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Quantitative Assessment of Coronary Stenosis by Harmonic Power Doppler With a Simple Pulsing Sequence and Vasodilator Stress in Patients

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OBJECTIVES We examined whether myocardial contrast echocardiography (MCE) with harmonic power Doppler (HPD) employing a simple ultrasound pulsing sequence enables estimation of the severity of coronary artery stenosis in patients.

BACKGROUND Contrast intensity (CI) during MCE with intravenous microbubble infusion is dependent on the myocardial blood flow velocity (MBFV) and pulsing interval (PI).

METHODS Based on an in vitro experiment, we devised the MBFV index calculated as the reciprocal of the magnitude of CI decay produced by abrupt PI shortening during intermittent imaging. In 68 coronary artery territories from 49 patients, myocardial HPD images were acquired during intravenous infusion of Levovist, while the long PI with 1:10 electrocardiographic gating was shortened to 1:1, both at baseline and during adenosine triphosphate infusion. The MBFV index in each coronary territory and MBFV reserve as the ratio between hyperemia and baseline were compared with the severity of corresponding coronary artery stenosis assessed by quantitative coronary angiography (QCA) or by pressure guide wire as the fractional flow reserve (FFR).

RESULTS Both the MCE-derived MBFV index during hyperemia and MBFV reserve exhibited significant negative correlations with the QCA-derived stenosis severity ($r = -0.56$ and $r = -0.64$, respectively). The MBFV reserve positively correlated with FFR ($r = 0.89$). By combining the cutoff values of the MBFV index during hyperemia and MBFV reserve, ≥75% of stenoses defined by QCA were determined, with a sensitivity of 77.3%, specificity of 93.4%, and accuracy of 88.3%.

CONCLUSIONS Shortening of PI during intravenous MCE with intermittent HPD imaging under vasodilator stress enables assessment of coronary artery stenoses in patients. (J Am Coll Cardiol 2003; 41:2060–7) © 2003 by the American College of Cardiology Foundation

Diagnostic ultrasound destroys contrast microbubbles (1). Contrast intensity (CI) is determined by the degree of microbubble destruction and the velocity at which blood containing undestroyed microbubbles refills the imaging field between transmitted pulses (2). Since the initial demonstration by Wei et al. (3) that microbubble destruction/refilling dynamics is applicable to quantification of myocardial blood flow (MBF), several ultrasound pulsing patterns have been proposed to quantitate myocardial perfusion with myocardial contrast echocardiography (MCE). Wei and colleagues programmed a pulsing pattern in which pairs of ultrasound pulses with progressively prolonged intervals were transmitted to describe the relationship between the pulsing interval (PI) and CI. An exponential equation was used for curve fitting, in which the peak plateau of CI defined the blood volume and the rate of CI increase represented MBF velocity (MBFV) (3). Villanueva et al. (4) altered electrocardiographic (ECG) gating intervals to obtain the relationship between CI and PI, which was fit to the same equation. Real-time MCE was also employed to apply flash-replenishment dynamics, which was based on the same quantitative model, to measure MBF in dogs (5).

Harmonic power Doppler (HPD) is a new imaging approach for detecting microbubbles by displaying signals derived uniquely from acoustically stimulated, non-linear microbubble oscillation or microbubble destruction (6). The applicability of HPD to the quantitative model to measure MBF was demonstrated in an animal experiment by Villanueva et al. (4). In the present study, we applied HPD combined with a simple pulsing pattern based on our initial finding that the CI difference between intermittent (long PI) and continuous (short PI) imaging correlates with flow velocity (2). We used 1:10 ECG gating for a long PI and 1:1 for a short PI to derive a new index for MBFV and investigated its feasibility in estimating the severity of coronary stenoses in patients.

METHODS

Principle of the model. Figure 1 depicts the principle of our model. The alterations in the number of microbubbles

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Abbreviations and Acronyms

ATP	= adenosine triphosphate
CI	= contrast intensity
ECG	= electrocardiogram, electrocardiographic, or electrocardiographically
FFR	= fractional flow reserve
HPD	= harmonic power Doppler
LAD	= left anterior descending coronary artery
MBF(V)	= myocardial blood flow (velocity)
MCE	= myocardial contrast echocardiography
PI	= pulsing interval
QCA	= quantitative coronary angiography

