Changes in Porcine Transmitral Flow Velocity Pattern and its Diastolic Determinants During Partial Coronary Occlusion

Steven B. Solomon, PhD, Paolo Barbier, MD, Stanton A. Glantz, PhD, FACC
San Francisco, California

OBJECTIVES
To define the mechanical determinants of transmitral flow and the effect of heart rate during regional ischemia.

BACKGROUND
Myocardial ischemia changes the transmitral flow velocity pattern due to disease-induced changes in the heart’s diastolic properties.

METHODS
Regional ischemia was produced in 12 pigs by partially occluding the left anterior descending coronary artery until segment-length shortening in the ischemic region fell by 20%. Transmitral flow velocity patterns and their determinants were measured under two conditions, baseline and ischemia, at two heart rates, 70 and 90 beats/min.

RESULTS
Regional ischemia had a significant effect on two determinants of filling: relaxation, which was slower, and chamber stiffness, which increased. These changes were associated with reduced contractility and increased myocardial stiffness, resulting in an early transmitral flow pattern that was flatter and narrower, but no change in the late flow pattern. Moderate increases in heart rate accelerated relaxation and decreased atrioventricular pressure gradient but had no effect on contractility or myocardial or chamber stiffness, resulting in an early transmitral flow pattern that was flatter and narrower and an increased late flow velocity.

CONCLUSIONS
This model of regional ischemia leads to a flatter and narrower early transmitral flow velocity pattern and no change in late flow due to a combination of slowed left ventricular relaxation and increased chamber stiffness. Reflex increases in heart rate that accompany ischemia tend to mask this effect. (J Am Coll Cardiol 1999;33:854–66) © 1999 by the American College of Cardiology

Previous studies have shown that myocardial ischemia results in changes in the pattern of flow through the mitral valve (1–6). These changes in transmitral flow velocity pattern are most likely determined by disease-induced changes in the underlying diastolic properties of the heart (3,6–8). Knowing which diastolic properties determine the transmitral flow velocity pattern and how interactions between diastolic properties modify the overall transmitral flow velocity pattern would be helpful in understanding the hemodynamics of patients with myocardial ischemia.

Several transmitral flow velocity patterns have been reported during myocardial ischemia (5,6,8–10). In one pattern, early flow velocity is decreased, deceleration time is increased, and late flow velocity is increased (5,6,8). The diastolic determinant changed in this pattern is left ventricular relaxation, which is slowed. Slowed left ventricular relaxation is observed in some patients with dilated cardiomyopathy (11,12) and coronary artery disease (1–3). In another transmitral flow velocity pattern, early flow velocity is increased, early acceleration and deceleration times are decreased, and late flow velocity is decreased (6,9,10). The diastolic determinants changed in this pattern are myocardial and left ventricular chamber stiffness which are increased. Increased myocardial and left ventricular chamber stiffness also occurs in restrictive cardiomyopathy (6) or diseases with a restrictive left ventricular filling physiology such as cardiac amyloidosis (13) and severe aortic regurgitation (14). It has been suggested, but not demonstrated, that during acute ischemia an intermediate transmitral flow velocity pattern may occur because of a concomitant alteration of left ventricular relaxation and chamber stiffness (15).

The precise determinants of the transmitral flow velocity pattern that may occur during partial occlusion model of myocardial ischemia are uncertain. The total coronary occlusion model of myocardial ischemia suggests changes in left ventricular relaxation and stiffness. Yet, it is not clear whether slowing of left ventricular relaxation or increases in myocardial and chamber stiffness or both are responsible for the transmitral flow pattern (5). The clinical relevance of
this observation lies in the fact that changes in left ventricular relaxation and chamber stiffness may occur at the same time during ischemia in the clinical setting, resulting in mixed transmitral flow patterns, which may be difficult to interpret in the absence of an adequate model. To identify the changes in the determinants of the transmitral flow velocity pattern seen in an animal model of regional ischemia produced by partial occlusion of the left anterior descending coronary artery, we observed the transmitral flow velocity pattern and identified the changes in its determinants during partial occlusion of the left anterior descending coronary artery. Once we identified the changes in the determinants that were responsible for the pattern observed during regional ischemia, we repeated the experiments at an increased heart rate to identify any heart rate–induced changes in the determinants of the transmitral flow velocity pattern during regional ischemia. This form of ischemia leads to a change in the transmital flow velocity pattern, with a flatter and narrower early filling wave (E-wave) and no change in late filling (A-wave), due to a combination of slowed left ventricular relaxation and increased chamber stiffness. Reflex increases in heart rate that accompany ischemia tend to mask this effect.

METHODS

Surgical preparation. Juvenile pigs (32–43 kg) were premedicated with a subcutaneous injection of ketamine (20 mg/kg) mixed with xylazine (2 mg/kg) and atropine (0.5 mg) given in the neck. After adequate sedation was observed, the pig was placed on the operating room table and then anesthetized via an ear vein with chloralose (100 mg/kg). The pig was intubated and mechanically ventilated with room air. The femoral vein was catheterized for introducing further anesthetics and for blood gas sampling. Anesthesia was maintained by the administration of fentanyl (30 μg) and pancuronium (4 mg) every 20 min or as needed. The pigs were placed in the supine position and a midline sternotomy was performed. The pericardium was opened widely to create a pericardial cradle. A sample of blood was then taken from the femoral artery for measurement of blood gases and a lidocaine drip (0.6 mg/min) was started. Blood gases were reassessed after each intervention curve over a wide range of end-diastolic volumes both before and after reduction of coronary flow. A pair of pacing wires was placed on the right atrial appendage.

