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Detection of Myocardial Perfusion in Multiple Echocardiographic Windows With One Intravenous Injection of Microbubbles Using Transient Response Second Harmonic Imaging

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Objectives. **The purpose of this study was to prove that transient response harmonic imaging could detect normal and abnormal myocardial perfusion in multiple echocardiographic windows with one intravenous injection of microbubbles in humans.**

Background. **Myocardial ultrasound contrast can be produced from intravenous perfluorocarbon-exposed sonicated dextrose albumin, and ultrasound can be significantly improved by briefly suspending the interval between frame rates. Whether this contrast can noninvasively quantify myocardial perfusion in humans is unknown.**

Methods. **In 28 patients, harmonic transient response imaging was used to image the heart in multiple different imaging planes after one intravenous injection of ultrasound contrast agent. Twenty-five of these 28 patients had a repeat injection during dipyridamole stress. In the primary view, the ultrasound transmission rate was one frame per cardiac cycle; in secondary and tertiary views, the transmission rate was once every multiple cardiac cycles. Regional myocardial contrast was visually assessed and quantified off-line. Quantitative rest thallium and dipyridamole stress sestamibi imaging was also performed.**

Results. **Perfusion abnormalities were evident in the secondary and tertiary views only with one frame every multiple cardiac cycles. Regional peak myocardial videointensity (PMVI) correlated closely with regional tracer uptake in individual patients both at rest ($r = 0.84$) and during stress ($r = 0.88$). A PMVI ratio (abnormal region divided by the region with highest nuclear uptake) <0.6 in any view had a 92% sensitivity and a 84% specificity in identifying a regional nuclear perfusion abnormality.**

Conclusions. **Transient response imaging produces myocardial contrast in multiple views with one intravenous injection of contrast agent and can accurately identify regional myocardial perfusion abnormalities.**

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Recent data (1,2) obtained in both animals and humans have demonstrated that visually detectable myocardial contrast can be produced from very low intravenous doses of perfluorocarbon-exposed sonicated dextrose albumin (PESDA) microbubbles when ultrasound transmission rates are reduced by triggering frame rates to once every cardiac cycle instead of the conventional 30-Hz frame rates. This phenomenon (referred to as *transient response imaging*) is especially pronounced when using harmonic imaging (1). Because intravenously injected PESDA microbubbles containing less soluble gases like perfluorocarbons have a delayed transit through the lung capillaries, there is often a prolonged left ventricular cavity contrast after one intravenous injection of microbubbles (3). Myocardial contrast can be produced in animals with this continuous left ventricular cavity infusion of microbubbles long after intravenous injection by extending the time interval between triggered frame rates to once every 5 to 10 cardiac cycles (4). This extended time interval permits the identification of myocardial perfusion abnormalities that are not evident with triggering just once every one cardiac cycle and could therefore be utilized in humans to detect perfusion abnormalities in different echocardiographic windows. The purpose of this study was to test whether one small intravenous injection of PESDA could produce myocardial contrast in multiple imaging planes and correlate the degree of contrast enhancement with myocardial perfusion determined by radionuclide imaging.

Methods

Preparation of PESDA. PESDA was prepared similar to previously described methods (1). Briefly, 8 ml of decafluorobutane (molecular weight 238 g/mol) was hand agitated with a 3:1 mixture of 5% dextrose and 5% human albumin. This mixture then underwent electromechanical sonication for 80 s. According to the manufacturer (Heat Systems Inc.) the sonication process involves the transference of electrical energy to mechanical energy (495 W). Energy output was manually

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Abbreviations and Acronyms

PESDA = perfluorocarbon-exposed sonicated dextrose albumin
PMVI = peak myocardial videointensity
SPECT = single-photon emission computed tomography (tomographic)

