Quantification of Myocardial Perfusion With Myocardial Contrast Echocardiography During Left Atrial Injection of Contrast

Implications for Venous Injection

Danny M. Skyba, MS; Ananda R. Jayaweera, PhD; Norman C. Goodman, BS; Suad Ismail, MD; Gustavo Camarano, MD; Sanjiv Kaul, MD

Background The purpose of this study was to determine whether myocardial perfusion can be quantified with myocardial contrast echocardiography using left atrial (LA) injection of contrast.

Methods and Results Based on a series of in vitro and in vivo experiments, the optimal dose of sonicated albumin microbubbles injected into the LA for establishing a linear relation between video intensity and blood volume in the anterior myocardium was determined. In 10 open-chest dogs, myocardial blood flow (MBF) was augmented by increasing myocardial blood volume (MBV) with an intravenous infusion of phenylephrine HCl. In the presence of this drug, left anterior descending artery stenosis was produced, followed by release of stenosis, to change MBF within the anterior myocardium. MBV was calculated by dividing radiolabeled microsphere-derived MBF by microbubble transit rate. There was close coupling between MBF and MBV in the anterior myocardium during LA injection of contrast (\(y=1.0x-0.03, \ SEE=1.07, \ r=.92, P<.001\). An excellent correlation was also noted between background-subtracted peak video intensity and MBV (\(y=0.24x+0.73, \ SEE=0.36, \ r=.88, P<.001\). On multivariate analysis, background-subtracted peak video intensity correlated best with MBV.

Conclusions Myocardial perfusion can be quantified from time-intensity curves derived from the anterior myocardium after LA injection of contrast. Background-subtracted peak video intensity in this situation correlates closely with MBV. When MBV and MBF are closely coupled, such as during inotropic stimulation of the heart, background-subtracted peak video intensity also correlates closely with MBF. Since there are similarities in the models of LA and venous injections, these data indicate that it may be feasible to quantify myocardial perfusion with myocardial contrast echocardiography after venous injection of contrast. (*Circulation. 1994;90:1513-1521.)*

Key Words • echocardiography • perfusion • contrast media

The quantification of myocardial perfusion remains a significant clinical problem. Myocardial contrast echocardiography (MCE) is a new technique with the potential of assessing myocardial perfusion from a venous injection of contrast.\(^1,2\) The opacification noted in the myocardium from venous injection with currently available contrast agents, however, is subtle, with a low signal-to-noise ratio.\(^1,2\) Since left atrial (LA) injection of contrast produces better myocardial opacification than right heart injection,\(^1,2\) and since there are similarities in the models of LA and venous injections, the purpose of this study was to determine whether myocardial perfusion could be quantified during LA injection of contrast.

We have previously demonstrated that when blood flow through a coronary artery is selectively altered without a concomitant change in myocardial blood volume (MBV), the transit rate of microbubbles on MCE correlates with regional myocardial blood flow (MBF).\(^3,4\) The model in which contrast is injected into the LA, however, differs substantially from that of the cannulated coronary artery, as depicted in Fig 1. Given that the concentration and rate of injection of microbubbles is constant during each LA injection, then the concentration of bubbles per milliliter of blood entering the aorta will be the same in any given dog so long as the volume of the left heart remains constant. In this situation, the number of microbubbles entering the coronary circulation will be determined by the fraction of cardiac output, which represents coronary blood flow. During hyperemia, this fraction increases, resulting in more microbubbles entering the myocardium. Thus, at any given time, the number of microbubbles within a cross section of the myocardium will be more during hyperemia than during baseline and will relate primarily to MBV.

To determine the relation between microbubble concentration and video intensity, we performed a series of in vitro and in vivo experiments. We used these data to determine the optimal dose of contrast needed for LA injection to obtain a linear relation between video intensity and microbubble concentration within the myocardium. Our aim was to correlate MBV with background-subtracted myocardial peak video intensity during LA injection of contrast in a beating canine heart in which MBF was altered by inducing changes in MBV.\(^5\) We achieved the latter through the administration of phenylephrine HCl.
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Animal

descending artery; adult vascular dilatation 4568C, Hewlett-Packard) catheter in this samples artery femoral as experiment Additional anesthesia ratus). Heart Association of Virginia conformed to the American Heart Association Guidelines for Use of Animals in Research. Ten adult mongrel dogs were anesthetized with 30 mg/kg sodium pentobarbital (Abbott Laboratories), intubated, and ventilated with a respirator pump (model 607, Harvard Apparatus). Additional anesthesia was administered during the experiment as needed. A 7F catheter was placed in the right femoral artery for withdrawal of arterial reference blood samples for radiolabeled microsphere blood flow analysis. This catheter was also connected to a multichannel recorder (model 4568C, Hewlett-Packard) via a fluid-filled transducer (model 1280C, Hewlett-Packard) to measure arterial pressure. An-

other 7F catheter was placed in the left femoral vein for the intravenous administration of fluids and drugs as needed.