A 7-Fr eight-electrode conductance catheter (Webster Laboratories, Baldwin Park, California) was placed in the left ventricle to measure left ventricular volume via an apical approach and connected to electronics (Leycom-Sigma 5, Leiden, The Netherlands) that convert the conductance signal into a volume. Proper positioning of the conductance catheter was checked echocardiographically and from the contours of the segment volume signals.

Echocardiographic measurements. To identify baseline transmitral flow velocity patterns, transmitral flow velocity was measured echocardiographically (epicardial approach)
after instrumentation and a steady-state had been reached. In addition, ventricular wall motion (an index of ventricular chamber function), and mitral orifice area (a determinant of the transmitral flow velocity pattern) were measured echocardiographically.

The echocardiographic studies of the transmitral flow velocity patterns were performed using a Hewlett-Packard ultrasound system (Sonos 2500) equipped with 2.5/3.5 MHz and 5.0 MHz transducers. An epicardial approach was used, positioning the transducer over the left ventricular apical dimple. Biplane recordings of the left ventricular cavity were obtained in the four- and two-chamber views, adjusting transducer angulation to maximize the left ventricular long axis, mitral valve annulus diameter and mitral valve leaflet excursion during diastole. In the four-chamber view, pulsed Doppler transmitral flow velocities were recorded by positioning the sample volume both at the level of the tip of the mitral valve leaflets and the mitral valve annulus. At the level of the tip of the mitral valve, the times to the beginning, peak and end of the components of the transmitral flow velocity pattern, the E-wave (early diastolic filling) and A-wave (late diastolic filling) were measured from the peak of the ECG R-wave. The acceleration and deceleration time of the E-wave was also measured. The deceleration time was calculated by extrapolating the deceleration slope to the baseline. At the level of the mitral valve annulus, diastolic filling time was determined as the duration of mitral flow. The flow during the E-wave and A-wave was calculated by planimetry of each velocity curve. The mean mitral orifice area was calculated by planimetry from a biplane view measured at the level of the mitral annulus. Each variable was quantified by averaging the measurements made from three consecutive steady-state beats. The occurrence of mitral regurgitation during ischemia was monitored using two-D color flow.

Hemodynamic measurements. Hemodynamic variables were measured from the left atrial and left ventricular pressure waveforms. Left ventricular end-diastolic pressure (LVEDP) was taken at the rapid upstroke in left ventricular pressure, which occurred at approximately 10% of dP/dt_max. Left atrial pressure crossover of the left ventricular pressure (PCO), which occurs when the mitral valve opens, was determined from the matched left atrial and left ventricular pressure traces. The pressures were matched by adjusting the left atrial and left ventricular pressure waveform during middiastole at the beginning of the experiment. An index of the driving atrioventricular pressure gradient was calculated as the time integral of the difference between left atrial and ventricular pressure from mitral valve opening until first pressure reversal. The mean atrioventricular pressure gradient was calculated from the atrioventricular pressure gradient integral divided by the time between mitral valve opening and first pressure reversal. Chamber stiffness at end-diastolic pressure was determined by calculating dP/dV at end-diastolic pressure. The time constant of isovolumic relaxation, \( \tau_e \), was determined by fitting the left ventricular pressure between the times of dP/dt_min and left atrial pressure crossover to \( P = P_0 e^{-\tau_e} \) (16,17).

A standard calibration function was used to correct left ventricular volume by adjusting for the parallel conductance volume and slope in each pig. This correction was estimated by comparing the conductance volumes with volumes measured using two-dimensional echocardiography during the four experimental conditions according to:

\[
V = (1/\alpha_e) V_c - V_p,
\]

where \( 1/\alpha_e \) is the slope of the relation between conductance volumes and two-dimensional echocardiographically measured volumes, \( V_c \) is the uncorrected conductance volume and \( V_p \) is the parallel conductance. The conductance volumes were used to calculate stroke volume and cardiac output.

We present the uncorrected chamber volumes (18) as well as the corrected chamber volumes. Since we were interested in the change in chamber volumes within a given pig, either uncorrected or corrected conductance volumes could be used to determine relative changes in chamber volumes (18,19). The parallel conductance volume was 53.6 ± 11.9 (SD) ml and \( \alpha_e \) was 0.77 ± 0.16.

Characterization of the passive left ventricular diastolic pressure-volume relation. The left ventricular diastolic pressure-volume relation was characterized using Nikolic’s approach (17):

\[
P = -S_p \ln[(V_m - V)/(V_m - V_0)];
\]

where \( S_p \) is a parameter that describes the curvature of the pressure-volume relation, \( V_0 \) is the equilibrium volume and \( V_m \) is the maximum attainable volume of the ventricular chamber before irreversible damage of tissue. The parameters \( S_p, V_m \) and \( V_0 \) were estimated using nonlinear regression with SigmaStat v2.0 (SPSS, Inc., Chicago, Illinois).

