DOI: 10.1111/echo.12138

Echocardiography

Novel Simple Approach for Detection of Regional Perturbations of Cardiac Function in Mouse Models of Cardiovascular Disease

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Aims: Transthoracic murine echocardiography is a cornerstone of small animal research, but conventional methods cannot detect regional perturbations in cardiac function. Reliable assessment of regional cardiac function would be of value in transgenic models of myocardial disease. Until now automatized algorithms for achieving this suffers from a number of drawbacks. We developed a simple algorithm for rapidly assessing the relative myocardial radial thickening that occurs between end-diastole and end-systole, that is, regional radial transmural end-systolic strain (RTESS). Methods and Results: Echocardiographic assessment was performed in mice at baseline (n = 8), 2 hours postintraperitoneal isoprenaline (ISO) injection (n = 8), and 10 days postmyocardial infarction (post-MI) (n = 6). A >1000 frames/sec cine loop was acquired by the ECG-gated Kilohertz visualization technique in the parasternal short-axis projection at 3 mm below the mitral annulus. Endo- and epicardial borders were traced at end-diastole and end-systole and RTESS was calculated for each of n segments by the algorithm. The intra- and inter-observer coefficients of variation for segmental RTESS assessment were 5.11 and 7.32, respectively. At baseline, average segmental RTESS was 56.75% and RTESS was similar in all cardiac segments regardless of how many segments the heart was divided into. In the akinetic myocardium of MI and ISO mice, 47.36% and 26.22% length of the endocardium, respectively, RTESS was near zero and significantly different from the remaining myocardium. Conclusion: We describe a simple and straightforward approach to quantify regional myocardial deformation in mouse models of cardiovascular disease. (Echocardiography 2013;30:843-849)

Key words: mouse echocardiography, regional transmural end-systolic radial strain, speckle tracking echocardiography

Increased availability of genetically modified mouse models of heart disease has established the mouse as a central small-animal model in cardiovascular research.[1] Although M-mode derived fractional shortening (FS) and ventricular dimensions have been validated in assessing global cardiac function, more sophisticated approaches are necessary for in-depth cardiac phenotyping, for example, detection and quantification of regional heterogeneity within the myocardium.[2]

Tissue Doppler imaging (TDI) and speckle tracking (ST) have emerged as promising means to study regional myocardial function. Although TDI is feasible in small animal echocardiography, it suffers from being angle dependent, that is, it can only reliably measure the velocity of tissue deformation that occurs parallel or near-parallel to the direction of the ultrasonic beam. ST-based algorithms on the other hand, are angle-independent and thus able to measure tissue deformation in all directions.[3]

Despite the fact that ST has been applied successfully in several clinical scenarios and reference values of myocardial strain and strain rate have been established for humans,[4,5] results in murine models are as yet not unequivocally convincing.[6,7] This may be due to too low frame rates in the cine loops used which leads to speckle decorrelation and inaccurate tissue tracking through the cardiac cycle.[8,9]

Cardiac deformation is complex, but, if simplified, it can be described to occur along 3 to each other perpendicular axes. Longitudinal deformation occurs along the long axis of the heart whereas circumferential deformation represents the deformation in the short axis and

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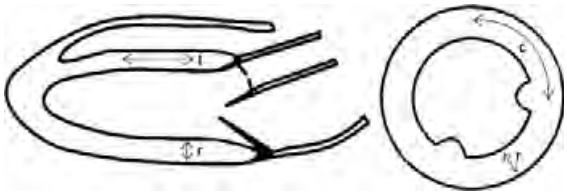

Figure 1. Radial, circumferential, and longitudinal deformation. During cardiac contraction, tissue deformation occurs along the radial (r), circumferential (c), and longitudinal (l) axes.

radial deformation pertains to deformation across the myocardial wall (Fig. 1).[3] Strain, as described above pertains to the relative deformation of one particle in relation to another. Radial transmural end-systolic strain (RTESS) thus represents the deformation of the endocardium relative to the epicardium, that is, fractional myocardial wall thickening, or the difference in myocardial wall thickness in end-systole compared with end-diastole.[3] RTESS is one of many parameters that theoretically can be derived by ST-based algorithms.

