EXPERIMENTAL PAPER

Multilayer radial systolic strain can identify subendocardial ischemia: An experimental tissue Doppler imaging study of the porcine left ventricular wall

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Received 4 January 2007; received in revised form 22 March 2007; accepted 31 March 2007
Available online 26 June 2007

KEYWORDS
Echocardiography; Pigs; Strain imaging; Ischemia; Myocardium; Color microspheres

Abstract  Aims: This study investigates whether subendocardial ischemia can be detected by measuring multilayer radial systolic strain from epicardial tissue Doppler imaging.
Methods: In 10 anesthetized open-chest pigs an extracorporeal shunt from the proximal brachiocephalic to the left anterior descending coronary artery was constricted in steps. Color microsphere injections and short axis Tissue Velocity Imaging (TVI) recordings were performed with open shunt, with a non-significant stenosis, and with 2 steps of shunt flow reduction.
Results: With open shunt and no transmural flow gradient, there was a gradient of peak ejection strain with high values subendocardially for both 4 and 2 layer measurements. For 2 layer measurement strain was $56.0 \pm 10.5\%$ subendocardially and $22.0 \pm 5.2\%$ subepicardially. A non-significant stenosis, not altering transmural flow distribution, reduced strain to $40.3 \pm 5.4\%$ in the endocardial half-layer. With reduced shunt flow resulting in subendocardial ischemia, peak ejection strain decreased further, primarily in inner wall layers, and postsystolic strain became evident. At severe stenosis ($52.4 \pm 1.8\%$ shunt flow reduction) strain was reduced to $3.8 \pm 3.6\%$ in the subendocardium and $0.0 \pm 2.6\%$ in the subepicardium.
Conclusion: Evaluation of myocardial function with multilayer radial systolic strain has a potential for detecting subendocardial ischemia.

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Introduction

Experimentally, the relationship between perfusion and function in myocardial segments is often evaluated by wall thickening, average velocity or average strain, even if perfusion can be measured with microspheres in several depths or layers.\textsuperscript{1–3} Function in myocardial wall layers can be assessed by sonomicrometry using crystals implanted at two depths of the wall, by using M-mode with implantable markers as well as implantable radiopaque beads.\textsuperscript{4–7} An alternative is the use of Tissue Velocity Imaging (TVI) avoiding any sensor implantation. Systolic function of different myocardial layers can be evaluated as the myocardial velocity gradient.\textsuperscript{8,9}

Strain is a measure of local deformation and is the preferred variable describing local function.\textsuperscript{10–13} Although a large number of experimental and clinical studies using Strain Rate Imaging (SRI) and Strain Imaging (SI) have been carried out\textsuperscript{14–17}, only few studies have addressed simultaneous multiple layer measurement.

Longitudinal strain measurements from apical TVI recordings enables assessments in one specific layer (subendocardium) or simultaneously in 3 layers across the myocardial wall.\textsuperscript{18,19} The results show highest strain in the subendocardial layer. Radial function can be evaluated by analysis of integrated backscatter from the myocardial wall and has been compared to simultaneous strain measurements using M-mode imaging.\textsuperscript{20–22} A recent experimental study shows that the maximal radial strain values in normal myocardium is shifting from the mid-wall to the subendocardium after pericardial opening.\textsuperscript{23} With a high frequency intracardiac ultrasound catheter, the highest work index is found in the non-ischemic subendocardial layer. If ischemia is introduced, postsystolic work index increase.\textsuperscript{24}

We have evaluated a method for experimental radial strain measurement in up to 4 myocardial layers in pigs using epicardial echocardiography, demonstrating a transmural gradient with higher levels of systolic strain in inner compared to outer wall layers.\textsuperscript{25} A significant coronary stenosis manifests itself by subendocardial ischemia, strongly correlating to reduced wall thickening.\textsuperscript{1} Our hypothesis was that alterations in transmural blood flow gradients during coronary stenosis would alter the radial strain gradient as measured with multi-layer SI. In an experimental model we investigated the effect of reducing coronary blood flow resulting in a gradually more severe subendocardial ischemia measured with microspheres and the corresponding changes in radial strain gradient measured in 4 and 2 wall layers.