Characterization of the passive myocardial stress-strain relation. To estimate the myocardial stress-strain relation, we used the ventricular pressure-volume relation based on a thick-walled version of the Laplace relation and the exponential stress-strain relation for the myocardium (20). The passive diastolic stress-strain relation that describes the myocardium’s nonlinear stiffness is (20):

\[
\sigma = \alpha(e^{\epsilon} - 1);
\]

where \( \sigma \) = stress (force/area in the myocardial wall); \( \epsilon \) = Lagrangian strain = \( (1 - 1/L) / L_0 \), where \( 1 - 1/L \) is length relative to the equilibrium length, \( L_0 \), which reflects the amount of sarcomere stretch (fractional extension from rest length); alpha and beta are parameters that describe muscle stiffness. Alpha and beta were calculated directly from the diastolic pressure-volume relation using (20):

\[
P = \alpha \eta (2 + \eta) \{\exp(\beta(2 + \eta)(3\pi^2/V /32)^{1/3} - x_0)) - 1\};
\]

where:
\[ \eta = h(4\pi/3V)^{1/3} \]
\[ x_0 = \pi(3V_0/4\pi)^{1/3} + h/2 \]

The equilibrium volume, \( V_0 \), is taken as a fixed value, obtained from fitting Nikolic’s equation to the data. To characterize the changes in the passive elastic properties of the myocardium, this equation was used to fit the end-diastolic pressure-volume data points from the vena caval occlusion performed before and after regional ischemia at 70 and 90 bpm. Inx was used as the parameter for estimation purposes to provide a better conditioned nonlinear parameter estimation using SigmaStat v2.0 (SPSS, Inc., Chicago, Illinois).

Systolic pressure-volume relation. The slope of the end-systolic pressure-volume relation, \( E_{max} \), an index of contractility, was determined by linear regression analysis of the end-systolic pressure (\( P_{es} \)) and volume (\( V_{es} \)) data obtained during inferior vena cava occlusions using:

\[ P_{es} = E_{max}(V_{es} - V_d); \]

where \( E_{max} \) is the slope and \( V_d \) is the volume-axis intercept of the end-systolic pressure-volume relation (21). \( E_{max} \) and \( V_d \) were estimated using Kono et al.’s method (22).

PROTOCOL. The transmitral flow velocity patterns and their determinants were measured under two conditions—baseline and ischemia, each during pacing at two heart rates—70 and 90 bpm.

Baseline hemodynamics needed to measure or calculate the determinants of transmitral flow velocity pattern were recorded. These determinants were left ventricular isovolumic relaxation rate, contractility, myocardial and ventricular chamber stiffness, and components of the atroventricular pressure gradient. To be able to calculate contractility and myocardial and chamber stiffness, a baseline inferior vena cava occlusion was performed to progressively decrease inflow so that a wide range of pressures and volumes could be observed.

Next, to identify the transmitral flow velocity pattern seen during regional ischemia and the determinants of this pattern, regional ischemia was induced and the pattern and the determinants were reassessed. To induce ischemia, the left anterior descending coronary artery was partially occluded by tightening a miniature C-clamp (LAD constrictor, Fig. 1) until absolute segment-length shortening decreased by 20% (for approximately 10–20 min). This degree of constriction decreased coronary artery blood flow by approximately 75% (from 45.8 ± 4.9 to 11.2 ± 4.9 (SEM) ml/min, \( p < 0.001 \)).

To determine the effect of increasing heart rate on the transmitral flow velocity pattern and the determinants of this pattern, baseline and ischemia experiments were performed during pacing at two heart rates, 70 and 90 bpm. Zatebradine (ULFS-49), a bradycardic agent that acts on the sino-atrial node without hemodynamic effects, was administered to maintain the lower rate (23). At each step, measurements were taken with the respirator turned off at end-expiration.

Statistical analysis. The ischemia protocols were analyzed using two-way repeated-measures analysis of variance to determine the effects of ischemia and heart rate. Because there are only two levels of each factor, we did not need to use a multiple comparison procedure. We report the least square means from the ANOVA and associated standard errors (Tables 1–3). The means for treatment reflect the main effect of ischemia independent of the heart rate, that is, the average at baseline (70 and 90 bpm) compared to the average during ischemia (70 and 90 bpm) and the means for heart rate reflect the main effect of heart rate independent of the presence or absence of ischemia, that is, the average at 70 bpm (baseline and ischemia) compared to the average at 90 bpm (baseline and ischemia). To determine the number of pigs required we used results from previous work in this laboratory on the effects of partial occlusion of the left anterior descending coronary artery. We would expect to see a change of \( 4 \pm 4.8 \) (SD) mm Hg in end-diastolic pressure in response to ischemia. To attain an 80% power to detect this difference with \( \alpha = 0.05 \), a minimum of 8 pigs was required. We used 12 pigs in this study to obtain unambiguous results. Computations were done with SigmaStat v2.0 for balanced data and SAS procedure GLM to account for missing values (24,25). There were six missing values in the echocardiographic data. E-wave deceleration at 90 bpm was not quantified because of the lack of separation with the A-wave at this heart rate. We considered differences significant when \( p < 0.05 \).

RESULTS

Changes in the transmitral flow velocity pattern due to regional ischemia. Regional ischemia induced by partial occlusion of the left anterior descending coronary artery changed the transmitral flow velocity pattern (Fig. 2). Specifically, the early filling wave (E-wave) became flatter and narrower; but peak velocity, timing and flow volume of the late filling wave (A-wave) were unchanged (Fig. 2, Table 1). The flattening of the E-wave was due to a decrease in peak E-wave velocity during ischemia (58.3 ± 4.2 vs. 46.9 ± 4.4 cm/s, \( p < 0.001 \)) (Table 1). The narrower E-wave was due to an increase in both the time to the beginning of the E-wave during ischemia (479 ± 12 vs. 507 ± 12 ms, \( p < 0.002 \)) and the isovolumic relaxation time (101 ± 7 vs. 113 ± 8 ms, \( p < 0.03 \)), and to a decrease in E wave deceleration time (122 ± 4 vs. 107 ± 5 ms, \( p < 0.045 \)). Times to the peak and end of the E-wave did not change. These changes in peak E-wave velocity and timing during ischemia resulted in decreased E-wave flow volume (34.8 ± 17 vs. 23.1 ± 17 ml, \( p < 0.001 \)) without a compensatory increase in A-wave flow, which led to decreased stroke volume and cardiac output (Table 2).