We aimed to establish a simple and reliable algorithm that allows to rapidly calculate the regional RTESS. Such an algorithm can be used in mouse model phenotyping to derive an index of regional cardiac function. It can also be used to validate the accuracy of more sophisticated ST-based algorithms. In this study, we demonstrate a conceptually straightforward and simple approach for quantification of regional myocardial function in mouse models of myocardial infarction and catecholamine-toxicity.

Methods:

The study protocol was approved by the Animal Ethics Committee at Gothenburg University and all animals were handled in accordance with the National Institutes of Health (NIH) guidelines for use of experimental animals. Twelve weeks old male C57BL/6 mice were housed in a temperature-controlled (25°C) facility with a 12-hour light/dark cycle, providing free access to food and water.

Echocardiographic assessment of cardiac function was performed at baseline, 10 days postmyocardial infarction (post-MI), and 2 hours postintraperitoneal isoprenaline (ISO) injection. Eight mice were included in each group. Eight mice were studied at baseline and then underwent ligation of the coronary artery and induction of MI. Two mice died within 10 days post-MI, whereas the other 6 mice were studied again. An additional 8 mice were studied 2 hours post-ISO. We choose the above specified timepoints because we have previously shown that ISO-induced cardiac dysfunction can be detected within 2 hours post-ISO and because at 10 days post-MI the heart is more easily visualized compared with the first few days following surgery. At 10 days post-MI the heart has also undergone significant remodeling.[7] These are the main 2 reasons why we have chosen 10 days as an important time point in our studies on post-MI remodeling. RTESS was calculated using the approach described below in a parasternal short-axis cine loop (frame rate >1000/sec). Inter- and intra-observer variability was assessed for RTESS assessment by the program in echocardiograms of 6 mice, that is, 36 segments, and expressed as $SD/\sqrt{2} \times 100/(x)$, where SD is the standard deviation and (x) is the pooled mean of the differences between the observers, as proposed by Bland and Altman.[10]

Algorithm for Determining Regional RTESS:

An image file containing 2 images (obtained in end-diastole and end-systole, respectively) in which the endo- and epicardial borders have been traced by green-blue color (e.g., VEVO 770 v3.0.0, Visualsonics, Toronto, ON, Canada) is loaded into the program, which identifies the pixels belonging to each of the 2 inner and outer walls. The center points are identified and are computed as the center of a bounding box surrounding the outer walls (Fig. 2). A given number of segments, each of equal length, are auto-generated. The user can then manually manipulate each individual segment to his or her liking (Fig. 2).

