EXPERIMENTAL STUDIES

Myocardial Washout of Sonicated Iopamidol Reflects Coronary Blood Flow in the Absence of Autoregulation

DANIELE ROVAI, MD, FESC, GIANNA GHELARDINI, MS, MASSIMO LOMBARDI, MD,
MARIA GIOVANNA TRIVELLA, MD, EDOARDO NEVOLA, MD, LUIGI TADDEI,
CLAUDIO MICHELASSI, ALESSANDRO DISTANTE, MD, FESC,
ANTHONY N. DeMARIA, MD, FACC, ANTONIO L'ABBATE, MD, FESC, FACC

Pisa, Italy

Objectives. The aim of the study was to evaluate the relation between measurements derived from myocardial contrast echocardiography and coronary blood flow.

Background. Contrast echocardiography has the potential for measuring blood flow.

Methods. In six open chest anesthetized dogs, the left circumflex coronary artery was cannulated and perfused with blood drawn from the left femoral artery. While adenosine was infused into the circuit, circumflex flow was generated by a calibrated roller pump to the point of abolishing coronary autoregulation. At each of 25 levels of coronary blood flow, paired bolus injections of sonicated iopamidol were performed proximal to a mixing chamber. The perfused area of the left circumflex coronary artery was labeled by radioactive microspheres injected into the perfusion line. Two-dimensional echocardiographic images of the left ventricular short axis were digitized off-line, and myocardial videodensity was measured in the area perfused by the left circumflex coronary artery to generate time-intensity curves.

Results. The washout slope of curves showed a good correlation with coronary blood flow, ranging from 0.5 to 12.5 ml/min per g of tissue. This correlation was good both in individual dogs (correlation coefficient [r] ranging from 0.78 to 0.96) and in the group of animals as a whole (r = 0.85). Washout slope also showed a good correlation with coronary diastolic pressure (r = 0.80), which ranged from 23 to 114 mm Hg, suggesting a possible primary effect of pressure on contrast washout. However, coronary blood flow appeared to be a stronger predictor of washout slope (partial F = 26.5, p < 0.001) than did perfusion pressure (partial F = 5.9, p < 0.05 by multiple regression). The injection to injection variability in myocardial washout slope appeared to be high (24%). The gamma variate fitting of curves did not improve the correlation with coronary flow (r = 0.78).

Conclusions. Myocardial washout of sonicated iopamidol reflects coronary blood flow in a model in which coronary autoregulation is abolished.

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The study of myocardial tissue perfusion is still an elusive goal in the clinical setting, and all available methods have significant limitations. Coronary arteriography provides information only on the anatomy of epicardial coronary vessels (1). Similarly, Doppler catheters measure coronary flow velocity in the largest coronary arteries without exploring the downstream tissue perfusion (2). Thallium scintigraphy is being semiquantitative and myocardial thallium uptake also reflects the metabolic/histologic state of cells (3). Positron emission tomography accurately measures myocardial tissue perfusion (4); however, high costs and complex organization limit its use.

In the last decade, efforts have been made to quantify myocardial perfusion by two-dimensional contrast echocar-

diography. The effects of experimental coronary stenoses on myocardial perfusion were initially evaluated, and a prolonged myocardial contrast echo disappearance rate ("washout") was found with increasing stenosis severity (5). The influence of different coronary blood flow rates on myocardial echo contrast washout was also studied: a shortening in washout with coronary hyperemia and a prolongation with coronary underperfusion were experimentally demonstrated (6). These results were also confirmed in the clinical setting, where a prolonged myocardial washout of contrast medium was found in patients with severe coronary stenoses (7). However, the correlation between myocardial contrast washout and coronary blood flow appeared to be weak. This weak correlation was later confirmed, whereas a good correlation was found between specific coronary blood flow and the width of time-intensity curves fitted by a gamma variate function (8).

In view of the clinical relevance of quantifying myocardial tissue perfusion and the growing clinical applications of contrast echocardiography (9-16), this study was undertaken to evaluate the relation between coronary blood flow

and several variables derived from myocardial contrast echocardiography. Recently, the pressure dependence of echocardiographic contrast agents was demonstrated (17,18). Specifically, an increase in the surrounding pressure was shown to reduce the diameter of microbubbles. Because the echo contrast effect is related to the diameter of bubbles (19), changes in the surrounding pressure may influence the intensity of the contrast effect. Therefore, an additional purpose of this study was to evaluate the relation between contrast echocardiographic variables and coronary perfusion pressure.

