Delayed Onset of Subendocardial Diastolic Thinning at Rest Identifies Hypoperfused Myocardium

Jianwen Wang, MD, PhD; Theodore P. Abraham, MD; Josef Korinek, MD; Stig Urheim, MD; Eileen M. McMahon, PhD; Marek Belohlavek, MD, PhD

Background—Onset of myocardial relaxation is highly energy dependent. Perfusion and therefore energy substrate delivery are predominantly reduced in the subendocardial myocardium in the early stages of progressive ischemia. We hypothesized that delayed onset of subendocardial diastolic thinning will functionally identify regionally hypoperfused resting myocardium.

Methods and Results—Progressive left anterior descending coronary artery stenosis was induced by an ameroid occluder and maintained for 1 or 2 weeks (end point) in 12 dogs. M-mode tissue Doppler images of the anterior apical and middle segments (testing region) and middle inferior segment (control region) were acquired selectively in the subendocardium and subepicardium. The time to the onset of thinning was measured with the use of tissue Doppler velocity (TOTv) and a thickness function (TOTt). At the end point in the testing region, myocardial flow was significantly lower in the subendocardial layer (P<0.05) in all animals, whereas viability staining showed preserved transmural viability in 10 dogs and thin subendocardial necrosis in 2 dogs. Both TOTv and TOTt were significantly (P<0.01) prolonged in the testing region. The mean difference between subendocardial and subepicardial TOTv values versus that in the control region identified the ischemic region, even when only dogs with hypoperfused but transmurally viable myocardium were considered (P<0.05). Systolic and diastolic myocardial velocities did not identify subendocardial hypoperfusion.

Conclusions—in resting myocardium subtended to progressive coronary stenosis, a delayed onset of subendocardial thinning suggests an early stage of hypoperfusion, before the development of local wall motion abnormalities. (Circulation. 2005;111:2943-2950.)

Key Words: myocardial relaxation ischemia regional blood flow echocardiography diastole

Impaired regional relaxation is one of the characteristics of locally ischemic myocardium.1 Flow-limiting coronary stenosis causes inadequate delivery of myocardial energy supplies.2 A decreased rate of energetically demanding calcium uptake by sarcoendoplasmic reticulum slows the decay of a calcium transient,3–6 prolongs myocardial contraction,7 and consequently delays the onset of regional relaxation. We have previously shown, in both an experimental8,9 and clinical10 setting, that a delayed onset of regional myocardial lengthening or thinning suggests an acutely developing ischemia. However, it has not been shown whether ischemia associated with early stages of slowly progressing coronary stenosis is signified by similar regional dysfunction in the subtended territory.

Transmural heterogeneity of myocardial contraction and relaxation has been well established,11,12 and it has been reported that myocardial hypoperfusion causes a "waveform" shift of this heterogeneity.13 However, it has not been entirely elucidated whether myocardial hypoperfusion results in transmurally different changes in timing of relaxation. In the initial stages of a developing coronary occlusion, the delayed onset of regional relaxation may occur predominantly in the subendocardial myocardium.

On the basis of this background, we hypothesized that progressive coronary stenosis will induce a delay to the transition from systolic contraction to diastolic relaxation, measurable as a delayed onset of regional thinning, and that this delay will predominantly occur in the subendocardium of the subtended myocardial territory before significant transmural dysfunction.

Methods

Study Animals and Dog Model of Progressive Coronary Stenosis

All experimental procedures conformed to the Position of the American Heart Association on Research Animal Use and were approved by the Institutional Animal Care and Use Committee of the Mayo Clinic. We studied 14 female hound dogs (weight, 20 to 28 kg) under sterile surgical conditions and general anesthesia. One side femoral artery was cannulated, and a 6F micromanometer catheter (Millar Instruments, Inc) was inserted for left ventricle (LV) pressure recordings. The chest was opened through the left fifth intercostal space. After the pericardium was opened and the left atrium (LA)
was exposed, a 16F venous tube was inserted into the LA, secured in position with a purse string suture, and exteriorized through a skin tunnel. This tube served as a permanently available access for intracardiac echocardiography, microsphere injections, and an alternative access for a micromanometer catheter. A left anterior descending coronary artery (LAD) segment, ~1 cm in length and located distal to the first diagonal branch, was dissected free, and an ameroid occluder (3- to 5-mm inner diameter; Research Instruments) was placed around the segment. In this setting, the occluder induces coronary stenosis with progressive severity within 2 weeks.14 Perioperative intracardiac echocardiography was used to monitor LV function to ensure that there was no immediate impairment of regional wall motion after placement of the ameroid occluder. The chest was then closed in layers, the pneumothorax was evacuated, transthoracic baseline scans were obtained, and the animals were revived. Antibiotics were administered daily. The permanent LA tube was flushed twice a day with heparin and antibiotics. At the end point (after 1 to 2 weeks) after ultrasound scans were obtained and microspheres were injected, each animal was euthanized with an overdose of pentobarbital, and the heart was removed for regional myocardial blood flow (MBF) analysis and viability staining.