There was no mitral regurgitation observed during ischemia.
### Table 1. Mitral Flow Patterns and Timing (in msec)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline</th>
<th>Ischemia</th>
<th>Means for Heart Rate</th>
<th>Means for Treatment</th>
<th>p Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>70 bpm</td>
<td>90 bpm</td>
<td>70 bpm</td>
<td>90 bpm</td>
<td>70 bpm</td>
</tr>
<tr>
<td>E-WAVE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to beginning</td>
<td>498 ± 12</td>
<td>460 ± 12</td>
<td>520 ± 12</td>
<td>492 ± 12</td>
<td>509 ± 12</td>
</tr>
<tr>
<td>Time to peak</td>
<td>570 ± 11</td>
<td>524 ± 11</td>
<td>595 ± 11</td>
<td>533 ± 12</td>
<td>583 ± 10</td>
</tr>
<tr>
<td>Time to end</td>
<td>681 ± 10</td>
<td>584 ± 10</td>
<td>690 ± 9</td>
<td>569 ± 23</td>
<td>686 ± 7</td>
</tr>
<tr>
<td>E-wave duration</td>
<td>204 ± 11</td>
<td>139 ± 11</td>
<td>189 ± 11</td>
<td>93 ± 21</td>
<td>196 ± 9</td>
</tr>
<tr>
<td>Peak velocity</td>
<td>61.8 ± 4.4</td>
<td>54.9 ± 4.4</td>
<td>51.7 ± 4.4</td>
<td>42.1 ± 5.1</td>
<td>56.8 ± 4.2</td>
</tr>
<tr>
<td>E-wave deceleration</td>
<td>122 ± 4</td>
<td>-</td>
<td>107 ± 5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E-wave flow</td>
<td>42.3 ± 3.2</td>
<td>27.1 ± 3.2</td>
<td>31.0 ± 3.2</td>
<td>15.3 ± 3.2</td>
<td>36.7 ± 3.2</td>
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<tr>
<td>A-WAVE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to beginning</td>
<td>743 ± 9</td>
<td>584 ± 9</td>
<td>738 ± 9</td>
<td>538 ± 20</td>
<td>740 ± 6</td>
</tr>
<tr>
<td>Time to peak</td>
<td>807 ± 7</td>
<td>618 ± 7</td>
<td>805 ± 7</td>
<td>613 ± 9</td>
<td>806 ± 6</td>
</tr>
<tr>
<td>Time to end</td>
<td>873 ± 7</td>
<td>688 ± 7</td>
<td>881 ± 7</td>
<td>687 ± 9</td>
<td>877 ± 6</td>
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<tr>
<td>A-wave duration</td>
<td>125 ± 4</td>
<td>106 ± 4</td>
<td>147 ± 4</td>
<td>141 ± 10</td>
<td>136 ± 2</td>
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<tr>
<td>Peak velocity</td>
<td>46.4 ± 3.8</td>
<td>66.7 ± 3.8</td>
<td>48.5 ± 3.8</td>
<td>68.5 ± 4.4</td>
<td>47.5 ± 3.5</td>
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<tr>
<td>A-wave flow</td>
<td>30.2 ± 2.0</td>
<td>36.3 ± 2.0</td>
<td>27.7 ± 2.0</td>
<td>40.5 ± 2.0</td>
<td>28.9 ± 2.0</td>
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<tr>
<td>Diastolic fill time</td>
<td>376 ± 13</td>
<td>228 ± 13</td>
<td>360 ± 13</td>
<td>195 ± 14</td>
<td>368 ± 13</td>
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<tr>
<td>Total flow</td>
<td>77 ± 2</td>
<td>57 ± 2</td>
<td>60 ± 2</td>
<td>50 ± 2</td>
<td>68 ± 9</td>
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<tr>
<td>Mean mitral area</td>
<td>7.2 ± 0.2</td>
<td>6.9 ± 0.2</td>
<td>7.7 ± 0.2</td>
<td>7 ± 0.3</td>
<td>7.5 ± 0.2</td>
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</tbody>
</table>

HR = heart rate; All values are least squares mean ± SEM for 12 pigs using repeated measures ANOVA.
### Table 2. Hemodynamic Measurements

