Assessment of Regional Myocardial Work in Rats

Emil K.S. Espe, MSc, PhD; Jan Magnus Aronsen, MD; Guro S. Eriksen, MSc; Lili Zhang, MSc, PhD; Otto A. Smiseth, MD, PhD; Thor Edvardsen, MD, PhD; Ivar Sjaastad, MD, PhD; Morten Eriksen, MD, PhD

Background—Left ventricular (LV) motion and deformation is dependent on mechanical load and do therefore not reflect myocardial energy consumption directly. Regional myocardial work, however, constitutes a more complete assessment of myocardial function.

Methods and Results—Strain was measured using high-resolution phase-contrast MRI in 9 adult male rats with myocardial infarction (MI) and in 5 sham-operated control animals. Timing of LV valvular events and LV dimensions were evaluated by cine MRI. A separate cohort of 14 animals (MI/sham=9/5) underwent measurement of LV pressure concurrent with identification of valvular events by Doppler-echocardiography for the purpose of generating a standard LV pressure curve, normalized to valvular events. The infarctions were localized to the anterolateral LV wall. Combining strain with timing of valvular events and a measurement of peak arterial pressure, regional myocardial work could be calculated by applying the standard LV pressure curves. Cardiac output and stroke work was preserved in the MI hearts, suggesting a compensatory redistribution of myocardial work from the infarcted region to the viable tissue. In the septum, regional work was indeed increased in MI rats compared with sham (median work per unit long-axis length in a mid-ventricular slice: 241.2 [224.1–271.2] versus 137.2 [127.0–143.8] mJ/m; P<0.001). Myocardial work in infarcted regions was zero. Additionally, eccentric work was increased in the MI hearts.

Conclusions—Phase-contrast MRI, in combination with measurement of peak arterial pressure and MRI-derived timing of valvular events, represent a noninvasive approach for estimation of regional myocardial work in rodents. (Circ Cardiovasc Imaging. 2015;8:e002695. DOI: 10.1161/CIRCIMAGING.114.002695.)

Key Words: magnetic resonance imaging ● myocardial contraction

A

alysis of myocardial deformation may provide important insight into left ventricular (LV) function. However, deformation parameters alone do not account for the variable mechanical load caused by LV pressure (LVP) variations.1 Regional myocardial work, however, has been shown to reflect regional metabolic demand and oxygen consumption and do therefore provide a more comprehensive assessment of myocardial function.2,3

See Clinical Perspective

Global LV work corresponds to the area of the LV pressure–volume loop, and regional work is accordingly reflected in the area of the local myocardial force–segment length loop.4 Myocardial force, however, is challenging to measure. Substitution of force with LVP and segment length with strain has been shown to constitute a suitable and powerful index of regional myocardial work.2,5 As shown previously in humans and dogs, intraventricular pressure can be estimated noninvasively by normalizing a standard LV pressure curve to peak arterial pressure and the duration of the isovolumic and ejection phases.2 Moreover, by incorporating regional radius of curvature and LV short-axis circumference, regional myocardial work can be calculated, facilitating comparison between regions within a heart and between hearts.

Small animal models play a vital role in the study of cardiac disease mechanisms, and there is a great need for methodology for evaluation of in vivo myocardial function in small animals. Several methods exist for assessment of myocardial deformation in small animals, including ultrasound speckle tracking and tissue Doppler, as well as a variety of MRI-based techniques. Challenges with 2D echocardiography include restrictions in available imaging projections and, in small animal hearts, limited spatial resolution. MRI provides superior geometric freedom and thus allows standardized projections, regardless of heart geometry. This is an absolute requirement when assessing regional function over time, for instance when evaluating disease progression. Phase-contrast MRI (PC-MRI) offers measurement of the geometry, motion,6,7 and

Received January 24, 2014; accepted January 15, 2015.

From the Institute for Experimental Medical Research (E.K.S.E., J.M.A., G.S.E., L.Z., I.S.) and Center for Cardiological Innovation (O.A.S., T.E., M.E.), Oslo University Hospital and University of Oslo, Oslo, Norway; KG Jeffs Cardiac Research Center and Center for Heart Failure Research, University of Oslo, Oslo, Norway (E.K.S.E., G.S.E., L.Z., I.S.); Bjerkestrøm College, Oslo, Norway (J.M.A.); Norwegian Institute of Public Health, Oslo, Norway (G.S.E.); and Department of Cardiology (O.A.S., T.E.) and Institute for Surgical Research (O.A.S., T.E., M.E.), Oslo University Hospital, Rikshospitalet, Oslo, Norway.


Correspondence to Emil K.S. Espe, Institute for Experimental Medical Research, Oslo University Hospital Ullevål, Kirkeveien 166, N-0407 OSLO, Norway. E-mail ekespe@medisin.uio.no

© 2015 American Heart Association, Inc.

Circ Cardiovasc Imaging is available at http://circimaging.ahajournals.org DOI: 10.1161/CIRCIMAGING.114.002695
strain of the myocardium with high spatiotemporal resolution. No studies have evaluated in vivo regional work in small animal hearts.