Myocardial radial thickening, that is, RTESS, is calculated as the difference in average thickness (or distance) between the inner and outer walls. For each segment, this average difference is computed and reported to the user. Algorithmically, the program computes the average thickness as follows:

```
For each segment,
for each pixel of the inner and outer wall segment,
find distance to closest pixel of opposite wall segment.
Return the average distance.
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The program was developed in C++ for Windows. Computation times were 0.2 seconds for 2 heart images, each of a square size of 330^2 pixels.

Echocardiography:

Echocardiography was performed using a VisualSonics 770 VEVO (Visualsonics) imaging station, which includes an integrated rail system for consistent positioning of the ultrasound probe. The hair from the chest was removed with an electrical clipper and a hair removal gel prior to the

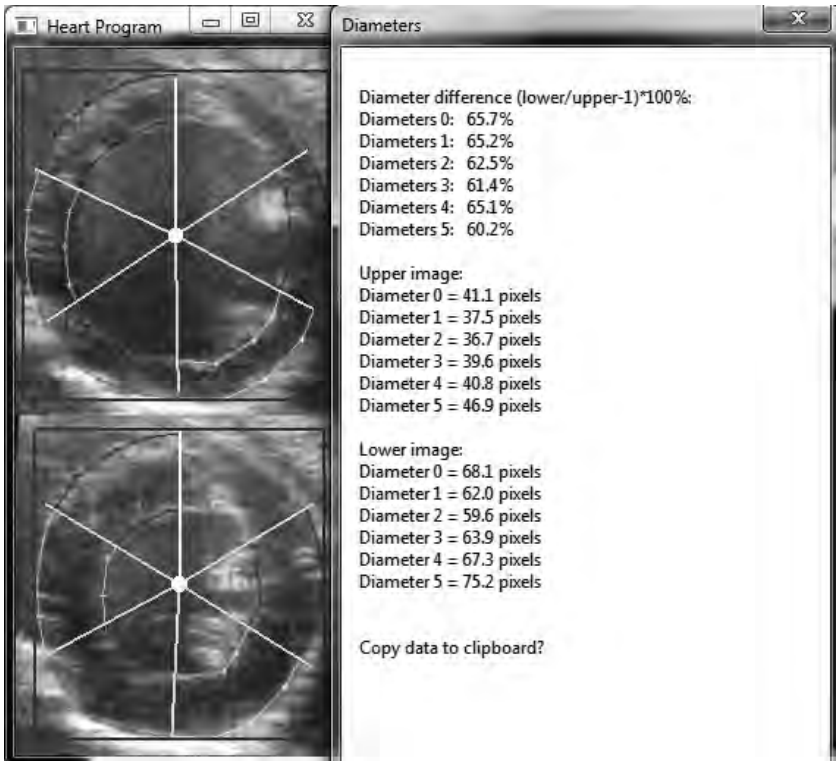

Figure 2. Demonstration of the method. The endo- and epicardial tissue borders are manually traced in end-diastole (upper) and end-systole (lower), respectively. The image is then imported by the program which identifies the pixels that belong to each of the 2 inner and outer walls. The center points (yellow) are computed as the center of a bounding box (blue) surrounding the outer walls. N equally long segments (n = 6 in this example) are auto-generated and the user can then manually drag the segments to his or her liking. Average thickness of each myocardial segment is then calculated at both time points and regional radial transmural end-systolic strain is derived. See text for details. The data can then be copied directly into a data handling program.

examination. The animals were anesthetized with isoflurane (1.0%), placed on a heating pad and connected to an ECG. Rectal temperature was monitored to maintain body temperature between 36 and 38°C. A 45 MHz linear transducer (RMV 707, Visualsonics) was used for imaging. An optimal parasternal long-axis (LAX) projection (i.e., visualization of both the mitral and aortic valves, and maximum distance between the aortic valve and the cardiac apex) was used for orientation. The probe was then rotated 90° and a parasternal short-axis (SAX) cine loops of >1000 frames/s were acquired at exactly 3 mm below the mitral annulus. In each SAX cine loop, endocardial and epicardial borders were traced in end-diastole and end-systole. Regional transmural end-systolic radial strain (RTESS) was calculated at each of the 3 SAX levels as described above. End-diastolic and end-systolic luminal areas (EDA and ESA, respectively) were derived by the endocardial border tracing. Fractional area change was estimated as FAC = (EDV − ESV)/EDV. Akinetic areas were traced in the cine loops and expressed as% of total left ventricular (LV) endocardial length (Fig. 2). Conventional echocardiographic indices were obtained from an M-mode tracing at 3 mm below the mitral annulus.[11]

Myocardial Infarction:

Myocardial infarction was induced as previously described in 8 mice,[12,13] with minor modifications to the protocol. Briefly, the mice were anesthetized with isoflurane (3%), intubated, connected to a mechanical ventilator, and ventilated on a mixture of isoflurane (2%) and air. The chest was shaved and electrodes were placed on the extremities. A left thoracotomy was performed through the fourth intercostal space and a 7-0 epsilon suture was passed below the left descending coronary artery at a point 3 mm below the left atrial appendage and the artery was permanently ligated. Induction of ischemia was verified by ECG and by observing a pale, akinetic ventricle below the suture. The chest was then closed and the animals were weaned off the ventilator and allowed to recovery in a partially heated cage. Per and postoperative analgesia was provided in the form of buprenorphine (Temgesic, 0.1 mg/kg).