**Intracardiac and Transthoracic Echocardiography Studies**

We used a Sequoia ultrasound scanner (Siemens Medical Solutions USA, Inc). We recorded intracardiac scans at 5.5 to 7 MHz with a 10F catheter–based transducer (AcuNav, Siemens Medical Solutions) and transthoracic views at 5 MHz with a standard hand-held transducer. Intracardiac scans were performed after the insertion of the ultrasound catheter into the LV through the permanent LA tube. Intracardiac M-mode tissue Doppler images (TDI) and M-mode anatomic (gray-scale) views15 of apical and middle segments of the anterior wall (ie, testing region) were obtained with a projection oriented similar to that of a parasternal view.

Cardiac function in the contralateral middle inferior segment (ie, control region) was evaluated with transthoracic scans in the same tissue Doppler and anatomic M-mode formats with the animal in the left decubitus position. Before initiating the present study, we performed an experimental pilot ultrasound study with transthoracic and intracardiac transducers. The anterior wall was located in the very near field of the imaging plane in the parasternal transthoracic short-axis view. The M-mode TDI of the anterior wall by transthoracic scanning was therefore suboptimal for further analysis. However, the inferior wall was located well within the scanning depth of the transthoracic transducer, and the M-mode TDI of the control region was of adequate quality. Intracardiac ultrasound provided unobstructed projections of the entire anterior wall, suitable for M-mode TDI analyses in subendocardial and subepicardial layers.15 Inferior wall projections, however, were difficult to obtain because the intracardiac ultrasound array, after insertion through the permanent tube, was in direct contact with the inferior wall and could also alter motion of the wall for functional analysis. Thus, similar to combined clinical transthoracic and transesophageal echocardiographic studies, we capitalized on the advantages of transthoracic and intracardiac ultrasound approaches in our animal model. During the pilot study, we also determined that it would take 1 to 2 weeks for the ameroid to induce LAD stenosis resulting in primarily subendocardial hypoperfusion in the testing region.

All data were recorded at baseline and at the end point and stored digitally for offline analysis.

**Echocardiographic Data Analysis**

Assessment of LV global function was based on LV ejection fraction (LVEF), calculated from 4-chamber and 2-chamber transthoracic views according to the biplane Simpson rule.

Assessment of the onset of myocardial relaxation in the testing and control regions was based on timing of the onset of regional thinning (TOT), which was measured with custom software (Quantbase, Siemens Medical Solutions) and 2 different methods.

In one method, the custom software partitioned the myocardium into 6 proportionally distributed sublayers after manual delineation of the endocardial and epicardial borders in M-mode TDI (Figure 1).16 Subendocardial and subepicardial myocardial velocity data were obtained as mean velocities within the innermost and outermost layers, respectively. We identified the onset of regional thinning as the point of the tissue velocity redirection in the end-ejection period (the red-blue transition; Figure 1). Then we measured the time to the onset of regional thinning as the interval from the ECG R wave to the point of velocity redirection (TOTv). If a bidirectional postejection velocity pattern was present,17 the second velocity redirection was used for the measurement. The difference between the subendocardial and subepicardial TOTv values, ie, TOTvdiff, was calculated as well. The layer-selective measurements also included peak systolic, peak early-diastolic (E′), and peak late-diastolic (A′) velocities.