The aims of this study are therefore to (1) investigate whether regional LV myocardial work can be calculated from MRI-derived myocardial strain and geometry in combination with an estimate of LVP and (2) as a proof-of-concept, evaluate regional myocardial work in a rat model of myocardial infarction and compare findings to data from sham-operated controls.

Methods

Animal Preparation
All animals were cared for according to the Norwegian Animal Welfare Act, and the use of animals was approved by the Norwegian Animal Research Authority (ID: 3284), conforming to ETS no. 123.

Anesthesia was induced in male Wistar rats (300 g) with 64% N2O, 32% O2, and 4% isoflurane, and they were subsequently ventilated on a Zöevent ventilator through endotracheal intubation. Anesthesia was maintained by 65% N2O, 32% O2, and 1.5% to 3% isoflurane. LV myocardial infarction (MI) was induced by proximal ligation of the left coronary artery, as previously described in detail. Sham-operated rats underwent the same procedure except ligation.

Six weeks after surgery, 28 rats (sham/MI=10/18) were randomly allocated to 2 equal cohorts; one to create a standard LVP curve and one to evaluate myocardial work.

Standard Pressure Curve Generation
For the purpose of generating a standard LVP curve, 14 rats (sham/MI=5/9) were anesthetized and ventilated as described earlier. To acquire LVP tracings, a 1.4F micrometer-tipped catheter (Millar Inc., USA) was inserted retrogradely via the right common carotid artery into the left ventricular cavity. Simultaneously, flow through the mitral and aortic valves were measured by Doppler echocardiography using a Vevo 2100 ultrasound scanner (VisualSonics, Canada), allowing determination of the timing of opening and closing of the mitral and aortic valves. Echocardiography allowed, in contrast to MRI, real-time recording of valvular events concurrent with measurement of LVP. ECG-synchronized measurements of LVP and timing of LV valvular events were recorded at 1 kHz. The individual pressure tracings were normalized to peak pressure and temporally matched to the valvular events, and the waveforms were averaged to produce a standard LVP curve.

MRI Experiments
To evaluate regional myocardial work, 14 rats (sham/MI=5/9) underwent a comprehensive MRI examination on a 9.4T MR system (Agilent Technologies, Inc., USA) as previously described. All MRI acquisitions were respiration-gated and prospectively triggered at the peak of the R-wave. Anesthesia was induced using a mixture of O2 and 4.0% isoflurane and maintained using O2 and 1.5% to 3.0% isoflurane, in freely breathing animals. ECG, respiration, and body temperature was monitored throughout the examinations; the latter maintained by heated air. In all MR experiments, temporal resolution was equal to the repetition time (TR).

Cine MRI Experiments
Stacked LV short-axis slices covering the whole LV were acquired using a radio frequency spoiled gradient echo cine sequence. Imaging parameters were TE=1.97 ms; TR=2.80 ms; field-of-view=45x45 mm; matrix 192x192 after 2x zero filling; slice thickness 1.5 mm; flip angle 15°; signal averaging=3x. Acquisition time for a complete cine stack was ≈20 minutes.

In addition, a single cine slice was oriented obliquely to include both LV valves (Figure 1A). Acquisition was set to cover >100% of the R-R interval to ensure complete coverage of whole cardiac contraction–relaxation cycle. Imaging parameters were as follows: TE=2.04 ms; TR=2.96 ms; field-of-view=50x50 mm; matrix 128x128; slice thickness 2.0 mm; flip angle 15°; signal averaging=3x. Acquisition time was 2 to 3 minutes.

PC-MRI Experiments
In the PC-MRI protocol, a 9-point velocity-encoded black-blood gradient echo cine sequence was used as previously described. Three short-axis slices were planned: basal, mid-ventricular, and apical (+3, 0, and −3 mm from the longitudinal midpoint of the ventricle), also covering >100% of the R-R interval. PC-MRI imaging parameters were TE=2.22 to 2.26 ms; TR=2.93 to 3.21 ms; field-of-view=50x50 mm; matrix 128x128; slice thickness 1.5 mm; flip angle 7°; venc=13.9 cm/s. Acquisition time for a complete PC-MRI slice (with 2x signal averaging using rotating field-of-view) was 15 to 20 minutes.

Arterial Pressure and Body/Organ Weighing
After MRI, peak arterial pressure was measured in the ascending aorta by catheterization as described earlier (1.4F Millar catheter introduced through the right common carotid artery). This was done >24 hours (~72 hours) after MRI using the same anesthesia setup and settings as in MRI experiments, allowing reproduction of the pressure conditions occurring during MRI acquisition of the strain data. The rats were then euthanized by neck dislocation, and body and organ...
weights were measured. The hearts were drained for blood before weighing.

