tracking based strain analysis represents a method for conducting relatively high-throughput and sensitive cardiac phenotyping and evaluating potential therapies in mice.
Echocardiographic Speckle-Tracking Based Strain Imaging for Rapid Cardiovascular Phenotyping in Mice

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EXPANDED METHODS

Experimental Myocardial Infarction

Left anterior descending (LAD) coronary artery ligation was performed as previously described. In brief, animals were anesthetized using pentobarbital (65mg/kg) and the onset of deep anesthesia was confirmed by the absence of hind-limb pain reflexes. Mice were intubated using a fine polyethylene cannula connected to a standard small rodent ventilator (Harvard 680, South Natick, MA) set to a volume of 160 ul at 140 strokes per minute. Each mouse was then positioned on its right side to expose the left chest. The skin was incised from the sternum to the left anterior axillary line at the level of the 5th intercostal space. The pectoralis muscles were incised and the chest opened at the 5th intercostal space. A small rib spreader was introduced to open the operation field and the pericardium was bluntly removed. Experimental MI was induced by permanent ligation of the LAD artery 2 mm from its origin between the pulmonary outflow tract and the edge of the left atrium, using a 6-0 prolene suture. Induction of acute MI was considered successful when the anterior LV wall turned pale and ST-segment elevation was observed on simultaneous electrocardiography. The chest was subsequently closed and the lungs inflated. Sham-operated animals underwent an identical procedure without coronary artery ligation. To maintain body temperature throughout the procedure, each mouse was positioned on a heating pad while anesthetized. Following chest closure, animals were extubated upon spontaneous recovery from anesthesia and resumption of spontaneous breathing. Buprenorphine (0.03-0.06 mg/kg) was then administered twice daily for 3 days to prevent post-operative discomfort. All procedures were approved by the Institutional Animal Care and Use Committee.

Echocardiography

For in vivo echocardiography, mice were lightly anesthetized using 1% isoflurane in oxygen and then fixed in the supine position on a heated platform. Electrode gel was applied to limb leads to obtain concurrent electrocardiographic recording during the exam. Isoflurane concentration was lowered as needed to allow heart rates to return to physiologic range (>500 bpm). Echocardiography was performed using an 18-38MHz linear-array transducer with a digital ultrasound system (Vevo 2100 Imaging System, VisualSonics, Toronto, Canada). Image acquisition was initiated with the transducer probe placed along the left sternal border to obtain the parasternal long axis view, which displays both the apex and the outflow tract of the left ventricle. Following acquisition of long-axis images, the probe was rotated 90 degrees to obtain and record short-axis images at the level of the mid-papillary muscles. An M-mode gate was placed through the center of the papillary level short-axis view to obtain standard M-mode recordings.

To ensure good quality images for speckle-tracking based strain analyses, all image acquisition was performed at a high frame rate (>200 frames per second), which provides optimal temporal resolution and reduces image analysis artifacts. Thus, frame rate was increased as needed for each echocardiographic view by decreasing the depth and/or narrowing the imaging sector width. Adjusting image sector size was performed also to improve spatial resolution and to minimize image dropout of various wall segments (particularly the basal and apical wall segments in the long-axis view, and the lateral wall segments in the short-axis view), while also ensuring that the endocardial and epicardial borders of the LV would be captured. Care was taken to record images (≥3 cardiac cycles per loop) where translational
motion or breathing artifacts were absent or minimized. Since speckle-tracking based strain analysis is susceptible to even small variations in how echocardiographic views are obtained, in addition to image tracing technique, all images were acquired and analyzed by the same blinded investigator (MB).

**Conventional Echocardiographic Measurements**

Conventional echocardiographic measurements were obtained from grayscale M-mode images acquired in the parasternal short-axis view at the mid-papillary (midwall) level of the LV, and also from 2D images acquired from the parasternal long-axis and short axis views. For measures of LV diameter and wall thickness, measurements were made from short-axis M-mode images for 3 consecutive cardiac cycles and then averaged. M-mode based measurements included LV end-diastolic diameter, LV end-systolic diameter, anterior wall [AW] and posterior wall [PW] thicknesses, LV fractional shortening ([LV end-diastolic diameter – LV end-systolic diameter]/LV end-diastolic diameter x 100), and wall thickening ([systolic wall thickness – diastolic wall thickness]/systolic wall thickness x 100). To obtain fractional area change (FAC), left ventricular endocardial area was traced from both short- and long-axis B-mode loops at end-diastole and at end-systole. LV end-systolic and end-diastolic volumes and LV ejection fraction (EF) were measured from 2-dimensional parasternal long-axis views. LV mass was calculated using end-diastolic epicardial and endocardial area according to the following formula: LV mass=1.05 x (5/6 x epicardial area x epicardial major axis + T) - (5/6 x endocardial area x endocardial major axis), where T = sqrt(epicardial area/π) – sqrt(endocardial area/π). At 7 weeks, the total epicardial and endocardial border lengths were measured from the parasternal long axis view, in addition to the lengths of epicardial and endocardial border affected by scar. Infarct size was calculated as a percentage, based on the mean of the epicardial and endocardial ratios of infarct-to-total length x 100.

**Pathologic Assessment of Cardiac Remodeling**

Following echocardiography at the 7-week time point, each animal was sacrificed. Hearts were fixed using a defined end diastolic pressure of 5mmHg. Briefly hearts were perfused using a Langendorff apparatus, a balloon was inserted into the left ventricle through the mitral valve. The pressure in the balloon was adjusted to reach an end-diastolic pressure of 5 mmHg. Hearts were arrested in diastole using KCl and further perfused for 15 minutes using 10% formaldehyde. Hearts were unmounted and stored in 10% formaldehyde overnight with the pressured balloon still in place. The balloon was removed and the hearts weighted. For histological assessment of infarct size, hearts were cut into 3 transverse slices (basal, middle, and apical) parallel to the atrioventricular groove, embedded in paraffin, and stained using Masson trichrome stain. Images of the whole heart were recorded and infarct size was calculated as the mean percentage of epicardial and endocardial circumference occupied by scar tissue, as measured from and averaged over 3 serial cross sections of the LV.

