Perfusion Versus Function: The Ischemic Cascade in Demand Ischemia
Implications of Single-Vessel Versus Multivessel Stenosis

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Background—We hypothesized that during demand ischemia, abnormal perfusion will precede abnormal function, the spatial extent of perfusion abnormality will be greater than that of functional abnormality, and the spatiotemporal disparity between abnormal perfusion and abnormal function will be more marked in the presence of single-vessel stenosis (SVS) versus multivessel stenosis (MVS).

Methods and Results—Nine dogs each underwent either SVS or MVS placement. These noncritical stenoses were classified as mild, moderate, or severe on the basis of the transstenotic pressure gradient (10 to 14, 15 to 20, or >20 mm Hg). Dobutamine was infused starting at 10 and reaching 40 µg/kg·min. Wall thickening (WT) and myocardial perfusion (myocardial contrast echocardiography) were assessed at each stage. Resting perfusion and function were normal in all dogs. In SVS, abnormal perfusion (delayed rate of microbubble replenishment) was seen at the lowest dose of dobutamine irrespective of the stenosis severity, whereas WT abnormality was seen only at high doses of dobutamine and was influenced by the stenosis severity. The spatial extent of abnormal perfusion exceeded that of WT abnormality at all but the highest dobutamine dose. This spatiotemporal discordance between abnormal perfusion and function was significantly less in MVS, where it was possible to identify separate regions with abnormal function at lower doses of dobutamine.

Conclusions—These data support the occurrence of the ischemic cascade during demand ischemia. They also explain the higher sensitivity of abnormal perfusion compared with abnormal function for the detection of coronary stenosis as well as the higher sensitivity of dobutamine echocardiography for MVS compared with SVS. (Circulation. 2002;105:987-992.)

Key Words: perfusion □ ischemia □ stenosis □ vessels

When a coronary artery is occluded, reduced perfusion, decline in function, and an abnormal ECG occur in quick succession,1 a phenomenon that has been termed the ischemic cascade.2 The same phenomenon has been postulated for demand ischemia to explain the higher sensitivity of perfusion abnormalities over inducible regional dysfunction and the higher prevalence of both over an abnormal ECG and angina during stress testing.3–8 There is, however, no direct evidence to support this notion in a rigorous experimental setting where perfusion and function have been evaluated simultaneously. Furthermore, a comparison of this phenomenon has not been performed in models of single-vessel stenosis (SVS) versus multivessel stenosis (MVS).

We hypothesized that during demand ischemia, abnormal perfusion will precede abnormal function and the spatial extent of perfusion abnormality will be significantly greater than that of functional abnormality. We also hypothesized that the spatiotemporal disparity between abnormal perfusion and abnormal function will be more marked in the presence of SVS versus MVS. We tested these hypotheses in open-chest canine models.

Methods

Animal Preparation
The study was approved by the Animal Research Committee at the University of Virginia and conformed to the American Heart Association guidelines for the use of animals in research. Eighteen anesthetized open-chest dogs were used for the study. Catheters were inserted into both femoral veins for administration of drugs, fluids, and microbubbles and in the aortic root and both atria for pressure measurements. The proximal sections of the left anterior descending (LAD) and left circumflex (LCx) coronary arteries were dissected from the surrounding tissue. Ultrasonic flow probes were placed on...
both arteries and connected to a digital flowmeter to monitor coronary blood flow (CBF). Screw occluders were used to create SVS in 9 dogs and MVS in 9 dogs. The severity of stenosis was judged by the transstenotic pressure gradient with the distal coronary pressure measured with a 20-g catheter. Mean pressures and CBF were measured digitally.

Ultrasound (US) imaging was performed using the HDI 5000 system (Phillips ultrasound). The transducer was fixed in position, and a saline bath served as an acoustic interface between the transducer and the heart. Imaging was performed at the midpapillary muscle short-axis level caudal to the occluders. The image depth and gains were optimized at the beginning of each experiment and were held constant during the study.