Materials and methods

Experimental preparation

Eleven young pigs (Norwegian Land Race) of either sex weighing $53 \pm 6$ (SD) kg and with a calculated body surface area of $1.29 \pm 0.09$ (SD) $m^2$ were anesthetized and instrumented. After premedication with ketamine/atropine, anesthesia was induced and maintained with loading doses and continuous infusions of fentanyl, midazolam, pancuronium and pentobarbital as previously described in detail.\textsuperscript{25,26} The animals underwent tracheotomy and intubation; ventilation was continued with a mixture of $N_2O$ (56–57%) and oxygen. Fluid substitution was given as $15 \, mL \, kg^{-1}$ of Ringer’s Acetate with $20 \, mmol \, L^{-1} \, KCl$ added.

The abdominal aorta was cannulated via the right femoral artery for arterial blood gas analyses and reference blood sampling; serum was prepared for troponin-T measurements. After midline sternotomy and pericardiectomy, a pressure-tip catheter (Millar MPC-500, Houston, TX), inserted through an apical stab, measured left ventricular (LV) peak systolic and end-diastolic pressure, LV $dP/dt_{max}$ and LV $-dP/dt_{min}$. Continuous cardiac output was measured with a $7.5F$ balloon floating catheter in the pulmonary artery (Vigilance, Baxter, Irvine, CA).

After heparinisation ($500 \, IU \, kg^{-1}$), an extracorporeal shunt was established from the proximal brachiocephalic artery to the proximal left anterior descending coronary artery (LAD). The shunt consisted of silicone rubber tubing, an in-line transit time probe connected to a flowmeter (Medi-Stim, Oslo, Norway) and two pressure ports with transducers (SensoNor, Horten, Norway), one on each side of a constrictor (Fig. 1). The procedures were registered and approved by the Norwegian Animal Research Authority (Project 2004220) and by the local responsible laboratory animal veterinarian, and were conducted in accordance with national and international laws.

Experimental protocol

Data were obtained after 10 min of stabilization in the following situations: (a) Open — basal registration with open shunt; (b) Mild — distal shunt pressure reduction without flow reduction in the shunt; (c) Moderate — approximately 20% flow reduction; and (d) Severe — approximately 50% flow reduction.
For each situation arterial blood gases, s-troponin-T, ECG and global hemodynamic measurements and echocardiography were performed as well as the injection of color microspheres via a catheter in the left atrium (Dye-Trak VII™, Triton Technology, San Diego, CA).

The heart was arrested by an injection of saturated KCl into the left atrium. The proximal part of the coronary shunt was occluded and methylene blue solution was injected into the shunt for marking of the ischemic tissue. The left ventricular anterior (LAD-region) and posterior (Cx-region) wall, at the level of short axis TVI recordings, were divided into 4 separate layers labeled Epi, Mid-Epi, Mid-Endo and Endo. Tissue blood flow rate was calculated as mL min⁻¹ g⁻¹ according to standard procedures.²⁷ By adding up sample weights and spheres for the two inner and the outer samples, tissue blood flow rate was re-calculated for two layers. This allowed the relation between strain and perfusion to be investigated for both 2 and 4 layers.

**Echocardiography**

A silicone rubber pad (1.5 × 3 cm) was applied between the probe and the exposed heart, acting both as an offset to avoid near field artifacts, and as a cushion for the moving heart. We used a digital combined echo/Doppler scanner (System-Five, GE Vingmed Ultrasound, Horten, Norway) and a 5 MHz phased array transducer. First, a short axis TVI image half way between the ventricular equator and apex, including both the anterior ischemic and posterior non-ischemic wall, was obtained. By reducing the image depth to 3 cm, a detailed image showing radial velocities of the anterior left ventricular wall was recorded (Fig. 2). The narrow depth setting and small beam sector resulted in a high frame rate, averaging 158 ± 29 (SD) frames s⁻¹.

Velocity resolution of the TVI image was approximately 0.4–0.8 mm in the radial direction and 1–2 mm in the lateral direction for this ultrasound frequency and depth. Since no averaging in the radial direction was used during acquisition of the TVI images, the radial resolution was therefore first of all determined by the Strain Length (distance between velocity points − SL) selected during analysis. Also no averaging across the beam was used, the averaging of velocities from several beams to increase the accuracy was carried out during data analysis. The beam width, which depends on several settings like sector and depth, was approximately 1.6 mm.