**Statistical Analysis**

All continuous data are presented as mean ± standard error (SE). After testing for inequality of variances, the difference between echocardiographic measurements before and after surgery was tested using one-way analysis of variance (ANOVA) for repeated measurements. If the results of analysis of variance were significant, paired Student's t tests were used. Comparison of echocardiographic parameters between groups of mice and groups of segments was analyzed using one-way ANOVA for repeated measurements. If the interaction of time and group was significant, unpaired Student's t tests were used to compare
echocardiographic parameters between groups at the same time point. Two-way ANOVA was used to assess for the possible interaction of treatment group on time from baseline to each of the LV measures. Spearman correlations were used to assess the association of early (3 week) measures of longitudinal strain and strain rate with later (7 week) measures of LV remodeling, as represented by percent change in LVEDD (from week 3 to 7). A 2-tailed P value of <0.05 was considered statistically significant. Statistical analyses were performed using R version 2.10.0 (The R Foundation for Statistical Computing, Vienna, Austria).
PROTOCOL FOR SPECKLE TRACKING BASED STRAIN ANALYSIS IN MICE

Image Acquisition

1. Perform 2D B-mode echocardiography on lightly anesthetized mouse in standard fashion.
2. Image parasternal long axis view in 2D. Optimize frame rate to >200 fps by narrowing imaging sector width and decreasing depth as needed.
3. Align focus depth with the LV posterior wall epicardium. Adjust both gain and dynamic range to optimize contrast.
4. Acquire cine loops in the parasternal long axis view that contains at least 3 consecutive cardiac cycles where there is complete and optimal visualization of both endocardial and epicardial borders, and where image artifacts (e.g. near field artifacts, breathing/translational motion artifacts, etc.) are avoided or minimized.
5. Image parasternal short axis view in 2D at the level of the mid-ventricle (papillary muscle level). Optimize frame rate to >200 fps by narrowing imaging sector width and decreasing depth as needed.
6. Acquire cine loops in the parasternal short axis view that contains at least 3 consecutive cardiac cycles where there is complete and optimal visualization of both endocardial and epicardial borders, and where image artifacts (e.g. near field artifacts, breathing/translational motion artifacts, etc.) are avoided or minimized.

Image Analysis

1. Review all acquired loops for quality. Select the best quality loop based on presence of the following factors: optimal frame rate, absence of artifacts, myocardial visualization, and contrast of endocardial and epicardial borders. If any of these factors is absent and/or if there is dropout of 2 or more myocardial segments in all acquired loops (where no 2 consecutive loops are deemed adequate in image quality), then exclude entire echocardiographic view from analysis. If at least 2 consecutive acquired loops are deemed to have adequate quality for analysis, open these select loops using the VevoStrain package (which will convert and import the loops into the strain analysis application).
2. For each good quality image, play the cine loop within the strain analysis application interface to assess location and relative motion of the endocardial border during systole and diastole.
3. Place an M-mode gate through the B-mode loop shown.
4. Specify the start and end of each cardiac cycle using the widest diameter of the endocardial border (as also indicated by M-mode tracings) and onset/upslope of the electrocardiographic R wave as reference points.
5. Narrow the selection to the 3 cardiac cycles with the best visualization of endocardial and epicardial borders and no breathing artifacts (2 cardiac cycles if only 2 loops were available for import). Once the selection has been made, advance to the analytical functions.
6. At end-diastolic frame of the 1st selected cardiac cycle, trace just within the endocardial border. Use 8-12 points total to trace the endocardium in its entirety (base to apex to base in the long-axis view; anterior to lateral to inferior to septal in the short-axis view). Papillary muscle or trabeculae should be excluded during endocardial border tracing. Place tracing points closer together along more curved segments and farther apart along straighter segments. In cases where the endocardial border is not optimally visualized at end-diastole, select a frame at an alternate time point in the cardiac cycle when the endocardial border is more optimally traceable, while favoring time points that are closer to end-diastole.
7. Process endocardial tracing. Inspect tracking of the processed tracings and, if tracking is poor (either endocardial border motion lags tracing, or tracing lags endocardial border motion), then re-trace as needed to optimize endocardial tracking.

8. After satisfactory endocardial tracking has been achieved, select the edit trace option and activate automated appearance of epicardial tracing. Adjust overall width between endocardial and epicardial tracings as needed. Next, adjust the epicardial tracing such that the tracing is just within the epicardial border.

9. In cases where sufficient quality tracings cannot be achieved for all 3 cardiac cycles (if 3 loops total were imported), alternate cycles may be selected (or the total number of cycles may be reduced to 2 cardiac cycles).

10. Process endocardial and epicardial tracings. Check tracking of the processed tracings and, if tracking is poor (either endocardial border motion lags tracing, or tracing lags endocardial border motion), then re-trace as needed.

11. Once optimal tracking has been achieved, activate tracking analysis of cine loop to generate both longitudinal (or circumferential) and radial strain and strain rate curves. Activate peak analysis and record average peak of strain curves for each of the 2 axes in each view (longitudinal and radial in the long-axis view; circumferential and radial in the short-axis view). Record average peak of strain rate curves for each of the 2 axes in each view (longitudinal and radial in the long-axis view; circumferential and radial in the short-axis view).

12. Digitally save all image analysis data.