A left lateral thoracotomy was performed, and the heart was suspended in a pericardial cradle. The left anterior descending coronary artery (LAD) was dissected free from the surrounding tissues, and a custom-designed reversible snare was placed loosely around it. A 2-mm Doppler flow probe (series SB, Transonic) was placed on the LAD proximal to the snare and was connected to a digital flow meter (model T206, Transonic). A 7F catheter was placed in the LA for injection of microbubbles and radiolabeled microspheres. A flotation catheter (Baxter-Edwards Laboratory) was placed in the pulmonary artery and was connected to a computer (model 9520A, Edwards Laboratories) for the measurement of thermodilution cardiac output.

Contrast Echocardiography

Both the in vitro and in vivo experiments were performed with a phased-array 2DE system (RTS000, General Electric Medical Systems) with a 5-MHz transducer. Gain settings were optimized initially and were held constant throughout the experiment. A maximal dynamic range of 72 dB was used. For the in vivo experiments, a saline bath served as an acoustic interface between the heart and the transducer, and imaging was performed at the mid–papillary muscle short-axis level. Data were recorded on 1.25-cm S-VHS videotape with a high-fidelity video recorder (Panasonic AG-7355, Matsushita Electrical Co).

For the in vitro studies, we used 17 doses of Albunex ranging from 0.001 to 1.0 mL. For the in vivo studies, we injected four doses of Albunex (0.5, 1.0, 2.0, and 4.0 mL) into the LA at each stage as a bolus (over 1 second) with a power injector (model 3000, Liebel-Flarsheim) and a saline flush so that the total volume of injection was 5 mL.

All images were analyzed with an off-line computer (Mi- pron, Kontron Electronics) as previously described7 and were transferred from videotape to image memory of the computer in a 244x244x8-bit format. For the in vitro experiments, a large region of interest (at least 3000 pixels) was defined over the image, carefully avoiding the edges of the beaker. The average pixel intensity within this region was calculated at 5 seconds after each injection to allow adequate mixing of the microbubbles with the saline in the beaker. The concentration of microbubbles was plotted against background-subtracted peak video intensity, and the plot was then fit to an exponential function: y = A(1-e^-m).

For the in vivo experiments, consecutive end-diastolic frames, encompassing the period from just before contrast injection until 10 seconds thereafter, were selected and aligned by computer cross-correlation as previously described by us.7,8 Fig 2 depicts examples of precontrast and postcontrast images. Note opacification in the anterior wall after injection of contrast into the LA along with posterior wall attenuation due

Methods

In Vitro Experiments

Various quantities of sonicated albumin microbubbles with a mean concentration of 0.5 billion/mL and mean size of 4.3 μm (Albunex, Molecular Biosystems Inc) were mixed with 4 L of 0.9% saline solution in a glass beaker. A magnetic stirrer at the bottom of the beaker ensured thorough mixing of the solution. The transducer was held in a fixed position in the center of the beaker, 2 cm into the solution, to minimize artifacts from the edges of the beaker and from the stirrer. Two-dimensional echocardiographic (2DE) images were acquired for 10 seconds beginning at the time of injection.

