Detection of Noncritical Coronary Stenosis at Rest Without Recourse to Exercise or Pharmacological Stress

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Background—Currently, the detection of noncritical coronary stenoses requires some form of stress. We hypothesized that these stenoses can be detected at rest without recourse to stress by assessing adaptive changes that occur distally in the microcirculation.

Methods and Results—Phasic changes in myocardial video intensity (VI) were measured at rest with continuous high-mechanical-index (MI) contrast echocardiography in 15 open-chest dogs. Data were acquired at baseline and in the presence of different degrees of noncritical coronary stenosis. In 6 of these dogs, capillary blood volume was also measured at baseline using high-MI intermittent imaging with triggering performed separately at both end diastole and end systole. During continuous high-MI imaging, a significant increase in systolic VI was noted with coronary stenoses that resulted in progressive increases in the systolic/diastolic VI ratio with greater degrees of stenosis (P=0.003), with a mildly quadratic relation noted between the two: $y = 1.3 \cdot 10^{-6} \cdot x^2 + 0.01x + 0.32$, $P<0.001$, $r=0.76$, SEE=0.14. There was no difference in capillary blood volume between end diastole and end systole at baseline.

Conclusions—Capillary blood volume does not change between diastole and systole in vivo. Phasic changes in VI are noted at baseline during high-MI continuous imaging. The systolic component is negligible at baseline but increases with increasing levels of noncritical coronary stenosis because of adaptive changes in the microcirculation distal to the stenosis. Thus, the measurement of phasic changes in myocardial VI has the potential to detect coronary stenosis at rest without recourse to any form of stress. (Circulation. 2002;105:218-223.)

Key Words: stenosis ■ echocardiography ■ blood volume

Because of autoregulation, coronary blood flow (CBF) remains normal at rest until a coronary stenosis exceeds $\approx 85\%$ in severity.$^{1,2}$ In the absence of a prior infarction, therefore, noncritical coronary stenoses cannot be detected by cardiac imaging at rest, and some form of stress is required to induce either regional dysfunction or a perfusion abnormality. The same is true even for electrocardiography.

When coronary arterioles dilate in the presence of a noncritical stenosis, their blood volume (aBV) increases.$^{3,4}$ A portion of these arterioles are intramyocardial.$^{5}$ We hypothesized that because the degree of dilation of the coronary arterioles is related to the severity of stenosis, aBV should increase in proportion to coronary stenosis severity. If this phenomenon could be measured noninvasively, the presence and severity of noncritical coronary stenosis could be measured at rest without recourse to any form of stress.

Whereas phasic changes in intramyocardial arteriolar dimensions have been documented during cardiac contraction, there is controversy regarding changes in capillary dimensions during this period.$^{8-11}$ We postulated that because arterioles and venules are larger than capillaries, their compression would occur early in systole, causing an increase in their resistance that would result in the functional isolation of capillaries. Capillary pressure would thus increase concurrently with intramyocardial pressure during systole, preventing appreciable changes in capillary dimensions.

Methods
Animal Preparation
The study was approved by the Animal Care and Use Committee at the University of Virginia and conformed to the American Heart Association Guidelines for Use of Animals in Research. Fifteen anesthetized, open-chest dogs were used. Catheters were placed in both femoral veins for administration of fluids and microbubbles. Catheters were also placed in the ascending aorta and left atrium to measure central aortic and left atrial pressure. A screw occluder was placed on the proximal portion of the left anterior descending coronary artery to produce stenoses of varying severity. An ultrasonic time-of-flight flow probe (series SC, Transonic) was placed distal to the screw occluder and connected to a digital flowmeter (model T206, Transonic) to measure mean epicardial CBF. A 20-gauge catheter was introduced into a side...
branch of the left anterior descending coronary artery for measurement of coronary artery pressure distal to the screw occluder.

Hemodynamic Data

All catheters and the flowmeter were connected to a multichannel recorder (model ES 2000, Gould). Simultaneous mean CBF, pressures, and ECG were acquired digitally and displayed continuously online. The severity of the stenosis was judged by the transstenotic pressure gradient (mean aortic pressure minus mean distal left anterior descending coronary artery pressure). Percent diameter stenosis was calculated by 1 – (R0/Rs)1/4, where R0 is the resistance offered by a coronary artery segment 0.5 cm in length with a 3-mm luminal diameter and Rs is stenosis resistance.12

Myocardial Contrast Echocardiography

Echocardiography was performed with the Sequoia system (Acuson-Siemens). Definity (DuPont Pharmaceuticals)13 was used as the ultrasound contrast agent. The ultrasound transducer was held in a fixed position, and a saline bath served as an acoustic interface between the transducer and the heart. Imaging was performed at the midapical short-axis plane caudal to the screw occluder. The transmit power was set at maximum, and the image depth and focal point were set at 6 cm to image primarily the anterior myocardium. All other system settings were optimized at the beginning of each experiment and held constant during each study.