(Fig. 1, top) and CI (Fig. 1, bottom) during the pulsing sequence in which a long PI is switched to a short PI are displayed. Let us assume that a single ultrasound pulse destroys a certain number of microbubbles and that new microbubbles enter the imaging field (indicated as rectangles in Fig. 1) with a flat front profile. During long PI imaging, because the replacement is complete, previous microbubble destruction does not affect the number of microbubbles subjected to the next pulse. Thus, CI during a long PI is independent of the transit velocity and is determined by the concentration of circulating microbubbles. At the onset of a short PI that allows only partial replenishment of microbubbles between the pulses, the microbubble concentration in the imaging field and corresponding CI decrease frame by frame. Then, equilibrium is gradually achieved between microbubble destruction and replenishment at a level dependent on the transit velocity (7) and initial concentration of microbubbles. Transit velocity is directly proportional to the rate of replenishment of microbubbles and inversely related to the number of ultrasound pulses to which a bolus is exposed within the interrogating beam. Therefore, the CI difference between long and short PIs, or the magnitude of CI decay after PI shortening, should cancel the effect of the concentration of circulating microbubbles and should represent the transit velocity of microbubbles in the imaging field.

A single exponential function, as follows, can describe such an alteration in CI after the shortening of PI (8):

$$CI = a \times Exp(-b \times f) + c$$

where "a" is the magnitude of the decay; "b" is the decay rate; "f" is the frame number after PI shortening; and "c" is a constant. The CI at a long PI is given as "a + c."

In vitro study. To devise an MCE index of flow transit velocity, we assembled a flow model in which a syringe pump controlled the transit flow velocity (0.25 to 3.3 mm/s) of microbubble solution (0.1% Optison) in a tubular lumen (4 mm in diameter). The CI from short-axis images of the lumen obtained with an S12 transducer (5 to 12 MHz) of the SONOS 5500 (Philips Medical Systems, Andover, Massachusetts) at the mechanical index of 0.8 was quantitated with on-board acoustic densitometry (8). Alteration of CI produced by shortening PI from 6 to 0.3 s was fit to the above equation. Then, the magnitude of CI decay corrected by the value before PI shortening "a/(a + c)" was calculated as a candidate for the index of flow transit velocity.

Study population. A total of 49 patients who had undergone elective coronary angiography were enrolled in the present study. Exclusion criteria included three-vessel coronary artery disease, atrial fibrillation, frequent ventricular arrhythmias, myocardial infarction, diabetes mellitus, and

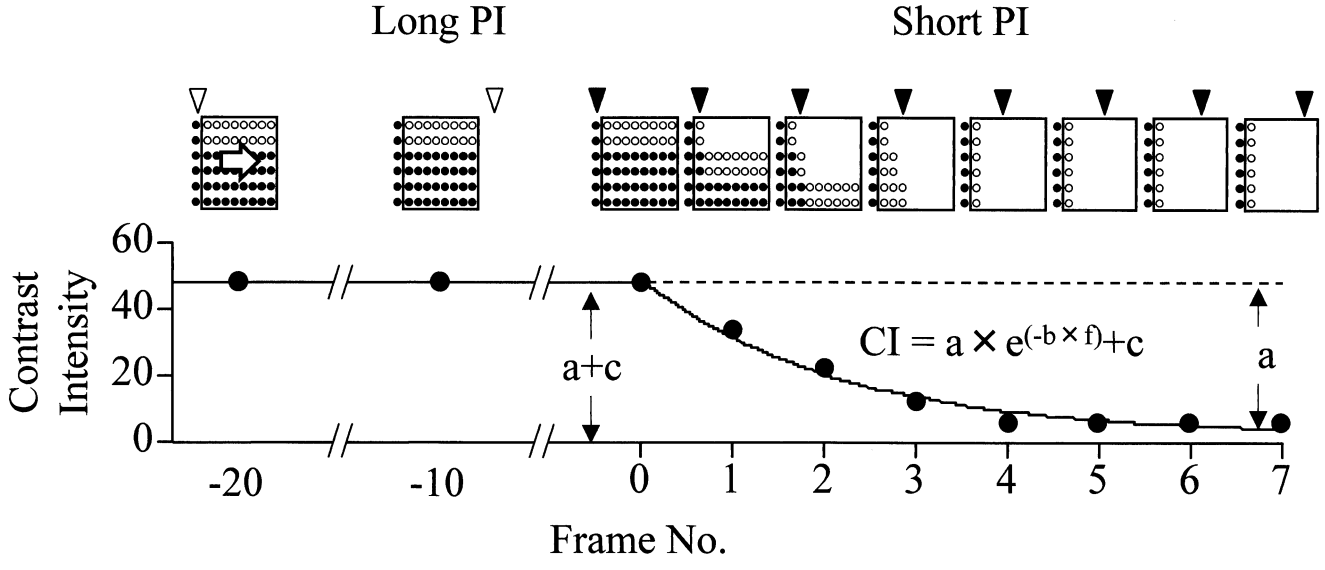

Figure 1. Principle of the model. At long pulsing intervals (PIs), complete replenishment of the imaging field **(boxes)** results in a plateau in contrast intensity (CI) in the frame–CI curve **(bottom)**. After the onset of short PI imaging (from frame no. 0), the microbubble concentration in the imaging field was gradually decreased until a constant level was reached, which can be fit to the decay function. **Circles in boxes** represent microbubbles; **open circles** are those to be disrupted during each frame. The **arrow in the first box** indicates the flow direction, whereas the **inverted triangles** indicate the advancing fronts of the flow.

bronchial asthma. All patients gave written, informed consent to participate in this study, which was approved by the institutional Review Board of our institute.