<table>
<thead>
<tr>
<th>Treatment Heart Rate</th>
<th>Baseline</th>
<th>Ischemia</th>
<th>Means for Heart Rate</th>
<th>Means for Treatment</th>
<th>p Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>70 bpm</td>
<td>90 bpm</td>
<td>70 bpm</td>
<td>90 bpm</td>
<td></td>
</tr>
<tr>
<td>EDP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.9 ± 1.4</td>
<td>10.3 ± 1.4</td>
<td>19.2 ± 1.4</td>
<td>13.7 ± 1.4</td>
<td>16.6 ± 1.2</td>
<td>12.1 ± 1.2</td>
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<tr>
<td>LVP_{peak} (mm Hg)</td>
<td>103 ± 5</td>
<td>107 ± 5</td>
<td>96 ± 5</td>
<td>99 ± 4</td>
<td>105 ± 4</td>
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<tr>
<td>MVO (mm Hg)</td>
<td>7.7 ± 0.7</td>
<td>6.4 ± 0.7</td>
<td>9.0 ± 0.7</td>
<td>7.8 ± 0.7</td>
<td>7.0 ± 0.7</td>
</tr>
<tr>
<td>LAP_{peak} (mm Hg)</td>
<td>8.7 ± 0.9</td>
<td>9.0 ± 0.9</td>
<td>10.4 ± 0.9</td>
<td>11.2 ± 0.9</td>
<td>9.6 ± 0.9</td>
</tr>
<tr>
<td>LVP_{min} (mm Hg)</td>
<td>6.3 ± 0.6</td>
<td>5.9 ± 0.6</td>
<td>7.5 ± 0.6</td>
<td>7.6 ± 0.6</td>
<td>6.9 ± 0.6</td>
</tr>
<tr>
<td>A-V grad (mm Hg-ms)</td>
<td>36.1 ± 6.7</td>
<td>33.3 ± 6.7</td>
<td>31.8 ± 6.7</td>
<td>31.5 ± 6.7</td>
<td>33.9 ± 6.7</td>
</tr>
<tr>
<td>EDV_v (ml)</td>
<td>105 ± 3</td>
<td>98 ± 3</td>
<td>107 ± 3</td>
<td>103 ± 4</td>
<td>106 ± 3</td>
</tr>
<tr>
<td>ESV_v (ml)</td>
<td>77 ± 4</td>
<td>75 ± 4</td>
<td>80 ± 4</td>
<td>79 ± 4</td>
<td>79 ± 4</td>
</tr>
<tr>
<td>EDV (ml)</td>
<td>55 ± 4</td>
<td>48 ± 4</td>
<td>58 ± 4</td>
<td>54 ± 4</td>
<td>56 ± 4</td>
</tr>
<tr>
<td>ESV (ml)</td>
<td>27 ± 4</td>
<td>25 ± 4</td>
<td>31 ± 4</td>
<td>30 ± 4</td>
<td>29 ± 4</td>
</tr>
<tr>
<td>SV (ml)</td>
<td>28.5 ± 2.9</td>
<td>24.3 ± 2.9</td>
<td>25.6 ± 2.9</td>
<td>21.4 ± 2.9</td>
<td>28.5 ± 2.9</td>
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<tr>
<td>CO (l/min)</td>
<td>2.0 ± 0.2</td>
<td>2.2 ± 0.2</td>
<td>1.8 ± 0.2</td>
<td>1.9 ± 0.2</td>
<td>1.9 ± 0.2</td>
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<tr>
<td>τ (ms)</td>
<td>45.9 ± 1.6</td>
<td>39.0 ± 1.6</td>
<td>52.2 ± 1.6</td>
<td>46.0 ± 1.6</td>
<td>49.0 ± 1.5</td>
</tr>
<tr>
<td>IVRT (ms)</td>
<td>104 ± 8</td>
<td>97 ± 8</td>
<td>112 ± 8</td>
<td>113 ± 8</td>
<td>108 ± 7</td>
</tr>
<tr>
<td>ΔSL (mm)</td>
<td>4.7 ± 0.5</td>
<td>4.0 ± 0.5</td>
<td>3.4 ± 0.5</td>
<td>3.6 ± 0.5</td>
<td>4.1 ± 0.5</td>
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<tr>
<td>RVP_{peak} (mm Hg)</td>
<td>31.5 ± 10.1</td>
<td>31.9 ± 10.1</td>
<td>33.7 ± 10.1</td>
<td>45.3 ± 10.1</td>
<td>32.6 ± 9.6</td>
</tr>
<tr>
<td>RVEDP (mm Hg)</td>
<td>6.0 ± 1.6</td>
<td>3.6 ± 1.6</td>
<td>6.2 ± 1.6</td>
<td>5.3 ± 1.6</td>
<td>6.1 ± 1.5</td>
</tr>
<tr>
<td>RVP_{mean} (mm Hg)</td>
<td>15.6 ± 5.4</td>
<td>17.1 ± 5.4</td>
<td>16.9 ± 5.4</td>
<td>25.2 ± 5.4</td>
<td>16.3 ± 5.1</td>
</tr>
</tbody>
</table>

A-V _grad_ = atrioventricular pressure gradient integral; CO _uncorrected_ cardiac output; ΔSL = systolic segment-length shortening; EDP = end-diastolic pressure; EDV_v = uncorrected end-diastolic volume; ESV_v = uncorrected end-systolic volume; EDV = corrected volumes; HR = heart rate; IVRT = isovolumic relaxation time; LAP_{peak} = peak left atrial pressure; LVP_{mean} = minimum left ventricular pressure; LVP_{min} = peak left ventricular pressure; MVO = pressure at mitral valve crossover; RVP_{peak} = peak right ventricular pressure; RVP_{min} = minimum right ventricular pressure; SV = corrected stroke volume; τ = rate of left ventricular pressure decay. All values are least squares mean ± SEM for 12 pigs using repeated measures ANOVA.
Changes in the determinants of the transmitral flow velocity pattern during ischemia. The changes observed in the transmitral flow velocity pattern during regional ischemia were associated with changes in five determinants of flow: relaxation, contractility, myocardial and left ventricular chamber stiffness, and atrioventricular pressure gradient but not to mitral orifice area, which did not change (Tables 2 and 3).

Left ventricular relaxation slowed during regional ischemia, quantified by an increase in $\tau$, the time constant of isovolumic relaxation ($42.4 \pm 1.4$ vs. $49.1 \pm 1.4$ ms, $p < 0.001$) (Table 2). The slowed relaxation was also manifest by an increase in isovolumic relaxation time ($101 \pm 7$ vs. $113 \pm 8$ ms, $p < 0.03$). The increase in $\tau$ affected the transmitral flow velocity pattern by increasing the time to the beginning of the E-wave (and isovolumic relaxation time).