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 Use of Animals in Research. Ten adult mongrel dogs were anesthetized with 30 mg/kg sodium pentobarbital (Abbott Laboratories), intubated, and ventilated with a respirator pump (model 607, Harvard Apparatus). Additional anesthesia was administered during the experiment as needed. A 7F catheter was placed in the right femoral artery for withdrawal of arterial reference blood samples for radiolabeled microsphere blood flow analysis. This catheter was also connected to a multichannel recorder (model 4568C, Hewlett-Packard) via a fluid-filled transducer (model 1280C, Hewlett-Packard) to measure arterial pressure. An-

```plaintext
# vessels open n 2n
myocardial blood volume v 2v
background-subtracted video intensity i 2i
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Fig 1. Model pertaining to quantification of myocardial perfusion from a venous or left atrial injection of contrast when myocardial blood flow is changed by altering myocardial blood volume. See text for details. Ao indicates aorta; LAD, left anterior descending artery; LV, left ventricle; and LA, left atrium. Although in this diagram myocardial blood volume is shown to increase because of recruitment of vessels, it can also increase by vascular dilatation or both.

Fig 2. Precontrast and postcontrast images depicting opacification of the anterior wall (top arrow) with intense enhancement of the left ventricular (LV) cavity (middle arrow) and attenuation of the posterior wall (bottom arrow) from the presence of contrast in the LV cavity.
to the presence of contrast in the left ventricular cavity. A transmural region of interest was then defined over the LAD bed. An attempt was made to include as much of the anterior myocardium as possible without including areas that were subject to attenuation from the presence of contrast in the left ventricular cavity. Care was also taken not to include the specular epicardial and endocardial borders. The region of interest was selected during LAD stenosis, since at this stage the LAD bed was likely to be the thinnest, and the same region was then placed over the LAD bed in all preceding and subsequent stages (Fig 3).

Time-intensity plots were generated from the region of interest by use of all end-diastolic aligned images, and background (average video intensity in the region of interest from before injection of contrast) was subtracted from all data in the time-intensity plot. A gamma-variate function, \( y = A e^{-\beta t} \), was then fit to the background-subtracted time-intensity plots, where \( A \) is a scaling factor, \( t \) is time, and \( \beta \) is an indicator of the transit rate of the tracer. The conventional definition of mean transit rate is the reciprocal of the average time it takes for an indicator to travel through a volume of distribution. Although it is not possible to directly measure the mean transit rate of a tracer in the in vivo beating heart, it is possible to determine its spread as it transits through the myocardium. A measure of this spread is the mean transit time calculated from the centroid of the time-intensity plot. The reciprocal of the mean transit time is the mean transit rate. Mean transit rate has a special meaning with reference to a two-compartment model, such as the one used in this experiment: when an indicator is injected as a bolus into the first compartment, its concentration in the second compartment varies according to a gamma-variate function, \( A e^{-\beta t} \), where \( \beta \) equals flow/volume of each compartment. It can be shown that the mean transit rate through both compartments, which is the reciprocal of the centroid of a gamma-variate function, equals \( \alpha/2 \). It is with respect to this definition that we chose to use \( \alpha \) as an indicator of microbubble transit rate in this study. Peak video intensity was derived by use of the equation \( A/\alpha \).

MBF Measurement

Approximately \( 2 \times 10^4 \) 11-µm radiolabeled microspheres (DuPont Medical Products), suspended in 4 mL of 0.9% saline solution/0.01% Tween 80, were injected into the LA at each stage. Reference samples were withdrawn from the femoral artery over a period of 130 seconds with a constant-rate withdrawal pump (model 944, Harvard Apparatus). The short-axis slice of the left ventricle corresponding to the MCE image was cut into 16 wedge-shaped pieces, and each piece was divided into endocardial, midwall, and epicardial portions. The papillary muscles were not included in the endocardial portions. The tissue samples and the arterial reference samples were counted in a well counter with a multichannel analyzer (model 1282, LKB Wallace). Corrections were made for activity spilling from one window to the next with a custom-designed computer program.

Flow to each sample was calculated by the equation \( Q_m = (C_m TQ_m)/C_e \), where \( Q_m \) is myocardial flow (mL/min), \( C_m \) is tissue counts, \( Q \) is rate of arterial sample withdrawal (mL/min), and \( C_e \) is counts in the arterial reference sample. Transmural MBF (mL·min⁻¹·g⁻¹) was derived by dividing the sum of flows to the individual segments by their combined weight. MBF to the LAD bed at each stage was calculated by averaging transmural flows in the segments that demonstrated reduced flows during LAD stenosis.