Assessment of coronary artery stenosis. Digital cineangiographic images were acquired in orthogonal projections after intracoronary injection of 2 mg isosorbide dinitrate. The percent diameter stenosis was obtained by quantitative coronary angiography (QCA). In seven patients, the functional severity of coronary stenosis was evaluated by obtaining the fractional flow reserve (FFR) (9,10), as previously described (9). In brief, a 0.014-in. sensor-tipped, high-fidelity pressure wire (RADI Medical, Uppsala, Sweden) was used to measure the pressure immediately distal to the stenosis during maximum hyperemia by an intracoronary bolus injection of 50 μg adenosine triphosphate (ATP). The FFR was calculated as the ratio of the mean distal intracoronary pressure to mean aortic pressure at peak hyperemia. A primary target coronary territory was chosen by a physician, based on catheterization data, and the patient was referred to the echocardiography laboratory for MCE.

MCE. Myocardial contrast echocardiography by HPD was performed by trained physicians blinded to the severity of coronary stenosis, using the SONOS 5500 and S4 transducers within seven days after cardiac catheterization. Levovist (Schering and Tanabe Seiyaku, Japan), an agent of air microbubbles with a galactose and palmitic acid surfactant (11,12), was prepared at the microbubble concentration of 300 mg/ml and continuously infused at 200 ml/h from the left antecubital vein. One-half of the maximal dose allowed for clinical use (7.5 g/test) was used for baseline recordings. The primary territory chosen by the referring physician was recorded first; then an additional territory was also recorded if possible. Apical long-axis four-, three-, or two-chamber views, depending on the perfusion territory evaluated, were digitally recorded on magnetooptical computer disks. The mechanical index was set at 1.4, compression at 60%, and lower Doppler gain at <65% to avoid "blooming" of the color signals. We employed color map "E" or "F," which are known to provide the most linear relationship between the microbubble concentration and CI. Care was taken to image the segment of interest in the middle regions of the imaging plane, with the focus set at the level of the center of the myocardial segment of interest. Images were recorded during a pulsing sequence in which the long PI with one to 10 end-systolic ECG gating (1:10) was shortened to 1 to 1 (1:1). At least three and 10 frames were captured during 1:10 and 1:1 ECG gating, respectively, which was repeated at least twice per territory. Recordings were repeated using the remaining microbubbles during hyperemia produced at least 5 min after the initiation of infusion of ATP (5 mg/ml) at 0.15 mg/kg per min.

Analysis. Digital images were converted to tag image file format by a digital storage and retriever converter provided by the manufacturer (Philips Medical Systems) and were analyzed with the NIH Image, version 1.69 (National Institutes of Health, Bethesda, Maryland). The average pixel intensity in 256 scales was measured in regions of interest drawn over the coronary territory in each frame and then plotted against the frame number after the onset of short PI imaging, which was fit to the decay function (see previous equation). The alteration of CI that showed the highest regression coefficient was selected for analysis. Then, based on in vitro data, we used the calculation "a/(a + c)" to define the MCE-derived index of MBFV as (a + c)/a, the reciprocal, so that a positive correlation would exist between the index and MBFV. The MBFV reserve was defined as the ratio of the MBFV index during hyperemia to that at baseline.

In 14 territories from seven randomly selected patients, two physicians analyzed the recordings to obtain the MBFV index both at baseline and during hyperemia to assess the inter-observer variability of the index. One of the two physicians repeated the analyses seven days after the first analyses to assess the intra-observer variability.

Statistics. Values were expressed as mean ± SD. For comparison between groups, one-way analysis of variance with a post hoc Bonferroni method was used. For intra-individual comparisons between baseline and hyperemia and between the two different territories in the same patients, the paired Student *t* test was used. All correlation analyses were performed with the least-squares fit regression method. A p value <0.05 was considered statistically significant.

RESULTS

In vitro study. The candidate of the MBFV index "a/(a + c)" (x) exhibited an excellent negative correlation with the flow transit velocity (y) as: $y = -3.29x + 3.30$, $r = 0.98$, $p < 0.001$, SEE = 0.24 mm/s, which provided the basis for employing (a + c)/a, the reciprocal of the magnitude of CI decay, as the positive index of MBFV in the study patients.