Catecholamine-Induced Cardiotoxicity:
The mice received ISO (400 mg/kg) intraperitoneally and were then placed back in their cages and monitored for signs of distress.[7]

Statistics:
IBM SPSS statistics software (version 19; SPSS Inc., Chicago, IL, USA) was used for standard statistical analysis of the data. Kolmogorov–Smirnov test was used to analyze the appropriateness of assuming Gaussian distribution of the variables. Two-way analysis of variance (ANOVA) and Student's *t*-test with Tukey's method were used to detect any significant difference in strain between the segments within or between the different groups. All comparisons were specified in advance. P-values <0.05 were considered statistically significant. Data are presented as mean $\pm$ SD in text and tables and as mean + SEM in figures.

Results:
A total of 16 mice were used. All animals developed decreased locomotor activity and hair erection within 15 minutes after administration of ISO or induction of MI. Two mice in the MI group died before the echocardiographic examination.

Conventional indices of global cardiac structure and function are presented in Table I. Conventional indices of cardiac function, that is, FS and FAC, were significantly decreased post-MI but not post-ISO.

Coefficients of Variation:
The intra- and inter-observer coefficients of variation for segmental RTESS assessment were 5.11 and 7.32, respectively.

Cardiac Deformation Indices:
RTESS values are displayed in Table II. At baseline, average segmental RTESS was 56.75% and RTESS was similar in all cardiac segments regardless of how many segments the heart was divided into. Inhomogeneity in baseline strain values were observed when the heart was divided into >6 different segments. Post-MI the anterolateral wall was affected whereas the posterior wall was most severely dysfunctional post-ISO (Fig. 3).

In the akinetic myocardium of MI and ISO mice (26.22% and 47.36% length of the endocardium, respectively), RTESS was near zero and significantly different from the remaining myocardium.

Discussion:
In this article we describe a simple and straightforward approach to quantify regional myocardial deformation in mouse models of cardiovascular disease. We show that the technique is associated with acceptable intra- and inter-observer agreement and can successfully detect local decreases in myocardial strain in 2 different models that have both been previously shown to be associated with regional cardiac dysfunction.[7,14] Indeed, if one is willing to accept the premises that an experienced echocardiographer is able to reliably trace the tissue borders at both end-systole and end-diastole, and that the present algorithm adequately calculates the average distance between the endocardial and epicardial tracings, it is feasible to expect that such an approach would be likely to reliably assess regional cardiac radial deformation in cine loops of sufficient quality and frame rate.

TABLE I

Conventional Echocardiographic Parameters

	Baseline (N = 8)		Isoprenaline (N = 8)		Myocardial Infarction (N = 6)	
	Mean	SD	Mean	SD	Mean	SD
Heart rate	468	60	563*	23	490†	26
Extent of akinesia (%)	–	–	26.22*	9.50	47.36*†	10.73
One-dimensional						
End-diastolic diameter (mm)	4.00	0.28	4.05	0.54	5.41*†	0.73
End-systolic diameter (mm)	2.74	0.39	2.65	0.49	4.95*†	0.81
Fractional shortening (%)	31.77	5.90	34.77	5.67	8.72*†	4.17
B-mode						
End-diastolic area (mm^2)	11.93	1.29	12.30	3.24	23.13*†	5.26
End-systolic area (mm^2)	5.71	1.84	5.56	2.51	18.74*†	5.69
Fractional area change (%)	52.71	12.57	56.30	10.05	20.01*†	7.19

*P < 0.05 versus baseline.
†P < 0.05 versus isoprenaline.

TABLE II

Regional Radial Transmural End-Systolic Strain

Segmental Division		Baseline (N = 8), %		Isoprenaline (N = 8), %		Myocardial Infarction (N = 6), %	