The slowing of left ventricular relaxation contributed to
the decrease in the atrioventricular pressure gradient which also decreased during regional ischemia, calculated from the integral of the difference between the left atrial and ventricular pressure waveforms during early filling (34.7 ± 6.7 vs. 31.7 ± 6.7 mm Hg-ms, p < 0.002) (Table 2). The decrease in the atrioventricular pressure gradient was accompanied by a decrease in the peak velocity of the E-wave producing a flattening of the E-wave.

Contractility, quantified by E\(_{\text{max}}\), the slope of the end-systolic pressure-volume relation, and dP/dt\(_{\text{max}}\) decreased during regional ischemia (5.0 ± 0.2 vs. 3.8 ± 0.4 mm Hg/ml, p < 0.008; 1960 ± 87 vs. 1478 ± 87 mm Hg/s, p < 0.001, respectively) (Table 3). The decrease in contractility was reflected as decreased left ventricular systolic segment shortening in the ischemic region (4.4 ± 0.5 vs. 3.5 ± 0.5 mm, p < 0.042), and a small increase in end-diastolic volume (52 ± 4 vs. 56 ± 4 ml, p < 0.042) and end-systolic volume (26 ± 4 vs. 30 ± 4 ml, p < 0.05) (Fig. 3). The increased end-diastolic volume was accompanied by an increase in end-diastolic pressure (12.1 ± 1.2 vs. 16.4 ± 1.2 mm Hg, p < 0.001). (Uncorrected volumes behaved similarly; see Table 2.)

Both myocardial and chamber stiffness increased during regional ischemia. The increase in myocardial stiffness was indicated by increases in ln \(\alpha\) (−4.2 ± 0.6 vs. −3.2 ± 0.6, p < 0.055) and \(\beta\) (11.0 ± 1.8 vs. 13.6 ± 1.8, p < 0.045), parameters that describe muscle elasticity (Table 3 and Fig. 4). The results are consistent with increased dP/dV at end-diastolic pressure, a measure of chamber stiffness at end-diastole (0.91 ± 0.27 vs. 1.4 ± 0.27 mm Hg/ml, p < 0.011). There was a systematic change in myocardial stiffness during ischemia, which was reflected as a stiffer left ventricular chamber.

The left ventricular chamber, like the myocardium, was stiffer during ischemia, as indicated by \(S_p\), a parameter that describes the curvature of the pressure-volume relation (4.4 ± 0.9 vs. 6.0 ± 0.9, p < 0.03) (Table 3 and Fig. 4). The other chamber property, \(V_0\), the diastolic equilibrium volume, did not change during ischemia. These increases in myocardial and chamber stiffness also contributed to the narrowing of the E-wave.

The other determinant of the transmitral flow velocity pattern, mitral orifice area, did not change during ischemia.

Mean right ventricular pressure, right ventricular end-diastolic pressure and peak right ventricular pressure also did not change with ischemia, showing no signs of right ventricular failure or a ventricular interdependence effect, both of which could have contributed to changes in the transmitral flow velocity pattern.

Changes in the transmitral flow velocity pattern due to increased heart rate. Increasing heart rate altered the transmitral flow velocity pattern, but differently than regional ischemia did. The early filling wave (E-wave) was flatter and narrower when heart rate was increased (Fig. 2). However, the peak velocity of the A-wave was increased, a change not seen during regional ischemia. These changes in the early and late filling wave are reflected by a decrease in the E/A ratio (1.36 ± 0.14 vs. 0.67 ± 0.14, p < 0.001).

The flattening of the E-wave was due to a decrease in peak E-wave velocity (Table 1). The narrowing of the E-wave was due to a decrease in the time to the end of the E-wave that
was greater than the decrease in the time to the beginning of the E-wave (Table 1). E-wave deceleration time could not be calculated due to difficulty in resolving the pattern at higher rates. These decreases in peak E-wave velocity and timing decreased E-wave flow volume (Table 2). The increase in peak A-wave velocity and the absence of a change in timing resulted in increased A-wave flow volume. Although the resulting stroke volume was decreased, this decrease was offset by the increase in heart rate, resulting in no change in cardiac output.

Changes in the determinants of the transmitral flow velocity pattern due to increased heart rate. Of the six determinants of transmitral flow velocity that we tested, only two—relaxation rate and the atrioventricular pressure gradient—were changed by increasing heart rate. Contractility, myocardial and chamber stiffness and mitral orifice area were unchanged.

Relaxation rate increased when heart rate was increased, as indicated by a decrease in $\tau$, the time constant of isovolumic relaxation (49.0 ± 1.5 vs. 42.5 ± 1.5 ms, p < 0.001) (Table 2). The faster relaxation resulted in a decrease in the time to the beginning of the early filling wave (E-wave). The faster relaxation combined with the heart being paced at a faster rate (90 bpm) decreased the duration of filling (from 196 ± 9 to 116 ± 13 ms, p < 0.001), resulting in a narrower E-wave.

The atrioventricular pressure gradient integral decreased when heart rate was increased (from 33.9 ± 6.7 to 32.4 ± 6.7 mm Hg·ms, p < 0.029) (Table 2). The decrease in the atrioventricular pressure gradient resulted in the flattening of the E-wave.

The increased heart rate also led to decreased end-diastolic pressure (16.6 ± 1.2 vs. 12.0 ± 1.2 mm Hg, p < 0.001). A decrease in end-diastolic pressure, which reflects a decrease in left atrial afterload, increased peak A-wave velocity and flow but was not sufficient to maintain total flow. Thus, end-diastolic volume decreased (56 ± 4 vs. 51 ± 4 ml, p < 0.004) but there was no significant change in end-systolic volume. (Uncorrected volumes behaved similarly; see Table 2).