Methods

General preparation. Adult male or female mongrel dogs (n = 6), weighing 19 to 25 kg were premedicated with morphine sulfate (2.5 mg/kg body weight subcutaneously) and anesthetized with alpha-chloralose (100 mg/kg intravenously). The dogs were intubated with an endotracheal tube and were ventilated with oxygen-enriched room air by a positive-pressure respirator (Harvard model 613) so that arterial oxygen tension was maintained at >90 mm Hg and carbon dioxide tension at 30 to 40 mm Hg. The anesthesia was maintained by supplemental injections of 500 mg of alpha-chloralose, as needed. The metabolic acidosis associated with chloralose anesthesia was prevented by bolus injections of 8.4% sodium bicarbonate, as required. A pressure-tip catheter (Millar 7ᶠ) was inserted into the abdominal aorta through the right femoral artery. Atrioventricular heart block was produced by formaldehyde (37%) injections, as described by Ito and Feigl (20), and the heart was paced by a pacing wire positioned in the right ventricular apex. A left thoracotomy was performed at the fourth intercostal space, the pericardium excised and the heart suspended in a pericardial cradle. The left circumflex coronary artery was dissected free from the surrounding tissues, and a double-lumen steel cannula was inserted into the vessel (Fig. 1). The dogs were given heparin sodium (1,000 U/kg intravenously) before coronary artery cannulation. The left circumflex coronary artery was perfused by a well calibrated roller pump (Sarns) through one lumen of the cannula. Arterial blood from the left femoral artery was used as the perfusion source and was maintained at body temperature by a thermostat system positioned along the extracorporeal circuit. Coronary flow was monitored by an electromagnetic flow transducer (Zepeda Instruments SWF-4RD). Coronary artery pressure was measured at the tip of the cannula through a strain gauge manometer (Statham P23 ID). A mixing chamber was positioned immediately above the coronary artery cannula. A partial right thoracotomy was also performed to facilitate the echocardiographic examination. Before and during injection of the contrast agent, coronary blood flow, coronary pressure, aortic pressure and two electrocardiographic (ECG) leads were monitored and recorded on paper at a speed of 25 mm/s.
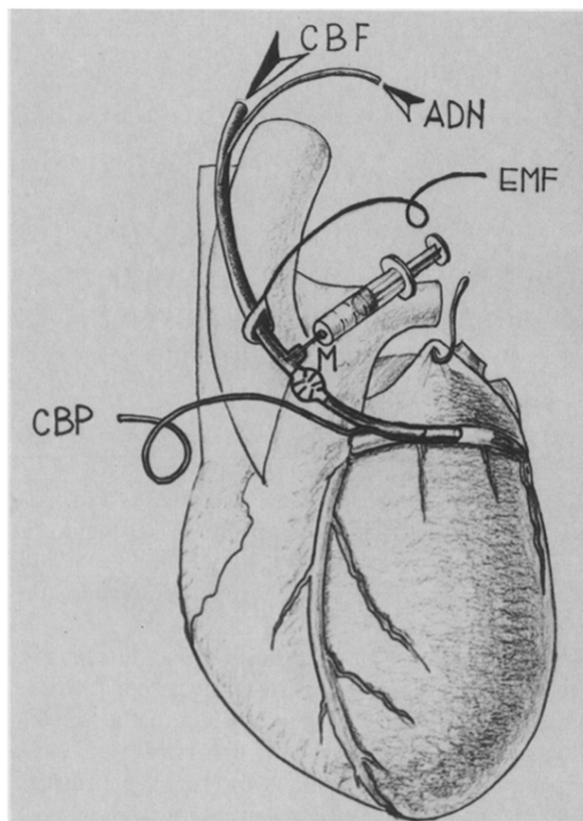

Figure 1. Drawing of the experimental model. A double-lumen steel cannula was inserted into the left circumflex coronary artery. One lumen of the cannula was used to perfuse the vessel, the other to measure coronary blood pressure (CBP). Coronary blood flow (CBF) was measured by an electromagnetic flow meter (EMF). A mixing chamber (M) was positioned between the coronary cannula and the injection point. Adenosine (ADN) was continuously infused.