We used 2 high-mechanical-index (MI) approaches for assessing myocardial perfusion with echocardiography. The first approach was used in 6 dogs to determine changes in capillary blood volume (cBV) between systole and diastole at baseline. Microbubbles (2 mL diluted in 23 mL of saline) were infused at a continuous rate (∼2 mL/min). Because their velocity in the capillaries is ∼1 mm/s and the ultrasound beam elevation measures roughly 5 mm, it normally takes ∼5 seconds after microbubble destruction to replenish the beam elevation.14 Because 90% of myocardial blood volume resides in capillaries, video intensity (VI) at a pulsing interval of >5 seconds mostly represents cBV. This approach was used to acquire intermittent images at increasing pulsing intervals separately at end diastole and at end systole. The background-subtracted plateau VIs from the pulsing interval–versus-VI plots from both data sets were compared.15

The second approach was used in all 6 dogs to detect phasic changes in aBV. Because the interval between frames during continuous imaging is only 30 ms, microbubbles fill only the intramyocardial arterioles before being destroyed by the next ultrasound pulse. Frames acquired over 3 entire cardiac cycles were transferred to an offline computer. A region of interest was placed over the midmyocardium of the anterior wall on an end-systolic frame. The midmyocardium from all other selected frames was then manually aligned to the same region of interest, from which myocardial VI was automatically derived. Precontrast VIs obtained from end-systolic and end-diastolic frames before microbubble administration were subtracted from their respective contrast-enhanced values to calculate the systolic/diastolic VI ratio.

Experimental Protocol

Hemodynamic and echocardiographic data were acquired at baseline and after creation of different degrees of stenosis. At least 2 levels of noncritical stenosis were created in each dog in a random order. CBF was monitored during creation of each stenosis to confirm that it remained essentially unchanged. Stenosis severity was categorized on the basis of the transstenotic pressure gradient as trivial (<5 mm Hg), mild (6 to 25 mm Hg), or moderate (26 to 45 mm Hg).

At the end of the experiment, the dogs were killed with an overdose of KCl and pentobarbital.

Statistical Methods

Data are expressed as mean±SD. Comparisons between stages were made by repeated-measures ANOVA. When differences were found between stages, Student’s t test was performed to determine inter-stage differences. Correlations were obtained with least squares fit regression analyses. Differences were considered significant at P<0.05 (2-sided).

Results

There was no difference in plateau VI between end diastole and systole when data were acquired by both high-MI intermittent imaging methods: triggering in end systole alone versus triggering in end diastole alone (57±11 versus 56±8, P=0.59). The plateau VI was measured at a mean of 5.5±1.0 seconds, well after the beam had saturated. These findings indicate that in the normal coronary circulation, cBV does not change between end diastole and end systole when cardiac function and CBF are normal. After stenosis placement, no significant differences were noted in left ventricular peak +dP/dt between baseline and stenosis stages, and no changes were noted visually in regional wall thickening between these stages. Figure 1 depicts images acquired during continuous high-MI imaging at baseline. Because a large dose of microbubbles is necessary to cause myocardial opacification during this form of imaging, far-field shadowing is seen from the bubbles in the left ventricular cavity. At end diastole, opacification is seen only in intramyocardial arterioles that were replenished with microbubbles 30 ms from the preceding destructive ultrasound pulse. The linear structures traversing the anterior myocardium represent the larger of these arterioles. The rest of the opacification is seen from smaller arterioles that have a velocity high enough to completely or partially fill in 30 ms. Systole results in compression of the larger intramyocardial arterioles so that they are no longer visualized, and overall myocardial opacification is also less from the overall decrease in aBV because of compression of the smaller arterioles as well.

A total of 29 stenoses were produced in the 15 dogs (Table). Although mean CBF declined minimally during severe stenosis, no changes in wall thickening were noted visually. We have previously shown that at these coronary pressures, total myocardial blood flow does not decrease because of collateral flow despite a modest decline in anterograde CBF.3

Myocardial VI plots obtained from baseline and 2 separate stenosis stages from 1 dog before background subtraction are shown in Figure 2. Figure 2A depicts the original data, whereas Figure 2B depicts the same data where the diastolic VI from all stages is normalized to baseline, as is the interval between cardiac cycles. Systolic myocardial VI was lowest in

A.  

