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METHODS

Myocardial Contrast Echocardiography in Humans. II. Assessment of Coronary Blood Flow Reserve

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The hypothesis that myocardial contrast echocardiography could be used to simultaneously assess coronary blood flow reserve and the size of the perfusion bed supplied by a coronary artery was examined in nine patients and six dogs. All patients were undergoing cardiac catheterization and had single vessel coronary artery disease (>85% stenosis of either the proximal left anterior descending or the left circumflex coronary artery); the six dogs had a critical stenosis of the left circumflex coronary artery. Three milliliters of sonicated Renografin-76 (mean microbubble size 6 μm) was injected into the left main coronary artery before and after intracoronary administration of papavarine, 6 to 9 mg. The beds supplied by the normal and stenotic vessels could not be differentiated during contrast echocardiography before injection of papavarine. However, after papavarine, the normal vascular bed showed significantly more contrast enhancement than did the bed supplied by the stenotic artery. This disparity in contrast enhancement made it possible to delineate the size of the bed perfused by the stenotic vessels.

When quantitative analysis of the time-intensity curves obtained from the echocardiograms was performed in the dogs, the absolute values for the area under the curve, peak contrast intensity and curve width did not correlate with absolute blood flows measured with radiolabeled microspheres. However, the ratios of the areas under the curves derived from the two vascular beds before and after papavarine correlated well with the ratios of blood flows between the two beds during the same stages ($r^2 = 0.73$ by linear regression and $r^2 = 0.85$ by an exponential function). In comparison, the ratios of peak amplitudes and curve widths before and after papavarine had poor correlations with ratios of flows from the two beds ($r^2 = 0.18$ and 0.02, respectively).

In conclusion, myocardial contrast echocardiography can be used to simultaneously assess coronary blood flow reserve and the size of the perfusion bed supplied by a stenotic vessel.

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Not only is there a poor correlation between antemortem cineangiographic and postmortem estimation of the degree of luminal narrowing of coronary arteries (1–3), but the visual interpretation of coronary angiograms is also subject to large intra- and interobserver variability and errors (4–8). Further, the ability of observers to predict the hemodynamic significance of coronary stenoses by visual interpretation is limited (9,10). Although quantitative coronary angiography has improved the ability to predict the severity of discrete coronary stenoses (11), this technique is still limited by difficulties in border recognition, inability to assess dynamic changes in stenoses and inability to determine the significance of complex multiple stenoses along the length of a diffusely diseased coronary artery (12,13). In addition, the degree of stenosis does not fully define the amount or distribution of the myocardial perfusion.

Quantitation of coronary blood flow reserve is an effective method for assessing the hemodynamic significance of coronary stenoses in the experimental model and in humans (14–16). Epicardial and intracoronary Doppler flow measurements of the changes in velocity after the intracoronary injection of a coronary vasodilator such as papavarine have been made in patients (16,17). The major disadvantages of

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this technique are: 1) it requires placement of the flow probes selectively in each coronary artery or on each vessel at the time of thoracotomy, 2) it can measure only changes in velocity and not changes in flow (for which measurement of intraluminal area is required), and 3) it does not provide information regarding the size of the perfusion bed supplied by a coronary artery.

Because myocardial contrast echocardiography can define both the perfusion bed of a coronary artery (19–22) and nutrient myocardial blood flow (23–26), we hypothesized that myocardial contrast echocardiography could be used to assess coronary blood flow reserve in humans. Before the technique was used in humans, it was validated in six mongrel dogs with myocardial blood flow measurements made with the use of radiolabeled microspheres. Studies were then performed in humans to determine the ability of this technique to assess coronary blood flow reserve in patients with single vessel coronary artery disease.

Methods

Animal Studies

Animal model. Six mongrel dogs weighing 26 ± 3 kg (mean ± 1 SD) were anesthetized with 30 mg/kg body weight pentobarbital sodium (Abbott Laboratories), intubated and ventilated with a dual phase control respirator pump (model 607, Harvard Apparatus). Additional anesthesia was given as needed during the experiment. An 8F catheter was placed in the left femoral vein for the administration of intravenous fluids and drugs. A similar catheter was placed in the left femoral artery and connected to a multichannel physiologic recorder (model 4568C, Hewlett Packard) by way of a fluid filled transducer (model 4568C, Hewlett Packard). This catheter was used for monitoring mean arterial pressure, measuring arterial blood gases and withdrawal of reference arterial samples during injection of radiolabeled microspheres.

A left thoracotomy was performed and the heart was suspended in a pericardial cradle. A 4F catheter was placed in the left atrium for the injection of radiolabeled microspheres. The left anterior descending coronary artery was dissected free from the surrounding tissues and a 22 gauge Teflon catheter (Travenol Laboratories) was inserted into the lumen of the artery through its anterior wall. The tip of this catheter was positioned at the bifurcation of the left main coronary artery (21). This catheter was used for the injection of the contrast agent during echocardiography. An appropriately sized electromagnetic flow probe (model EP406, Carolina Medical Electronics Inc.) was attached to the left circumflex coronary artery to measure coronary blood flow. A fluid-filled hydraulic occluder was placed snugly around this artery just distal to the electromagnetic flow probe (Fig. 1). The flow probe was connected to a flowmeter (model FM501, Carolina Medical Electronics), which in turn was connected to the physiologic recorder.

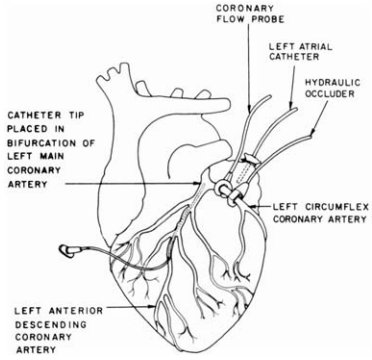


Figure 1. Animal preparation used for the experiments (see text for details).