In the other method, we manually outlined endocardial and epicardial borders in M-mode anatomical images (yellow and green lines in Figure 1), and the custom software generated a wall thickness function from the endocardial and epicardial border displacements over the duration of a cardiac cycle. We measured the time from the ECG R wave to the point of maximal thickness, ie, time interval to the onset of thinning (TOTtP). In addition, to measure the temporal relationship between the onset of regional thinning and global LV relaxation, we introduced a TOTtP parameter as the time interval between the peak negative dP/dt and the maximal thickness. Because the timing parameters include ejection duration, which is affected by variations in heart rate, we also report corrected TOTvdiff values. The corrected TOTvdiff was calculated as the unitless ratio of TOTvdiff to the square root of the corresponding ECG R-R interval.18

**Hemodynamic Data Analysis**

Values of the peaks of positive time derivative of pressure (+dP/dt) and negative time derivative of pressure (−dP/dt) were calculated from LV pressure tracings. Time constant of the LV isovolumic pressure decline (τ) was calculated as described by Weiss et al.19
Regional Blood Flow Analysis
Approximately 5 million color-labeled fluorescent microspheres (Interactive Medical Technology, Ltd) were administered into the LA through the established LA access to the beating heart in situ under hemodynamically stable state conditions. Femoral arterial blood was withdrawn as reference samples over 2 minutes at a rate of 7.5 mL/min starting 5 seconds before the injection of microspheres. We determined in the pilot studies that the microspheres were distributed at an average concentration of 1970 microspheres per gram of perfused myocardium. Tissue samples with lodged microspheres were obtained, and MBF was calculated separately in subendocardial and subepicardial myocardium in both the testing and control regions.20

Viability Analysis by Tissue Staining
The heart was dissected into ~1-cm-thick transverse slices that were incubated in 2% triphenyltetrazolium chloride (TTC) solution at 37°C for 5 minutes. Viable myocardium became brick red, whereas necrotic myocardium remained unstained.

Experimental Protocol
Intracardiac and transthoracic echocardiography scans were performed at the beginning and end point of the study. The images were obtained during brief (~10 seconds) animal apnea controlled by the respirator. LV pressure was recorded simultaneously with each recording of echocardiographic data acquisition. Fluorescent microspheres were injected after the echocardiography scans.

Statistical Analysis
The results are presented as mean±SD. All data were found normally or close to normally distributed with the use of StatView (SAS, Ltd) statistical software. Therefore, a 2-tailed, paired t test was used for comparisons of the end point versus baseline status, and a 2-tailed unpaired t test was used to compare subendocardial versus subepicardial layers within the same region or for comparisons of the end point versus baseline status. A P value of <0.05 was considered statistically significant.

Results

Animal Survival
Two animals died within 24 hours after the surgery because of internal bleeding. Data from 12 animals were used for analyses. In 4 animals, the protocol had to be ended within 7 to 10 days because of endocarditis (2 animals), displacement of the tube from the LA (1 animal), and paroxysmal ventricular tachycardia (1 animal). The complications were eliminated during the course of the study by the optimization of therapy and surgical procedures. In 8 animals, the protocol was conducted in full length (ie, 2 weeks).

Thus, for analyses of TOTv and TOTv, we had 24 segmental observations for the testing region (2 segments per region) and 12 segmental observations for the control region. For analyses of TOTt, we had 20 testing and 10 control observations available because in 2 animals the definition of myocardial borders was inadequate for analysis.

Viability Staining
At the end point, TTC staining detected a very thin (<2 mm) nonviable layer in the subendocardium of the anterior wall in only 2 dogs. In the remaining 10 dogs, viability was completely (transmurally) preserved, and we calculated TOTv for this selected group as well.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>End Point</th>
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</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>109±11</td>
<td>119±15</td>
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<tr>
<td>LVEDP, mm Hg</td>
<td>12±6</td>
<td>11±6</td>
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<tr>
<td>+dP/dt, mm Hg/s</td>
<td>1413±330</td>
<td>1969±556*</td>
</tr>
<tr>
<td>−dP/dt, mm Hg/s</td>
<td>1828±370</td>
<td>2111±762</td>
</tr>
<tr>
<td>( \tau )</td>
<td>30.30±6.36</td>
<td>28.75±6.00</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>65.66±6.64</td>
<td>62.76±11.59</td>
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</tbody>
</table>

LVEDP indicates LV end-diastolic pressure; \( \tau \), time constant of LV isovolumic pressure decline. Data from all 12 animals are presented.

*P<0.01 vs baseline.