Apical long axis 2 Ch and 4 Ch images were recorded to calculate the ejection fraction (EF). TVI images with reduced sector size were recorded of the ventricular septum for evaluation of longitudinal function with a frame rate of 100 ± 12 (SD) frames s⁻¹. Finally, apical pulsed Doppler velocity recordings in the aortic orifice identified aortic valve opening and closure. All recordings were carried out during brief stops (4–6 beats) of the respirator at end-expirium. No hemodynamic changes could be seen during these short respirator stops.

**Data analysis**

The TVI and B-mode images were analyzed using dedicated software (EchoPac PC 4.0, GE Vingmed Ultrasound, Horten, Norway). This program enables the adjustment of the SL during SI analysis as well as placing single or multiple Region of interest (ROI) in different depths of the myocardial wall. The user can control these ROI’s to track one specific region of the wall during the heart cycle and the Langrangian strain within each ROI can be displayed (Fig. 2). The ROI’s were evenly distributed across the wall, for 2 layer measurement (as shown in Fig. 2) the ROI’s were placed with a distance approximately ½SL from the epicardium and ½SL from the endocardium and a slightly larger distance between the ROI’s. This was done because this software also includes velocities outside the ROI in the strain rate and strain estimates.
The same principle was used for the 4 layer measurement.

In this open chest, open pericardium study we did not analyze strain during isovolumetric contraction phase. Some thickening occurs during the isovolumetric phase and thus the wall can more easily include several ROI’s when starting the integration at the aortic valve opening. Some overlap was unavoidable for 4 layer measurement during ischemia.

The images including both the anterior and posterior wall were analyzed with SL 6 mm and ROI 6 × 6 mm (first number denotes dimension along the beam), giving average strain values across the wall with SL approximately half the wall thickness. The short axis images of the anterior wall, only, were analyzed with 4 elliptically shaped ROI’s with size 2 × 6 mm and SL 2 mm as well as 2 elliptically shaped ROI’s with size 4 × 6 mm and SL 4 mm. Using a ROI of 6 mm across the beam enables averaging over several ultrasound beams in the lateral direction. This size of ROI’s enabled the ROI’s to be separated during ejection for both 2 and 4 layer, thus corresponding to the perfusion measurements. After careful adjustment of the ROIs and the start of integration, peak (maximum) ejection strain and postsystolic strain were reported (Fig. 2). Postsystolic strain was calculated as the difference between early diastolic and end systolic strain if the postsystolic strain was higher than end systolic strain. Postsystolic strain was set to zero if a relaxation occurred after end systole.

From the 4 chamber long axis images of the septum, longitudinal septal strain was estimated using SL 8 mm and ROI 8 × 6 mm. Care was taken to record only velocities at 90° to the ventricular wall surface for short axis views and parallel to the surface for long axis views. The EF was calculated by the modified biplane Simson’s method using the 2 and 4 chamber views.

### Statistical analysis

Unless otherwise noted, all data are expressed as mean ± SEM. Data were analyzed using one-way analysis of variance (ANOVA) for repeated measurements with post hoc Newman–Keuls multiple range tests when justified by the preceding ANOVA. A \( P < 0.05 \) was considered significant. The interobserver agreement was calculated from 20 randomly selected 4 layer measurements of peak ejection strain by two independent observers, and given as the mean difference ± SD and the intraclass correlation coefficient (Ric).

### Results

One animal was discarded from the study; a blood clot in the shunt caused a severe reduction of blood flow. Results from 10 animals are reported.

Repeated arterial blood gas analyses throughout the protocol revealed stable respiratory conditions with normal values for this pig model. This was also the case for respiratory gases, hemoglobin, rectal temperature and diuresis.

With mild stenosis heart rate significantly increased from 94 ± 6 to 105 ± 9 beats min⁻¹ with
no other hemodynamic changes (Table 1). With moderate stenosis of the shunt the left ventricular peak systolic pressure (LVSP\textsubscript{max}), dP/dt\textsubscript{max} and left ventricular ejection fraction (EF) decreased and −dP/dt\textsubscript{min} increased significantly. Long axis ejection strain changed from −19.5 ± 1.9% to −12.7 ± 1.2%, corresponding to reduction in longitudinal shortening in the septal wall during moderate stenosis. When a severe stenosis was applied, the global cardiac function deteriorated further, demonstrated as a further decrease of LVSP\textsubscript{max}, increased left ventricular end-diastolic pressure (LVEDP) and reduction of dP/dt\textsubscript{max}, cardiac index (CI) and EF and less negative long axis septal strain (−7.5 ± 1.1%).