Regional Function Analysis
Regional function analysis was performed with fundamental imaging at 50 Hz. A representative digital loop consisting of frames from end diastole to end systole was analyzed for each stage using custom-designed software. Nine to 12 epicardial and endocardial targets were defined in each frame, and these points were automatically connected using cubic-spline interpolation to derive the epicardial and endocardial contours. To correct for cardiac rotation, the junction of the left ventricular (LV) posterior and right ventricular free walls was defined in each frame. The computer generated 100 equidistant chords starting at this junction point, with each chord representing the shortest distance between the epicardial and endocardial contours. The observer identified different regions of the myocardium for derivation of wall thickening (WT). The observer then identified the number of chords that fell below this value and expressed them as the percent of the LV short-axis slice. The computer computed percent WT (%WT). Reduced %WT was defined as 2 SD below the %WT of the normal bed during that stage. The computer then identified the number of chords that fell below this value and expressed them as the percent of the LV short-axis slice. The interobserver and intraobserver correlations for our method of WT analysis are \( r = 0.85, P < 0.001 \) and \( r = 0.93, P < 0.001 \), respectively.

Myocardial Perfusion Analysis
Myocardial perfusion was assessed using power pulse inversion imaging with the focal point placed below the posterior wall to obtain an even beam profile. Optison (Amersham Health) was diluted 1:6 in normal saline and administered intravenously at a rate of 0.5 mL/min. After steady state was reached, 4 US pulses were transmitted at a mechanical index of 1.2 to destroy all the myocardial bubbles within the US beam elevation. This was followed by imaging with a mechanical index of 0.1 at a pulse-repetition frequency of 2500 Hz for at least 15 consecutive cardiac cycles.

Images were analyzed offline. Separate representative loops were created for each stage consisting of only end-systolic frames after bubble destruction. After alignment, large transmural regions of interest (ROIs) were placed over the perfusion bed supplied by the stenosed artery (defined during their separate transient occlusions, Figure 1) as well as the region not supplied by either of these vessels, which was considered to be normal. AI versus time plots were generated from these regions and fitted to an exponential function, \( y = A(1 - e^{-at}) \), where \( y \) is AI at time \( t \), \( A \) is the plateau AI representing myocardial blood volume (MBV), and \( \beta \) reflects the rate of rise of AI or mean microbubble velocity. The product \( A \cdot \beta \) reflects MCE-derived MBF. Abnormal perfusion at each stage was defined as an \( A \cdot \beta \) value of <2 SD of that seen in the normal bed. The interobserver and intraobserver correlations for this value are \( r = 0.83, P < 0.001 \) and \( r = 0.80, P < 0.001 \), respectively. Perfusion defect (PD) size at each stage was assessed by planimetry in the frame where the disparity in AI between beds was maximal and expressed as a percent of the total LV short-axis area.

Experimental Protocol
For each dog, up to four different degrees of noncritical stenosis, placed in random order, were examined. Using the resting transstenotic pressure gradient (mm Hg), the stenosis stages were divided into mild, \( 10-14 \) moderate, \( 15-20 \) and severe (>20 mm). For each stenosis level, a standard dobutamine stress protocol was performed with a baseline and up to four incremental 3-minute dobutamine stages (10 to 40 \( \mu \)g/kg \( \cdot \) min \(^{-1} \) infused intravenously). At baseline and each dobutamine stage, hemodynamic as well as regional function and perfusion data were recorded. After each stage, the stenosis was relieved and hemodynamics were allowed to return to normal resting levels before proceeding to the next stage. At the end of the experiment, the dog was euthanized with an overdose of pentobarbital and KCl.

Statistical Methods
Data are expressed as mean±SD. Comparisons between stages were made using repeated-measure ANOVA. When differences were found, interstage comparisons were performed using Student’s \( t \) test. Differences were considered significant at \( P < 0.05 \) (two-sided).