Hemodynamic and Global Functional Data
The values of heart rate, LV end-diastolic pressure, −dP/dt, \( \tau \), and LVEF did not change significantly at the end point compared with those at baseline (Table 1; Figure 2A). However, +dP/dt was significantly higher than baseline at the end point (P<0.01).

Regional Peak Systolic and Diastolic Velocities
At baseline, in both the testing and control regions, the peak systolic, E’, and A’ velocities (Table 2; Figure 2B) were significantly higher (P<0.01) in the subendocardial layer than in the subepicardial layer, except for P=0.11 for A’ in the testing region. At the end point, these differences remained similar. Compared with baseline, there was no consistent change in the peak systolic, E’, or A’ parameters.

MBF Data
Table 3 summarizes the MBF data and shows that subendocardial perfusion decreased significantly at the end point compared with baseline in both testing regions (both P<0.05). In contrast, subepicardial MBF did not change significantly in these 2 regions. As a consequence, the ratio of MBF in the subendocardium to that in the subepicardium decreased significantly in testing regions (P<0.01 for apical segment and <0.05 for middle segment). Subendocardial MBF, subepicardial MBF, and their ratio did not change significantly in the inferior wall (all P>0.05). Even when data from the 2 dogs with subendocardial necrosis in the testing region were excluded from analysis and therefore only the remaining 10 dogs with transmurally viable myocardium were considered, the MBF measurements remained similar.

These results demonstrate relative hypoperfusion of the subendocardial layer of myocardium in the LAD territory, whereas hemodynamic and global measures of cardiac function did not change except for +dP/dt.

Onset of Myocardial Thinning
The time to onset of thinning based on tissue velocity, ie, TOTv, showed a strong trend (P=0.065) to an increase from baseline to the end point in the subendocardial layer of the testing region (Table 4; Figure 2B). When TOTv was calculated, the value in the testing region was significantly higher at the end point compared with that at baseline as well as compared with baseline and the end-point in the control region. Even when data from only those animals in which
transmural viability was preserved in the testing region were used, the statistical relationship remained the same.

Timing based on the analysis of the myocardial thickness function, ie, TOTt (Figure 2C), corroborated the velocity timing intervals by a significant increase at the end point in the testing region. However, the TOTt value in the testing segment was significantly higher than the control segment both at baseline and at the end point; therefore, solely comparing the testing TOTt value with the control TOTt value at the end point would not necessarily be indicative of pathology. In addition, the baseline TOTt interval was shorter than baseline TOTv intervals in the control region, but the TOTt and TOTv intervals in the testing region were similar. TOTIP measurements were also consistent with the velocity timing intervals and indicated a significant delay of the onset of regional thinning with respect to the start of global relaxation. The negative TOTIP values in the control region reflected the end of inferior wall thickening occurring shortly before the onset of global relaxation.

After we corrected for R-R interval, TOTv significantly increased at the end point in the subendocardial layer compared with baseline (11.07±1.55 at baseline versus

![Figure 2. Timing intervals at baseline and at end point in the same animal as in Figure 1. A, LV pressure and its temporal derivative (dP/dt). B, Myocardial velocities from subendocardial and subepicardial layers. TOTv indicates time from ECG R wave to onset of thinning velocity; TOTvdiff, difference of subendocardial TOTv and subepicardial TOTv; Vs, peak systolic velocity; E', peak early-diastolic velocity; and A', peak late-diastolic velocity. C, Myocardial thickness. TOTt indicates time interval from R wave to maximal thickness; TOTIP, time interval between peak negative dP/dt and maximal thickness. Increase in both TOTvdiff and TOTIP at end point is noticeable despite difference in R-R interval. Adequate comparison of time intervals between baseline and end point required correction for R-R duration.]