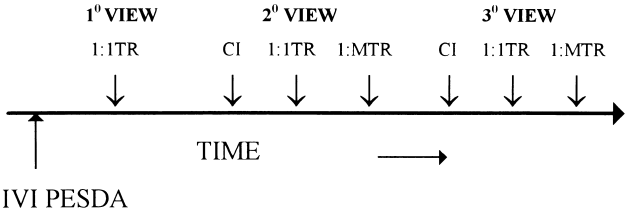

Figure 1. Schematic for the acquisition of myocardial contrast images after one intravenous injection of PESDA. Myocardial contrast in the second (2^0) and third (3^0) views was obtained by switching from conventional (30 Hz) frame rates to one frame rate every multiple cardiac cycles. See text for details. CI = conventional imaging; IVI PESDA = time at which intravenous injection of perfluorocarbon-exposed sonicated dextrose albumin was given; 1^0 = first; 1:MTR = triggering ultrasound frame rates to one point every 5 to 10 cardiac cycles; 1:1 TR = triggering ultrasound frame rates to one point every cardiac cycle.

adjusted using a digital scale during sonication to achieve 25 ± 3% (mean ± SD) of this maximal output during the entire sonication time (124 ± 15 W). Because this energy is emitted vertically from the circular 0.5-in. horn tip, the output from the horn tip was estimated to be 98 ± 11 W/cm^2. The mean microbubble size of PESDA using this method has been shown (2) to be 4.7 ± 0.2 μm, with a mean concentration of 1.3 ± 0.1 × 10^9 microbubbles/ml.

Study patients. This study included 28 patients (mean age 61 ± 12 years, range 39 to 82; 18 men, 10 women [36%]) who were undergoing stress echocardiography and who agreed to also undergo a rest thallium uptake and dipyridamole stress dual-isotope study (stress sestamibi/rest thallium) at the University of Nebraska Medical Center. The patients were selected from the routine cohort of patients undergoing stress echocardiographic studies. There were no differences in age (61 ± 12 years for both those in the study and those patients not in the study undergoing stress echocardiography in a similar time period) or gender (45% women from patients not in the study). However, the study group had a higher incidence of abnormal study results (16 [57%] of 28 had a fixed or inducible abnormality vs. 20 [38%] of 53 with stress echocardiographic abnormalities in a similar time period). Left ventricular systolic function at rest was normal in 19 (68%) of 28 study patients and 42 (79%) of 53 of patients undergoing stress echocardiography in a similar time period.

Contrast echocardiography was performed the same day as dipyridamole stress dual-radioisotope studies in 25 patients, and within 1 week of the rest thallium studies (n = 3). The indications for the stress studies were evaluation of symptoms suggestive of coronary artery disease in all patients. The rest studies were all done in patients with a previous myocardial infarction (two inferoposterior, one anteroseptal). All patients provided written informed consent, and the entire protocol was approved by the Institutional Review Board of the University of Nebraska Medical Center, as well as the Food and Drug Administration as an Investigational Device Exemption.

Ultrasound image acquisition and analysis of contrast intensity. Ultrasound images were obtained with the use of a prototype harmonic transducer (Hewlett-Packard) that was available on a limited basis. Harmonic imaging has been shown (1,2) to significantly enhance the improvement in myocardial contrast achieved with transient response imaging. The transmit frequency was 2.0 MHz (4.0-MHz received frequency) for all studies.

In this study, the *primary view* was defined as the first standard ultrasound window (usually the parasternal short-axis or apical four-chamber view) being imaged after intravenous injection of PESDA. These primary views were chosen mainly because they would make it easiest to switch to other views after the peak period of contrast in the myocardium. Echocardiograms from the primary view were routinely obtained by triggering ultrasound transmission once per cardiac cycle (end-systole). The background-subtracted peak myocardial video intensity at end-systole observed with this triggering technique has correlated closely with coronary blood flow changes in animals (1). After obtaining primary views, secondary views were obtained by switching back to conventional 30-Hz frame rates and obtaining a second window (Fig. 1). The ultrasound frame rate was then switched initially back to one frame every cardiac cycle and then to once every three to five cardiac cycles. If triggering once every three to five cycles produced myocardial contrast enhancement, it was utilized to measure peak myocardial videointensity (PMVI) in this view. If this triggering did not produce sufficient enhancement, it was reduced to once every 10 cardiac cycles, and PMVI was measured with this frame rate interval. This same protocol was then used to obtain myocardial contrast in a third, or tertiary, view, if possible (Fig. 1). The terms "one frame every multiple cardiac cycles" or "extended time intervals between frames" will subsequently be used to refer to this process.