**MRI Postprocessing**

**Cine MRI Analysis**

In the MRI cine loops, the endo- and epicardial borders were manually tracked in all slices in end-diastole and end-systole. Myocardial and blood pool volumes, stroke volume, ejection fraction, and cardiac output were calculated. Also, LV long-axis diameter and myocardial volumes were calculated, and the size of the infarcted regions, defined as regions of akinesis and by its lack of radial thickening in end-systole, was measured. All myocardial mass was found from myocardial volume using a conversion factor of 1.05 g/cm³. The timing of aortic valve opening and closure and mitral valve opening and closure were identified by visual examination of the valve cine loop (Figure 1A). LVP was then estimated by scaling the standard curve (Figure 2B) to peak arterial pressure and temporally matched to the 4 LV valvular events.

Additionally, total LV stroke work was calculated in the sham rats as stroke volume multiplied by mean systolic LVP. Stroke work per unit muscle mass was found by dividing stroke work by myocardial mass.

**PC-MRI Analysis**

PC-MRI analysis included manual tracing of the endo- and epicardium as previously described. All operator inputs, including MR imaging segmentation, were done under identical brightness and contrast settings to ensure consistency between subjects. Each of the 3 slices was divided into 32 sectors in which regional circumferential strain was calculated as previously described. From the segmentation masks, end-diastolic wall thickness and mid-myocardial circumferential length (see Figure 1C for definitions) were found in each sector and filtered using a 5-sector running average. LV in-slice circumference was found as the sum of the circumferential length from all sectors. Time-resolved sector-wise radius of curvature was found the following way: first, the in-plane point that minimized the variance in distance to 5 neighboring sectors was calculated as the mean distance from the 5 sectors to this point.

Because both the valve- and PC-MRI data sets covered >100% of the R-R interval, the mean heart rate during acquisition could be calculated in these data sets. Any subtle fluctuation in heart rate between acquisitions was corrected by matching the duration (using cubic spline interpolation) of the individual R-R intervals.

In addition, the thickness of the right ventricular (RV) free wall and the RV short-axis diameter was measured at the level of the basal LV slice.

**Calculation of Regional Myocardial Work**

Sector-wise myocardial work was calculated from strain, radius of curvature, and the estimated LVP (using the Young–Laplace equation; see the Data Supplement). Because only the circumferential component of deformation was included, we labeled this parameter circumferential regional myocardial work (cRMW).

Subsequently, work per unit long-axis (ie, through-plane) length (cRMW_L), eccentric work (cRMW_EW), and work per unit muscle mass (cRMW_M) were calculated in each of the 32 sectors in each slice from cRMW, circumferential length, and wall thickness (see the Data Supplement).

In each individual slice, the septal and lateral sectors were identified to allow comparison of regional strain and work between groups. In addition, in the MI hearts, the sectors representing infarcted regions (identified by substantial thinning and akinesia) were identified. Finally, whole-slice strain, cRMW, and cRMW_M were calculated, and LV stroke work was approximated by multiplying the average whole-slice cRMW from each rat with the long-axis diameter of the ventricle found from the cine MRI data.

To explore the effect of curvature to the calculations, supplemental calculations were performed where the effect of curvature was omitted.

**Myocardial Collagen Content**

Mid-septal samples (collected midway along both the long and short axis of the septum) from 3 representative sham and MI hearts were fixed in 4% formaldehyde and embedded in paraffin, and 4 µm sections were stained with Masson’s trichrome and photographed using 40x objectives. Myocardial collagen content was determined by an automated ImageJ-based protocol calculating the fraction of the images representing collagen/fibrosis.

**Data Analysis and Statistics**

All data analysis and statistics were done in Matlab (The MathWorks, USA) and ImageJ (NIH, USA). Results are reported as median (1st quartile–3rd quartile). The 2-sided Mann–Whitney U-test was used to evaluate statistical significance between groups. P < 0.05 was considered statistically significant. There were not any adjustments for multiple comparisons.

**Results**

**Animal Characteristics**

In the MI rats, heart and lung weights were increased by ~50% and 135%, respectively, compared with sham (Table 1). Neither body weight nor peak arterial pressure was significantly different between groups.

**Standard LVP Curve**

Figure 2A shows the LVP–time curves with valvular events from each of the 14 rats. In Figure 2B, the resulting ensemble-averaged standard LVP curve is shown with valvular events denoted. Although the diastolic pressures vary noticeably, reflecting the diversity of the cohort, the pressure curves from the individual animals exhibit similar shapes when the valvular events are aligned, and the curves are scaled to the same systolic peak pressure.

**Figure 2.** Normal pressure curves for rats.

A, Time-left ventricular pressure (LVP) curves were measured in 14 rats. Concurrently, the timing of the LV valvular events was determined by echocardiography. Both LVP and valvular events were coregistered with ECG tracings, allowing offline synchronization. ○, MVC; □, AVo; ●, AVc; ×, MVo.

B, The individual pressure tracings (grey) were normalized to peak pressure and temporally fitted to match valvular events. A normal time–LVP pressure curve (black) was generated as the average of the individual normalized tracings. AVc indicates aortic valve closure; AVo, aortic valve opening; MVC, mitral valve closure; and MVo, mitral valve opening.
All MI rats had medium-sized infarctions (median infarct size was 33.1% [31.2%–39.6%]) localized in the anterolateral wall (Figure 1B).