**TABLE 2. Regional Myocardial Velocity Data**

<table>
<thead>
<tr>
<th>Region/Layer</th>
<th>Baseline</th>
<th>End Point</th>
<th>Baseline</th>
<th>End Point</th>
<th>Baseline</th>
<th>End Point</th>
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</thead>
<tbody>
<tr>
<td>Testing</td>
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<tr>
<td>Endo</td>
<td>4.58±1.38†</td>
<td>5.05±1.98†</td>
<td>13.54±4.37†</td>
<td>11.55±5.81†</td>
<td>1.99±1.17</td>
<td>2.71±1.33†</td>
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<tr>
<td>Epi</td>
<td>2.93±1.15</td>
<td>3.87±1.60*</td>
<td>9.08±2.84</td>
<td>6.52±2.84*</td>
<td>1.67±1.02</td>
<td>2.21±0.98</td>
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<tr>
<td>Control</td>
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<tr>
<td>Endo</td>
<td>7.52±2.72†</td>
<td>8.26±2.05†</td>
<td>8.82±2.25†</td>
<td>9.74±4.20†</td>
<td>3.60±1.15‡</td>
<td>5.33±2.42†</td>
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<tr>
<td>Epi</td>
<td>5.46±2.55</td>
<td>6.05±1.63</td>
<td>5.41±1.88</td>
<td>7.30±3.05</td>
<td>2.21±1.16</td>
<td>4.04±2.13*</td>
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</table>

Vs indicates maximal systolic velocity; E’, maximal velocity during early diastole; A’, maximal velocity during late diastole; Endo, subendocardial layer; and Epi, subepicardial layer. Data from all 12 animals are presented.

*P<0.05 vs baseline; †P<0.01 vs Epi.
12.61 ± 1.65 at end point; P<0.01). Corrected TOT\textsubscript{v,ref} in the testing region was still significantly higher at the end point compared with baseline (−0.14 ± 0.24 at baseline versus 0.44 ± 0.87 at the end point; P<0.01) in 10 dogs without necrosis.

**Discussion**

The main finding of our study is that a selectively (ie, subendocardially) delayed onset of LV wall thinning can signify resting hypoperfusion when the subtended myocardium is transmurally viable (10 of the 12 experimental dogs). The severity of the induced perfusion deficit was obvious from the development of thin subendocardial necrosis in the 2 remaining animals. However, even in these animals, the LV remained regionally functional on the basis of selectively measured tissue velocities.

**TABLE 3. MBF Data**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>End Point</th>
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<tbody>
<tr>
<td>MBF, mL·min(^{-1})·g(^{-1})</td>
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<tr>
<td>Subendocardial layer</td>
<td>0.90 ± 0.35</td>
<td>0.68 ± 0.32*</td>
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<tr>
<td>Subepicardial layer</td>
<td>0.97 ± 0.52</td>
<td>0.92 ± 0.37</td>
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<td>Middle anterior segment</td>
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<td>Subendocardial layer</td>
<td>0.90 ± 0.32</td>
<td>0.61 ± 0.31*</td>
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<td>Subepicardial layer</td>
<td>0.89 ± 0.52</td>
<td>0.83 ± 0.24</td>
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<tr>
<td>Subendocardial layer</td>
<td>0.93 ± 0.54</td>
<td>0.87 ± 0.43</td>
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<tr>
<td>Subepicardial layer</td>
<td>1.04 ± 0.68</td>
<td>1.02 ± 0.44</td>
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<tr>
<td>Apical anterior wall</td>
<td>0.99 ± 0.21</td>
<td>0.73 ± 0.30†</td>
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<tr>
<td>Middle anterior wall</td>
<td>1.07 ± 0.24</td>
<td>0.74 ± 0.34*</td>
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<tr>
<td>Control region</td>
<td>0.91 ± 0.17</td>
<td>0.85 ± 0.21</td>
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*P<0.05, †P<0.01 vs baseline.

**Conventional Global and Regional Functional Parameters**

We demonstrate that global parameters, such as LVEF, −dP/dt, and r, as well as magnitudes of regional systolic and diastolic tissue Doppler velocities may not significantly change at the early stage of progressive ischemia. The increases of ventricular +dP/dt as well as trends to higher values or significant increases of peak systolic velocity and A’ in both testing and control regions at the end point are explainable by the effect of a higher sympathetic tone on nonischemic regions during induced progressive ischemia. The baseline data show that both systolic and diastolic myocardial velocities are higher in the subendocardium than in the subepicardium in a given testing or control region. This functional heterogeneity is consistent with clinical\(^{16}\) and experimental\(^{21}\) reports.