Patient demographics. The age of the study population was 65 ± 9 years (range 40 to 79). Eight patients had normal coronary arteries, 28 had one-vessel disease, and 13 had two-vessel disease, but there were no patients with three-vessel disease. Sixty-eight territories from 49 patients were free from artifacts and were analyzed. Only one coronary territory was evaluated in each of 30 patients, whereas both primary and secondary territories were evaluated in 19 patients. There were 39, 14, and 15 territories of the left anterior descending coronary artery (LAD), left circumflex artery, and right coronary artery, respectively. The FFR was obtained in seven coronary arteries in seven patients; all territories were assessable by MCE.

Representative recordings. Figure 2 displays representative recordings from a patient without significant coronary stenosis. At baseline, the color signal in the anterior interventricular septum gradually decreased for three frames until it reached a constant intensity (Fig. 2, top). During ATP infusion, the

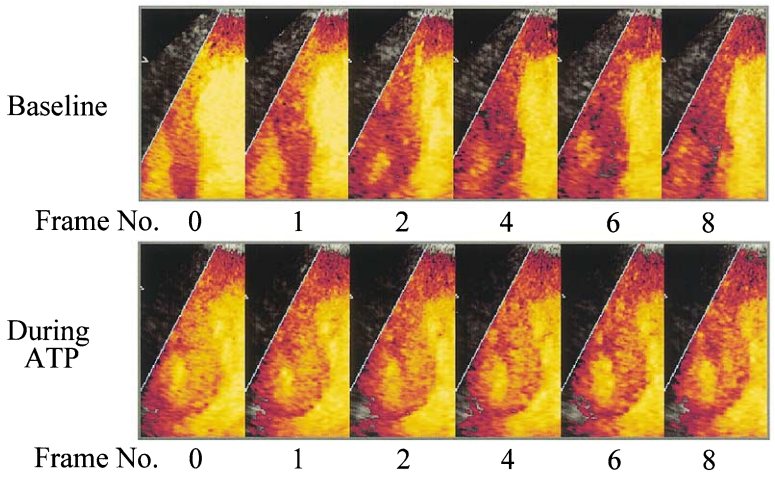

Figure 2. Representative recordings from a normal coronary territory. Opacification in the anterior septum was decreased frame by frame until the fourth frame and was constant thereafter **(top row)**. The magnitude of decay was less during infusion of adenosine triphosphate (ATP) **(bottom row)** than at baseline. The **numbers** indicate the frame numbers after the onset of short pulsing interval imaging.

signal decreased for several frames to a constant level that was higher than that at baseline. Figure 3 displays recordings from a patient with 99% stenosis in the LAD. Opacification in the anterior septum at baseline decreased after the PI shortening for several frames and then reached a constant level (Fig. 3, top). During ATP infusion, the decay of CI became swift and great. Plots of CI (y) obtained from the anterior septum against the frame number (x) fit well to the decay function both at baseline and during ATP infusion in both territories (Fig. 4). The magnitude of CI decay was greater at baseline (Fig. 4, open circles) than during hyperemia (Fig. 4, closed circles) in the normal territory (Fig. 4, left panel). In contrast, a greater magnitude of decay during ATP infusion than at baseline was noted in the severely stenosed coronary territory (Fig. 4, right panel). The initial value for CI was lower during hyperemia than at baseline in this case.

The MBFV index and its change by hyperemia versus severity of coronary stenosis. Figure 5 plots the MCE-derived MBFV index both at baseline (open circles) and during hyperemia (solid circles) against QCA-derived severity of stenosis. The MBFV index at baseline was essentially unaffected by the severity of coronary stenosis. However, during hyperemia the index was increased in the territories perfused by normal to mildly stenosed coronary arteries, was essentially unchanged in the moderately stenosed coronary territories, but was substantially decreased in the severely stenosed territories (Table 1). There was a significant negative correlation between the MBFV index during hyperemia and percent diameter stenosis ($r = -0.53$, $p < 0.01$). Therefore, as displayed in Figure 6, the MCE-derived MBFV reserve exhibited a significant negative correlation with percent diameter stenosis by QCA ($r = -0.64$, $p < 0.01$).