Quantitative measurements of myocardial contrast were performed off-line from high fidelity videotape images. Myocardial videointensity was measured using gray-scale software (Tom-Tec Review Station) that quantitates videointensity (scale of 1 to 255) versus time. PMVI from the anteroseptal, inferoposterior, lateral and apical regions (four regions per injection) was measured in both the primary view and from the secondary and tertiary views using a 30-pixel region of interest placed in the mid or distal portion of each region. The apical region of interest was placed in the middle of the end-systolic apical myocardial segment (Fig. 2). Images were manually aligned before analysis of video intensity to correct for heart motion due to respiration. If the PMVI was measured in more than one view (e.g., anteroseptal region in the parasternal short-axis view and then again in the parasternal or apical

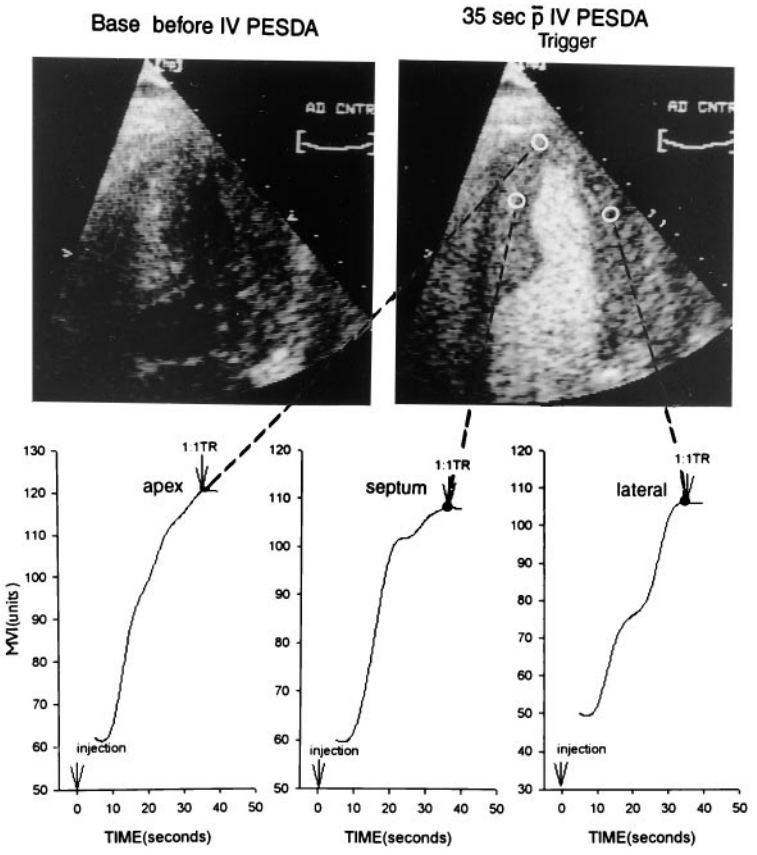

Figure 2. Myocardial contrast enhancement in the apex, septum and lateral walls in a patient who exhibited normal contrast enhancement with transient response imaging in the primary view (apical four chamber). This patient also had normal tracer activity in these regions. IV = intravenous; MVI = myocardial videointensity; $\bar{p}$ = after; other abbreviations as in Figure 1.

long-axis view), the highest background-subtracted video intensity was used for comparison with quantitative thallium uptake. PMVI in secondary and tertiary views was measured using the myocardial videointensity during 30-Hz frame rate imaging as background intensity because no myocardial contrast was evident at this frame rate in these views. Instrumentation settings (transmit power, time and lateral gain compensation) were kept constant when switching to different views. The PMVI ratio was calculated for all studies by dividing the PMVI in the region with the lowest quantitative tracer uptake by the PMVI in the region with highest tracer uptake.