The mean shunt pressure distal to the artificial stenosis decreased gradually with the severity of the stenosis (Fig. 3, upper panel). Shunt flow was unaltered with mild stenosis, decreased by 23.4 ± 2.4% during moderate stenosis and by 52.4 ± 1.8% during severe stenosis (Fig. 3, middle panel). With open shunt, radial peak ejection strain averaged over the entire wall was 32.6 ± 3.9% in the anterior wall perfused by the shunt and gradually decreased with the severity of stenosis to 1.1 ± 1.8% with severe stenosis (Fig. 3, lower panel). In the posterior wall of the left ventricle the corresponding strain averaged 19.2 ± 1.4% with open shunt and was not affected. After shunt instrumentation serum troponin-T was below the reference value (<0.03 µg L\textsuperscript{-1}) in 3 animals and averaged 0.05 ± 0.02 µg L\textsuperscript{-1} in the remaining 7 animals. Both with mild and moderate stenosis troponin-T averaged 0.07 ± 0.02 µg L\textsuperscript{-1} in 8 animals, and were below the reference value in 2. With severe stenosis troponin-T averaged 0.10 ± 0.2 µg L\textsuperscript{-1} (n = 10).

With the LAD shunt fully open and with a non-significant (Mild) stenosis applied, there were no substantial transmural gradients of blood flow rates between inner and outer myocardial wall layers (Table 2). Both with a moderate and a severe stenosis applied to the shunt, transmural myocardial perfusion gradients with low tissue blood flow rates in inner compared to outer layers appeared in the left ventricular anterior wall (LAD region). This gradient became more evident with increasing severity of the stenosis, irrespective of whether the wall was divided and blood flow rate calculated for 4 layers or 2 layers. In the posterior left ventricular wall (Cx region), blood flow rate and transmural distribution was unaffected by the shunt stenosis and changes in global cardiac function.

There was a transmural gradient of peak ejection strain with high levels in inner and lower levels in outer wall layers. This was particularly evident when the wall was divided into 4 layers (Table 3). With moderate stenosis peak ejection strain decreased significantly compared to corresponding

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**Table 1** Left ventricular function in pigs with graded stenosis of an extracorporeal shunt to the LAD artery