Estimation of MBV

Since in a two-compartment model, flow = volume × transit rate, MBV was calculated by dividing MBF derived by use of radiolabeled microspheres by the myocardial microbubble transit rate derived by use of MCE. These values of blood volume were then normalized to baseline values such that all values were expressed as fractions of the baseline values.

Protocol

Each dog was studied at baseline and during coronary hyperemia induced by an intravenous infusion of 0.4 to 0.8 mg·kg⁻¹·min⁻¹ ofephedrine HCl (Schein Pharmaceuticals). Coronary hyperemia under these circumstances results from an increase in MBV. A stenosis was created on the LAD with the reversible snare, whereby LAD flow was reduced by approximately one half under the continuous influence ofphenylephrine HCl. The degree of stenosis was monitored with the Doppler flow probe. The stenosis was then reversed under the continuous influence ofphenylephrine HCl, the dose of which was not adjusted to compensate for tachyphylaxis, a phenomenon noted after about 1 hour following the initiation of the infusion. At each stage, by random order, radiolabeled microspheres and microbubbles were injected into the LA. The dog was then killed, the heart was excised, and the slice corresponding to the image on MCE was processed to determine radiolabeled microsphere–derived MBF.

Statistical Methods

All data were analyzed with RS/1 (Bolt, Beranek, and Newman) resident on a minicomputer (VAX 4000, Digital Equipment Corp) and were expressed as mean±SD. Comparisons between MCE and other measurements were made by linear regression analysis. Statistical significance was defined as \( P < .05 \) (two-sided).

Results

Relation Between Microbubble Concentration and Video Intensity

Fig 4 illustrates the relation between microbubble concentration and video intensity by use of the RTS5000 system at 5 seconds after each dose of microbubbles was
introduced into the beaker during the in vitro experiments. As expected, the relation is exponential, with a linear relation noted at lower concentrations (see inset). A unique feature of this 2DE system compared with other systems is that in its linear range, for a given increase in the concentration of microbubbles, the increase in video intensity is severalfold greater.

In the in vivo experiments, when the dose of contrast injected into the LA is plotted versus peak video intensity obtained from the LAD bed at baseline, an exponential trend similar to that seen in the in vitro experiment is noted (Fig 5). Up to the 2-mL dose, the relation is linear (despite log compression of the video data), and beyond that it becomes flat. Similarly, Fig 6 indicates that when the dose of contrast injected into the LA is plotted against microbubble transit rate through the LAD bed, the transit rate decreases as the dose increases and reaches a plateau at 2 mL.

The transit rate of a tracer should be independent of the dose injected, provided that the detector operates over a linear range. The results from our in vivo experiments indicate that because of a threshold effect of the 2DE system, the time-intensity plots are truncated at lower doses (Fig 7), making the curves narrower and hence resulting in artificial increases in transit rates. The effect of thresholding decreases as the dose of microbubbles increases, and as Fig 6 indicates, it is minimal at more than 2 mL.

**Relation Between MBV and Video Intensity**

On the basis of the in vitro and in vivo experiments, we determined that the optimal dose for establishing a linear relation between peak myocardial video intensity and MBV from an LA injection is 2 mL of Albunex. This dose also provides maximal signal-to-noise ratio

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**Concentration of bubbles (thousands/ml)**

![Graph showing relation between microbubble concentration and video intensity.](image)

**Fig 4.** Curve showing relation between microbubble concentration and video intensity at 5 seconds after microbubbles were introduced into the beaker during the in vitro experiments. This is a smoothed curve obtained with a moving average filter from 17 data points. The relation is exponential, with a linear range occurring at lower concentrations (inset). The slope of the linear portion of the curve is such that for a given increase in the concentration of microbubbles, the increase in video intensity is severalfold greater. See text for details.

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**Fig 5.** Plot showing relation between amount of Albunex injected (in mL) into the left atrium and background-subtracted peak video intensity obtained from the anterior myocardium in all dogs. The data are depicted as boxplots: in each boxplot, the horizontal line denotes the median; the upper and lower margins of the box denote the upper and lower quartiles (levels containing 25% of the data above and below the median); and the tails depict the upper and lower ranges of the data, respectively. As in the in vitro experiments, the relation is exponential, with "flattening" occurring over the 2-mL dose. See text for details.