Visual grading of myocardial contrast enhancement produced by transient response imaging in each of these views was also assessed by two independent reviewers (T.R.P., S.L.). Each reviewer evaluated whether the regional myocardial contrast produced after one intravenous injection of PESDA was normal or abnormal in the primary, secondary or tertiary views. A visually evident contrast defect was considered *present* when there was either 1) a relative decrease in contrast enhancement in one region compared with others in the primary view; or 2) a lack of contrast enhancement in one region compared with others in the secondary or tertiary views when switching from triggering once every one cardiac cycle to once every multiple cardiac cycles. Interobserver and intraobserver variability in image interpretation of visual myocardial contrast was calculated by comparing the percent agreement between the two independent observations in all patients.

Acquisition and quantitative interpretation of rest thallium stress sestambi images. To test the validity of the myocardial contrast observed with triggered imaging, we performed rest thallium studies in 26 patients on the same day as their contrast injections. The remaining two patients had rest thallium studies performed within 1 week of the rest contrast injections. After an overnight fast, patients received an injection of 3 to 4 mCi of thallium-201 on the same day as the ultrasound contrast studies. One patient received 8 mCi of technetium (Tc)-99m sestamibi (Cardiolyte). Twenty-five patients received a 20- to 25-mCi injection of Tc-99m sestamibi at peak dipyridamole stress (3 min after completion of a 4-min 0.56-mg/kg body weight infusion). SPECT images were obtained after 10 min for thallium and after 30 min for Tc-99m sestamibi injections. A triple-headed rotating gamma camera (Picker 3000) equipped with a low energy, high resolution parallel-hole collimator was used and centered on 73- and 166-keV photopeaks with 30% and 20% windows, respectively, for thallium studies and 140-keV photo peaks with a 15% bandwidth for Tc-99m studies. The triple-headed camera was rotated over a 120° arc at 3° increments for 25 s each. This combined rest thallium and dipyridamole stress SPECT sestamibi protocol has been demonstrated (5) to have high sensitivity and specificity for detection of angiographically significant coronary artery disease in patients with a low or intermediate probability of having disease. Furthermore, this sequence of injections (rest thallium followed by stress sestamibi) was utilized be-

cause there is minimal crosstalk (<4%) of thallium into the sestamibi window when given in this order (6).

From the raw scintigraphic data, the rest and stress images were reconstructed by backprojections using a low pass ramp filter. The images were oriented in the three standard orthogonal planes of the left ventricle (horizontal long-axis, vertical long-axis and short-axis views) using the Odyssey VP program. Radioisotope uptake was quantified using a computer algorithm (STEPS 10, Odyssey VP Program, Picker, Inc.), which grades the percent uptake in each region (anteroseptal, inferoposterior, lateral and apical) on a 1 (no uptake) to 10 scale (maximal thallium uptake) in relation to the region of highest uptake. Any percent uptake <70% of the highest region was considered abnormal, similar to previously described protocols (7). The percent uptake in each region (anteroseptal, lateral, inferoposterior and apical) in each individual patient was then compared with the PMVI in each region produced with transient response imaging.

Study protocol. After thallium acquisition at rest, described previously, baseline intravenous injections of PESDA were given. The starting dose of intravenous PESDA for evaluation of myocardial contrast enhancement in multiple views was 0.00125 ml/kg and was increased to either the 0.0025-, 0.005- or 0.01-ml/kg doses as needed to produce myocardial contrast using the previously described triggering protocol. Before, during and after each injection, blood pressure, heart rate, oxygen saturation, respiratory rate and heart rhythm were recorded. Any patient symptoms during and after injection were also recorded.