Intracoronary adenosine infusion. After coronary cannulation, adenosine was continuously infused into the circuit by means of a perfusion pump at pharmacologic doses ranging from 0.6 to 1.8 mg/min. To test the efficacy of the adenosine dose in abolishing coronary autoregulation, coronary perfusion was stopped for 20 s: the lack of hyperemic response when perfusion was restored was considered to reflect maximal vasodilation. If moderate coronary hyperemia occurred, the dose of adenosine was increased and the coronary perfusion was again stopped.

Echocardiographic examination. Two-dimensional echocardiograms were obtained by using a commercial sector scanner (77020, Hewlett-Packard) operating at 5.0 MHz. The transducer was positioned on the right ventricle by way of the right thoracotomy, and a short-axis view of the left ventricle was obtained (Fig. 2). Gain setting controls were adjusted to obtain optimal images at the onset of the experiment and were maintained constant throughout the duration of the study. Echocardiographic images were recorded on a 0.5-in. (1.27 cm) VHS videotape recorder for subsequent playback and analysis.

Echocardiographic contrast agent. Sonicated iopamidol (Iopamiro 370, Bracco, Italy) was used as the echocardio-

Figure 2. Two-dimensional echocardiographic image of left ventricular short-axis. The perfusion territory of the left circumflex coronary artery, enhanced by the contrast effect, is included between the 4 o'clock and 10 o'clock positions. LV = left ventricle; RV = right ventricle.

graphic contrast agent in all experiments. Sonication was performed according to a well established protocol (21,22). The microbubbles produced by this procedure are reported to have a mean diameter of 5.5 ± 3 μm (23). One milliliter of the contrast agent was hand-injected as a bolus immediately above the mixing chamber. If the myocardial echo contrast effect appeared inadequate to visual examination, the dose of the contrast agent was doubled.

Experimental protocol. A wide range of coronary blood flow rates was tested in each animal by adjusting the pump rate. At each flow level, the radiolabeled microspheres (and subsequently the echocardiographic contrast agent) were injected at the same injection point proximal to the mixing chamber. Because echocardiographic contrast agents may transiently alter the physiology of the coronary circulation (24), the microspheres were always injected before the contrast agent. To test injection to injection variability, two contrast injections, separated by >5 min, were performed at each flow rate. To minimize heart motion, the respirator was turned off immediately before injection of the contrast agent.

Myocardial blood flow measurement. Absolute flow values of the cannulated left circumflex artery were obtained by the calibrated perfusion pump. In addition, beat by beat blood flow was monitored by an external electromagnetic flow probe. To normalize absolute flow values for the mass of perfused tissue, approximately 1×10^5 radioactive microspheres (15-μm diameter) were injected into the circumflex artery at three to six different flow rates. The microspheres used were labeled with [153]gadolinum, [113]tin, [103]rubidium, [56]cobalt, [51]chromium, [46]scandium (Du Pont, New England

Nuclear). At the end of the experiment, the animals were killed by intravenous potassium chloride, the heart was removed and Evans blue was injected into the circumflex coronary artery to visualize the perfused territory. The heart was then fixed in 10% formalin. The stained myocardium was removed and cut into five to six transverse sections (from the base to the apex). Each slice was then divided into wedges and each wedge was cut into an inner, a middle and an outer layer. The rest of the heart was also divided into small pieces. Each piece was weighed and then counted. The radioactivity was analyzed with a two-channel gamma counter (LKB Ultrogamma, model 1280). The sample weights and counts were transferred to a computer (Hewlett-Packard 1000) to obtain counts per gram for each piece. Flow per gram was then calculated according to the formula $f = F \times c/ D$, where F is the pump flow, c is the counts per gram in the central portion of the stained area and D is the total counts for the whole heart, that is, the dose of injected microspheres (25).