**TABLE 4. Timing of Onset of Regional Myocardial Thinning**

<table>
<thead>
<tr>
<th>Region/Layer</th>
<th>TOT\textsubscript{v} ms</th>
<th>TOT\textsubscript{v,ref} ms</th>
<th>TOT\textsubscript{v,ref,*} ms</th>
<th>TOT\textsubscript{t} ms</th>
<th>TOT\textsubscript{TP} ms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Testing</strong></td>
<td></td>
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<tr>
<td>Endo</td>
<td>259 ± 37</td>
<td>285 ± 43</td>
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<tr>
<td>Epi</td>
<td>262 ± 38</td>
<td>272 ± 45</td>
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<tr>
<td><strong>Control</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Endo</td>
<td>291 ± 48</td>
<td>275 ± 30</td>
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</tr>
<tr>
<td>Epi</td>
<td>293 ± 47</td>
<td>280 ± 34</td>
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</table>

TOT\textsubscript{v} indicates time to onset of regional myocardial thinning based on tissue velocity redirection; TOT\textsubscript{v,ref} mean difference of TOT\textsubscript{v} in subendocardial (Endo) minus subepicardial (Epi) layers; TOT\textsubscript{t} time to onset of regional myocardial thinning based on wall thickness change; and TOT\textsubscript{TP}, TOT\textsubscript{t} minus time from R wave to peak negative time derivative of LV pressure.

*Data from the 10 animals without subendocardial necrosis. In all other columns, data from all 12 animals are presented.

†P<0.01 vs baseline, ‡P<0.05 vs control.
Peak systolic velocities in the control region are higher than those in the testing region in our study. This is in agreement with observations by McNerney et al.\textsuperscript{122} on closed-chest dogs. These authors described significantly greater displacement and velocity of the posterior LV epicardium during systole compared with those of the anterior wall. After isovolumic contraction, when the LV in dogs becomes more elliptical at large volumes and more spherical at small volumes,\textsuperscript{23} vigorous posterior wall displacement in the anterior direction facilitates blood ejection through the LV outflow tract into the aorta.

### Potential Mechanisms of Subendocardial Delay in Myocardial Thinning

Our results indicate that the delayed onset of subendocardial thinning is present selectively in the hypoperfused subendocardial layer of the region at ischemic risk even when the myocardium is still transmurally viable. Henein et al.\textsuperscript{24} demonstrated that the onsets of both subendocardial contraction and relaxation are significantly delayed in the ischemic region. Garcia-Fernandez et al.\textsuperscript{1} showed a prolonged isovolumic relaxation period in ischemic myocardium. However, no one has compared the onset of regional thinning selectively in the subendocardial and subepicardial layers, nor had we found an attempt to explore whether progression of this heterogeneity, while segmental function is preserved, could be a functional alert of a preclinical stage of progressive coronary disease. Ischemia results in a prolonged action potential of Purkinje’s fibers and myocytes\textsuperscript{25} and prolonged calcium uptake by reticulum calcium ATPase.\textsuperscript{26} Because our study suggested a selective impairment of subendocardial myocardial perfusion and an impaired delivery of energy supplies\textsuperscript{27} in subendocardium, a delay of the thickening-thinning transition resulting from the prolonged action potential and calcium uptake should be localized in the subendocardium as well. This selective delay may appear counterintuitive when the tethering effect across the myocardial wall is considered. In other words, the persistent inward movement of subendocardium should have pulled the subepicardium to move inward as well. However, subepicardial myocardial fibers are in a different alignment compared with those in the subendocardium.\textsuperscript{28,29} In addition, the tension of subepicardial myocardium during diastole may be higher than that of subendocardial myocardium,\textsuperscript{30} ie, weakened but persisting subendocardial thickening may not necessarily tether the subepicardial myocardium.

Some investigators observed that decay of both the action potential and calcium transient is slower and lasts longer in subendocardial myocytes.\textsuperscript{31,32} These differences between subendocardial and subepicardial myocytes underlie physiological transmural functional heterogeneity within the LV wall.\textsuperscript{33} One could speculate that the onset of regional diastolic thinning would be normally delayed in the subendocardium compared with the subepicardium, which our baseline measurements in Tables 3 and 4 do not show. Later depolarization of subepicardium\textsuperscript{34} may represent a plausible explanation. In particular, depolarization starts in anterior endocardium first and spreads to the other wall and epicardium.\textsuperscript{34} Although the action potential duration and decay are longer in the subendocardium than in the subepicardium, an earlier depolarization in subendocardial myocardium may counteract this difference.