End-diastolic and end-systolic LV volumes were markedly increased and ejection fraction was reduced by nearly 60% in the MI hearts compared with sham (Table 2). Stroke volume and heart rate, and thus cardiac output, were not significantly different between the groups; however, in the MI rats, long-axis ventricular diameter and LV myocardial mass were increased by 25% and 10%, respectively (Table 2).

Wall Thickness, Circumference, and Radius of Curvature

The end-diastolic wall thickness in septum was not significantly different in the MI rats compared with sham, but the lateral wall (ie, in the ischemic regions) was thinner in all slices in MI (Table 2). Also, in the MI rats, the in-slice LV circumference was increased by 28% to 52% in all slices compared with sham, and the septal radius of curvature (measured midway between aortic valve opening and aortic valve closure) was increased by 49% to 98% (Table 2).

RV free wall thickness was not significantly different between groups (1.03 mm [0.93–1.19 mm] versus 1.08 mm [0.90–1.12 mm] in sham/MI, P = 0.61), nor was RV short-axis diameter (2.97 mm [2.70–3.35 mm] versus 3.45 mm [2.29–3.96 mm] in sham/MI, P = 0.80).

### Table 1. Animal Data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham (N=5)</th>
<th>MI (N=9)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart weight, g</td>
<td>1.30 [1.08–1.45]</td>
<td>1.97 [1.83–2.04]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lung weight, g</td>
<td>1.40 [1.27–1.50]</td>
<td>3.29 [2.27–3.94]</td>
<td>0.009</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>403 [388–439]</td>
<td>422 [403–490]</td>
<td>0.32</td>
</tr>
<tr>
<td>Peak arterial pressure, mmHg</td>
<td>120 [116–140]</td>
<td>118 [111–126]</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Data from the rats included in MR analysis. Values are median [1st quartile–3rd quartile]. MI indicates myocardial infarction.

### Table 2. MRI Geometry Data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham (N=5)</th>
<th>MI (N=9)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>End-diastolic volume, mL</td>
<td>0.46 [0.43–0.50]</td>
<td>1.02 [0.93–1.17]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>End-systolic volume, mL</td>
<td>0.14 [0.10–0.15]</td>
<td>0.72 [0.62–0.83]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stroke volume, mL</td>
<td>0.34 [0.29–0.35]</td>
<td>0.34 [0.31–0.35]</td>
<td>1</td>
</tr>
<tr>
<td>Ejection fraction</td>
<td>0.72 [0.69–0.76]</td>
<td>0.31 [0.28–0.33]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean heart rate during acquisition, bpm</td>
<td>418 [400–427]</td>
<td>362 [339–419]</td>
<td>0.14</td>
</tr>
<tr>
<td>Cardiac output, mL/min</td>
<td>136 [123–144]</td>
<td>136 [107–145]</td>
<td>0.70</td>
</tr>
<tr>
<td>Long-axis diameter, mm</td>
<td>12.0 [11.6–13.5]</td>
<td>15.0 [14.6–16.5]</td>
<td>0.006</td>
</tr>
<tr>
<td>LV myocardial mass, mg</td>
<td>659 [648–690]</td>
<td>723 [694–782]</td>
<td>0.007</td>
</tr>
<tr>
<td>Stroke work, mJ</td>
<td>4.80 [4.43–5.18]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke work per unit muscle mass, mJ/g</td>
<td>7.29 [6.73–7.65]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wall thickness, mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral (3-sector mean)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1.81 [1.57–1.89]</td>
<td>0.95 [0.78–1.20]</td>
<td>0.012</td>
</tr>
<tr>
<td>MV</td>
<td>1.85 [1.70–2.05]</td>
<td>0.82 [0.74–0.92]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>A</td>
<td>1.84 [1.62–1.92]</td>
<td>0.78 [0.74–1.01]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Septum (3-sector mean)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1.47 [1.47–1.64]</td>
<td>1.68 [1.53–2.00]</td>
<td>0.15</td>
</tr>
<tr>
<td>MV</td>
<td>1.47 [1.21–1.51]</td>
<td>1.72 [1.50–2.02]</td>
<td>0.05</td>
</tr>
<tr>
<td>A</td>
<td>1.37 [1.27–1.45]</td>
<td>1.60 [1.38–1.93]</td>
<td>0.24</td>
</tr>
<tr>
<td>Mid-systolic septal radius of curvature, mm</td>
<td>4.5 [4.5–4.6]</td>
<td>6.7 [6.1–6.9]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B</td>
<td>4.3 [4.1–4.6]</td>
<td>6.7 [6.4–7.7]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MV</td>
<td>3.1 [2.7–3.9]</td>
<td>6.1 [5.8–7.0]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>A</td>
<td>20.8 [20.7–21.7]</td>
<td>32.3 [29.5–36.4]</td>
<td>0.012</td>
</tr>
</tbody>
</table>

LV data derived from the cine MRI experiments and PC-MRI segmentation masks. Values are median [1st quartile–3rd quartile]. B, MV, and A refers to the basal, mid-ventricular, and apical slices. LV indicates left ventricle; MI, myocardial infarction; and PC-MRI, phase-contrast MRI.
Regional Myocardial Work

Strain, LVP traces, and surface tension–strain loops from 2 representative rats (one sham and one MI) are shown in Figure 3. Peak regional strain in the lateral wall was reduced in the MI hearts, as was peak whole-slice strain (Table 3). Peak septal strain was not significantly altered. Septal $cRMW_L$ was larger in MI than in sham in the basal and mid-ventricular slices (Table 3 and Figure 4). Lateral-wall work was reduced in the MI rats, and the total work in the infarcted regions alone was not significantly different from zero. The work done per slice was not significantly different between groups.