Image analysis. Echocardiographic images were digitized off-line by using an array proce or-based system for medical image processing (Mipron). A 256×256-pixel matrix with 256 gray levels was used. Images were digitized at 25 frames/s and stored in random access memory. A total of 256 frames could be stored in the computer at one time. End-diastolic images were identified on the basis of the largest left ventricular cavity size and were stored on disk. Digitization included at least four beats preceding visual myocardial contrast appearance and lasted for 20 s. To verify the accuracy of data acquisition, the sequences of end-diastolic images corresponding to each contrast injection were reviewed in cine loop format, and questionable images were again digitized. The endocardial and epicardial edges of each image were delineated by the operator and the center of gravity of the left ventricular cavity was automatically identified. Starting from this point, the myocardium was automatically divided into 16 sectors of equal angle (Fig. 3). In each of these sectors, the summated videodensity (in gray levels) and the area (in square pixels) were measured to obtain the mean videodensity (in gray levels/pixel). These values were transferred to a personal computer (Macintosh SE, Apple) for subsequent analysis (26).

Analysis of curves. Although videodensitometric analysis was performed in all sectors of the left ventricular short axis, only data corresponding to the central portion of the left circumflex perfusion territory were sampled. In the echographic projection used (corresponding to a parasternal short-axis view), these sectors were posteriorly located (sectors 4 and 5 of Fig. 3). This sampling avoided attenuation artifacts, which are commonly present in lateral regions (27). A mean videodensity value of the area, corresponding to the sum of sectors 4 and 5, was obtained for each image and these values were plotted against the corresponding times to generate myocardial time-intensity curves (Fig 4). These curves exhibited a rapid ascending phase, a peak and a slower descending phase. Both background and peak con-
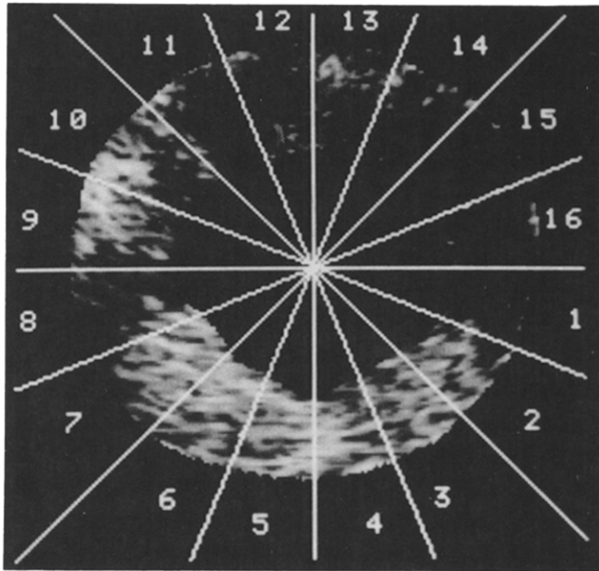
Figure 3. Computerized analysis of the echocardiographic image shown in Figure 2. The ventricular myocardium is automatically divided into 16 sectors of equal angle.

$$y = Ate^{(-\alpha t)}$$

Figure 5. Myocardial time-intensity curve fitted by a gamma variate function. A = scaling factor; t = time; e = base of natural logarithms; α = measure of curve width.

trast intensity of each curve were measured. Visual examination showed that the initial portion of the washout phase was linear. The observation of a rectilinear decay phase when data were plotted on a linear scale was interpreted as indirect evidence of the logarithmic compression that exists in commercial sector scanners (28). The relation between videodensity and time was tested in the linear portion of the washout phase of the curves by using least squares linear regression analysis. The washout slope was then derived from the regression equation. Each curve was fitted by a gamma variate function according to the equation $y = Ate^{-\alpha t}$, where A is a scaling factor, t is time, e is the base of natural logarithms and α is the measure of curve width (Fig.

5) (29). Peak contrast intensity, initial washout slope and the alpha coefficient of curves were then compared with coronary blood flow rates.

Statistical analysis. Least squares linear regression analysis was used to test the relation between videodensity and time in the washout phase of curves, to study the correlation between the variables derived from curve analysis and coronary blood flow or pressure and to evaluate injection to injection variability. Multiple linear regression analysis was used to evaluate the relative effect of coronary blood flow and perfusion pressure on myocardial echo contrast washout.