Dipyridamole infusion was then initiated at a rate of 0.14 mg/kg per min over 4 min or up to a maximal dose of 60 mg. At 6 and 8 min after completion of the dipyridamole infusion, respectively, two repeat doses of intravenous PESDA (using the same dose as the baseline injection) were given, and the imaging protocol shown in Figure 1 was repeated. At 7 min after the initiation of dipyridamole, 20 to 25 mCi of sestamibi was given for subsequent SPECT images using the previously described protocol.

Statistical analysis. Differences in PMVI ratios in the patients with normal findings on dual-isotope imaging (≥70% uptake in all regions) and patients that had either fixed or inducible defects by dual-isotope imaging were compared with analysis of variance (Student-Newmann-Keuls multiple comparison procedure). A p value <0.05 was considered significant. The Pearson correlation coefficient was used to determine the correlation between each patient's regional PMVI and regional quantitative thallium or sestamibi uptake. Both interobserver and intraobserver agreement on PMVI measurements in the different regions were compared by computing the standard deviation of 45 measurements made by the two different observers divided by the mean PMVI measured by both observers (coefficient of variation). Injection to injection variability was also calculated by repeating the same PMVI measurements in the primary, secondary and tertiary views in 21 regions of seven patients after a second injection of the same intravenous dose of PESDA.

Results

The dose of intravenous PESDA used to produce myocardial contrast was 0.00125 ml/kg in 6 patients, 0.0025 ml/kg in 18, 0.005 ml/kg in 2 and 0.01 ml/kg in 2. The primary view was either the parasternal short-axis (after 30 injections) or apical four-chamber view (20 injections). The apical two-chamber view was the primary view in one patient and the apical long-axis view in two patients. Myocardial contrast could be visually observed by both reviewers in all 28 patients when triggered imaging was used. With this technique myocardial contrast enhancement was visually evident in at least three different windows with one intravenous injection of contrast agent in 26 of 28 patients and in two views in all patients.

Myocardial contrast in the second and third views was evident only when extended time intervals between frame rates were used. In 21 patients, production of myocardial contrast required one frame every three to five cardiac cycles in the secondary and tertiary views, whereas contrast enhancement was not achieved in the remaining 7 until the frame rate was reduced to once every 10 cardiac cycles. Of the 89 regions that were compared twice (at rest and again during dipyridamole stress), 51 had PMVI measured from the same view (primary, secondary or tertiary) at rest and during stress. In the remaining 38 regions, rest PMVI measurements were obtained using a different view from that used during dipyridamole stress. Seven regions in six patients (lateral in five, inferoposterior in two) could not be assessed because of persistent attenuation of this region in all views and at all frame rates.

Dipyridamole infusion induced a slight increase in heart rate in the 25 patients studied (67 ± 12 to 81 ± 13 beats/min) and a slight decrease in systolic blood pressure (from 141 ± 23 mm Hg at rest to 132 ± 19 mm Hg after dipyridamole). There was no significant change in diastolic blood pressure (from 79 ± 11 mm Hg at rest to 77 ± 18 mm Hg after dipyridamole).