Both septal and whole-slice eccentric work ($cRMW_{LW}$) were higher in MI rats in all slices (Table 3). Work per unit mass ($cRMWM$) in the septum was not significantly different between the groups.

Estimated LV stroke work from mean whole-slice work multiplied with long-axis diameter was not significantly different between groups (3.70 mJ [2.97–3.95 mJ] versus 3.76 mJ [3.28–4.38 mJ] in sham/MI, $P=0.70$).

In the supplemental calculations omitting the effect of curvature, septal work was no longer significantly different between groups.

As described in more detail in the Data Supplement, supplemental experiments were performed comparing invasive LVP measurements with systolic pressure measured by tail-cuff, confirming that systolic pressure can be measured non-invasively in rats.

Collagen Content

The septal myocardial samples revealed no difference in interstitial or perivascular fibrosis between groups (6.87% [6.02%–7.34%] versus 6.70% [5.95%–7.71%] in sham/MI, $P=0.96$; Figure 5).

Discussion

We have previously shown distinct alterations in regional myocardial motion and strain in infarcted rat hearts compared with normal controls.6 In the present study, we evaluate a technique for MRI-based assessment of myocardial work...
and demonstrate that this provides additional value compared with strain alone in revealing a compensatory redistribution of work in the infarcted heart, compared with control.

A method for estimation of LV work based on echocardiography has been applied in dogs and humans. However, in rodents, echocardiography suffers from limitations caused by restrictions in available projections, variability in segmentation, and acoustic access and exhibits a considerable interobserver variability. In the present work, we therefore used MRI which circumvents the mentioned limitations and offers a supreme ability in standardization of the geometry.

This facilitates assessment of regional function over time, for instance in a remodeling heart.

Regional work has previously been estimated in canine hearts using 3D tagged MRI, reporting stroke work per myocardial mass in normal hearts to be 2.42 to 2.96 mJ/g. Another study on canine hearts reports 1.3 to 1.5 kJ/m³, equivalent to 1.2 to 1.4 mJ/g using a myocardial weight/volume ratio of 1.05. In humans, LV work per mass has been reported to be 3.1 to 7.0 mJ/cm³, equivalent to 3.0 to 6.7 mJ/g. In the current study, we found work per unit mass in healthy rodent myocardium to be in the range 4.3 to 7.6 mJ/g (Tables 2 and 3), higher than in dogs but lower than in humans.