By employing the values of mean minus 1 SD among the territories with coronary stenosis <75% as the cutoff values, a positive test for the severe coronary stenosis ≥75% by QCA was defined as the MBFV index during ATP <4.89 or MBFV reserve <0.78. This MCE method exhibited a

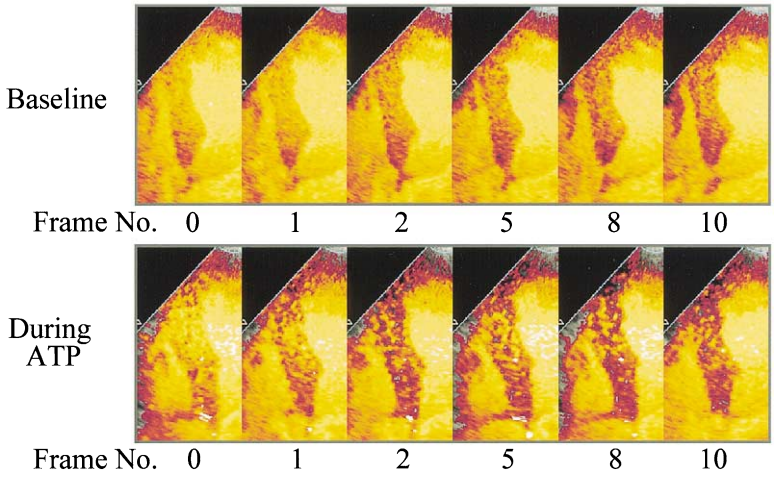

Figure 3. Representative recordings from a territory of a severely stenosed coronary artery. Opacification in the anterior septum was decreased frame by frame until the eighth frame and was constant thereafter at a relatively higher level **(top row)**. The magnitude of decay was greater during hyperemia by adenosine triphosphate (ATP) **(bottom row)** than at baseline. The **numbers** indicate the frame numbers after the onset of short pulsing interval imaging.

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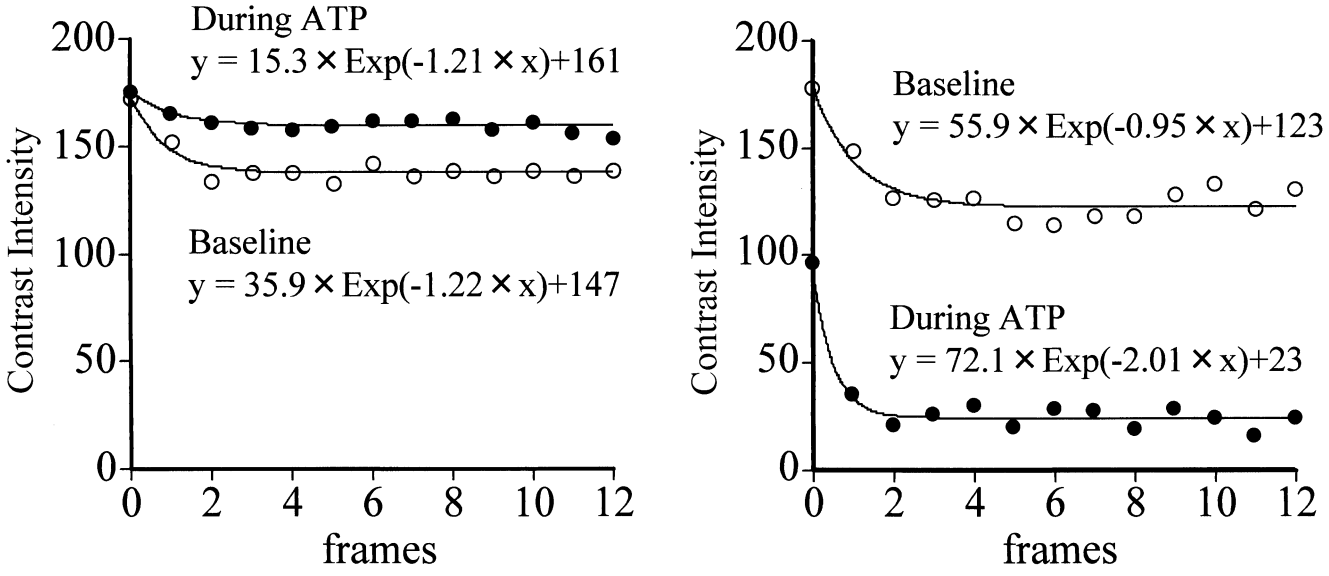

Figure 4. The contrast intensity (CI)–frame number curves from the anterior interventricular septum in the patient shown in Figure 2 **(left panel)** and the patient in Figure 3 **(right panel)**. The alteration in CI fit well with the decay function both at baseline **(solid circles)** and during adenosine triphosphate (ATP) infusion **(open circles)** in both territories. Although the magnitude of decay was less during ATP infusion than at baseline in a normal coronary territory **(left)**, it was greater in a severely stenosed coronary territory **(right)**.

sensitivity of 77.3% (17/22), specificity of 93.4% (43/46), and diagnostic accuracy of 88.3% (60/68).