Two-dimensional echocardiography. Two-dimensional echocardiography was performed with a commercially available sector scanning system equipped with a 5 MHz mechanical transducer (Mark III, Advanced Technology Laboratories). Images were recorded on a 0.5 in (2.54 cm) video tape with the use of a commercially available VHS recorder (Panasonic NV-8950, Matsushita Electrical Industrial Co.). The transducer was placed to obtain short-axis views at the mid-papillary muscle level. Its position was fixed at the same level throughout the experiment by a clamp attached to the procedure table (21). Gain settings were optimized at the beginning of each experiment and kept constant throughout the recording period. A saline bath acted as an acoustic interface between the transducer and the heart (21). The injection sequence was timed with a character generator (model G58, Medical Diagnostic Services) that projected the time in hundredths of a second onto the ultrasound video image. A standard limb lead electrocardiogram was also recorded with the echocardiographic images on video tape.

The echo contrast agent used for these experiments comprised 4 to 10 μm microbubbles (mean size 6 μm) produced by sonication of Renografin-76 as previously described (27). Eight milliliters of Renografin-76 (diatrizoate meglumine and diatrizoate sodium, 18.5 g/ml, E.R. Squibb and Sons Inc.) was sonicated at 20,000 Hz with a power output of 75 W for 30 s with the use of a commercially available sonicating system (model W-375, Heat Systems Ultrasonics Corp.). The half-life of the microbubbles is 2.7

min and the concentration is 500,000 ± 200,000 microbubbles/ml (28).

Images were analyzed on an off-line image analysis computer (Mipron System, Kontron Electronics) as previously described (24). Images were transferred from video tape to the computer in a 340 × 240 × 8 format. End-diastolic frames, beginning six beats before contrast injection and extending until contrast disappearance from the myocardium, were identified. An acetate sheet was placed over the video monitor and regions of interest over myocardial beds supplied by the left anterior descending and left circumflex coronary arteries were defined on this sheet. These beds were defined from a contrast injection performed during a transient total occlusion of the left circumflex artery that was performed in each animal before termination of the experiment. Other landmarks such as the epicardium, endocardium and right ventricular free wall–posterior left ventricular wall junction were also marked on the acetate sheet. This acetate sheet was then positioned over each end-diastolic frame to achieve proper registration of these landmarks to account for cardiac translation and rotation. A hand-held digitizer was then used to trace the myocardial regions of interest in each end-diastolic frame corresponding to those drawn on the acetate sheet. The regions of interest were defined in the middle of the myocardium, thereby avoiding the epicardial and endocardial echo bands. The computer then determined the average gray level (range 0 to 255) in each region of interest. The resultant values were written to an ASCII file and transferred to a minicomputer (VAX 8200, Digital Equipment Corp.) by means of an RS-232 interface. Background subtraction and least square curve fitting of a gamma-variate function ($y = At e^{-\alpha t}$) were performed, where A is a scaling factor, α is a variable of curve width and t is time (29). α is represented in seconds^{-1}; the greater the value of α, the narrower the width of the curve. The following variables were also calculated: curve amplitude (A/αe) and area under the curve (A/α^2).

Measurement of regional myocardial blood flow. Myocardial blood flow was measured before and after intracoronary injection of papaverine, as previously described (24). Approximately 2.0×10^6 15 μm size, radiolabeled microspheres (New England Nuclear Corp.) were injected into the left atrium. These microspheres were agitated in 4 ml of 0.9% saline–0.01% Tween-80 solution before injection. The two sets of spheres used and their corresponding energy windows were: ^{51}chromium, 280 – 360 KeV and ^{113}tin, 362 – 440 KeV. The catheter through which the spheres were injected was flushed immediately after the injections with 5 ml of 0.9% saline solution. Reference arterial blood sampling through the aortic catheter was begun at a rate of 6 ml/min just before microsphere injection and continued for a total of 90 s with the use of a constant-rate withdrawal pump (model 944, Harvard Apparatus).

At the end of the experiment, the left circumflex coronary artery was ligated at the site of the hydraulic occluder. Myocardial contrast echocardiography was performed to define the perfusion beds. Forty milliliters of saturated monastral blue solution (0.5% Monastral Blue dye, Sigma Chemical Co., in phosphate buffer solution mixed with 5% dextran and 0.9% saline solution) was injected into the catheter placed in the left anterior descending coronary artery, after which the animal was killed (21). In this manner, the left anterior descending artery bed was stained blue and the left circumflex artery bed remained unstained. The heart was removed from the thorax, and the great vessels, atria, right ventricular free wall and epicardial fat were removed. The heart was cut into a 1 cm thick slice corresponding to the level of echocardiographic interrogation (19). This slice was then cut into 16 equal wedge-shaped pieces, and each piece was divided into inner, middle and outer portions. The myocardial and reference blood samples were placed in preweighed plastic tubes and reweighed on an electronic balance (model 1601, Sartorius Corp.) that was connected to the minicomputer (VAX 8200) by way of an RS-232 interface allowing automatic transfer of data into a preconfigured file. The samples were counted in a well counter with a multichannel analyzer (model 5986, Auto-gamma Scintillation Counter, Packard Corp.). These data were also automatically transferred to the preconfigured file in the minicomputer containing the sample weights. A computer program was used to correct for the activity spilling from one window to the next and for determining myocardial blood flow in milliliters per minute in each of the samples with the use of the following equation: Qm = (Cm × Qr)/Cr, where Qm = myocardial blood flow, Cm = tissue counts (cts/min), Qr = rate of withdrawal of arterial sample (ml/min) and Cr = counts in reference arterial sample. Transmural blood flow (milliliters per minute per gram) was calculated by dividing the total flow to all three myocardial segments by their combined weight.