Results

Hemodynamic conditions. Data were collected at 25 different levels of coronary blood flow, representing 3 to 6 levels in each dog. A wide range of coronary flows rates was examined: from 0.5 ml/min per g up to 12.5 ml/min per g of myocardium. A wide range of coronary blood pressures was also explored: from 35 to 153 mm Hg in systole and from 23 to 114 mm Hg in diastole. With high flow rates, coronary pressure was equal to or slightly greater than aortic pressure; conversely, with low flow rates, coronary pressure decreased below aortic pressure. Thus, the gradient between aortic and coronary pressure ranged from +39 to −68 mm Hg.

Feasibility. Because two injections of contrast agent were performed at each of the 25 flow rates, a total of 50 contrast injections were given. Two contrast injections were not suitable for analysis owing to either the poor quality of the echocardiographic images or inadequate contrast effect;

Figure 4. Myocardial contrast echo time-intensity curve. Both intensity and time are displayed in a linear scale. The initial descending phase of contrast disappearance (washout) appears to be rectilinear.
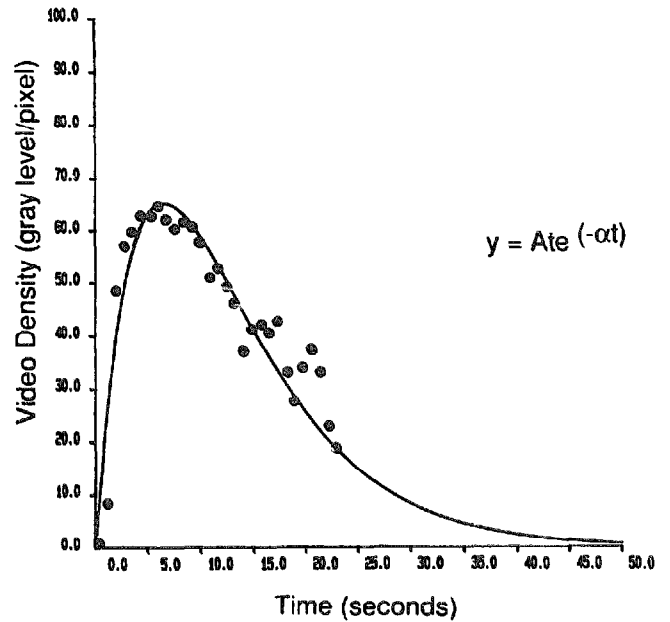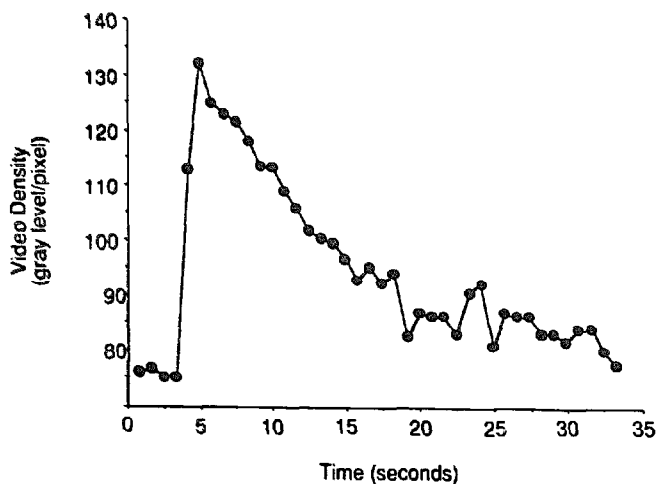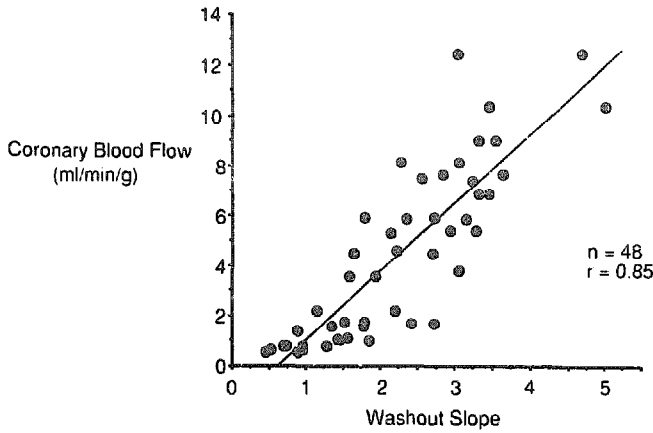
Figure 6. Correlation between the slope of initial myocardial washout and coronary blood flow.
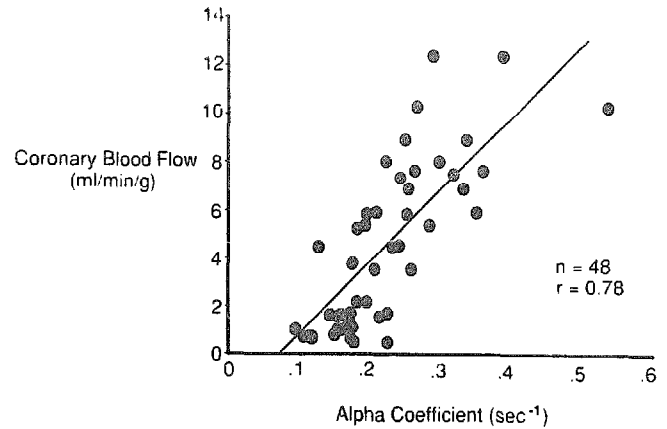