Correlation between myocardial contrast observed with transient response imaging and tracer uptake. Of 25 patients with both rest and dipyridamole stress studies, 5 patients had quantitative evidence of a defect present at rest and during stress with dual-isotope imaging (<70% uptake at rest and during stress; inferoposterior region in 4, lateral region in 1). Ten regions in eight patients exhibited new uptake abnormalities during dipyridamole infusion (inferoposterior in 5, lateral in 1, anteroseptal in 3, apical in 1). One patient had a mild decreased uptake of thallium (60%) at rest that was quantitatively normal during dipyridamole stress. The remaining regions were quantitatively normal (≥70% uptake) during both rest thallium and stress sestamibi. In the three patients with rest thallium studies only, uptake abnormalities in the anteroseptal, apical and inferoposterior regions were present in one and inferoposterior uptake abnormalities in the other two. Therefore, there was an overall total of 11 regional uptake abnormalities at rest and 15 uptake abnormalities during dipyridamole stress. One patient with a rest inferoposterior thallium defect could not be evaluated with transient response

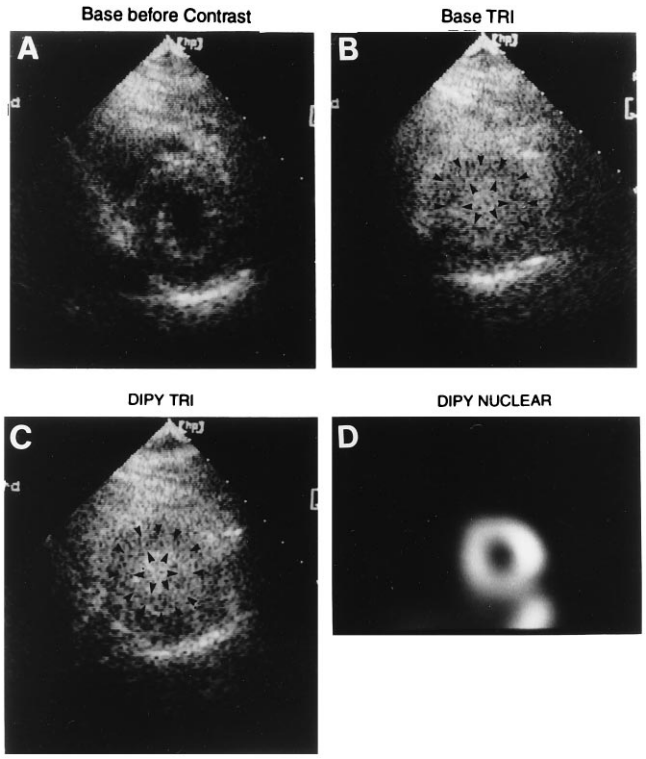

Figure 3. Myocardial contrast enhancement from the parasternal short-axis view in another patient (primary view in this patient). **Arrows** enclosing endocardial and epicardial borders depict normal contrast enhancement under rest conditions and during peak dipyridamole stress. The corresponding stress tracer uptake is also shown and was also quantitatively normal. DIPY = images obtained during dipyridamole stress; TRI = triggered imaging (one frame every cardiac cycle in this primary view).

imaging because of persistent inferoposterior attenuation after intravenous PESDA. Thus, 10 rest and 15 stress tracer uptake abnormalities remained for comparison studies.

The PMVI ratio produced with transient response imaging in the regions that exhibited both normal rest and normal dipyridamole tracer uptake was 0.76 ± 14 at rest and did not change during dipyridamole stress (0.70 ± 0.11). Figure 2 is an example from the apical four-chamber view (primary view) of a patient with both normal contrast enhancement at rest and normal thallium uptake. Figure 3 demonstrates normal contrast enhancement in another patient at rest and during dipyridamole stress from the parasternal short-axis view (primary view in this patient).

In the regions with stress-induced tracer uptake abnormalities, the PMVI ratio decreased significantly during stress (from 0.78 ± 18 U baseline to 0.41 ± 0.20 U during dipyridamole stress, $p < 0.05$). In regions that had quantitatively abnormal tracer uptake both at rest and during stress, there was a significantly lower PMVI ratio during both rest studies (0.48 ± 0.14) and dipyridamole stress (0.35 ± 0.11). This ratio decreased slightly during dipyridamole stress and corresponded to a decrease in nuclear tracer activity in this region. Overall, a PMVI ratio >0.6 was seen in 154 of 168 regions that had normal tracer uptake at rest or during dipyridamole stress. In comparison, only 3 of the 25 abnormal regions had >0.6 PMVI ratio during rest or stress. Therefore, the sensitivity and specificity of a PMVI ratio <0.6 in detecting an abnormal tracer uptake at rest or during dipyridamole stress were 92% and 84%, respectively.