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**Fig 6.** Plot showing relation between amount of Albunex injected (in mL) into the left atrium and microbubble transit rate obtained from the anterior myocardium in all dogs. As in Fig 5, the data are depicted as boxplots. The transit rate decreases exponentially, with "flattening" occurring when the 2-mL dose is exceeded. See text for details.
without producing significant attenuation within the anterior myocardium. The absence of attenuation in the anterior myocardium was noted by visual inspection of both the 2DE data and the time-intensity plots. Even if attenuation is not seen on the 2DE data, it can be determined from the time-intensity plots, which appear flattened and widened.\textsuperscript{13} We were able to obtain adequate time-intensity plots in 36 of the 40 stages in the in vivo experiments; in two, artifacts occurred from entrapment of air under the saline bath, and in two, the images could not be aligned properly.

The Table depicts the mean MBF and MBV in the LAD bed at different stages. MBF in the LAD bed increased more than threefold during phenylephrine HCl-induced hyperemia. In the presence of this drug, when a stenosis was placed on the LAD, MBF decreased by approximately one half, and after the release of the stenosis, MBF rebounded to levels below those achieved before the stenosis was placed. Similarly, the estimated relative MBV of the LAD bed showed a 3.5-fold increase during phenylephrine HCl-induced hyperemia and an approximately twofold increase during the stenosis. It rebounded to prestenosis levels during reflow.

The Table also lists arterial pressures and cardiac outputs at the different stages. It is evident that after release of the LAD stenosis, the mean arterial pressure was lower compared with the other stages during the infusion of phenylephrine HCl. Similarly, since afterload increases in the presence of phenylephrine HCl, cardiac output was lower during its infusion compared with baseline.

Fig 8 illustrates the relation between normalized MBF measured with radiolabeled microspheres and normalized MBV in the LAD bed in all 10 dogs. It is apparent that there is close correlation between MBF and MBV, with a slope that approximates the line of identity. These results demonstrate that an increase in MBV by a certain factor was paralleled by a similar increase in MBF.

At higher flows, microbubbles transit the myocardium faster and, since only end-diastolic frames are analyzed to measure transit rates, only a few frames exhibit myocardial opacification. We have previously demonstrated that as the number of frames analyzed decreases, the likelihood of error introduced on curve fitting increases.\textsuperscript{4} Since MBV in the LAD bed was calculated from microbubble transit rates through that bed, greater dispersion is noted at higher flows in Fig 8.

Fig 9 depicts time-intensity plots obtained from the LAD bed in one dog during four different LAD flows when 2 mL of Albunex was injected into the LA. It can be noted that the background-subtracted peak video intensity from the LAD bed corresponds well with derived MBV of the LAD bed at each stage. Fig 10

Myocardial Blood Flow, Blood Volume, and Hemodynamics During Various Stages of the Experiment

<table>
<thead>
<tr>
<th>Phenylephrine HCl Infusion</th>
<th>Baseline</th>
<th>Hyperemia</th>
<th>Stenosis</th>
<th>Reflow</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAD MBF*</td>
<td>1.1±0.6</td>
<td>3.4±1.4</td>
<td>1.7±0.9</td>
<td>2.6±2.1</td>
</tr>
<tr>
<td>LAD MBV\dagger</td>
<td>1.0±0.0</td>
<td>3.6±3.0</td>
<td>1.9±1.9</td>
<td>3.5±3.6</td>
</tr>
<tr>
<td>Mean AoP\dagger</td>
<td>89±33</td>
<td>99±53</td>
<td>97±40</td>
<td>82±38|$</td>
</tr>
<tr>
<td>CO$</td>
<td>2.4±1.5$</td>
<td>1.3±0.9</td>
<td>1.4±1.0</td>
<td>1.3±0.9</td>
</tr>
</tbody>
</table>

\*LAD indicates left anterior descending arterial bed; MBF, myocardial blood flow (mL·min\textsuperscript{-1}·g\textsuperscript{-1}); MBV, myocardial blood volume (estimated by dividing MBF by microbubble transit rate and normalized to that at baseline); AoP, aortic pressure (mm Hg); and CO, cardiac output (L/min).
\dagger All stages are significantly (P<.01) different from each other.
\dagger All stages are significantly (P<.01) different from hyperemia and reflow.
\\dagger All stages are significantly (P<.05) different from each other.
\$Baseline and stenosis are significantly (P<.01) different from each other.
FIG 9. Time-intensity plots (with gamma-variate fits) obtained from the anterior myocardium from one dog during four different left anterior descending artery flows when 2 mL of Albunex was injected into the left atrium. It is apparent that the background-subtracted peak video intensity corresponds well with regional myocardial blood volume (MBV) normalized to that at baseline. See text for details. Hyperemic indicates hyperemic.

illustrates the relation between background-subtracted normalized peak video intensity and estimated MBV obtained from the LAD bed in all dogs when 2 mL of Albunex was injected into the LA. The relation is linear over a wide range of MBV, with an excellent correlation \((r=0.88)\).

Fig 11 illustrates the relation between background-subtracted normalized peak video intensity and MBF from the LAD bed in all dogs when 2 mL of Albunex was injected into the LA. Although the relation is good \((r=0.75)\), more scatter is noted at higher LAD flows. Furthermore, the correlation is not as good as that between peak video intensity and MBV.