Figure 7. Correlation between the alpha variable of myocardial time-intensity curves, fitted by a gamma variate function, and coronary blood flow.

data from 48 injections were therefore analyzed. The variables of peak contrast effect, slope of the initial washout phase and alpha coefficient of myocardial time-intensity curves were derived from each of these 48 curves.

Effect of different coronary blood flow rates. The peak contrast effect (mean $\pm$ SD 49 $\pm$ 13 gray levels/pixel [range 25 to 80]) did not show any significant correlation with coronary blood flow per gram of tissue (r = 0.13). The slope of initial myocardial echo contrast washout (2.24 $\pm$ 1.06, range 0.46 to 5.00 gray levels/pixel per s) was higher with higher flow rates, thus showing a direct relation with coronary flow. The correlation of washout slope and coronary flow was good (r = 0.85 for the total group [Fig. 6] and r = 0.78 to 0.96 in individual dogs [Table 1]). The correlation with coronary blood flow per gram of tissue was not improved by fitting the time-intensity curves with a gamma variate function. In fact the alpha coefficient derived from this analysis (mean 0.24 $\pm$ 0.09 s^{-1} [range 0.09 to 0.58]) showed a correlation with coronary blood flow of r = 0.78 for the total group (Fig. 7), and r = 0.61 to 0.86 in individual dogs (Table 1).

Effect of different coronary blood pressures. The slope of the initial echo contrast washout also showed a good correlation with coronary diastolic pressure (r = 0.80) (Fig. 8). To evaluate the relative weight of coronary blood flow and perfusion pressure in influencing myocardial washout of sonicated iopamidol, these measures were compared with washout slope by multiple linear regression analysis. Both variables showed a significant correlation with the slope of initial myocardial washout. However, coronary blood flow

was much greater (partial F = 26.5, p < 0.001) than that of diastolic coronary blood pressure (partial F = 5.9, p < 0.05), in influencing myocardial washout.

Variability of the measurements. To evaluate the injection to injection variability of the measurements obtained in this study, the difference in values from two consecutive injections was computed. We found a mean difference of 9% for peak contrast intensity, 24% for washout slope and 25% for the alpha coefficient. To minimize the impact of this variability, the measurements from the two injections were averaged, and this value was then correlated with coronary blood flow. The results of this comparison yielded a superior correlation between flow and both washout slope (r = 0.91) and alpha coefficient (r = 0.90). Finally, with the exclusion of the very high nonphysiologic rates of coronary blood flow (>6 ml/min per g of myocardium) from the data analysis, the correlation of myocardial echo contrast washout with coronary blood flow still remained significant, (r = 0.74 for contrast injections and 0.82 for the average of two paired injections).