Table 1 compares the ability of visual analysis and measurement of a PMVI ratio <0.6 in any view after intravenous PESDA to detect perfusion abnormalities in patients with either a rest or stress-induced tracer uptake abnormality <70%. A PMVI ratio <0.6 was seen in 22 of the 25 regions with either a thallium or sestamibi uptake <70%. In comparison, a visually evident myocardial contrast abnormality was identified by at least one of the reviewers in only 12 of the 25 regions with tracer uptake abnormalities (Table 1).

On a patient by patient basis, there was good correlation between regional thallium uptake (anteroseptal, lateral, inferoposterior and apical regions) and regional PMVI at rest (mean r value 0.84, range 0.53 to 0.97). The correlation between regional sestamibi uptake and regional PMVI during dipyridamole stress was also good, with a mean correlation coefficient of 0.88 (range 0.77 to 0.99). Figure 4 demonstrates an inferoposterior defect during dipyridamole stress seen in a secondary view (parasternal long axis) that was visually and quantifiably evident only with one frame every multiple cardiac cycles. Figure 5 illustrates an anteroseptal contrast defect seen in the apical long-axis image using an extended time interval between frame rates. This was the tertiary view both at rest and during dipyridamole stress in this patient. The secondary or tertiary view was required to either detect or confirm the perfusion abnormality in 15 contrast defects (4 during rest, 11 during stress).

The interobserver variability for measurements of background-subtracted PMVI was 18.8%, with a correlation coefficient between the two observers of 0.91 (SE 12 U). Intraobserver variability for PMVI measurements was 14%, with a correlation coefficient between the two measurements of 0.96 (SE 8 U). The injection to injection variability in PMVI measurements was 13%, with a correlation between the PMVI measurements from two separate injections of 0.97 (SE 7 U). These are all well below the 32% difference in PMVI from baseline to peak dipyridamole stress in normal regions and the 59% difference in PMVI that we observed between normal and abnormal regions during dipyridamole stress. The percent agreement between the two independent observers on whether myocardial contrast enhancement was visually normal or abnormal in the 25 patients undergoing rest and stress contrast studies was 93%.

Discussion

This study demonstrated two important advances in the noninvasive assessment of myocardial perfusion with intravenous ultrasound contrast: 1) We could detect myocardial contrast enhancement in multiple views with one intravenous

Table 1. Comparison of Nuclear Imaging and Transient Response Imaging in Detecting Perfusion Abnormalities*

				Quantitative			Visual		
Pt No./ Gender	Age (yr)	ND Location	Timing	PMVI Ratio <0.6	Location	Timing	Visual MCD	Location	Timing
1/M	63	IP	Stress only	Yes	IP	Stress only	Yes	IP	Stress only
2/M	65	AS	Stress only	Yes	AS	Stress only	No	—	—
		IP	Stress only	No	—		No	—	—
3/M	53	IP	R+S	Yes	IP	R+S	Yes	IP	R+S
4/M	58	AS	Stress only	Yes	AS	Stress only	Yes	AS/apex	R+S
5/F	51	AS	Stress only	Yes	AS/apex	R+S	Yes	Apex only	R+S
		Lat	R+S	Yes	Lat	R+S	No	—	—
6/M	73	IP	Rest only	Yes	IP	Rest only	No	—	—
7/M	73	IP	R+S	Yes	IP	R+S	Yes	IP	R+S
8/M	71	IP	R+S	Yes	IP	R+S	No	—	—
9/F	68	Apex	Stress only	Yes	Apex	Stress only	No	—	
10/M	78	Lat	Stress only	Yes	Lat	Stress only	No	—	
		IP	Stress only	Yes	IP	Stress only	Yes	IP	R+S
11/M	79	IP	Stress only	No	—	—	No	IP	
12/M	64	IP	R+S	Yes	IP	R+S	No	—	
13/F	62	IP	Stress only	Yes	IP	R+S	Yes	IP	R+S
14/M	55	AS/apex	Rest only	Yes	AS	Rest only	Yes	AS	Rest only
		Apex	Rest only	Yes	Apex	Rest only	Yes	Apex	Rest only
		IP	Rest only	No	—	—	Yes	IP	Rest only
15/M	54	IP	Rest only	Yes	IP/Lat	Rest only	Yes	IP	Rest only