Protocol. Arterial blood gases were monitored throughout the experiment; the respiratory rate and the concentration of expired oxygen in the air (FIO_2) were adjusted and intravenous sodium bicarbonate was given accordingly. Coronary blood flow and arterial blood pressure were recorded on the physiologic recorder at a paper speed of 10 mm/s, which was calibrated before each injection. A critical stenosis of the left circumflex coronary artery was created by tightening a micrometer attached to the hydraulic occluder until a 6 mg intracoronary injection of papaverine hydrochloride (Ely Lilly and Co.) no longer produced an increase in blood flow as measured by the electromagnetic flow probe. Myocardial blood flow measurements, obtained with the use of radiolabeled microspheres, and myocardial contrast echocardiography were performed in a random order 5 min apart. After hemodynamic equilibration, myocardial contrast echocardiography and myocardial blood flow measurements were

repeated 5 min apart 45 s after separate intracoronary injections of 6 mg of papavarine hydrochloride.

Statistical analysis. All data analysis (including curve fitting) was performed with RS/1 (Bolt, Beraneck, and Newman). Data were expressed as mean ± 1 SD. Data acquired before papaverine injection were compared with those acquired after papaverine injection with the Student's *t* test for paired data. Transmural blood flow data (absolute and ratios) were correlated with variables derived from the time-intensity curves during contrast echocardiography (absolute and ratios) with the use of linear regression analysis or an exponential function: $f(x) = a - be^{(-cx)}$. Intraobserver variability for the assessment of the degree of coronary stenosis was estimated with the use of a components of variance model (BMDP:8V, Department of Biomathematics, University of California, Los Angeles), and error was expressed as the square root of the variance between two sets of observations.

Human Studies

Patient selection. Nine patients (five men and four women, mean age 55 ± 12 years) with angiographically documented single vessel coronary artery disease involving either the left anterior descending or the left circumflex coronary artery were studied. Such patients were selected because it was planned to have one of the vascular beds supplied by the left main coronary artery act as a control for the other bed. Four of the nine patients had disease of the left anterior descending and five of the left circumflex coronary artery. All patients had ≥85% stenosis at a proximal site in the vessel; the mean percent stenosis was 90%.

Patients with conditions other than epicardial coronary artery stenosis known to affect coronary blood flow reserve, such as prior myocardial infarction or left ventricular hypertrophy, were excluded (30,31), as were patients with valvular heart disease and unstable angina pectoris. All patients included in the study gave informed consent to the protocol approved by the Human Investigation Committee at the University of Virginia School of Medicine.

Coronary angiography. Cardiac catheterization was performed with the Judkins technique (32). Biplane selective coronary angiography and left ventricular cineangiography were performed in a routine manner. The coronary angiograms were reviewed by two independent observers. Stenotic lesions were quantitated by measuring the diameter of the coronary artery lumen proximal to (D_N) and at the site of (D_S) the stenosis with hand-held calipers in two orthogonal views. The percent stenosis was equal to $1-(D_S/D_N)$. The intraobserver variability of our method of assessing coronary stenosis severity was small (2%). All patients had severe stenosis of one vessel (either the left anterior descending or the left circumflex coronary artery) and <50% stenosis in the remaining vessels. Stenoses of <50% should not have significant effects on coronary blood flow reserve (11,14).

Contrast echocardiography. Two-dimensional echocardiography was performed with use of a sector scanning system with a 2.5 MHz phased-array transducer (model 77020AC, Hewlett Packard Corp.). All images were recorded on 0.5 in. (2.54 cm) video tape with a commercially available VHS video recorder (model AG6300, Panasonic Corp.). During the injection of contrast medium, a single parasternal short-axis view at either the mid-papillary or apical level was obtained in seven patients, and a single parasternal long-axis view was obtained in two patients. The echocardiograms were analyzed by two independent observers. The intensity of contrast enhancement in the myocardial bed supplied by the left anterior descending coronary artery (anterior septum and anterior wall) was visually compared with the enhancement seen in the left circumflex bed (posterolateral walls). This assessment was made on contrast injections performed before and after papaverine injection in all patients.

Protocol. After routine coronary angiography, a foam wedge was placed behind the patient's back so as to tilt the chest to a 30° lateral position. The Judkins catheter was then engaged in the left main coronary artery and 3 ml of sonicated Renografin-76 injected into the catheter by hand. Echocardiographic recordings were obtained simultaneously until the contrast medium disappeared from the myocardium. The Judkins catheter was then disengaged from the left main ostium and flushed with 0.9% saline solution. Five minutes later, the catheter was again engaged in the left main ostium and 9 mg of papavarine hydrochloride injected through it. The catheter was immediately flushed with 5 ml of 0.9% saline solution. Forty-five seconds later (during the maximal coronary vasodilator effects of papavarine), 3 ml of sonicated Renografin-76 was injected through the catheter and echocardiographic recordings were obtained until the contrast medium disappeared from the myocardium. Because the dead space of the catheter is approximately 1 ml, only 2 ml of sonicated Renografin-76 entered the myocardium during each injection. We have previously demonstrated (32) that this amount of sonicated Renografin-76 is safe in humans. The addition of this protocol added no more than 10 to 15 min to the cardiac catheterization procedure.