The correlation between MBF and microbubble transit rate \((\alpha)\) obtained from the LAD bed was poor \((r=0.10)\), as was the relation between microbubble transit rate \((\alpha)\) and peak video intensity \((r=0.35)\). The relations between peak video intensity and mean arterial pressure and cardiac output were also poor \((r=0.09\) and \(r=0.08\), respectively). When multivariate regression was performed using peak video intensity versus MBF, MBV, mean microbubble transit rate \((\alpha)\) through the LAD bed, mean arterial pressure, and cardiac output, the only significant correlate of peak video intensity was MBV of the LAD bed. When this variable was removed from the analysis, MBF to the LAD bed was the best correlate of peak video intensity.

**Discussion**

**Quantification of Myocardial Perfusion With MCE**

When microbubbles are injected as a bolus directly into a coronary artery and flow through it is selectively altered without a concomitant change in MBF, the mean transit rate of the microbubbles correlates with regional MBF.\(^3\)\(^4\) When contrast is injected into a peripheral vein, however, the bolus spreads as it passes through the right heart, lungs, and left heart. Furthermore, it is detected in the myocardium during only a small portion of the time while it is in the left ventricular cavity. This occurs because, whereas the number of bubbles within the left ventricular cavity is high enough to be detected (and even cause attenuation), the number in the myocardium is low, since only a small fraction of stroke volume constitutes MBF (Fig 12).\(^1\)\(^4\) This thresholding effect, therefore, is related principally to the paucity of bubbles

FIG 10. Graph showing relation between estimated myocardial blood volume obtained from the left anterior descending artery bed \((x\) axis) and background-subtracted normalized peak video intensity \((y\) axis) in all 10 dogs when 2 mL of Albunex was injected into the left atrium. The correlation is excellent and is linear over a wide range of blood volumes.

FIG 11. Graph showing relation between normalized myocardial blood flow to the left anterior descending (LAD) artery bed \((x\) axis) and background-subtracted normalized peak video intensity obtained from that bed \((y\) axis) in all 10 dogs when 2 mL of Albunex was injected into the left atrium. The correlation is not as good as that seen in Fig 10 because of more scatter noted at higher blood flows. See text for details.

FIG 12. Diagram depicting left ventricular and myocardial time-intensity plots after a venous injection of contrast. The \(y\) axis on the left indicates left ventricular cavity video intensity, and the \(y\) axis on the right denotes myocardial video intensity. Myocardial opacification is detected only during a small portion of the time when contrast is present in the left ventricular cavity and when the left ventricular contrast effect is maximum. Because of the paucity of bubbles per unit volume of the myocardium compared with unit volume of the left ventricular cavity, the degree and duration of myocardial opacification are significantly less compared with left ventricular cavity opacification. See text for details.
per unit volume of myocardial tissue compared with unit volume of blood in the left ventricular cavity, and the microbubbles are detected within the myocardium only when their concentration in the left ventricular cavity exceeds a certain value (Fig 12).

Unlike after a bolus injection directly into a coronary artery, the shape of the myocardial time-intensity plot after venous injection is influenced not only by MBF but also by the dispersion of the bolus as it passes through the cardiac chambers and pulmonary circulation and by the dilution of the bubbles during their transit through these structures. Furthermore, as depicted in Fig 12, since the input function (time-intensity plot from the left ventricular cavity) is longer than the output function (time-intensity plot from the myocardium), myocardial microbubble transit rate cannot be derived by deconvolving the input from the output function. Thus, the measurement of microbubble transit rate through the myocardium may not be a feasible approach for quantifying myocardial perfusion when microbubbles are injected into the peripheral vein or the right heart.

Another approach for the quantification of myocardial perfusion is the assessment of MBV rather than MBF. MBV denotes the volume of blood within the myocardial microvascularature (arterioles, capillaries, and veins) and under resting conditions constitutes approximately 6 to 15 mL/100 g of the left ventricular myocardium. Most current clinical techniques used to assess myocardial perfusion cannot measure MBV because the tracers used do not remain in the intravascular space but either are extracted by myocytes (agents used for single-photon

Finding of the Present Study

In the present study, we elected to increase MBV by augmenting myocardial oxygen consumption by use of phenylephrine HCl, a drug that principally increases afterload. We found a close coupling between MBV and MBF, with the slope of the equation approximating unity, which substantiated that the increase in MBF in our experiments was closely associated with an increase in MBV.