N	Segment	Mean	SD	Mean	SD	Mean	SD
2	Hypo/akinetic	–	–	−1.29	4.78	−0.17	2.44
	Normokinetic	–	–	69.9	5.97	44.63	6.91
2	Lateral	57.08	6.66	62.84	5.60	6.98	2.85
	Septal	55.94	7.75	38.84	8.86	46.27	8.30
3	Anterolateral	57.10	6.66	96.73	10.17	−0.43	2.68
	Posterior	56.45	8.40	12.15	5.76	38.27	10.21
	Anteroseptal	56.03	7.36	61.21	12.87	42.1	6.12
4	Antrolateral	56.49	6.18	107.33	11.90	1.2	3.38
	Posterolateral	57.65	9.08	33.41	6.66	12.62	5.24
	Posteroseptal	56.45	8.40	11.98	8.59	56.07	11.15
	Anteroseptal	55.64	7.33	74.85	11.80	37.53	6.33
5	1	56.61	6.64	112.59	10.96	2.65	3.66
	2	56.59	9.18	60.95	8.00	−0.10	3.54
	3	57.80	9.08	48.38	7.27	40.15	13.78
	4	56.10	7.86	26.98	14.33	56.75	6.75
	5	55.96	7.93	83.53	10.93	35.28	6.33
6	Anterolateral	57.31	6.51	115.81	10.62	3.08	4.27
	Lateral	56.93	7.30	81.43	11.07	1.42	6.95
	Posterolateral	57.28	8.85	20.40	7.36	24.50	9.17
	Posteroseptal	55.71	9.34	4.83	6.05	56.17	13.91
	Septal	56.30	7.75	40.10	17.36	49.72	8.32
	Anteroseptal	56.98	8.01	88.52	10.26	33.96	7.41
7	1	58.14	7.20	117.95	10.30	4.52	4.96
	2	56.08	8.43	93.23	14.55	−3.13	4.26
	3	57.41	12.87	41.60	7.62	5.58	6.03
	4	58.05	10.57	2.55	7.26	41.10	15.18
	5	55.84	7.73	13.80	9.94	61.40	8.06
	6	54.33	8.39	49.89	17.01	48.8	6.01
	7	57.30	7.87	91.64	9.73	31.35	7.71
8	1	59.20	7.50	118.83	9.91	5.45	5.55
	2	53.93	9.22	99.58	15.20	−2.42	4.88
	3	57.06	11.56	58.86	7.33	−1.16	5.10
	4	59.73	8.50	14.03	8.74	26.02	8.52
	5	56.31	8.60	2.11	15.35	54.27	16.76
	6	57.21	10.35	24.94	15.89	59.02	6.05
	7	52.80	8.49	58.34	15.89	46.57	5.22
	8	57.75	9.17	94.28	8.98	30.75	8.72
9	1	59.53	7.90	119.1	9.3	6.45	6.24
	2	53.03	9.50	104.9	14.5	−2.17	4.39
	3	59.70	8.18	74.1	9.8	−4.02	3.94
	4	57.81	12.06	29.4	8.5	12.97	6.99
	5	58.26	11.75	0.8	6.7	41.40	15.39
	6	55.06	8.13	7.7	7.2	59.8	12.37
	7	57.94	8.79	32.6	17.7	56.30	6.94
	8	52.35	8.93	64.5	15.1	45.55	4.68
	9	59.20	8.98	95.7	8.6	30.50	9.24

RTESS values at baseline and postmyocardial infarction or isoprenaline administration for each myocardial segment. The heart can be divided into n number of segments (see Fig. 2). Furthermore, the segment borders can be manipulated to divide to heart into, for example, akinetic and normokinetic regions.

In this study we show that regional perturbations of myocardial function occur post-MI as well as post high dose ISO. MI was associated with significantly decreased indices of global cardiac function whereas ISO was not. ISO induces regional posterior wall myocardial dysfunction that is clearly visible to the naked eye. However, because anterolateral regions are

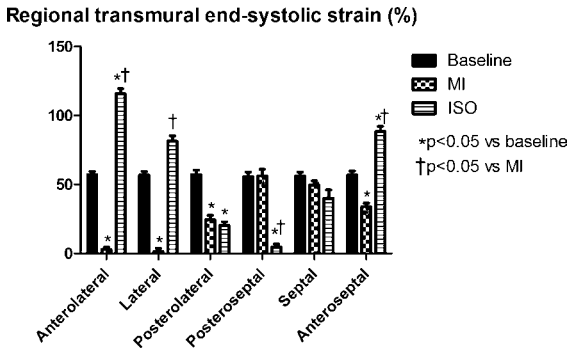

Figure 3. Regional radial transmural end-systolic strain (RTESS). When the heart was divided into 6 segments, RTESS was uniform at baseline. Post–myocardial infarction (post-MI) RTESS decreased to near zero in lateral segments whereas the posterior wall was most severely affected postisoprenaline (post-ISO) administration. Significant within group differences, defined as $P < 0.05$, were found in the MI (anterolateral, lateral, and posterolateral vs. septal) and ISO (anterolateral vs. posterolateral, posteroseptal, and septal; lateral vs. posterolateral and posteroseptal; groups; anterolateral vs. posterolateral, posteroseptal, and septal).

hyperkinetic due to the positive inotropic actions of ISO, conventional indices of global cardiac function are normal.[7]