MCE-derived MBFV reserve and FFR. A significant linear positive relation was observed between the index of functional stenosis, FFR, and MCE-derived MBFV reserve ($r = 0.86$, $p < 0.02$) (Fig. 7).

Intra-individual analysis. An intra-individual paired comparison between the territories with different severities of stenosis was possible in 18 of 19 patients in whom both the primary and secondary territories were evaluated, except for one patient in whom both measurements were performed in the insignificantly stenosed coronary territories. In eight patients, the MBFV reserve was 2.34 ± 1.59 in the insignificantly stenosed (<50%) coronary territory and 1.30 ± 0.62 in the moderately stenosed (50% to 75%) coronary territory. In 10 patients, the MBFV reserve was 1.95 ± 0.79 in the territory of stenosis <50% and 0.88 ± 0.33 in the severely stenosed (≥75%) coronary territory ($p < 0.01$).

Reproducibility of analysis of CI decay. The MBFV indexes obtained by the two observers agreed well ($y = 1.06x - 0.39$, $r = 0.93$, $p < 0.001$). The average of the measurements by the two observers ranged from 4.1 to 30.6, whereas the mean ± 2 SD of the difference between the measurements was 0.3 ± 5.2. Similarly, the results of the repeated analyses by one observer correlated well ($y = 0.94x + 0.84$, $r = 0.97$, $p < 0.001$). The average of the repeated measurements ranged from 3.4 to 25.3, whereas the mean ± 2 SD of the difference was 0.1 ± 2.9.

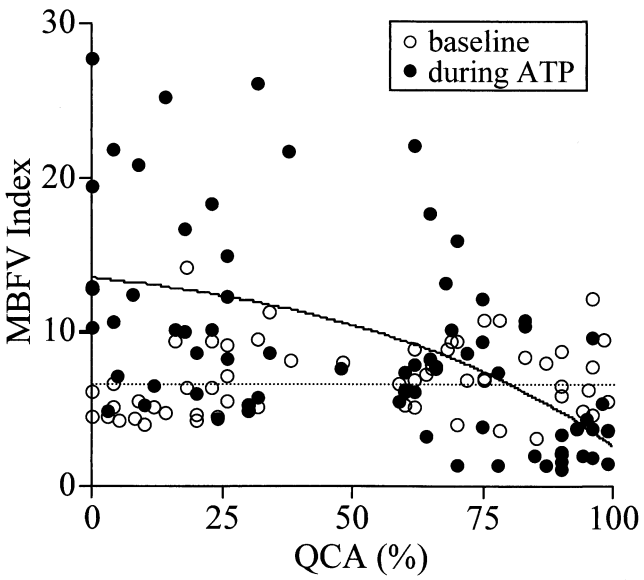

Figure 5. Plots of the myocardial contrast echocardiography-derived myocardial blood flow velocity (MBFV) index at baseline **(open circles)** and during hyperemia **(solid circles)** against quantitative coronary angiography (QCA)-derived percent diameter stenosis. The **dotted line** denotes the average value at baseline, whereas the **solid line** indicates the regression line for the data during hyperemia ($y = 15.5\{1 - \text{Exp}(0.019[x - 109.8])\}$, $r = 0.53$, $p < 0.01$). ATP = adenosine triphosphate.

DISCUSSION

In the present study, HPD with a simple pulsing sequence was applied to the assessment of coronary stenoses in patients. The magnitude of CI decay after PI shortening from 1:10 to 1:1 ECG gating was decreased by ATP in normal myocardial beds but was increased in the territories of severely stenosed coronary arteries. The MBFV reserve that was estimated as the ratio of the new MCE-derived index for MBFV exhibited a significant correlation with the severity of coronary stenosis as assessed by QCA and FFR.

Table 1. The Myocardial Blood Flow Velocity Index at Baseline and During Hyperemia and Their Ratio in the Territories of Coronary Arteries of Various Percent DS

DS	n	Baseline	Hyperemia	Reserve
≤50%	31	6.57 ± 2.67	12.51 ± 6.98†	1.98 ± 1.05
50% to 75%	15	7.16 ± 1.62	9.53 ± 5.58	1.27 ± 0.58
≥75%	22	6.70 ± 2.72	4.78 ± 3.58†‡	0.71 ± 0.39‡
p Value*		0.75	< 0.001	< 0.001

*By one-way analysis of variance. †$p < 0.01$ vs. baseline by the paired Student *t* test. ‡$p < 0.05$ vs. both ≤50% and 50–75% by the post hoc Bonferroni test. Data are presented as the mean value ± SD.
DS = diameter stenosis.