Results

Animal Studies

Regional myocardial blood flow. Mean transmural blood flows to the myocardial beds supplied by the left anterior descending and the left circumflex coronary arteries were similar before papaverine (0.9 ± 0.4 and 0.7 ± 0.3 ml/min per g, respectively). After papaverine, approximately a 3.5-fold increase in flow was noted to the arterial bed supplied by the left anterior descending artery (2.6 ± 0.7 ml/min per g [$p <$

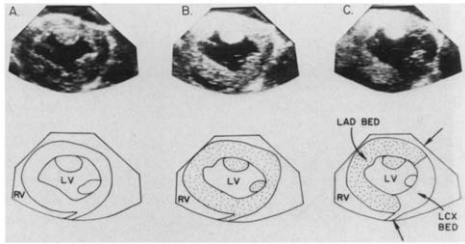


Figure 2. End-diastolic echocardiographic images obtained at the mid-papillary muscle short-axis level in a dog with a critical stenosis of the left circumflex coronary artery: **A,** Before injection of contrast medium; **B,** After injection of contrast medium; and **C,** After injection of both intracoronary papavarine and contrast medium. The area not showing contrast enhancement in panel C (arrows) represents the perfusion bed of the left circumflex (LCX) coronary artery (see text for details). LAD = left anterior descending coronary artery; LV = left ventricle; RV = right ventricle.

0.01]), whereas the flow to the left circumflex artery bed did not change significantly (0.8 ± 0.4 ml/min per g).

Echocardiographic data. Echocardiographic images obtained before (A) and during (B and C) contrast injection are shown in Figure 2. Contrast medium appears throughout the myocardium before injection of papaverine despite a critical left circumflex artery stenosis (Fig. 2B). After injection of papaverine, there is enhanced contrast in the entire left anterior descending coronary artery bed compared with the left circumflex artery bed (Fig. 2C). The size of the myocardial bed supplied by the left circumflex artery is also clearly delineated because of the disparity of contrast enhancement between the two beds (area subtended by arrows).

Because of large baseline variations noted in the gray scale intensities caused by respiration in one animal, quantitative data analysis was performed in five of the six dogs. Figure 3 illustrates the results of the quantitative analysis of the contrast injections shown in Figure 2. Before injection of papaverine, the curves for the left anterior descending and left circumflex artery beds are similar (Fig. 3A). However, after the injection of papaverine, the peak intensity and area under the curve are greater in the left anterior descending compared with the left circumflex artery bed; the curve width also appears to be greater despite increased flow to that bed.

The absolute values derived from quantitative curve analysis for the five dogs are shown in Table 1. All three variables were similar in the left anterior descending and left circumflex artery beds before the injection of papavarine. After the intracoronary injection of papavarine, the area under the curve increased, whereas there were small, insignificant decreases in α and peak amplitude in the left anterior descending artery bed. In comparison, the area under the curve and peak amplitude decreased while α increased in the left circumflex artery bed after papavarine. The difference in these values between the two beds after intracoronary papavarine reached borderline significance in the case of the areas under the curves ($p = 0.07$) but attained statistical significance in the case of peak amplitude ($p = 0.03$). There was no significant change in α. The correlations between these absolute values and actual transmural blood flows were poor with r^2 values of 0.01, 0.03 and 0.002 for areas under the curves and transmural blood flow, peak amplitude of the curves and transmural blood flow and α and transmural blood flow, respectively.

Because the concentration of injected microbubbles and the gain settings were not constant between experiments, the ratios of myocardial blood flow in the left anterior descending and left circumflex artery beds were compared with the ratios of all three curve variables derived from these beds before and after papavarine (Table 2). The ratio of areas under the curves was significantly greater ($p < 0.05$) after than before papavarine. In contrast, although the ratio of the amplitudes of the curves from the two beds was higher after papavarine, the difference was not significant. The ratio of α derived from the two beds remained unchanged after papavarine. The areas under the curves derived from the two beds during myocardial contrast echocardiography correlated well with the blood flow ratios (Fig. 4). With linear regression, the r^2 value was 0.73 ($p < 0.001$) and SEE 0.44. When curve fitting was performed with the exponential function, this value increased to 0.85 ($p < 0.001$) (SEE 0.35). There was a poor correlation between both the ratios of the peak amplitudes of the time-intensity curves and α to the ratios of the blood flows derived from the two vascular beds before and after papavarine ($r^2 = 0.18$ and $r^2 = 0.02$, respectively).

Human Studies

Echocardiographic data. Figure 5 shows end-diastolic frames at the apical level from myocardial contrast echocardiographic images taken from a patient with a 90% stenosis of the left anterior descending coronary artery. Panel A represents a baseline echocardiogram before contrast injection, panel B represents contrast enhancement before papaverine and panel C demonstrates the change in the pattern of contrast enhancement produced by papaverine-induced

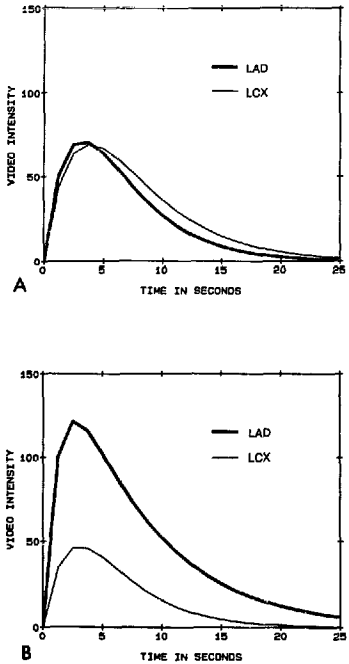


Figure 3. Time-intensity curves obtained after fitting a gamma-variate function to the data obtained from the stages of the experiment in the dog with critical stenosis of the left circumflex coronary artery indicated in Figure 2. **A,** Before injection of papavarine; the curves from both arterial beds are nearly identical. **B,** After injection of papavarine; the peak contrast effect and area under the curve are significantly greater in the left anterior descending (LAD) compared with the left circumflex (LCX) artery bed (see text for details).