Table 3. Strain and Work

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Region</th>
<th>Slice</th>
<th>Sham (N=5)</th>
<th>MI (N=9)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak circ. strain, %</td>
<td>Septum (mean)</td>
<td>B</td>
<td>−20.2 [−20.4 to −17.3]</td>
<td>−19.5 [−20.7 to −17.3]</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MV</td>
<td>−18.6 [−22.1 to −18.3]</td>
<td>−18.8 [−20.6 to −17.1]</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>−18.0 [−20.1 to −15.4]</td>
<td>−14.1 [−17.7 to −11.7]</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Lateral wall (mean)</td>
<td>B</td>
<td>−18.1 [−18.5 to −17.7]</td>
<td>−0.3 [−3.2 to 0.3]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MV</td>
<td>−19.5 [−19.9 to −18.6]</td>
<td>−1.5 [−2.9 to 0.5]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>−19.2 [−19.9 to −16.9]</td>
<td>0.2 [−2.4 to 2.3]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Infarction (mean)</td>
<td>B</td>
<td>0.1 [−1.3 to 2.1]</td>
<td>1.0 [−0.8 to 1.8]</td>
<td>1.7 [0.41 to 2.5]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MV</td>
<td>201 [176 to 219]</td>
<td>37.1 [21.7 to 92.5]</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>97.5 [84.9 to 170.6]</td>
<td>26.8 [−2.2 to 65.0]</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Whole slice (mean)</td>
<td>B</td>
<td>−18.8 [−19.4 to −17.4]</td>
<td>−9.5 [−10.7 to −8.6]</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MV</td>
<td>−19.7 [−20.1 to −18.8]</td>
<td>1.5 [−9.8 to −8.8]</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Work, mJ/m</td>
<td>Septum (sum)</td>
<td>B</td>
<td>161 [147 to 181]</td>
<td>161 [198 to 257]</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>MV</td>
<td>137 [127 to 144]</td>
<td>241 [224 to 271]</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>76.5 [65.7 to 82.0]</td>
<td>118 [76 to 165]</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lateral wall (sum)</td>
<td>B</td>
<td>201 [176 to 219]</td>
<td>37.1 [21.7 to 92.5]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>MV</td>
<td>201 [184 to 224]</td>
<td>39.3 [13.2 to 82.2]</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>97.5 [84.9 to 170.6]</td>
<td>26.8 [−2.2 to 65.0]</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Infarction (sum)</td>
<td>B</td>
<td>25.8 [6.9 to 44.6]</td>
<td>8.6 [−0.7 to 46.1]</td>
<td>10.5 [5.0 to 45.9]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MV</td>
<td>6.07 [5.08 to 7.21]</td>
<td>7.55 [6.17 to 9.29]</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>Whole slice (sum)</td>
<td>B</td>
<td>380 [352 to 406]</td>
<td>318 [237 to 347]</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MV</td>
<td>362 [338 to 386]</td>
<td>288 [270 to 341]</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Eccentric work, mJ/m</td>
<td>Septum (sum)</td>
<td>B</td>
<td>−5.7 [−7.0 to −4.6]</td>
<td>−21.5 [−70.1 to −12.0]</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>MV</td>
<td>−2.4 [−3.2 to −1.8]</td>
<td>−7.7 [−19.71 to −4.3]</td>
<td>0.042</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>−2.5 [−5.1 to −0.9]</td>
<td>−9.1 [−13.1 to −5.9]</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>Whole slice (sum)</td>
<td>B</td>
<td>−6.2 [−12.4 to −5.9]</td>
<td>−97 [−108.5 to −74.4]</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MV</td>
<td>−3.4 [−5.2 to −2.2]</td>
<td>−64.5 [−88.3 to −58.7]</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>−4.5 [−10.5 to −2.7]</td>
<td>−56.4 [−91.4 to −46.6]</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Work, mJ/g</td>
<td>Septum (mean)</td>
<td>B</td>
<td>6.89 [5.17 to 7.35]</td>
<td>5.43 [4.78 to 5.86]</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>MV</td>
<td>6.07 [5.08 to 7.21]</td>
<td>7.55 [6.17 to 9.29]</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>4.30 [4.06 to 4.85]</td>
<td>5.24 [4.43 to 5.89]</td>
<td>0.24</td>
<td></td>
</tr>
</tbody>
</table>

Strain and work parameters derived from the PC-MRI experiments. Values are median [1st quartile–3rd quartile]. B, MV, and A indicate the basal, mid-ventricular, and apical slices; cRMWL, accumulated circumferential regional myocardial work (cRMW) per unit long-axis length; cRMWL EW, eccentric cRMW per unit length during systole and isovolumic relaxation; cRMWM, cRMW per unit mass; MI, myocardial infarction; and PC-MRI, phase-contrast MRI.
closely agreeing with the human estimates. Although this might reflect actual differences between the species, the discrepancy could also be ascribed methodological differences.

Calculation of Work
Regional work equals the area of the myocardial force–segment length loop. In this study, LVP served as a substitute for myocardial force. Replacing force with pressure directly in the loop–area calculation results in an index of regional myocardial work, which has been shown to correspond closely with myocardial metabolism and supports its use as an adequate replacement for myocardial work. However, this index does not allow direct comparison within and between hearts with varying radius of curvature. In the present study, infarcted hearts with a considerable variation in regional geometry were used, and regional mechanical power per unit surface could be estimated by implementing a measure of the local radius of curvature. cRMWL and cRMWM were subsequently calculated by incorporating myocardial circumferential length and wall thickness, respectively.

The presented method is, in principle, applicable to any source of myocardial strain, for instance from myocardial tagging MRI. Although the latter often is considered the gold standard in MR assessment of myocardial function, we chose PC-MRI because it provides a higher data yield (thus potentially more complete characterization of the myocardium from a single acquisition) by offering, in addition to regional strain, both regional velocities and (nearly) uninterrupted magnitude images (see for instance, Figure 1B).

Regional Work
In porcine models of myocardial infarction, peak circumferential strain in the remote zone has been shown to be not significantly different to control animals. This is in line with our findings of unchanged strain in the septum. Furthermore, in the MI rat model, a hypertrophic response in myocardium remote to the infarction is well-documented in animals with small- to medium-sized infarctions, and regional defects in glucose metabolism have previously been demonstrated using positron emission tomography. In humans, it has also previously been shown that regional blood flow in the remote zone is increased in infarcted hearts and that those regions exhibit hyperfunction compared with controls. Similarly, we found that the pattern of work done by the infarcted hearts was prominently different than in control hearts. cRMWL in the lateral wall

![Figure 4. Regional work. Regional work per unit length (cRMWL) measured in the 6 regions selected, allowing comparison of septal and lateral regions. The work done in the lateral wall of the myocardial infarction (MI) rats, where the infarction was localized, was reduced in all slices. An increase in regional myocardial work was found in the septum in the basal and mid-ventricular slices, related to increased septal mass and increased radius of curvature. Data are shown as median with 1st and 3rd quartiles, and P values are relative to corresponding region in sham; (N=5/9 for sham/MI).](http://circimaging.ahajournals.org/)