A new index for MBFV. We devised a new index for MBFV that is obtainable with a simple pulsing sequence. Our method is different from that of Wei et al. (3,13), although both methods are based on the microbubble destruction/replenishment dynamics. Wei and colleagues used a pair of frames, one to eliminate all microbubbles in the imaging field and the other with a certain delay from the first, to evaluate the reappearance of the signal during a constant infusion of microbubbles. By using multiple pairs with varying PIs, they obtained a "replenishment curve" relating CI to PI and observed that the rate of increase in CI per increase in PI (β value) closely correlated with MBFV.

In contrast, we used only two PIs. Recordings were obtained while the long PI, which allows complete replacement of insonated volume between the frames, was switched to the short PI, which allows partial volume replacement during the intervals. We observed a gradual decay in CI to a constant value rather than an immediate decrease to a plateau level after PI shortening, which suggested that only partial destruction of the microbubbles was achieved by a single frame of HPD, at least in the clinical settings examined in our study. In an imaging field where pulsed ultrasound is transmitted successively, an equilibrium is gradually achieved between microbubble destruction and replenishment. Thus, CI can be dependent on not only the replenishment rate but also the number of pulses encountered by the bubbles during the passage through the imaging plane, both of which are determined by the transit velocity when *successive* imaging is performed at a short interval. Thus, in contrast to Wei's method hypothesizing that microbubble destruction by a single frame is complete or sufficient to produce no signals, our method does not require complete microbubble destruction but, rather, utilizes the incompleteness of destruction at each frame. The pertinence of the principle of our method was supported by flow model experiments by Ugolini et al. (7), who exhibited that CI reduction after PI shortening was greater for the dialyzer cartridge perfused at a slower rate. Because the CIs during both long and short PIs are affected by the concentration of circulating microbubbles that enter the imaging field, we normalized the extent of CI decay with the CI during long PI imaging, so that the results might represent the transit velocity more accurately. Our in vitro experiment has clearly shown that the calculation thus obtained closely correlates with the transit velocity of the microbubbles through the imaging plane.

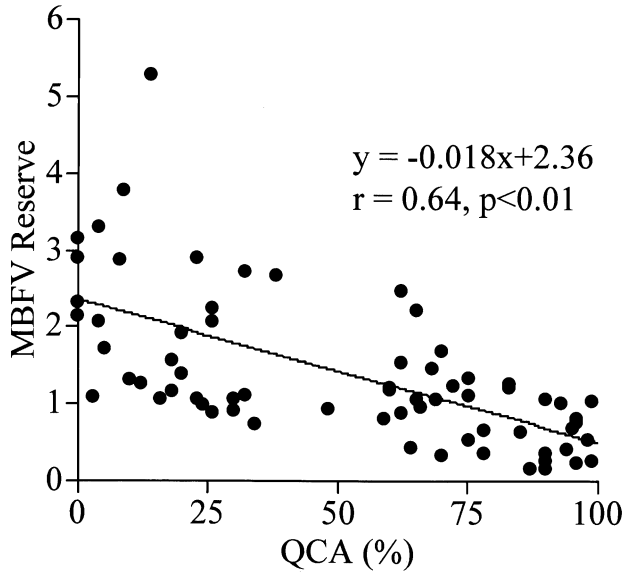

Figure 6. Plots of the myocardial contrast echocardiography-derived myocardial blood flow velocity (MBFV) reserve against quantitative coronary angiography (QCA)-derived percent diameter stenosis. A significant inverse correlation was noted.

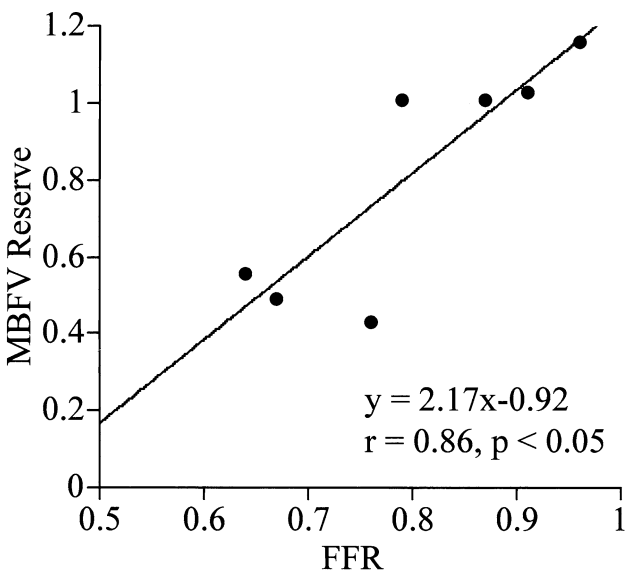

Figure 7. Correlation of myocardial contrast echocardiography-derived myocardial blood flow velocity (MBFV) reserve with fractional flow reserve (FFR). The MBFV reserve exhibited a good negative correlation with the functional severity of coronary stenosis.