Discussion

Correlation of myocardial echo contrast washout and coronary blood flow. This study shows that variables derived from myocardial contrast echocardiography are related to coronary blood flow. Specifically, initial myocardial echo contrast washout appears to be related to coronary flow. These results are in agreement with indicator-dilution prin-

Table 1. Correlations Between Contrast Echographic Variables and Coronary Blood Flow in the Six Study Dogs

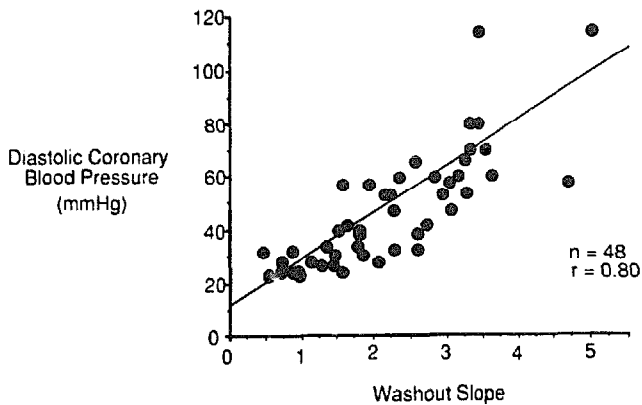| Correlation Coefficient	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5	Dog 6	Total Group
Peak contrast intensity	0.69	0.80	0.71	0.19	0.44	0.39	0.13
Washout slope	0.92	0.97	0.86	0.79	0.78	0.93	0.85
Alpha coefficient	0.87	0.72	0.85	0.79	0.77	0.61	0.78

Figure 8. Correlation between the slope of initial myocardial washout and coronary blood pressure.

ciples (30–33) and with previous studies on coronary blood flow based on the myocardial washout of radioactive tracers (34). They are also consistent with previous contrast echo studies, in which a prolonged myocardial washout was shown in the presence of either coronary underperfusion or severe coronary stenoses (5–8).

The correlation between myocardial echo contrast washout and coronary blood flow was closer in this study than in previous studies (6,8). A major factor in this improved accuracy might be the experimental model, in which continuous intracoronary infusion of adenosine permitted study of a wide range of coronary blood flow rates. Adenosine infusion might also have minimized changes in intramyocardial blood volume, thus favoring more accurate flow quantitation (35). In addition to a wider range of coronary flow rates than those in previous studies, more contrast injections were performed. Finally, the improved technology during the last several years enhanced the accuracy of both data acquisition and analysis.

In a previous study, the alpha coefficient, derived from the gamma variate fitting of curves, has been shown to have a correlation with coronary blood flow closer than that obtained by myocardial echo contrast washout (8). A good correlation of the alpha coefficient with coronary blood flow has been confirmed in this study; however, the correlation was not superior to that with washout slope, probably owing to different electronic signal processing implemented in the different scanners. To display a wide range of echo contrast effect intensities, the electronic signal is logarithmically amplified in current echo cardiographic equipment. Indirect evidence of this operation is shown in Figure 4, where the slope of the initial contrast washout appears to be rectilinear in a linear scale. According to the indicator-dilution theory, this downslope should be rectilinear in a semi-logarithmic scale, suggesting that the logarithmic compression has already occurred inside the instrument. A logarithmically compressed curve is probably not the ideal candidate for gamma variate fitting. In addition, logarithmic compression also varies with the gain setting controls.

As recently demonstrated, blood pressure influences the intensity of the echo contrast effect (17,18). The intensity of the reflected ultrasound signal is exponentially related to the diameter of microbubbles (19), which is reduced by increasing pressure. With these considerations in mind, a parallel goal of our study was to assess whether coronary perfusion pressure could be a source of error in measuring flow, through influence on myocardial echo contrast washout. We found a good correlation between initial contrast washout and coronary blood pressure. However, this correlation can be largely attributed to the experimental model, in which changes in flow are paralleled by changes in pressure. By multiple linear regression analysis, coronary blood flow appeared to more powerful than perfusion pressure in influencing myocardial echo contrast washout.

Limitations of the study. Some limitations of the study should be mentioned. The first relates to the experimental model of continuous coronary vasodilation. Because myocardial washout rate of the contrast agent could be influenced by changes in intramyocardial blood volume, all contrast injections were performed after abolishing coronary autoregulation by intracoronary adenosine infusion. Data collected in this model, however, cannot be directly applied to different situations. Under physiologic conditions, in the presence of coronary autoregulation, this approach might be challenged by changes in intramyocardial blood volume, secondary to changes in arteriolar tone. Additionally, even a continuous adenosine infusion does not ensure constant intramyocardial blood volume, because different degrees of perfusion pressure and intraventricular pressure can influence vascular capacitance (36–38).