Results
Single-Vessel Stenosis
A total of 27 stages were performed with SVS. No differences were noted in the hemodynamics between the stenosis stages at any dobutamine dose. Baseline, predobutamine %WT, and perfusion were normal in all dogs. Although all segments supplied by a stenosis exhibited PDs starting at the smallest doses of dobutamine irrespective of the severity of stenosis, this was not observed for WT abnormality until the dose of dobutamine reached 30 \( \mu \)g/kg \( \cdot \) min \(^{-1} \). Only half of the segments supplied by a severe stenosis and none supplied by mild to moderate stenoses exhibited abnormal WT at a dose of 10 \( \mu \)g/kg \( \cdot \) min \(^{-1} \). At 20 \( \mu \)g/kg \( \cdot \) min \(^{-1} \), the percent of segments exhibiting WT abnormality varied from 50% to 100% depending on the severity of stenosis. This value increased to 100% at 30 \( \mu \)g/kg \( \cdot \) min \(^{-1} \) and higher.

Function and perfusion data from 1 dog with a mild LCx stenosis during administration of 10 \( \mu \)g/kg \( \cdot \) min \(^{-1} \) of dobutamine are shown in Figure 2A. At this low dose, although %WT is still normal, a PD is apparent early during microbubble replenishment in the LCx bed (arrows), which fills in late. Figure 2B shows the corresponding time versus AI curves obtained from the LAD and LCx beds. \( \beta \) is reduced in the stenosed LCx compared with the LAD bed.

Another interesting observation was that the circumferential extent of PD was similar for all coronary stenosis...
severities at all dobutamine doses (Figure 3A). The circumferential extent of abnormal WT, however, was related to both the severity of stenosis as well as the dobutamine dose used (Figure 3B). The more severe the stenosis, the greater the extent of abnormal WT, and for each level of stenosis, the extent of WT abnormality increased with increasing doses of dobutamine, with a plateau effect seen beyond a dose of 30 μg/kg·min⁻¹.

Multivessel Stenosis
A total of 27 stages were performed with MVS. Six combinations of stenoses were produced, ranging from both stenoses being mild to both being severe. As in SVS, baseline predobutamine %WT and perfusion were normal in all dogs. There were no differences in hemodynamics at any dobutamine dose for any of the stenoses combinations. PDs were seen in both the LAD and LCx beds in all stenoses combinations at all dobutamine doses, starting at the lowest dose. In comparison, WT abnormality was seen in both beds at this dose in only 1 instance, a dog with severe stenoses on both arteries. Unlike the setting of SVS, however, the occurrence of WT abnormality in both beds was seen in all stenoses combinations at dobutamine doses of 20 μg/kg·min⁻¹ and higher.

Figure 4A shows images from 1 dog with a mild LAD and a severe LCx stenosis during administration of 10 μg/kg·min⁻¹ of dobutamine. Although %WT is mildly reduced in the LCx bed, it is normal in the LAD bed. However, abnormal perfusion is seen in both beds with lower A and β values compared with the normal interventricular septum (Figure 4B). Thus, although only a single stenosis is detected by %WT analysis during low-dose dobutamine, both stenoses were detected on perfusion imaging. Images from another dog with severe stenoses on both the LAD and LCx are shown in Figure 5. Although pronounced reductions in end-systolic %WT in both beds do not become visually apparent until a higher dobutamine dose (30 μg/kg·min⁻¹), perfusion abnormalities are readily apparent in both beds at even the lowest dose and worsen at higher doses.

As in SVS dogs, the circumferential extent of abnormal WT was significantly less than the extent of PD at the 10 μg/kg·min⁻¹ of dobutamine in MVS (Figure 6A). However, unlike SVS dogs, it was equivalent to that of PD at all other dobutamine doses (Figure 6B).