Interaction between ischemia and heart rate. In addition to the main effects of regional ischemia and increased heart rate on the transmitral flow velocity pattern and its determinants in the two way analysis of variance, there were interaction effects for several variables. In other words, the effects of ischemia were different depending on the heart rate.

A significant interaction was noted between the effects of regional ischemia and heart rate on total transmitral flow (Table 1). When heart rate was increased from 70 bpm to 90 bpm during ischemia, the 10 ml decrease in total flow (60 ± 2 vs. 50 ± 2 ml) was less than the 20 ml decrease in total flow when heart rate was increased from 70 bpm to 90 bpm at baseline (77 ± 2 vs. 57 ± 2 ml, p < 0.001) (Fig. 5). To determine where the specific changes occurred, we looked at the changes in flow and duration during early (E-wave) and late (A-wave) filling. During early filling, flow decreased in parallel when heart rate was increased from 70 bpm to 90 bpm during ischemia and when heart rate was increased from 70 bpm to 90 bpm at baseline (both by 15 ml) (Fig. 5). During late filling, flow increased more when heart rate was increased during ischemia than when heart rate was increased at baseline (by 13 vs. 6 ml) (Fig. 5). Thus, the late filling wave (A-wave) was more sensitive to increased heart rate during ischemia, resulting in less of a decrease in total flow after heart rate was increased during ischemia.

A significant interaction was found between the effects of regional ischemia and heart rate on the atrioventricular pressure gradient integral (Table 2). When heart rate was increased from 70 bpm to 90 bpm during ischemia, the 1.5 mm Hg decrease in the atrioventricular pressure gradient integral (33.9 ± 6.7 vs. 32.4 ± 6.7 mm Hg·ms) was less than the decrease in the atrioventricular pressure gradient (3.1 mm
Hg-ms) when heart rate was increased from 70 bpm to 90 bpm at baseline (34.7 ± 6.7 vs. 31.6 ± 6.7 mm Hg/ms) (Fig. 5).

**Changes in regional function: ischemic versus nonischemic region.** During ischemia, an increase in end-diastolic segment length was found in the ischemic region whereas no change was found in the nonischemic region (11.1 ± 0.9 vs. 17.4 ± 0.9 mm in the ischemic region; 11.5 ± 0.6 vs. 11.5 ± 0.6 mm in the nonischemic region; p < 0.04 for ANOVA interaction). The increase in the end-diastolic segment length was observed in the pressure-segment length loop and is consistent with an increase in end-diastolic length of the pressure-volume relation observed early in acute myocardial infarction (26,27). This rightward shift (increased end-diastolic segment length) in the pressure-segment length loop corresponds to severe hypokinesis of the apex, mid-to-distal interventricular septum and distal anterior wall determined by echocardiographic left ventricular segmental wall motion analysis. Moderate increases in heart rate lead to a leftward shift in the pressure-segment length loop due to a decrease in the end-diastolic segment length in both the ischemic and nonischemic regions (15.2 ± 0.7 vs. 13.4 ± 0.7 mm in the ischemic region, p < 0.05 comparing segment length at 70 vs. 90 bpm; 12.1 ± 0.6 vs. 10.9 ± 0.6 mm in the nonischemic region, p < 0.006 comparing segment length at 70 vs. 90 bpm). Although the end-diastolic segment length decreases with moderate increases in heart rate, the ischemic region still operates at an increased end-diastolic segment length when compared with baseline.

**DISCUSSION**

In this model of regional ischemia, the transmitral flow velocity pattern is different from patterns described in other models that used total coronary occlusion (28,29). Specifically, early flow velocity and duration are decreased during regional ischemia due to partial coronary occlusion as indicated by both a flatter and narrower E-wave (with a shorter deceleration time). The decrease in early flow velocity and duration is secondary to slowed ventricular relaxation and increased left ventricular chamber (and myocardial) stiffness, respectively. This result shows that the effect of increased stiffness on E-wave deceleration (shortening of deceleration time) overcomes the effect of slowed ventricular relaxation (prolonged deceleration time) (4,30). Late flow velocity (A-wave) is unchanged, which is in contrast to the expected compensatory increase in response to reduced early filling due to increased left atrial afterload caused by increased left ventricular chamber stiffness.

Previous studies, using an acute occlusion of the coronary artery to produce regional ischemia, reported a pattern consisting of a decrease in early flow velocity (as seen in our study) but also an increase in deceleration time and an increase in late flow velocity (5,6,8). This transmitral flow velocity pattern was attributed primarily to a slowed left ventricular relaxation. Other studies (29,31) reported a pattern consisting of an increase in early flow velocity rather than a decrease, a decrease in both acceleration and deceleration time and a decrease in late flow velocity. This transmitral flow velocity pattern was attributed primarily to an increase in left ventricular stiffness.
What caused the flow pattern we observed? We attribute the pattern we saw during this model of regional ischemia to a combination of the changes in the determinants previously reported, slowed left ventricular relaxation and increased myocardial and left ventricular chamber stiffness. Though this combination has been suggested in previous noninvasive clinical studies (15,32) and simulated in a mathematical model (33), it has never been experimentally demonstrated. The increase in chamber stiffness was due, in part, to an increase in myocardial stiffness. In addition, contractility fell during regional ischemia resulting in an increase in end-diastolic volume. This increase in end-diastolic volume caused the heart to function on a stiffer portion of the end-diastolic pressure-volume relation, also contributing to increased left ventricular chamber stiffness. The increase in chamber stiffness masked the effects of a slowed left ventricular relaxation decreasing deceleration time to produce a narrowing of the E-wave and opposed the expected compensatory increase in atrial contribution to ventricular filling (the A wave). The increase in chamber stiffness and slowed left ventricular relaxation resulted in a decreased atrioventricular pressure gradient which drives left ventricular filling, producing a flatter E-wave.