<table>
<thead>
<tr>
<th></th>
<th>Open (A)</th>
<th>Mild (B)</th>
<th>Moderate (C)</th>
<th>Severe (D)</th>
<th>ANOVA-P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Global myocardial function</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>HR (beats min\textsuperscript{-1})</td>
<td>94 ± 6</td>
<td>105 ± 9\textsuperscript{a}</td>
<td>109 ± 10\textsuperscript{a}</td>
<td>117 ± 11\textsuperscript{a}</td>
<td>0.002</td>
</tr>
<tr>
<td>LV-SP\textsubscript{max} (mmHg)</td>
<td>114 ± 4</td>
<td>110 ± 5</td>
<td>106 ± 5\textsuperscript{a}</td>
<td>99 ± 4\textsuperscript{a,b,c}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LV-EDP (mmHg)</td>
<td>10.4 ± 1.1</td>
<td>9.2 ± 1.3</td>
<td>10.7 ± 0.8</td>
<td>13.3 ± 1.0\textsuperscript{a,b,c}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>dP/dt\textsubscript{max} (mmHg s\textsuperscript{-1})</td>
<td>1572 ± 67</td>
<td>1598 ± 94</td>
<td>1436 ± 65\textsuperscript{a,b}</td>
<td>1370 ± 68\textsuperscript{a,b}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>−dP/dt\textsubscript{min} (mmHg s\textsuperscript{-1})</td>
<td>−1795 ± 80</td>
<td>−1742 ± 87</td>
<td>−1522 ± 92\textsuperscript{a,b}</td>
<td>−1376 ± 70\textsuperscript{a,b,c}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CI (L min\textsuperscript{-1} m\textsuperscript{-2})</td>
<td>4.4 ± 0.2</td>
<td>4.3 ± 0.3</td>
<td>4.3 ± 0.3</td>
<td>3.8 ± 0.2\textsuperscript{a,b,c}</td>
<td>0.002</td>
</tr>
<tr>
<td>SV\textsubscript{i} (ml m\textsuperscript{-2})</td>
<td>48 ± 3</td>
<td>43 ± 3\textsuperscript{a}</td>
<td>42 ± 3\textsuperscript{a}</td>
<td>35 ± 3\textsuperscript{a,b,c}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EF (%)</td>
<td>46 ± 3</td>
<td>45 ± 3</td>
<td>37 ± 3\textsuperscript{a,b}</td>
<td>28 ± 3\textsuperscript{a,b,c}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Local myocardial function</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT\textsubscript{dia} (mm)</td>
<td>11.5 ± 0.5</td>
<td>11.3 ± 0.6</td>
<td>10.7 ± 0.6</td>
<td>10.3 ± 0.5\textsuperscript{a,b}</td>
<td>0.021</td>
</tr>
<tr>
<td>WT\textsubscript{sys} (mm)</td>
<td>16.9 ± 0.6</td>
<td>16.0 ± 0.7</td>
<td>12.7 ± 0.4\textsuperscript{a,b}</td>
<td>10.4 ± 0.3\textsuperscript{a,b,c}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WT (%)</td>
<td>47 ± 3</td>
<td>44 ± 6</td>
<td>22 ± 9\textsuperscript{a,b}</td>
<td>2 ± 4\textsuperscript{a,b,c}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LA peak systolic strain (%)</td>
<td>−19.5 ± 1.9</td>
<td>−16.7 ± 1.5</td>
<td>−12.7 ± 1.2\textsuperscript{a,b}</td>
<td>−7.5 ± 1.1\textsuperscript{a,b,c}</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Mean values ± SEM, n = 10. HR = heart rate; LV-SP\textsubscript{max} and -EDP = left ventricular peak systolic and end-diastolic pressure, respectively; dP/dt\textsubscript{max} and −dP/dt\textsubscript{min} = peak positive and peak negative first derivative of left ventricular pressure; CI = cardiac index; SV\textsubscript{i} = indexed stroke volume, EF = left ventricular ejection fraction; WT\textsubscript{dia} = wall thickness end diastole, WT\textsubscript{sys} = wall thickness end systole, WT = wall thickening, LA = long axis. ANOVA-P = probability from analysis of variance for repeated measurements. Lower-case superscript letters denotes significant difference from value in column with corresponding upper-case letter by post-hoc multiple contrasts.
values with open shunt in the two inner layers. With severe stenosis strain levels decreased further and were low in all wall layers. Postysstolic strain was low in all wall layers with open shunt and increased when a moderate and severe stenosis was applied. For two layers the decrease in peak ejection strain was significant in the subendocardial half already during the mild stenosis situation and decreased further together with an increase of postysstolic strain in both wall layers during moderate and severe stenosis.

Fig. 4 presents mean radial peak ejection strain in: (A) four transmural wall layers; and (B) two transmural wall layers plotted against mean blood flow rate in corresponding layers. For 4 layers there was no relation between low peak ejection strain levels and corresponding blood flow rates for the Epi layer. From Mid-Epi and inwards towards the endocardium a relationship between strain and blood flow rate was seen. In 2 layers there was a relationship for both transmural myocardial halves.

The interobserver variation for the 4 layer measurement was low with a mean difference for peak ejection strain of $-0.87 \pm 5.96$ (SD)% ($n = 20$) and $R_{ic} = 0.979$.

Discussion

This study demonstrates a relationship between changes in the transmural distribution of blood flow and radial strain in myocardial wall layers during gradually aggravating hypoperfusion. With a non-significant stenosis (Mild), no systematic changes could be observed in the transmural pattern of tissue blood flow and systolic strain for the 4 layer measurements. The high levels of radial peak ejection strain normally found in inner wall layers were reduced by lowering pressure and flow in the extracorporeal shunt to the LAD artery (Table 3). The concomitant reduction of inner wall tissue blood flow rate was paralleled by reduction of radial strain levels in inner wall layers (Fig. 4A). Based on this study, a less pronounced transmural gradient of radial systolic strain with low values in inner wall layers can be used as a sign of subendocardial ischemia caused by a significant coronary stenosis.