Detection and quantification of regional myocardial dysfunction is becoming increasingly important in a variety of cardiovascular disease states. As clinicians implement sophisticated echocardiographic equipment in their search for meaningful indices of regional myocardial function that can reliably detect subclinical disease, evaluate response to therapy and/or predict outcome, small animal echocardiographers look to these modalities and algorithms for more detailed characterization of various experimental models of heart disease and interventions. Due to its independence of the incident angle, ST echocardiography has emerged as a potential means of achieving these goals.[3] Although several attempts have been made to implement these techniques in mice, published deformation data are not always convincing.[15–18] Among the most important concerns raised is the dependency on excellent image quality and the necessity of excluding animals where sufficient image quality is not achieved.[17,18]

In contrast to our previous attempts at quantifying regional cardiac deformation by manually tracing tissue borders, intra- and inter-observer variability were acceptable with the present approach.[7]

Manual tracing is dependent on an experienced echocardiographer to trace the tissue border both in diastole and systole. However, also automatized techniques are likely to require experienced handlers to ensure appropriate tissue tracking. Furthermore, the fact that manual approaches rely only on manually traced tissue borders allows for assessment of cine loops where a given segment is poorly visualized for a given number of frames, as long as the tissue borders are clearly visualized at end-diastole and end-systole, respectively, or as long as the echocardiographer is able to reliably extrapolate the tissue contour across that segment. This is of relevance as the cine loops sometimes, as is discussed above, may be of imperfect quality, especially when echocardiography is performed postthoracic surgery.[18,19]

Although sophisticated ST based algorithms have the potential to derive many useful indices of cardiac function beyond merely radial wall strain and are likely to play an important role in future experimental models of heart disease, until these techniques have been thoroughly validated and are widely available, simpler approaches for estimating regional myocardial function may be of value. Indeed, many of the present echocardiographic modalities, including the VEVO 770, allow for quantification of myocardial wall thickness after manual tracing of the endo- and epicardial borders. This can be done in systole as well as diastole and differs from the approach presented here mainly in that it does not calculate transmural strain, that is, ratio of systolic to diastolic wall thickness, and in that it does not allow for division of the heart into various cardiac segments. We believe that such minor upgrades would be of value in assessment of murine regional myocardial function.

Limitations:

The major limitation of this approach is that it allows for assessment of only radial strain and uses only endo- and epicardial reference points, that is, no points within the myocardium. It cannot quantify longitudinal or circumferential strains and does not derive instantaneous strain or strain rate. Furthermore, an experienced echocardiographer is required to reliably trace the tissue borders. In a >1000 frames/s cine loop, it may be difficult to decide which exact frame that best represents end systole.

This algorithm was not tested in severely dysmorphic hearts, that is, large bulging aneurysms. In these circumstances, the "center point" derived by the algorithm may not be satisfactory.

The absence of a gold standard method against which this algorithm could be validated can be considered a major limitation. A relatively small number of animals were used in this study.

Conclusion:

We describe a simple and straightforward approach to quantify regional myocardial deformation in mouse models of cardiovascular disease and suggest that similar approaches be included in the software.

Acknowledgments: The study was supported by grants from the Swedish Heart and Lung Foundation, the Swedish Research Council, Gothenburg Medical Society, and the Swedish Medical Society.

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