What, then, accounts for the discrepancy between the transmitral flow velocity pattern and its determinants that we found in this model of regional ischemia due to partial coronary occlusion and the patterns and determinants previously reported (4–6)? Part of the explanation is due to the use of different models of regional ischemia. In the model of regional ischemia used in this study, coronary flow was reduced whereas most of the previous studies used total coronary occlusion.

Effects of heart rate change. In previous models of regional ischemia, heart rate was allowed to increase reflexively. A reflex increase in heart rate often results from the decrease in cardiac output associated with regional ischemia. Harrison et al. (34) showed that an increase in heart rate in normal subjects resulted in no change in early filling velocity but an increase in late filling velocity. A reflex increase in heart rate was observed in several studies investigating changes in the transmitral flow velocity pattern during ischemia (31,35). The reflex increase in heart rate in and of itself decreases the velocity and duration of early flow, thus flattening and narrowing the E-wave, and increases the velocity of late flow (the A-wave). In contrast, we controlled heart rate.

The changes in the transmitral flow velocity pattern due to moderate increases in heart rate we found are as follows: The early flow velocity and duration decrease but late flow velocity increases. This transmitral flow velocity pattern is consistent with the findings of Harrison et al. (34). The determinants of this transmitral flow velocity pattern are a decreased atrioventricular pressure gradient and a faster left ventricular relaxation rate. The decrease in the atrioventricular pressure gradient decreased the early flow velocity because of a decrease in the driving pressure, which determines flow velocity. A faster left ventricular relaxation rate in combination with an increased heart rate decreased the duration of early flow. Thus, the decrease in early flow velocity and duration resulted in a compensatory increase in late flow velocity.

Interaction between ischemia and heart rate changes. To identify the changes in the transmitral flow velocity pattern occurring during increases in heart rate that depend on the presence or absence of ischemia, we tested for an interaction effect between ischemia and heart rate. An interaction effect existed for total flow (Fig. 5). The decrease in total flow during ischemia in response to increasing heart rate was less than the decrease in total flow at baseline in response to the same increase in heart rate. Thus, the effect of regional ischemia on total flow was altered by increasing the heart rate.

We wanted to determine what component of total flow produced this interaction effect. The altered component was late flow; early flow was not affected. Specifically, the increase in late flow in response to increased heart rate during ischemia was larger than that seen at baseline. In contrast, the decrease in early flow in response to increased heart rate during ischemia was parallel to the decrease at baseline. The increase in late flow during regional ischemia which exceeded the increase during baseline in response to moderate increases in heart rate, in turn reduced the decrease in total flow during regional ischemia. This interaction effect suggests that the increase in the late flow component of the transmitral flow velocity pattern observed in previous studies (31,35,36) was due to a reflex increase in heart rate. The change in transmitral flow velocity pattern in response to moderate increases in heart rate is an important consideration when determining the underlying diastolic properties that are involved in regional ischemia.

The observed changes in the atrioventricular pressure gradient also reflect an interaction effect. The atrioventricular pressure gradient decreased when heart rate increased during ischemia less than when heart rate increased at baseline. This decrease in the atrioventricular pressure gradient when heart rate increased during ischemia explains the flattening in the early filling wave during ischemia with no increase in myocardial or chamber stiffness.

Other determinants of mitral flow. The effect of other determinants on the transmitral flow velocity pattern—atrioventricular conduction interval (37), left ventricular afterload (38), and preload (39,40)—were also considered in this study. The atrioventricular conduction interval did not change during ischemia at different heart rates. Similarly, left ventricular afterload approximated by left ventricular systolic pressure did not change. Preload was taken into account by our analysis because it was included in the effect of the atrioventricular pressure gradient on the transmitral flow velocity pattern. This study also minimized the effect of interventricular interaction since the pericardium was open.
An open pericardium is also a limitation since the role of interventricular interaction on the determinants of the transmural flow is not quantified. Myocardial ischemic preconditioning, which is common in studies using patients with preexisting disease at baseline, could be excluded as an explanation for the changes found since the hearts in this experiment had normal coronary arteries and left ventricular systolic function at baseline.

Regional ischemia was produced in this study as reflected by decreasing segment-length shortening by 20% in the ischemic region. This regional dysfunction resulted in this region of the myocardium being functionally stiffer without affecting the nonischemic regions of the myocardium. Thus, small increases in regional stiffness can affect ventricular relaxation and overall chamber stiffness.

Conclusions. In conclusion, this study shows that regional ischemia due to partial coronary occlusion has a significant effect on three of the primary mechanical determinants of filling: relaxation, which is slower, and myocardial and chamber stiffness, which increase. Decreased contractility contributed to the changes in these primary mechanical determinants resulting in a decreased atrioventricular pressure gradient. The flatter and narrower early flow pattern with no change in late flow was due to a combination of slowed left ventricular relaxation and increased chamber stiffness which can be masked by a moderate change in heart rate. In addition, this study shows that the atrioventricular pressure gradient is not as sensitive to increases in heart rate during ischemia, as reflected by a reduced decreased atrioventricular pressure gradient during ischemia resulting in a decreased early flow velocity.

Acknowledgments
We thank James Stoughton for technical assistance; Mimi Zeiger for instructing scientific writing; William Grossman and Nelson Schiller for criticism of the manuscript.

Reprint requests and correspondence: Dr. Stanton Glantz, Box 0130, University of California, San Francisco, San Francisco, California 94143-0130. E-mail: glantz@medicine.ucsf.edu.

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