For 2 layer measurements a decreased strain was already seen for a mild stenosis and with a further decrease for moderate and severe stenosis. The reason for this early detection of subendocardial dysfunction is most probably related to the significant increase in heart rate from 94 ± 6 to 105 ± 9 beats min$^{-1}$ and the concomitant reduction of stroke volume. Shorter cardiac cycles leads

Figure 3: Mean shunt pressures, shunt blood flow and peak ejection strain in 10 animals with an extracorporeal shunt to the LAD artery. Bars represent SEM. Prox./Dist. = pressures from ports proximal and distal to a graded stenosis of the shunt, respectively. LAD = strain in myocardium perfused by the shunt (anterior wall); Cx = values from myocardium not affected by the shunt (posterior wall); * = denotes significant difference from corresponding value(s) to the left.
Measuring strain, a delayed onset of diastolic 
ent has been used as a marker of ischemia.\textsuperscript{9,28,29} 
layer and changes in the myocardial velocity gradi-
systolic velocity is found in the subendocardial 
several layers of the myocardial wall. The highest 
function include experimental models with coro-
obtained is a function of time of ejection. 

Mean values ± SEM, n = 10. Epi and Endo = subepicardium and subendocardium; ANOVA-P = probability from analysis of variance 
for repeated measurements. Lower-case superscript letters denotes significant difference from value in column with correspond-
ing upper-case letter by \textit{post-hoc} multiple contrasts.

Studies dealing with myocardial ischemia and 
function include experimental models with coro-
mary occlusion; few studies report data from 
several layers of the myocardial wall. The highest 
systolic velocity is found in the subendocardial 
layer and changes in the myocardial velocity gradi-
et has been used as a marker of ischemia.\textsuperscript{9,28,29} 

Table 2  Regional myocardial tissue blood flow in 4 and 2 layers in the left ventricular anterior wall in pigs with 
graded stenosis of an extracorporeal shunt to the LAD artery

<table>
<thead>
<tr>
<th>Open</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>ANOVA-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 layer measurement (ml min(^{-1})g(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epi</td>
<td>(1.05 \pm 0.09)</td>
<td>(0.90 \pm 0.12)</td>
<td>(0.92 \pm 0.10)</td>
<td>(0.67 \pm 0.09)\textsuperscript{a}</td>
</tr>
<tr>
<td>Mid-Epi</td>
<td>(1.03 \pm 0.09)</td>
<td>(1.03 \pm 0.09)</td>
<td>(0.75 \pm 0.09)\textsuperscript{a,b}</td>
<td>(0.50 \pm 0.09)\textsuperscript{a,b,c}</td>
</tr>
<tr>
<td>Mid-Endo</td>
<td>(0.96 \pm 0.07)</td>
<td>(0.99 \pm 0.10)</td>
<td>(0.57 \pm 0.08)\textsuperscript{a,b}</td>
<td>(0.29 \pm 0.04)\textsuperscript{a,b,c}</td>
</tr>
<tr>
<td>Endo</td>
<td>(0.86 \pm 0.05)</td>
<td>(0.86 \pm 0.06)</td>
<td>(0.41 \pm 0.06)\textsuperscript{a,b}</td>
<td>(0.21 \pm 0.03)\textsuperscript{a,b,c}</td>
</tr>
</tbody>
</table>

Mean values ± SEM, \(n = 10\). Epi and Endo = subepicardium and subendocardium; ANOVA-P = probability from analysis of variance 
for repeated measurements. Lower-case superscript letters denotes significant difference from value in column with correspond-
ing upper-case letter by \textit{post-hoc} multiple contrasts.

Table 3  Local radial strain in 4 and 2 layers in the left ventricular anterior wall in pigs with graded stenosis of an 
extracorporeal shunt to the LAD artery

<table>
<thead>
<tr>
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<th>ANOVA-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 layer measurement: Peak ejection (%)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Epi</td>
<td>(3.1 \pm 4.5)</td>
<td>(6.2 \pm 5.1)</td>
<td>(3.1 \pm 6.6)</td>
<td>(1.3 \pm 2.8)</td>
</tr>
<tr>
<td>Mid-Epi</td>
<td>(35.6 \pm 8.6)</td>
<td>(32.2 \pm 3.6)</td>
<td>(16.3 \pm 9.8)</td>
<td>(-3.7 \pm 6.2)\textsuperscript{a,b,c}</td>
</tr>
<tr>
<td>Mid-Endo</td>
<td>(57.3 \pm 14.2)</td>
<td>(37.7 \pm 6.4)</td>
<td>(25.0 \pm 8.3)\textsuperscript{a}</td>
<td>(-4.0 \pm 5.1)\textsuperscript{a,b,c}</td>
</tr>
<tr>
<td>Endo</td>
<td>(61.4 \pm 10.9)</td>
<td>(44.3 \pm 10.0)</td>
<td>(30.6 \pm 8.6)\textsuperscript{a}</td>
<td>(2.5 \pm 3.5)\textsuperscript{a,b,c}</td>
</tr>
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</table>