Before examining the relation between background-subtracted myocardial peak video intensity and MBV, we defined the relation between microbubble concentration and video intensity both in vitro and in vivo, for two major reasons. First, since we wanted to correlate peak video intensity with MBV, it was imperative to define the portion of the relation between microbubble concentration and video intensity, which was most linear. Second, since we planned to use microbubble transit rate to estimate MBV, we wanted to define the dose of microbubbles at which transit rates would not be affected by the "thresholding" effect of the 2DE system (Fig 7) and at which attenuation would not occur within the anterior myocardium. Wieneck and colleagues also observed this phenomenon and its effect on microbubble transit rate. From our experiments (Figs 4 through 6), we determined that 2 mL of Albunex injected into the LA would provide optimal results. At this dose, we found a linear relation between peak video intensity and MBV.

Since MBV was closely coupled to MBF in these experiments, as expected, we also found a linear relation between peak video intensity and MBF. It can be seen in the Table that after release of the LAD stenosis, while the MBV rebounded to that before placement of the LAD stenosis, MBF did not. At this stage, MBV was not influenced solely by an increase in myocardial oxygen demand induced by phenylephrine HCl but also by hyperemia induced after the release of endogenous adenosine after the period of ischemia. Consequently, maximal coronary vasodilatation had occurred, resulting in maximal MBV. However, since the endogenous release of adenosine, by causing maximal vasodilatation, also abolishes autoregulation, MBF was influenced by the coronary driving pressure.

The Table shows that aortic pressure was lower after the release of LAD stenosis compared with other stages during phenylephrine HCl infusion. It is probably for this reason that the relation between peak video intensity, which can be used as a measure of MBV, and MBF was not as good as that between background-subtracted peak video intensity and MBV. Since the purpose of stressing the heart is to test its ability to maximize coronary vascular reserve, an estimation of MBV may be superior to an estimation of MBF in a situation in which flow and volume may not be closely coupled, such as during postischemic hyperemia or during the infusion of adenosine or diprydamole.

An interesting observation in our study was the decrease in MBV during LAD stenosis. Even though the stenosis was not flow limiting at rest, it was placed in the presence of phenylephrine HCl–induced hyperemia. Consequently, if MBV is determined primarily by resistance vessels, one would expect the MBV distal to the bed to be similar with or without a stenosis. We can offer two possible explanations for the decrease in MBV after placement of the LAD stenosis. First, since MBF was reduced in the presence of the stenosis, fewer microbubbles entered the myocardium, resulting in less myocardial video intensity. In this situation, the thresholding effect of the 2DE system could have resulted in an artificial narrowing of the time-intensity curve and an increase in microbubble transit rate (Fig 7), which would have resulted in apparent decrease in MBV.

The other explanation is related to the microcirculation itself. During MCE, it is not only the resistance vessels, such as the arterioles, that are imaged but also the venules, which are much more compliant. It is therefore possible that the drop in distal pressure after placement of the stenosis resulted in partial collapse of the venules and an actual decrease in MBV. Although the linear relation between peak video intensity and calculated MBV even during LAD stenosis favors the
latter explanation, further studies are required to determine whether the decrease noted in MBV after placement of a stenosis is apparent or apparent.

**Critique of Our Methods**

Rather than directly measuring MBV, we derived it from MBF calculated from radiolabeled microspheres and microbubble transit rate calculated from MCE. Previous attempts to measure MBV in vivo have also used dye dilution techniques with derivation of transit rates of diffusible and nondiffusible tracers.15,16,22 Another approach could entail the derivation of MBV by the method of Wu et al.17 in which the density of contrast in a chamber of known volume, such as the aorta, is used to derive regional MBV in vivo. This approach, using ultrafast cine-computed x-ray tomography, is not possible with MCE simply because the injection of bubbles into the LA in numbers great enough to produce myocardial opacification results in attenuation of the LA, left ventricle, and aorta. An independent assessment of MBV by a method such as ultrafast cine-computed tomography would, nonetheless, be desirable.