Stenosis estimation by response of the MBFV index to vasodilator stress. Wei et al. (13) found that the index for MBFV, rather than that for myocardial blood volume, provided a more accurate estimate of coronary blood flow reserve in the clinical setting. In our study, the responses of the MBFV index to the coronary vasodilator exhibited significant correlations with the stenosis severity of the corresponding coronary arteries (Figs. 6 and 7). The combination of cutoff values for the MBFV index during hyperemia and its reserve was able to discriminate severe coronary stenosis ≥75% by QCA with a diagnostic accuracy (88.3%) equivalent to that of exercise stress myocardial scintigraphy (14). The moderate sensitivity of our method may be partially due to the limitation of QCA to assess the actual resistance produced by the stenosis. In fact, the MBVF reserve exhibited a good correlation with FFR, one of the indexes of functional severity of coronary stenosis (9,10).

HPD. Until recently, HPD has been used in qualitative or semi-quantitative assessments of myocardial perfusion (11,15–17). Because CI by HPD is determined by the magnitude of variance between successive pulses, it is susceptible to motion artifacts and has a narrower dynamic range than that of B-mode harmonic imaging. A linear relationship between microbubble concentration and CI by HPD is not necessarily guaranteed in in vivo settings. Nevertheless, HPD has been shown to be more capable of delineating flow-limiting stenoses in dogs than B-mode imaging when end-systolic ECG gating is used (17). Contrast intensity with HPD has been found useful in quantitative assessment in in vitro experiments (7) and also in the application of microbubble destruction/replenishment dynamics to the assessment of coronary stenoses in dogs (4). The present study further extends the applicability of ECG gated HPD imaging to the estimation of coronary stenosis in patients.

Study limitations. This study has several limitations. Inhomogeneous distribution of myocardial perfusion produced by coronary vasodilators might have been better assessed if MBFV or its reserve had been compared among different myocardial segments (4). However, the extent of microbubble destruction and the relationship of CI to microbubble concentration may be inhomogeneous among the myocardial segments. With a phased-array sector, the scan line density and ultrasound energy delivered are less on the sides and in the far field than in the near field and center (15), which produces false defects in the lateral and anterior walls in apical four- and two-chamber views, respectively (11,15,16). In contrast to Wei's method, which solely observed the refill with microbubbles, our method, which employed the extent of microbubble destruction in addition to refill, may be more susceptible to the inhomogeneity of acoustic energy distribution. Therefore, we chose to focus on a single territory that we imaged in the center of the imaging field (12). Because the maximal dose of Levovist allowed for a patient at one time was limited, we were able to record the CI alteration in another segment in the center of the imaging field in only 19 (39%) of 49 patients. Nevertheless, we found a consistent trend in these patients: the MBFV reserve in a stenosed coronary territory was reduced compared with that in the normal coronary territory of the same persons. However, there may be limitations in applying our method to the detection of unknown coronary stenoses, although it may allow estimation of the severity of stenoses of selected coronary arteries based on other stress tests or those susceptible to post-intervention restenosis.

Because the reserve derived from MCE is functional, a comparison with FFR, a functional parameter of coronary stenosis, was performed in part of our study population. It remains unknown, however, whether our MBFV index better correlates with the functional parameter in a larger population.

Although patients with diabetes mellitus were excluded from our study, other possible risk factors might impair microcirculation in the absence of epicardial coronary stenosis. This may be a reason for the substantial variation of MBFV reserve seen among the patients with insignificant coronary stenoses. Although CI at a long PI is equivalent to the plateau intensity (A value) of Wei's method, which reflects relative myocardial blood volume, we did not use it to derive the volumetric MBF. Although the decrease in CI at a long PI observed in severely stenosed coronary territories, such as shown in Figure 3, might represent the decrease in myocardial blood volume, influences of change in circulating microbubble concentration by vasodilator stress are not excluded. Because we did not measure volumetric MBF reserve by MCE or by other methods in the present study, it remains unclear whether this variation among insignificantly stenosed coronary territories was a limitation of our index or the nature of MBF reserve, which are determined by both epicardial coronary resistance and the presence of microangiopathy (18).

Finally, neither Levovist nor ATP injection is readily available in all countries. However, because nearly all contrast agents follow microbubble destruction/replenishment dynamics and adenosine may be a good substitute for ATP, our method should be feasible with other agents.

Conclusions. We have demonstrated that quantitative assessment of coronary stenoses using a model based on microbubble destruction and replenishment is possible in patients by employing a simple pulsing sequence during HPD imaging, with pharmacologic stress and continuous microbubble infusion.

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