### Ultrasound Approaches to Timing of Myocardial Thinning

In the present study we used 2 approaches for quantitative timing of regional thinning, ie, TDI and wall thickness function. TDI has become the primary method of choice for clinical analysis of localized systolic and diastolic myocardial velocities.\textsuperscript{17,35,36} In our study we used TDI for the timing of myocardial postejection motion redirection assessed selectively in the subendocardial and subepicardial layers. A short-lived isovolumic biphasic pattern usually can be seen in normal conditions, especially by transthoracic scanning.\textsuperscript{17} We used the second redirection for the TOTv measurement because it may mark the end of postejection thickening.

Considering that wall thickness may provide important information during progressive ischemia,\textsuperscript{37} we used the thickness function for measuring the onset of regional thinning. We found retardation of the onset of wall thinning at the end point compared with baseline in the testing region; the thickness function, however, did not allow for further layer-selective analysis of thinning. TOTP values corroborated these findings. The TOTP interval is independent of the duration of the ejection phase. However, the interval analysis requires timing of the peak $-dP/dt$ from an invasively measured LV pressure.

Our previous clinical research suggested that dobutamine stress would further increase and transmurally expand the delay in diastolic thinning.\textsuperscript{16} In the present study we explored and documented that an early stage of myocardial hypoperfusion can be detected at rest, thus avoiding a possible progression of an energy metabolic debt.

Importantly, TOTv$_{diff}$ can identify the presence of subendocardial hypoperfusion without comparison with the baseline status. The subtended subepicardial myocardium, which remains functionally intact, can serve as an internal reference factor for the subendocardial myocardium. Such an approach facilitates detection of early hypoperfusion under resting conditions when regional velocities are not measurably changed. Consequently, baseline measurements, which would not be available in a clinical setting of ischemia, would not be needed to identify subendocardial myocardium at ischemic risk.

The results showed that baseline TOTv was similar to TOTi in the testing anterior wall but longer than TOTi in the control inferior wall. This may be due to a more frequent occurrence of biphasic isovolumic relaxation velocity patterns observed in TDI from the inferior wall compared with isovolumic relaxation velocity patterns from the testing region, which were frequently unidirectional.

TDI-measured velocity magnitude can be confounded by the tethering effect of the surrounding myocardium and heart translational motion. Velocity gradient or strain rate measurements by Doppler echocardiography, which minimize these effects, were clinically validated.\textsuperscript{38} We rec-
ordered tissue velocities within subendocardial and subepicardial layers selectively, capitalizing on high spatial and temporal resolution of TDI. However, we showed that timing (rather than velocity magnitude) of local myocardial thinning identifies resting subendocardial hypoperfusion in viable myocardium. Comparisons within the myocardial segment (ie, TOTvdiff) further contribute to canceling the effects of heart translation or segment tethering and support prospective application of the timing parameter in clinical TDI.

Limitations
Placement of the LA tube represents a highly invasive procedure, acceptable only for an experimental setting. However, intracardiac ultrasound is used in minimally invasive clinical cardiology. In addition, human chest anatomy is less limiting for transthoracic approaches than that of the dog, and a clinical study by Marcos-Alberca et al16 on intramyocardial analysis of regional systolic and diastolic function in patients with LV ischemia documents that adequate quality images for layer-selective analysis are obtainable by transthoracic scanning.

The present animal model simulates progressive stenosis only in a single coronary artery. Additionally, the rate of stenosis progression may vary widely.

Conclusions
On the basis of our experimental model, progressive coronary stenosis is characterized in early stages, when segmental velocities are preserved, by a delayed onset of diastolic thinning. The delayed onset occurs selectively in the subendocardial myocardium, represents an early functional correlate of subendocardial hypoperfusion, and is detectable by high-resolution echocardiography in a resting heart. Transmural heterogeneity in the onset of diastolic thinning is induced by subendocardial hypoperfusion even when the myocardium at risk is still transmurally viable.

Acknowledgments
This work was supported by the American Heart Association Established Investigator Award and in part by HL 68555 and HL 70363 grants and by Siemens Medical Solutions USA, Inc. We thank Kay D. Parker and Nancy L. Peters for veterinary assistance and Jennifer L. Milliken for secretarial help.

References


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Jianwen Wang, Theodore P. Abraham, Josef Korinek, Stig Urheim, Eileen M. McMahon and Marek Belohlavek

_Circulation_. 2005;111:2943-2950; originally published online May 31, 2005;
doi: 10.1161/CIRCULATIONAHA.104.482984
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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