B.  

Figure 1. End-diastolic (A) and end-systolic (B) images obtained during continuous high-MI contrast echocardiography. VI is significantly higher in end diastole. See text for details.
the absence of stenosis and progressively increased as stenosis severity increased. Although diastolic VI decreased progressively with more severe stenosis in this example, no significant differences in mean background-subtracted end-diastolic VI were noted with increasing levels of stenosis when data from all 15 dogs were pooled (Table). The background-subtracted end-systolic VI, however, increased in proportion to the severity of stenosis not only in the example shown here but also in all dogs, resulting in an increase in the systolic/diastolic VI ratio. Figure 3 shows box plots of the systolic/diastolic VI ratios versus the transstenotic pressure gradient from all dogs. The ratio demonstrates a stepwise increase with increasing levels of stenosis, with a mildly quadratic relation demonstrating a best fit between the 2 variables: $y = 1.3 \times 10^{-6} \cdot x^2 + 0.01x + 0.32$, $P<0.001$, $r=0.76$, SEE=0.14.

**Discussion**

In this study, we have shown for the first time that phasic changes in myocardial VI with continuous high-MI contrast echocardiography allow the detection and quantification of coronary stenosis at rest without need for pharmacological or exercise stress. This method represents a novel approach that is based on the ability of contrast echocardiography to image adaptive changes in the myocardial microcirculation distal to a stenosis.

**Phasic Changes in cBV**

Our results are consistent with the hypothesis that because of increased resistance in intramyocardial arterioles and venules during systole, the capillaries are functionally isolated from the 2 sets of larger vessels and, therefore, cBV remains unchanged between systole and diastole. There is considerable controversy regarding the status of cBV between systole and diastole. To the best of our knowledge, most measurements were made from postmortem examinations after the heart was arrested with barium. The cardiac contraction induced by barium is far greater than what is seen physiologically. It is also possible that removing and handling the nonbeating heart displaces blood volume from one compartment to another. Capillary topography is very complex, and histological studies may not provide an accurate assessment of the 3-dimensional layout of the capillaries. Finally, capillaries are not passive vessels with a fixed number or distribution. Both vary dynamically, even during the same hemodynamic conditions, and are unlikely to be accurately defined with postmortem techniques.

We used an in vivo method for measuring cBV in which a continuous infusion of microbubbles was administered at a dose that results in a linear relation between microbubble concentration and VI. After their concentration in blood had reached a steady state, we destroyed the microbubbles within the ultrasound beam and measured their rate of replenishment by progressively increasing the pulsing interval between microbubble destruction and imaging. Imaging was gated separately at both end diastole and end systole. When the beam was completely replenished, we saw no difference in the background-subtracted myocardial VI between end diastole and end systole, which indicates that cBV between systole and diastole remains unchanged. Because blood velocity in capillaries is $\approx 1$ mm/s, it would take a long time to replenish a 0.5-mm-long capillary if it were partially emptied in systole. Furthermore, if the capillary were depleted of blood for a significant portion of the cardiac cycle, it could interfere with oxygen delivery. Conversely, sequestration of blood within the capillaries during systole may allow more efficient oxygen unloading to tissue at the time of cardiac contraction. Therefore, even from a teleological perspective, cBV should not change during the cardiac cycle.
Phasic Changes in aBV

Arteriolar vasodilation is the principal mechanism that maintains normal resting CBF in the presence of a noncritical stenosis.6 Because aBV constitutes a small fraction of the total myocardial blood volume (blood present within the myocardium rather than the entire coronary tree),5 changes in aBV will not be seen when myocardial blood volume is measured. Therefore, low-MI continuous imaging cannot be used for this purpose, nor can high-MI intermittent imaging, because the plateau VI by both methods reflects cBV rather than aBV.

We therefore used continuous high-MI imaging, in which microbubbles within the myocardium were destroyed by pulses of ultrasound at a sampling rate of 30 Hz. Any opacification could therefore occur only from partial or complete filling of vessels whose velocity would allow significant replenishment at 33 ms. In this manner, there is no backscatter from capillaries and venules, and the phasic VI changes reflect only changes in aBV.