vasodilation. The appearance of contrast is similar in the left anterior descending and left circumflex beds before the injection of paraverine. After papaverine, the left circumflex artery bed demonstrates enhanced contrast opacification. The area supplied by the left anterior descending artery is also delineated after the intracoronary injection of papavarine (area subtended by arrows in Fig. 5C) because of the obvious disparity in contrast enhancement between the two beds. Similar results were noted in all patients. As would be expected, none of these patients with chronic stable angina exhibited less contrast enhancement of the bed supplied by the stenotic artery during myocardial contrast echocardiography before the injection of papavarine.

Discussion

In the present study we have demonstrated that myocardial contrast echocardiography can be used to assess coronary blood flow reserve in patients with coronary artery disease. We did not validate the technique in humans using an independent standard because we feel that there is no "gold standard" for assessing coronary blood flow reserve in humans. However, we validated this technique for the measurement of blood flow reserve against the method of radiolabeled microspheres in dogs with critical coronary stenoses. We showed that the ratios of areas under the curves derived from time-intensity plots generated during myocardial contrast echocardiography correlate well with blood flow reserve measured using radiolabeled microspheres. We, therefore, believe that myocardial contrast echocardiography has promise for the assessment of coronary blood flow reserve during coronary angiography in patients with coronary artery disease.

Variables of time-intensity curves and coronary blood flow reserve. The rate of turnover of an inert tracer from a tissue is proportional to the flow through the tissue as long as the volume of distribution is constant. We have previously demonstrated (24) that the width of the time-intensity curves generated during myocardial contrast echocardiography relates directly to regional myocardial blood flow measured using radiolabeled microspheres. These findings are consistent with the indicator-dilution principles (33). In the same experiments, when vasoactive substances were not used to alter flow, we were unable to show a relation between peak

Table 1. Quantitative Contrast Curve Analysis With Critical Coronary Stenosis of the Left Circumflex Artery in Five Dogs

	LAD		LCx	
	Before Papaverine	After Papaverine	Before Papaverine	After Papaverine
Area under curve (arbitrary units)	299 ± 209	536 ± 426*	339 ± 296	215 ± 141*
Curve amplitude (arbitrary units)	77 ± 25	71 ± 35†	45 ± 28	30 ± 16†
Curve width $= \alpha\ (s^{-1})$	0.46 ± 0.15	0.45 ± 0.18	0.42 ± 0.17	0.50 ± 0.45

*$p = 0.07$ between the two beds; †$p = 0.03$ between the two beds. LAD and LCx = left anterior descending and left circumflex coronary artery, respectively.

Table 2. Ratios of Contrast Washout Variables and Regional Myocardial Blood Flow From the Left Anterior Descending and Left Circumflex Artery Beds in the Presence of Critical Stenosis of the Left Circumflex Artery in Five Dogs

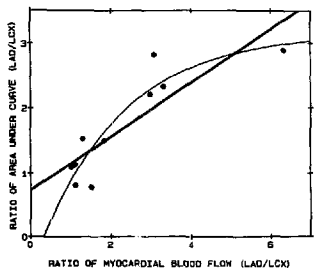
	Ratio of α (LAD/LCx)	Ratio of Curve Amplitude (LAD/LCx)	Ratio of Area Under the Curve (LAD/LCx)	Ratio of Blood Flow (LAD/LCx)
Baseline	0.93 ± 0.22	1.16 ± 0.28	1.1 ± 0.3*	1.2 ± 0.2*
After papaverine	0.99 ± 0.48	3.01 ± 2.10	2.4 ± 0.6*	3.5 ± 1.7*

*$p < 0.05$ before and after papaverine. Abbreviations as in Table 1.

amplitude of the curves and regional flow; the relation between the areas under the curves and blood flow was not measured in those experiments.

In the present study, we used intracoronary papaverine which, as a potent vasodilator, can change the myocardial volume as well as concentration and velocity of red blood cells within the myocardium (34,35). Because sonicated microbubbles behave like tracers of red blood cells (36), their flux through the myocardium represents the in vivo fate of red blood cells rather than the total blood volume. Our data suggest that the number of red blood cells per unit volume of tissue is significantly higher in the normal arterial bed after papavarine. Therefore, a measure of the number of microbubbles (areas under the curves) relates best to regional perfusion. These findings are consistent with those of Klitzman and Duling (34), who found a four-fold increase in local arteriolar hematocrit in the hamster cremaster muscle during superfusion of 0.1 m*M* adenosine. Because of the variability in the concentration and rate of injection of the microbubbles during each stage of the experiment, the ratios of the areas under the curves from the left anterior descending and left circumflex artery beds, rather than the absolute areas of the curves derived from these two beds, correlate with blood flow reserve as measured with the use of radiolabeled microspheres. Perhaps the standardization of microbubble size, concentration and rate of injection and improvements in the methods of acquiring echocardiographic data will make it possible to measure absolute changes in flow induced by vasoactive substances.