In this study, we assumed that there is a linear relation between MBV and myocardial microbubble concentration. It is well known that the hematocrit within the microvasculature is lower than that in the large vessels15,51 and is inversely related to the size of the blood vessels. If the increase in MBV is due to recruitment of vessels identical in size to those already present, then the hematocrit effect should be negligible. On the other hand, if the recruitment is principally of vessels that are significantly different in size from those already present, then the hematocrit effect needs to be considered. Because the relation between MBV and MBF in our study approximated the line of identity (Fig 8), it is likely that the average size of the vessel recruited during phenylephrine HCl–induced hyperemia was similar to those that were already patent.

The radiolabeled microsphere method for the estimation of MBF is well validated and state of the art.11 Our method of analysis of 2DE data to estimate mean microbubble transit rate has also been validated and used extensively by us and others.3,4,32 We could have used an agent such as dobutamine or epinephrine instead of phenylephrine HCl to increase myocardial oxygen consumption and MBV, but these agents cause tachycardia and an exaggerated swinging motion of the heart. We could also have used a coronary vasodilator such as adenosine or dipyridamole to increase MBV, but as stated earlier, in that situation, since autoregulation is lost, MBF is also influenced by the coronary driving pressure and not by MBV alone.30 Since we could accurately measure regional MBF and since we had no independent means of quantifying MBV, we opted to use a tactic by which changes in MBV and MBF would be closely coupled.5

Although in individual dogs, we found a close relation between actual background-subtracted peak video intensity and estimated regional MBV, we found a good correlation between peak intensity and MBV in all dogs only after normalizing data for individual dogs. Since the peak myocardial video intensity in any dog was influenced not only by the MBV but by the left heart blood volumes as well, different volumes of the left heart in different dogs could have resulted in variable amounts of microbubble dispersion and dilution and hence, variable degrees of myocardial opacification. Thus, because of the interanimal variability, peak video intensity in different myocardial beds after LA injection of contrast can be used only to express relative MBV. Our results suggest that in individual dogs, LA and left ventricular volumes remained reasonably constant during the experiment. It is for the same reason that the myocardial input function also remained constant in each dog and that the width of the myocardial time-intensity plot (α) truly corresponded to myocardial microbubble transit rate.

Myocardial video intensity will be affected by even more factors when a venous injection of contrast is used. In addition to LA and left ventricular blood volumes, pulmonary and right heart blood volumes will also affect the concentration of microbubbles that will enter the coronary circulation. The dose of microbubbles injected into the peripheral veins to produce optimal myocardial opacification will have to be determined. Despite use of a standard dose, however, interindividual variability in pulmonary blood volume35 and other determinants of microbubble concentration will allow only a relative estimation of MBV within different myocardial beds from video intensity measurements within the same individual.

A way to minimize interdog variability would be to normalize microbubble intensity to a chamber of known volume, such as the left ventricle. However, attenuation and errors in calculating left ventricular volumes could be major problems. If these can be overcome, it may be possible to derive absolute regional MBV. The advantage of relative over absolute MBV estimation in the clinical setting will, however, need to be demonstrated.

Finally, we did not find a correlation between cardiac output and myocardial peak video intensity. These results imply that within the cardiac output range in our study (twofold increase or decrease), mixing of microbubbles with blood was not affected. It is, however, possible that in inordinately high cardiac outputs, the bubbles would become more diluted, especially if the rate of injection was also considerably slower.

**Conclusions**

In this study, we demonstrate that myocardial perfusion can be quantified from time-intensity curves derived from the LAD bed after LA injection of contrast. Background-subtracted peak video intensity in this situation correlates closely with MBV. Our results indicate that when MBV and MBF are closely coupled, such as during inotropic stimulation of the heart, background-subtracted peak video intensity also correlates closely with MBF. In the situation in which MBV and MBF are not coupled, such as after maximal coronary vasodilation (either after reflow or with a coronary vasodilator), background-subtracted peak video intensity may not correlate with MBF. In this setting, however, assessment of MBV, which quantifies actual microvascular reserve, may be superior to the assessment of MBF, since the latter is also influenced by the coronary driving pressure.30,34 Since there are similarities in the models of LA and venous injections, these data also indicate that it may be feasible to quantify myocardial perfusion from venous injection when images with high signal-to-
noise ratio become available. Newer contrast agents hold promise for such an eventuality.35,36

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