Post-systolic (%) |
| Epi  | \(7.0 \pm 5.9\) | \(3.4 \pm 1.4\) | \(19.9 \pm 5.4\)\textsuperscript{a} | \(17.2 \pm 3.3\)\textsuperscript{b} | 0.015 |
| Mid-Epi | \(10.4 \pm 7.7\) | \(11.8 \pm 5.6\) | \(31.6 \pm 8.1\)\textsuperscript{a,b} | \(36.2 \pm 4.8\)\textsuperscript{a,b} | 0.003 |
| Mid-Endo | \(21.1 \pm 12.8\) | \(16.8 \pm 8.2\) | \(16.8 \pm 3.8\) | \(35.3 \pm 4.4\) | 0.188 |
| Endo  | \(10.6 \pm 6.0\) | \(10.8 \pm 5.2\) | \(11.6 \pm 2.4\) | \(31.0 \pm 6.0\)\textsuperscript{a,b,c} | 0.005 |

2 layer measurement: Peak ejection (%) |
| Epi  | \(22.0 \pm 5.2\) | \(17.1 \pm 4.3\) | \(9.5 \pm 6.7\)\textsuperscript{a} | \(0.0 \pm 2.6\)\textsuperscript{a,b} | <0.001 |
| Endo  | \(56.0 \pm 10.5\) | \(40.3 \pm 5.4\)\textsuperscript{a} | \(24.4 \pm 7.1\)\textsuperscript{a,b} | \(3.8 \pm 3.6\)\textsuperscript{a,b,c} | <0.001 |

Post-systolic (%) |
| Epi  | \(7.6 \pm 2.3\) | \(15.1 \pm 6.5\) | \(22.6 \pm 5.0\)\textsuperscript{a} | \(25.9 \pm 3.6\)\textsuperscript{a} | 0.020 |
| Endo  | \(3.1 \pm 1.3\) | \(3.8 \pm 1.8\) | \(10.6 \pm 2.4\)\textsuperscript{a,b} | \(29.1 \pm 2.1\)\textsuperscript{a,b,c} | <0.001 |

Mean values ± SEM, \(n = 10\). Epi and Endo = subepicardium and subendocardium; ANOVA-P = probability from analysis of variance 
for repeated measurements. Lower-case superscript letters denotes significant difference from value in column with correspond-
ing upper-case letter by \textit{post-hoc} multiple contrasts.
in sheep with open pericardium compared to 8, 38 and 57% in pigs using the present method. Thus, with opened pericardium both methods demonstrate similar strain patterns despite different species and different tracking methods.

Radial strain reported in some studies are either averaged over most of the myocardial wall or measured in the mid-wall or in one layer. Thus, values varies between studies from 18% in the normal adult human left ventricle to 60% in pigs. Furthermore, some studies report natural strain and not Langrangian strain as in the present study. Also the integration period of strain rate is important for the obtained values. In this study

**Figure 4** Regional myocardial tissue blood flow vs. radial peak ejection strain in four wall layers (A) and two layers (B) of the anterior left ventricular wall with graded constriction of an extracorporeal shunt to the LAD artery. Mean values of 10 animals, bars represent SEM. Epi = subepicardium; Endo = subendocardium; open symbols = Open shunt; light gray = Mild; dark gray = Moderate; black = Severe stenosis, respectively.
peak ejection strain is reported. If one relatively small ROI is used, the resulting strain value depends highly on the relative placement in the wall because of the strain gradient. This is also the case in a heterogeneously perfused wall (Table 2, Moderate and Severe) and may explain the large variation in reported radial strain values.