Figure 4. Relation between ratios of transmural blood flows (with the use of radiolabeled microspheres) noted in the left anterior descending (LAD) and left circumflex (LCX) artery beds before and after injection of papavarine to the ratios of the areas under the curves during myocardial contrast echocardiography during the same stages. By linear regression (**thin line**), $y = 0.4x + 0.7$ ($r^2 = 0.73$, $p < 0.001$, SEE = 0.44). By an exponential function (**bold line**), $y = 3.2 - 3.7xe^{-(0.5x)}$ ($r^2 = 0.85$, $p < 0.0001$, SEE = 0.35).



In both Figures 2 and 5, the myocardium supplied by the stenotic vessels (left circumflex artery in Figure 2 and left anterior descending artery in Figure 5) shows almost total lack of contrast enhancement compared with the normally perfused bed after the intracoronary injection of papavarine (panel C in both figures). There are two possible explanations for this phenomenon. First, it is possible that intracoronary injection of papavarine causes "coronary steal" from a bed supplied by a severe stenosis, resulting in reduced blood flow to that bed (37). For example, in the dog whose echocardiogram is illustrated in Figure 2, the mean transmural blood flow to the left circumflex artery bed after the intracoronary injection of papavarine decreased by 50%. However, "coronary steal" was noted in only two of the six dogs, while the postpapavarine echocardiograms appeared similar in all the dogs. Although it is more difficult to document "coronary steal" in humans, the patient whose echocardiogram is depicted in Figure 5 did not exhibit ischemic ECG changes or experience chest pain during the intracoronary injection of papavarine. However, some of the patients did demonstrate such findings after the intracoronary injection of papavarine.

The second explanation is related to the flux of microbubbles to the bed supplied by the stenotic artery versus the normal bed during intracoronary injection of contrast. Before the injection of papavarine, the flow to both vascular beds was equal. After the injection of papavarine, the average flow increased by 3.5-fold in the bed supplied by the normal artery, compared with the bed supplied by the stenotic vessel. Assuming that an equal number of microbubbles was injected at each stage, the number of microbubbles entering the normal bed would be 3.5 times greater than that entering the bed supplied by the stenotic vessel. This

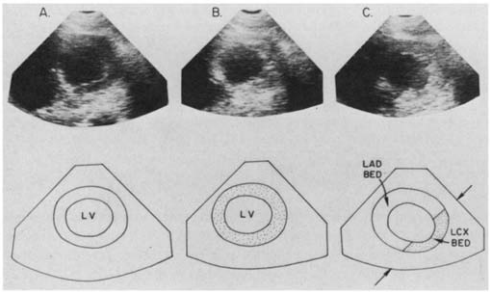


Figure 5. End-diastolic echocardiographic images obtained at the apical short-axis level in a patient with a 90% stenosis of the left anterior descending (LAD) coronary artery. **A,** Before injection of contrast medium; **B,** After injection of contrast medium; and **C,** After injection of both intracoronary papavarine and contrast medium. The area not showing contrast enhancement in panel C (**arrows**) represents the perfusion bed of the left anterior descending coronary artery (see text for details). Abbreviations as in Figure 2.

"microbubble steal" could result in the large disparity of contrast noted between the two beds. It is likely that in several instances a combination of both "coronary steal" and "microbubble steal" caused a disparity in contrast enhancement between the different myocardial beds.

It can also be noted in both Figures 2 and 5 that because of the disparity of contrast enhancement in the beds supplied by the normal and stenotic vessels, the area perfused by the stenotic vessel was clearly defined after the intracoronary injection of papavarine (panel C in both figures). This observation is an added advantage of myocardial contrast echocardiography in comparison with other techniques used to measure coronary blood flow reserve in the clinical setting (16–18). Therefore, information on both the coronary blood flow reserve and the size of the perfusion bed of a coronary artery are available. In all our patients this information was available to us on-line and could have been used to determine the need for a coronary revascularization procedure.

Qualitative versus quantitative assessment of coronary blood flow reserve. In both our human and animal studies, the disparity between the contrast effect noted in the bed supplied by a critically stenosed vessel and that supplied by a normal vessel was so great that it was easily observed visually. This disparity suggested that in comparison with the control bed, coronary blood flow reserve was reduced in the bed supplied by the stenosed vessel. Quantitative information confirmed these findings in the dogs. However, when all myocardial beds are supplied by vessels with significant disease, visual assessment will only indicate the bed supplied by the most critical stenosis. Being a relative comparison between beds, it will not necessarily indicate the reduction in flow reserve in other beds. In contrast, the comparison of the areas under the curves before and after papavarine will provide an estimation of coronary blood flow reserve. If the area under the curve derived from any one myocardial bed does not increase by a certain amount after papavarine, a critical stenosis supplying that bed can be suspected. However, if an absolute estimation of coronary blood flow reserve is desired, false positive results could be obtained in the presence of prior infarction and hypertrophy (30,31). Furthermore, self-attenuation of ultrasound occurs when the microbubble concentration increases above a certain threshold (38). Therefore, it is possible that beyond a certain ratio of blood flows between two myocardial beds, the ratio of the areas under the curves will not increase. This phenomenon might explain why an exponential function shows a better fit to our data than a linear regression (Fig. 4). However, the data are too few to derive any definitive conclusions.

Comparison with previous studies. Lang et al (39) noted an increase in contrast enhancement of myocardial beds after coronary angioplasty that was greater than that in the normal beds. They attributed this phenomenon to hyperemic blood flow in the bed supplied by a stenotic artery immediately after coronary angioplasty. Cheirif et al. (40) also observed an increase in the mean contrast intensity after papavarine compared with baseline in a perfusion bed supplied by a vessel that had undergone successful angioplasty. They suggested that myocardial contrast echocardiography could be used in conjunction with papavarine for assessing the success of coronary angioplasty. Our data are in agreement with these reports and further suggest that quantitation of time-intensity curves generated during contrast echocardiography relates directly to coronary blood flow reserve. Our data also explain the underlying reasons for using different variables derived from time-intensity curves in the presence and absence of coronary vasoactive drugs.