![Figure 5. Myocardial collagen content (40x magnification). No increased extracellular volume or fibrosis was found in midseptal LV biopsies from post–myocardial infarction (MI) rat hearts. Top, Masson’s TC staining of 3 representative sham hearts. Bottom, 3 representative post-MI hearts.](http://circimaging.ahajournals.org/)
(coincident with the infarcted region) was eliminated, and septal cRWMv was increased. This suggests a compensatory mechanism for the work lost in the lateral wall and is consistent with our finding of sustained stroke work in the MI hearts. Both LV circumference and long-axis diameter were increased, and wall thickness was unaltered in the septum of the MI hearts, implying increased septal myocardial mass. This is consistent with earlier results. Increased septal cRWMv is therefore attributed to the combination of increased in-slice myocardial mass in the septum of the MI hearts and sustained septal cRWMv rather than increased work per unit mass of viable tissue.

We did not observe negative work in the infarcted regions, which may be explained by tethering of the myocardium to the chest wall following compromised pericardium after surgery, restricting passive stretching during systole.

The supplemental calculations omitting the effects of curvature did not demonstrate any significant difference in septal work between the groups. This suggests that septal flattening in the MI animals is an important contributor to our finding on increased work in the septum in the MI rats. This is in accordance with previous results. The histological images indicated no increase in extracellular volume in the MI septum, suggesting that the mass–muscle volume ratio was not altered in the diseased hearts. Furthermore, there was no indication of increased fibrosis in the MI septal myocardium, which could have affected the work done by the cardiomyocytes.

**Young–Laplace Equation**

The Young–Laplace equation is in principle only valid for thin membranes with negligible inner forces. However, the information required to do more precise calculations is not easily acquired. Nevertheless, comparison of end-systolic circumferential stress in infarcted sheep hearts with finite element calculations has demonstrated that the Young–Laplace approximation is a reasonable approximation in regions remote to the infarction. The work calculation also relies on the assumption that there are no external forces acting on the myocardium. This condition may be compromised in regions close to the infarction because we did observe a tethering effect between the myocardium and the chest wall in the MI rats. Furthermore, in circumstances with right ventricular hypertension, septal work would be overestimated because the LVP estimate used for calculation of septal wall tension would be higher than the actual trans-septal pressure gradient. A low trans-septal pressure gradient could also have caused the septal radius of curvature to approach infinity, which this model would interpret as an isometric tension also approaching infinity. However, there were no signs of RV hypertrophy in the infarcted hearts in the present study. Thus, we concluded that there were no significant alterations in RV geometry or any RV hypertrophy because of increased RV pressure.

**Blood Pressure Measurement and Organ Weights**

Our study used invasive pressure measurements. However, by using a noninvasive measure of peak arterial pressure, the method of work calculation can be completely noninvasive as previously validated. We chose an invasive blood pressure approach because it is considered the gold standard for pressure assessment. Nevertheless, a noninvasive tail-cuff technique has been validated in mice, and comparison with invasive blood pressure measurements in our laboratory confirmed that peak arterial pressure can be measured by tail-cuff also in rats and is therefore expected to produce similar results (see the Data Supplement). This allows for longitudinal studies on regional myocardial work.

Noticeably, the relative increase in whole-heart weight (Table 1) was greater than the relative increase in LV myocardial mass (Table 2). This is attributed to the hypertrophic and dilated left atrium which contributed largely to the organ weight, in addition to the presence of clotted blood in the atrium that was not removed before weighing.

**Limitations**

The presented method lacks a reference technique for calculation of regional myocardial work. However, the 2 different estimations of stroke work (from cine MRI and PC-MRI) in sham rats agreed closely, and the distribution of regional work found in infarcted hearts corresponded to expected values and previous findings. The present calculations of myocardial work did not incorporate longitudinal strain or longitudinal radius of curvature, which would constitute a more complete representation of myocardial work. Furthermore, estimating LVP from a normal curve introduces uncertainties, especially in the diastole of diseased animals.

Some of the (negative) work during diastole caused by passive filling, represented by the area between the loop and the x-axis in Figure 3, might be stored as elastic stretching of the myocardium, which in part will be returned in the following systole. Supplementary calculations of work only during systole and isovolumic relaxation (not shown) resulted in higher absolute values, but the same differences.

**Conclusions**

Work analysis allows comprehensive evaluation of myocardial function. In this study, we have used MRI and LVP estimates to calculate regional myocardial work in the rat LV. We demonstrate distinct alterations in regional work in the infarcted rodent hearts, compared with control. In the infarcted hearts, we demonstrate increased regional work in viable tissue, and our findings suggest that this can be attributed to an increase in muscle mass in the septum rather than increased work per unit mass of viable tissue. By applying a noninvasive measure of peak arterial pressure, this method constitutes a noninvasive technique for evaluation of regional myocardial work.

**Sources of Funding**

This work was supported by the South-Eastern Norway Regional Health Authority, Anders Jahre’s Fund for the Promotion of Science, the Research Council of Norway, and Familien Blix’ Fond Til Fremme Av Medisinsk Forskning.