Discussion
In this study, we have shown that similar to supply ischemia, regional perfusion abnormalities precede regional function abnormalities during demand ischemia. Although PDs are seen distal to a mild stenosis even at low doses of dobuta-
mine, WT abnormalities occur at higher doses. Furthermore, the circumferential extent of a PD is greater than that of WT abnormalities at all except the highest doses of dobutamine. The temporal separation between the development of PDs and WT abnormalities as well as the discordance between the circumferential extent of these abnormalities is more marked in the setting of SVS compared with MVS. These findings may explain both the higher sensitivity of perfusion compared with function imaging for the detection of coronary artery stenosis as well as the superiority of DE for the detection of MVS compared with SVS.

Mechanism of Action of Dobutamine
The main mechanism by which dobutamine increases CBF is by enhancement of myocardial O₂ demand. At low doses, however, the increase in CBF exceeds the increase in myocardial O₂ demand from a direct coronary vasodilatory effect of dobutamine. For instance, we have previously shown that whereas CBF doubled and myocardial vascular resistance (MVR) was reduced to half the baseline value at 10 μg/kg⁻¹ · min⁻¹ of dobutamine, LV dP/dt increased by only 1.5-fold. At the level of the microvasculature, only 75% of the increase in CBF was associated with increases in myocardial blood volume, whereas the remainder occurred from increases in red blood cell velocity.

Figure 4. Images from a dog with a moderate LAD and a severe LCx stenosis during 10 μg/kg⁻¹ · min⁻¹ of dobutamine. A, End-diastolic and end-systolic images showing reduced WT in the LCx bed. B, End-systolic MCE images at increasing time points after the destructive flash showing slower filling of both the LAD and LCx bed (the latter filling the slowest) compared with the normal bed. C, Corresponding time vs Al curves. NOR indicates normal bed.

Figure 5. End-systolic short-axis MCE images obtained at the same time point after the destructive flash from a MVS dog. There was severe stenosis on both the LAD and LCx during each of the following doses of dobutamine (in μg/kg⁻¹ · min⁻¹): A, 10; B, 20; C, 30; and D, 40.

Figure 6. Relation between combinations of stenosis severity (y-axis), dobutamine dose (x-axis), and the circumferential extent of PD (A) and abnormal WT (B) in MVS. 1 indicates both mild stenoses (n=3); 2, both moderate stenoses (n=4); 3, both severe stenoses (n=4); 4, one moderate and one mild stenosis (n=6); 5, one severe and one mild stenosis (n=7); and 6, one moderate and one severe stenosis (n=3).
Therefore, MVR decreases significantly at low doses of dobutamine, an effect that is similar to that noted with low doses of a direct coronary vasodilator. We have previously shown that when coronary arteriolar resistance decreases in the presence of a noncritical stenosis, capillary resistance increases to maintain a constant hydrostatic pressure.13 Because capillaries have no smooth muscle, the increase in capillary resistance occurs from capillary derecruitment, which is the main reason for seeing a reversible PD on both MCE13 as well as 99mTc-sestamibi imaging.14 Results from the present study would imply that the mechanism of a reversible PD with low-dose dobutamine is also the same. Thus, actual ischemia is not a prerequisite for seeing a PD with dobutamine, which can explain the higher sensitivity of PDs compared with WT abnormalities for the detection of coronary stenosis with this agent.

At higher doses of dobutamine, the increase in CBF is not commensurate with the increase in myocardial O2 demand in regions subtended by stenoses, leading to ischemia that is evidenced as a WT abnormality. The dose of dobutamine required to produce ischemia is related to the severity of stenosis as seen in this and other studies,15,16 a finding not seen with regard to PDs that were produced at the lowest dose of dobutamine irrespective of the degree of stenosis. When both abnormalities are present, the circumferential extent of abnormal WT is usually less than that of the PD at almost all dobutamine doses. It has been shown that more than approximately 10% of the myocardial circumference has to exhibit reduced perfusion before WT abnormality can be noted.17 Myocardial blood flow within the ischemic bed is higher at the borders than at the center, which may prevent the borders from exhibiting significant dysfunction compared with the center.