Comparison with other techniques of assessing coronary blood flow reserve. Other methods of assessing coronary blood flow reserve in patients also measure relative changes in flow. For instance, the epicardial and intracoronary Doppler techniques measure the rate of change of velocity (16,17). Because of its inability to measure changes in coronary dimensions, the Doppler ultrasound technique is

not capable of measuring changes in flow. In addition, it does not assess changes induced in myocardial blood flow or the size of the perfusion bed supplied by the stenotic vessel. In comparison, myocardial contrast echocardiography provides information on coronary blood flow reserve, myocardial blood flow and the size of the perfusion bed of a coronary artery. Digital subtraction angiography is another technique with the potential for assessing coronary blood flow reserve. Using this technique, Nissen et al. (18), like us, found that the areas under the curves obtained from myocardial regions of interest correlate best with changes induced in coronary blood flow after intracoronary injection of papavarine. Because digital subtraction angiography is not a tomographic technique, however, it cannot provide a precise estimation of the size of the perfusion bed supplied by a coronary artery.

Limitations. The limitations of the current study pertain to the limitations of assessing coronary blood flow reserve, our method of measuring blood flow reserve and the patients selected for this study. The limitations of assessing coronary blood flow reserve are common to all techniques and are summarized by Hoffman (30) and Klocke (31). In addition, vasoactive drugs such as papaverine might change the caliber of the stenotic vessels resulting in potential underestimation of the flow limitations produced by a stenosis during maximal myocardial oxygen demand (41). The greatest limitation of our method of assessing flow reserve, however, is related to the lack of standardization of the size, concentration and method of injection of microbubbles. In addition, even 2 ml of Renografin-76 alters coronary blood flow (28). Use of the newly described albumin microbubbles, under current investigation in our laboratory (28), should eliminate these problems. Finally, we selected patients with severe single vessel disease. Whether similar results could be obtained in patients with less severe critical stenoses or in patients with multivessel disease needs to be investigated.

Conclusions. Our study describes the use of myocardial contrast echocardiography to assess coronary blood flow reserve in humans. It also validates the technique against radionuclide microspheres in a canine model. We have demonstrated that quantitative assessment of coronary blood flow reserve is possible by measuring the area under the curves generated during myocardial contrast echocardiography before and after an intracoronary injection of papavarine. We have previously shown that this technique is safe in humans (32). Assessment of coronary blood flow reserve with the use of this technique adds no more than 15 min to the cardiac catheterization procedure. Compared with other techniques utilized to measure coronary blood flow reserve, this technique provides additional information on myocardial blood flow and the size of the perfusion bed supplied by a stenotic artery. Studies with larger populations, patients with a large array of coronary stenoses and patients with multivessel disease are required to determine whether the measurement of coronary blood flow reserve is superior to assessment of coronary angiograms for determining prognosis in patients with coronary artery disease.

References

1. Vlodaver Z, French R, Van Tassel RA, Edwards JE. Correlation of the antemortem angiogram and the postmortem specimen. Circulation 1973;47:162–9.
2. Arnett EN, Isner JM, Redwood DR, et al. Coronary artery narrowing in coronary artery disease: comparison of cineangiography and necropsy findings. Ann Intern Med 1979;91:350–6.
3. Schwartz JN, Kong Y, Hackel DB, Bartel AG. Comparison of angiocardiographic and postmortem findings in patients with coronary artery disease. Am J Cardiol 1975;36:174–8.
4. Bjork L, Spindola-Franco H, Van Hooten FX, Cohn PF, Adams DF. Comparison of observer performance with 16 mm cinefluorography and 70 mm camera fluorography in coronary arteriography. Am J Cardiol 1975;36:474–8.
5. DeRouen TA, Murray JA, Owen W. Variability in the analysis of coronary arteriograms. Circulation 1977;55:324–8.
6. Detre KM, Wright E, Murphy ML, Takaro T. Observer agreement in evaluating coronary angiograms. Circulation 1975;52:979–86.
7. Myers MG, Shulman HS, Saibil EA, Naqvi SZ. Variation in measurement of coronary lesions on 35 and 70 mm angiograms. Am J Roentgenol 1978;130:913–5.
8. Zir LM, Miller SW, Dinsmore RE, Gilbert JP, Harthorne JW. Interobserver variability in coronary angiography. Circulation 1976;53:627–32.
9. Klocke FJ. Measurements of coronary blood flow and degree of stenosis: current clinical implications and continuing uncertainties. J Am Coll Cardiol 1983;1:31–41.
10. White CW, Wright CB, Doty DB, et al. Does visual interpretation of the coronary arteriogram predict the physiologic importance of a coronary stenosis? N Engl J Med 1984;310:819–24.
11. Wilson RF, Marcus ML, White CW. Prediction of the physiologic significance of coronary arterial lesions by quantitative lesion geometry in patients with limited coronary artery disease. Circulation 1987;75:723–32.
12. Gould KL, Kelley KO. Physiologic significance of coronary flow velocity and changing stenosis geometry during vasodilation in awake dogs. Circ Res 1982;50:695–704.
13. Gould KL, Kelley KO, Bolson EL. Experimental validation of quantitative coronary arteriography for determining pressure-flow characteristics of coronary stenosis. Circulation 1982;66:930–7.
14. Gould KL, Lipscomb K, Hamilton GW. Physiologic basis for assessing critical coronary stenosis: instantaneous flow response and regional distribution during coronary hyperemia as measures of coronary blood flow reserve. Am J Cardiol 1974;33:87–94.
15. Gould KL, Lipscomb K. Effects of coronary stenosis on coronary flow and resistance. Am J Cardiol 1974;43:48–55.
16. Wilson RF, White CW. Intracoronary papaverine: an ideal coronary vasodilator for studies of the coronary circulation in conscious humans. Circulation 1986;73:444–51.
17. Greene ER, Reilly PR, Miranda IP. Doppler echocardiographic assessment of left internal mammary grafts in humans (abstr). Circulation 1986;74(suppl II):II-308.
18. Nissen SE, Elion JL, Booth DC, Evans J, DeMaria AN. Value and limitations of computer analysis in the assessment of coronary flow reserve. Circulation 1986;73:562–71.
19. Sakamaki T, Tei C, Meerbaum S, et al. Verification of myocardial contrast two-dimensional echocardiographic assessment of perfusion defects in ischemic myocardium. J Am Coll Cardiol 1984;3:34–8.
20. Kemper AJ, O'Boyle JE, Cohen CA, Taylor A, Parisi AF. Hydrogen peroxide contrast echocardiography: quantification in-vivo of myocardial