There is substantial variation in systolic strain in each layer between animals. This is to be expected since local events are not necessarily synchronized and the left ventricular contraction pattern is not homogeneous even in normally perfused myocardium. Changes in local strain are not influenced by changes in local perfusion alone. Overall, there was a significant correlation \((r = 0.472, p = 0.002, n = 40)\) between stroke volume and systolic strain in the anterior wall. The amount of myocardium affected by the shunt and the global hemodynamic consequences of regional ischemia differ between animals. Increased heart rate and preload and reduced afterload and stroke volume would also influence local strain. Strain in the non-ischemic posterior wall was, however, not affected by the changes in global hemodynamic variables (Fig. 3, lower panel). Furthermore, without global hemodynamic changes and subendocardial ischemia with progressive stenosis, subendocardial radial strain decrease.30

These variations between animals does not, however, conflict with the main conclusion from this study; there is a gradient of strain through the myocardial wall and this gradient changes most clearly for the moderate and severe degree of stenosis resulting in subendocardial ischemia. The relatively large variance in strain values also points at another problem with SI for local radial strain measurement. Spatial averaging over a larger ROI removes some of this variance. This is evident both from 2 layer measurements (Table 3) and from measurements averaged over most of the wall (Fig. 3, lower panel). The variance in strain values between animals could be caused by either real inter-animal difference, difference in timing or errors in placing of ROIs when analyzing. Due to the low interobserver variation in this study, we believe that most of the variance in the observed strain values represents actual strain differences between animals.

We found a large variation in the changes in positive postsystolic strain for all situations, also with open shunt (Table 3). This variation was not related to afterload. Severe ischemia (52% LAD flow reduction) caused a substantial increase in postsystolic strain. One study reports an increase in postsystolic shortening (as % of end-diastolic dimension) from 1.1 to 4.2% during a 47% reduction in LAD flow and a further increase to 8.2% at total occlusion.33 Another study shows the occurrence of postsystolic shortening in one third of normal segments and that this is not always a marker of disease, up to 20% longitudinal postsystolic shortening was demonstrated in normal segments.34 This is consistent with this study showing postsystolic radial strain between 7.0 and 21.1% in the 4 layers for open shunt increasing to 17.2–36.2% for severe stenosis (Table 3). When studying postsystolic strain, a 2 layer model is probably more suitable as shown by the reduced variance in postsystolic strain for these measurements (Table 3). Postsystolic strain increased significantly in both wall layers when ischemia was evident in the subendocardium, only.

Clinical applications

This method has the potential to measure radial strain in multiple layers and to detect subendocardial ischemia in humans. A recent study has shown its potential for detecting abnormal strain profiles in patients.35 Also by using a phase-tracking method, it is possible to measure thickening in two layers in the fetal heart.36 The 4 layer measurements in this epicardial experimental study is beyond clinical use. In transthoracic applications a frequency 5 MHz is too high for most measurement in adults. However, the 2 layer measurements should be tested in a clinical setting.

Study limitations

This open chest/open pericardium model will generate isovolumic contracting and relaxing phases with variable and sometimes higher strain readings than for a closed chest model. We did not analyze these raised velocities; the reported strains are peak ejection strain during ejection, only. An element of myocardial stunning following the necessary brief LAD occlusion during cannulation could reduce the systolic strain values. However, the time of no-flow is short and non-ischemic strain values do not differ from those reported by us in pigs without such instrumentation.25 Furthermore, the increase in s-Troponin-T levels after instrumentation (in 7 out of 10) was very moderate, and could be explained by the apical snare used for fixation of the LV pressure catheter.

Conclusions

Changes in multilayer function during ischemia can be measured with SI using high frequency and
carefully adjusted acquisition and analysis. The normal heterogeneous pattern with high levels of radial systolic strain in inner compared to outer wall layers in non-ischemic myocardium in the anterior left ventricular wall is altered during a gradually more severe coronary stenosis. Reduced endocardial peak systolic strain together with increasing postsystolic strain in both inner and outer wall layers is observed during subendocardial ischemia. These characteristic changes in strain distribution during subendocardial ischemia can be detected by measuring radial strain with SI in 2 or 4 separate transmural wall layers.

Acknowledgements

Financial support was obtained from the Locus for Cardiac Research, University of Bergen and from the Western Norway Health Authority. We acknowledge the technical assistance from Lill-Harriet Andreasen, Cato Johnsen, Gry-Hilde Nilsen, Anne Aarsand, Inger Vikey and the staff at the Vivarium, University of Bergen.

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