**Disclosures**

None.
References


CLINICAL PERSPECTIVE

The motion and deformation of the left ventricle is dependent on mechanical load and do therefore not directly reflect myocardial energy consumption. An expression of regional myocardial work, however, would allow a more comprehensive evaluation of myocardial function. In this study, we have used MRI and an estimate of intraventricular left ventricular pressure based on standard left ventricular pressure curve, normalized to valvular events and a measurement of peak systolic pressure, to calculate regional myocardial work in the left ventricle of rats. We demonstrate distinct alterations in regional work in the hearts of rats with myocardial infarction compared with controls. In the infarcted hearts, the presented method reveals increased regional work in viable tissue, and our findings suggest that this can be attributed to an increase in muscle mass in the septum. By applying a noninvasive measure of peak arterial pressure, this method constitutes a noninvasive technique for evaluation of regional myocardial work.
Assessment of Regional Myocardial Work in Rats
Emil K.S. Espe, Jan Magnus Aronsen, Guro S. Eriksen, Lili Zhang, Otto A. Smiseth, Thor
Edvardsen, Ivar Sjaastad and Morten Eriksen

_Circ Cardiovasc Imaging_. 2015;8:
doi: 10.1161/CIRCIMAGING.114.002695
_Circulation: Cardiovascular Imaging_ is published by the American Heart Association, 7272 Greenville Avenue,
Dallas, TX 75231
Copyright © 2015 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-9651. Online ISSN: 1942-0080

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circimaging.ahajournals.org/content/8/2/e002695

Data Supplement (unedited) at:
http://circimaging.ahajournals.org/content/suppl/2015/02/11/CIRCIMAGING.114.002695.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation: Cardiovascular Imaging_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation: Cardiovascular Imaging_ is online at:
http://circimaging.ahajournals.org//subscriptions/
Supplemental Material

APPENDIX

Calculation of work

In each sector, the surface tension was calculated using the Young-Laplace equation from LVP(t) (in units Pa) and R(t) (the local myocardial radius of curvature; in units m). Through multiplication with the dimensionless circumferential strain rate \( \dot{\varepsilon} \), instantaneous regional mechanical power per unit surface was found:

\[
P_S(t) = -LVP(t) \cdot R(t) \cdot \dot{\varepsilon}(t) \quad [W/m^2] \quad [1]
\]

The time integral of Eq. 1 over the whole cardiac cycle equals the area of the surface tension-strain loop, representing regional myocardial work per unit surface (Fig 3).

Multiplying \( P_S(t) \) with the circumferential length at zero strain \( L(0) \) yields the instantaneous mechanical power per unit of (long-axis) length, and its time integral represents sector-wise regional myocardial work per unit of length in the long-axis direction:

\[
cRMW_L = \int_0^{T_{ed}} P_S(t) \, dt \cdot L(0) \quad [J/m] \quad [2]
\]

where \( t=0 \) was the first time point after r-peak and \( T_{ed} \) was the end-diastolic time point.

Eccentric work was defined as the work performed during elongation while the contractile apparatus was activated. Eccentric work per unit length was found from the time integral of negative-valued \( P_S(t) \), during systole and isovolumic relaxation only:

\[
cRMW_{LEW} = \int_{T_{MVc}}^{T_{MVo}} P_{S\neg}(t) dt \cdot L(0) \quad [J/m] \quad [3]
\]

where \( T_{MVo} \) and \( T_{MVe} \) was the time point for mitral valve opening and closing, respectively.
Finally, total work per unit mass of muscle tissue in each sector was found by dividing the surface work by the regional wall thickness at zero strain, and dividing by a conversion factor (1.05 g/cm³):

\[ cRMW_M = \frac{1}{1.05} \cdot \int_0^{T_{ed}} P_s(t) \, dt \cdot D(0)^{-1} \quad [\text{mJ/g}] \]  

[4]

**Comparison of Tail-cuff Based and Invasive Blood Pressure Measurement**

To investigate whether tail-cuff could be used to measure peak systolic pressure in rats, we compared tail-cuff based measurement of blood pressure with measurement of LVP using catheterization in a separate cohort of six rats (three sham and three MI).

The rats were anesthetized and mask ventilated using the same setup and settings as during standard pressure curve generation. Body temperature was maintained using a combination of a heated table and heating lamp. Non-invasive peak systolic blood pressure was recording using a tail-cuff system dedicated for blood pressure measurement in rats (Kent Scientific, USA). Seven consecutive recording cycles were acquired, each lasting about 10 s. The mean systolic pressure was calculated from the cycles automatically approved by the tail-cuff system (typically 5-6). Immediately after, without changing depth of anaesthesia, peak LVP was measured by catheterisation using the same procedure as during standard pressure curve generation.

The median and interquartile range of peak systolic blood pressure measured by tail-cuff was 143.3 [130.6 - 153.1] mmHg, while median and interquartile range of peak LVP measured by catheterization was 145.0 [134.0 - 155.0] mmHg. This yielded no significant difference between methods (p=0.94, N=6).