**Single-Vessel Versus Multivessel Stenosis**

It is well known that the sensitivity of DE for the detection of coronary stenosis is significantly lower for SVS compared with MVS.15,18 This finding can be rationalized on the basis of Bayesian analysis. It can be argued that a diagnostic test will more readily pick up extensive compared with minimal disease. However, there could also be a physiological basis for this finding. For instance, it has been shown that the amount of regional dysfunction is less during demand compared with supply ischemia.6 Also, because stenosis on one artery is likely to compromise collateral flow to the other artery, ischemia may be more prone to occur at lower doses of dobutamine in MVS compared with SVS, thus minimizing the temporal separation between the occurrence of PD and WT abnormality.

Whereas dobutamine echocardiography has been reported to be more accurate in the setting of MVS, there are no reports showing that it can consistently identify different myocardial regions subtended by coronary stenoses. The reasons for this are several. The test is usually stopped when an inducible WT abnormality occurs, at a time when another region with less severe stenosis may still exhibit normal function. Chest pain and other abnormalities (such as arrhythmias) associated with inducible regional dysfunction in one bed may also necessitate discontinuation of the test. Our results show that multiple regions of dysfunction can be detected in the setting of physiologically significant MVS when moderate to high doses of dobutamine are used, and in this regard regional function analysis is equivalent to regional perfusion analysis in the setting of MVS but not SVS.

**Comparison With Previous Studies**

Our results support the results of a clinical study where both myocardial perfusion and wall motion were examined during dobutamine stress and PDs were observed in the absence of wall motion abnormalities.5 In some patients, WT abnormalities were seen to occur at higher doses of dobutamine than PDs. A recent experimental study also reported the occurrence of PDs at lower doses of dobutamine compared with WT abnormalities.19 This study, however, was not specifically designed to explore the ischemic cascade and evaluated only SVS. Both studies used power-pulse inversion imaging and a qualitative assessment of WT. The lower temporal resolution of this technique is not suited for optimal WT analysis.

**Critique of Our Methods**

We used fundamental imaging with a high temporal resolution (50 Hz) to assess WT. The algorithm used for measuring regional function takes into account the entire contraction sequence. Consequently, although tardokinesia and mild forms of hypokinesia can be detected with this technique, they can be missed when only end-diastolic and end-systolic frames are used.20,21 Furthermore, we compared WT in every bed to that of the normally perfused bed at each dose of dobutamine. Thus, a unique threshold for abnormality was used separately for each dog at each dobutamine dose, which allowed detection of subtle WT abnormalities that were not readily observed visually. This approach also allowed us to demarcate the circumferential extent of WT abnormality using objective criteria. In this regard, our analysis was different than that performed in the clinical setting, where the same myocardial region is qualitatively compared at different doses of dobutamine to determine whether the myocardial response to dobutamine is normal or abnormal. Furthermore, we performed multiple stress tests in each dog and cannot exclude the possible effect of ischemic preconditioning on our results.

In this study, objective criteria were also used to assess myocardial perfusion. One limitation of the real-time method is the low dynamic range. Hence, in some instances we saw reduction in β values without a concomitant reduction in A values. The latter are more likely to be influenced by the dynamic range. Thus, the β value may be more reliable when this modality is used, and this was the principal indicator of perfusion in this study. The higher dynamic range of B-mode imaging permits evaluation of the A value with a greater degree of precision and provides an additional parameter to determine the presence of stenosis.10

The lowest dose of dobutamine used in our study that produced perfusion abnormalities at all stenosis levels was 10 μg/kg·min. Because anesthetized dogs are particularly sensitive to dobutamine, this resulted in a relatively large increment in myocardial O2 demand (30% to 40% increases
in rate-pressure product) at this dose. In the clinical situation, higher doses of dobutamine may be necessary to produce similar effects. Because lower doses of dobutamine (<10 μg/kg⁻¹·min⁻¹) were not evaluated, we could not determine the minimal dose required to produce a PD. This study was conducted in the experimental setting, where optimal imaging was possible in open chest animals. Although we believe our results can be translated to the clinical setting, it remains important to confirm these findings in patients.

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