risk area during coronary occlusion and of the necrotic area remaining after reperfusion. Circulation 1984;70:309–17.

21. Kaul S, Glasheen W, Ruddy TD, Pandian NG, Weyman AE, Okada RD. The importance of defining left ventricular "area at risk" in-vivo during acute myocardial infarction: an experimental evaluation utilizing myocardial contrast 2D-echocardiography. Circulation 1987;75:1349–60.

22. Kaul S, Pandian NG, Guerrero JL, Gillam LD, Okada RD, Weyman AE. The effects of selectively altering the collateral driving pressure on regional perfusion and function in the occluded coronary bed in the dog. Circ Res 1987;61:77–85.

23. Tei C, Kondo S, Meerbaum S, et al. Correlation of myocardial echo contrast disappearance rate ("washout") and severity of experimental coronary stenosis. J Am Coll Cardiol 1984;3:39–46.

24. Kaul S, Oliner J, Kelly P, Watson D. Measurement of regional myocardial blood flow in-vivo using myocardial contrast two-dimensional echocardiography (abstr). J Am Coll Cardiol 1987;9:2A.

25. Kemper AJ, Force T, Kloner R, et al. Contrast echocardiographic estimation of regional myocardial blood flow after acute coronary occlusion. Circulation 1985;72:1115–24.

26. Armstrong WF, Kinney EL, Mueller TM, Tickner EG, Dillon JC, Feigenbaum H. Assessment of myocardial perfusion abnormalities with contrast-enhanced two-dimensional echocardiography. Circulation 1983;66:166–73.

27. Keller MW, Feinstein SB, Brilter RA, Powsner SM. Automated production and analysis of echo contrast agents. J Ultrasound Med 1986;5:493–8.

28. Keller MW, Glasheen W, Teja K, Gear A, Kaul S. Myocardial contrast echocardiography without significant hemodynamic effects or reactive hyperemia: a major advantage in the imaging of regional myocardial perfusion. J Am Coll Cardiol 1988;12:1039–47.

29. Thompson MK, Starmer CF, Whoten RE, McIntosh MD. Indicator transit time as a gamma variate. Circ Res 1964;14:502–15.

30. Hoffman JIE. A critical view of coronary reserve. Circulation 1987;75(suppl I):I-1–6.

31. Klocke FJ. Measurements of coronary flow reserve: defining pathophysiology versus making decisions about patient care. Circulation 1987;76:1183–9.

32. Moore CA, Smucker ML, Kaul S. Myocardial contrast echocardiography in humans. I. Safety—a comparison with routine coronary arteriography. J Am Coll Cardiol 1986;8:1066–72.

33. Bassingthwaighte JB. Physiology and theory of tracer washout techniques for the estimation of myocardial blood flow: flow estimation from tracer washout. Prog Cardiovasc Dis 1977;20:165–89.

34. Kiiteman B, Duling BR. Microvascular hematocrit and red cell flow in resting and contracting striated muscle. Am J Physiol 1979;237:H481–H90.

35. Crystal GJ, Downey F, Bashour FA. Small vessel and total coronary blood volume during intracoronary adenosine. Am J Physiol 1981;241:H194–H201.

36. Keller MW, Segal SS, Kaul S, Duling BR. The behavior of sonicated albumin microbubbles in the microcirculation: a basis for their use as myocardial echo contrast agents (abstr). J Am Coll Cardiol 1988;10:75A.

37. Gross GJ, Warltier DC. Coronary steal in four models of single or multiple vessel obstruction in dogs. Am J Cardiol 1981;48:84–92.

38. Powsner SM, Keller MW, Sanile J, Feinstein SB. Quantitation of echo contrast effects. Am J Physiol Imag 1986;1:124–8.

39. Lang RM, Feinstein SB, Feldman T, Newmann A, Chua KG, Borow KM. Contrast echocardiography for evaluation of myocardial perfusion: effects of coronary angioplasty. J Am Coll Cardiol 1986;8:232–5.

40. Cheirif J, Zoghbi WA, Minor ST, et al. Assessment of myocardial perfusion in humans by contrast echocardiography. I. Evaluation of regional coronary reserve by peak contrast intensity. J Am Coll Cardiol 1988;11:735–43.

41. Zijlstra F, Reiber JHC, Serruys PW. Does intracoronary papaverine dilate epicardial coronary arteries? Cathet Cardiovasc Diagn 1988;14:1–6.