Although data were acquired at a fixed pulsing interval, because we used destructive pulses, VI was influenced by the velocity of blood. Therefore, only vessels with a high enough velocity could be detected at the high sampling rate used. These vessels are relatively large and do not participate in autoregulation, so no significant change in their volume should be seen in diastole even in the presence of stenosis. In the normal coronary circulation, when these vessels are compressed in systole, their blood flow velocity is too low to be detected within 33 ms, and thus VI is low. This low flow velocity despite an increase in coronary driving pressure during systole occurs because of the increased myocardial vascular resistance,15,16 as well as retrograde flow from endocardial to epicardial "milking" of blood.17,18

The magnitude of retrograde milking increases in the presence of stenosis based on 2 mechanisms: an increase in aBV in smaller arterioles due to autoregulation3,4 and a decrease in coronary arterial afterload because of a drop in coronary driving pressure.17 This is supported by the observation that retrograde flow velocity in systole increases with dipyridamole (which increases aBV of smaller arterioles),18 as well as during a decrease in coronary driving pressure.17,19 The increase in velocity is enough to be detected at 30 Hz. Because aBV in the smaller resistance arterioles increases and coronary afterload decreases in proportion to the severity of stenosis,3,4,17,19 the amount of blood milked retrogradely also increases proportionately. Because the filling of the ultrasound beam is not influenced by the direction of microvascular flow,13 even retrograde flow is detected by our method, resulting in the increased systolic VI seen in the presence of stenosis.

Critique of Our Approach

Because microbubbles are sensitive to ambient pressure,20 changes in intramyocardial pressure during the cardiac cycle could potentially affect phasic changes in myocardial VI. Because we used a high MI, the signals emanated from microbubble destruction and not from backscatter from intact bubbles. Thus, the influence of bubble size on the signal was probably negligible. If changes in bubble size were an important determinant of ultrasound signal, one would not expect a relation between phasic changes and the degree of coronary stenosis. Furthermore, we did not see any difference in VI when measuring cBV at either end diastole or end

Figure 2. A, Myocardial VI plots obtained by continuous high-MI imaging before background subtraction. After background subtraction, end-diastolic and systolic VI values and systolic/diastolic VI ratios were as follows: 85, 32, and 0.39 for baseline (C1; transstenotic pressure gradient 5 mm Hg); 73, 35, and 0.48 for stenosis 1 (S1, •; transstenotic pressure gradient 17 mm Hg); 78, 43, and 0.55 for stenosis 2 (S2, ▷; transstenotic pressure gradient 20 mm Hg); and 57, 42, and 0.72 for stenosis 3 (S3, ◇; transstenotic pressure gradient 30 mm Hg). B, Same data after normalization in both y-axis (VI) and x-axis (time).
systole, which lends support to the notion that microbubble dimension was not the major contributor to the ultrasound signal.

If the heart were not moving, the use of high-MI continuous imaging would have resulted in the continuous destruction of all bubbles in the ultrasound beam, with none reaching the more distal vessels. Because the beating heart exhibits base-to-apex motion, rotation, as well as respiration-induced translation, the ultrasound beam intersects myocardial tissue at different locations during each pulse, which precludes complete microbubble destruction and allows them to reach distal portions of the coronary microcirculation. These microbubbles are then propelled retrogradely with the blood that is milked from the endocardium to the epicardium in systole and can be detected on ultrasound imaging. The lack of change in cBV from diastole to systole in the present study implies that blood in the capillaries is not milked; only the blood in arterioles and venules is milked. Because flow velocity in venules is always low (even in systole), it is unlikely that our method detected any changes in venous blood volume.

Although we found a good correlation between stenosis severity and systolic/diastolic VI ratio, other factors also affect this ratio, even in the absence of a stenosis. Incomplete myocardial filling during diastole secondary to tachycardia has been shown to result in a decrease in diastolic CBF. Because phasic changes in CBF are related to cardiac contraction, catecholamines can increase the ratio, whereas regional dysfunction can decrease it.

**Clinical Implications**

Currently, the detection of coronary stenosis requires some form of stress, which is tedious, time-consuming, and inconvenient for patients. Detection of coronary stenosis (and its physiological relevance) at rest would have a major impact on daily clinical practice. Our study provides proof of principle toward this end and demonstrates that stenoses can be detected at rest by imaging the adaptive changes in the coronary microcirculation that occur secondary to coronary stenosis.

This approach is not currently feasible in the clinical setting until further technological developments occur. We had to inject a large dose of microbubbles to be able to detect aBV. This dose caused intense far-field shadowing that precluded any assessment of the posterior wall. The sensitivity of the technique has to improve by an order of magnitude before we can obtain the same information from a continuous replenishment of microbubbles without producing far-field artifact and do not reflect any physiological phenomena. Furthermore, the low dynamic range of low-MI imaging may be unable to detect any true changes in myocardial blood volume. Physiological information regarding changes in myocardial blood volume can currently be obtained only when high-MI imaging is performed with a large dynamic range.

**References**


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