The significance of myocardial echo contrast washout is still unknown. It is unclear whether the decrease in myocardial echo contrast intensity only reflects the passage of microbubbles through the coronary circulation or is also influenced by their decay. A reduction in the diameter of bubbles on the arterial side might reduce myocardial signal intensity as the bubbles pass through the microcirculation. Small albumin-encapsulated bubbles were shown to cross the microcirculation (39). However, it is unlikely that larger bubbles, such as the ones used in this study, can freely cross the microcirculation without a modification in diameter.

In this study, the principles of the indicator-dilution theory were applied to contrast echocardiography. It should be remembered that sonicated iopamidol cannot be considered an intravascular free-passing tracer (40), as indirectly demonstrated by the prolonged myocardial transit times shown in Figure 4. Furthermore, the indicator-dilution theory requires a linear correlation between tracer concentration and signal intensity that cannot be achieved with current sector scanners (28), in which the received ultrasound signal is heavily distorted at various stages of signal processing and display (27). Owing to these limitations, myocardial echo contrast washout does not provide an exact flow measurement; rather, it is a variable related to coronary blood flow.

The area of myocardium perfused by any given vessel

varies according to functional factors such as coronary perfusion pressure and resistance (41). Thus, the postmortem assessment of a perfusion territory by dye injection differs from that obtained in vivo. For this reason, in the present study, staining of the left circumflex coronary artery with Evans blue only served as an anatomic reference for specimen preparation and well counting. The perfused territory was traced in vivo by variously labeled radioactive microspheres and varied in size in each animal with variable rates of coronary blood flow from 1% to 13%.

To measure specific coronary blood flow (in ml/min per g of myocardium), the entire perfusion territory of the left circumflex coronary artery was sampled by radiolabeled microspheres. Conversely, to assess coronary blood flow by contrast echocardiography, only a portion of the circumflex artery perfusion territory was analyzed and, because of the tomographic nature of two-dimensional echocardiography, only a cross-sectional image of the left ventricle was analyzed. Additionally, only a region of this tomographic image, in the center of the area of contrast effect was sampled. This portion of the left artery perfusion territory was assumed to be representative of the average flow to the entire area. This assumption may not be true at very low rates of coronary blood flow; with severe ischemia, myocardial perfusion may become inhomogeneous, both transmurally and horizontally.

Finally, the analysis of curves in this study was somewhat arbitrary with regard to identification of the rectilinear washout phase. Considerable injection to injection variability was also observed, which is in agreement with reported findings (42). This low reproducibility can be partly attributed to the use of manual injections and could be overcome by using standardized power injectors able to generate a constant input function. This limitation can also be partly overcome by averaging data relative to several contrast injections, as is currently done when measuring cardiac output by thermodilution (43). The limited accuracy of contrast echocardiography in the quantitation of coronary blood flow is evidenced by the observed variability in the computed flow values of 1 to 1.5 ml/min per g; washout slope ranged between 0.87 and 1.85 gray levels/pixel per s and the alpha coefficient between 0.099 and 0.188 s^{-1}.

Clinical implications. Myocardial contrast echocardiography currently provides information relating to the spatial distribution of myocardial perfusion. In particular, it permits evaluation of the presence and extent of collateral circulation (13,14), the presence of viable myocardium (44) and the efficacy of angioplasty procedures (10) and coronary bypass grafts (15,16). This study in dogs shows the possibility of using the technique of contrast echocardiography to derive quantitative information on coronary blood flow. This method requires direct injection of contrast medium into the coronary circulation and provides only an assessment (not a measure) of coronary flow. However, it is proposed as a complement to routine coronary arteriography and the contrast agent it uses is already being used in humans (7). In the

cardiac catheterization laboratory and in the operating room, this method might be useful in assessing the physiologic significance of coronary stenosis and in evaluating the efficacy of coronary reperfusion procedures.

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