*A total of 25 nuclear perfusion defects were identified (10 rest, 15 stress). AS = anteroseptum; F = female; IP = inferoposterior; Lat = lateral; M = male; MCD = myocardial contrast defect; ND = nuclear defect; PMVI= peak myocardial videointensity with transient response imaging after intravenous perfluorocarbon-exposed sonicated dextrose albumin; Pt = patient; R+S = rest and dipyridamole stress; — = no defect by this method.

injection of PESDA in the majority of patients when using harmonic transient response imaging with an extended time interval between frame rates. 2) We observed close agreement between quantitative thallium and sestamibi uptake and regional PMVI measured with transient response imaging at rest and during dipyridamole stress. The sensitivity of PMVI measurements was better than visual analysis in differentiating regions with normal and abnormal tracer uptake. These two findings demonstrate the potential of harmonic transient response imaging for the noninvasive detection of myocardial perfusion abnormalities in humans in a wide variety of clinical settings.

Optimizing detection of myocardial contrast with transient response imaging. Visually evident myocardial contrast was produced with transient response imaging in secondary and tertiary views in the majority of patients by prolonging the time interval between frame rates. The reason for this prolonged myocardial contrast can be attributed to two factors: 1) prolonged pulmonary "hangup" of PESDA microbubbles, creating a situation in which a continuous infusion of microbubbles into the left ventricular cavity occurs; and 2) the ability of transient response imaging to still detect these smaller concentrations of fluorocarbon-containing microbubbles as they pass through the myocardial microcirculation.

The lungs appear to act as a filter or "sieve" when large concentrations of microbubbles enter the pulmonary circulation (8,9). They not only prevent the passage of bubbles that are >8 μm from reaching the left atrium, but also delay the passage of smaller microbubbles (8). This filtering mechanism may transiently become defective with the initial bolus of microbubbles, briefly allowing a larger volume of bubbles into the left ventricular cavity (10). However, after this initial bolus effect, the remaining microbubbles transit at a slower rate, sometimes taking up to an entire circulation time to pass through the pulmonary circuit (8). This prolonged left ventricular cavity contrast was most likely not due to recirculation because of its continuous nature. With recirculation, one would expect a disappearance of left ventricular cavity contrast and reappearance. Furthermore, no reentry of microbubbles into the right ventricular cavity was ever observed.

Reducing the ultrasound frame rate or pulse repetition frequency has been shown (11,12) to prevent the destruction of PESDA microbubbles. When triggering at one frame every cardiac cycle, we observed a significant increase in PMVI in normally perfused regions immediately after injection that would not have been observed with a more rapid frame rate (13). This increase corresponded to the initial bolus of microbubbles reaching the left ventricular cavity from the pulmonary circulation. Although the concentration of microbubbles reaching the left ventricular cavity after this initial bolus began decreasing as the lung regained its filtering capabilities, the ratio of microbubbles reaching each perfusion bed did not change. Because a more prolonged time interval between frame rates should destroy even fewer microbubbles than one frame every cardiac cycle, the end result was an equivalent degree of myocardial contrast enhancement in the secondary and tertiary views comparable to that seen with the initial bolus

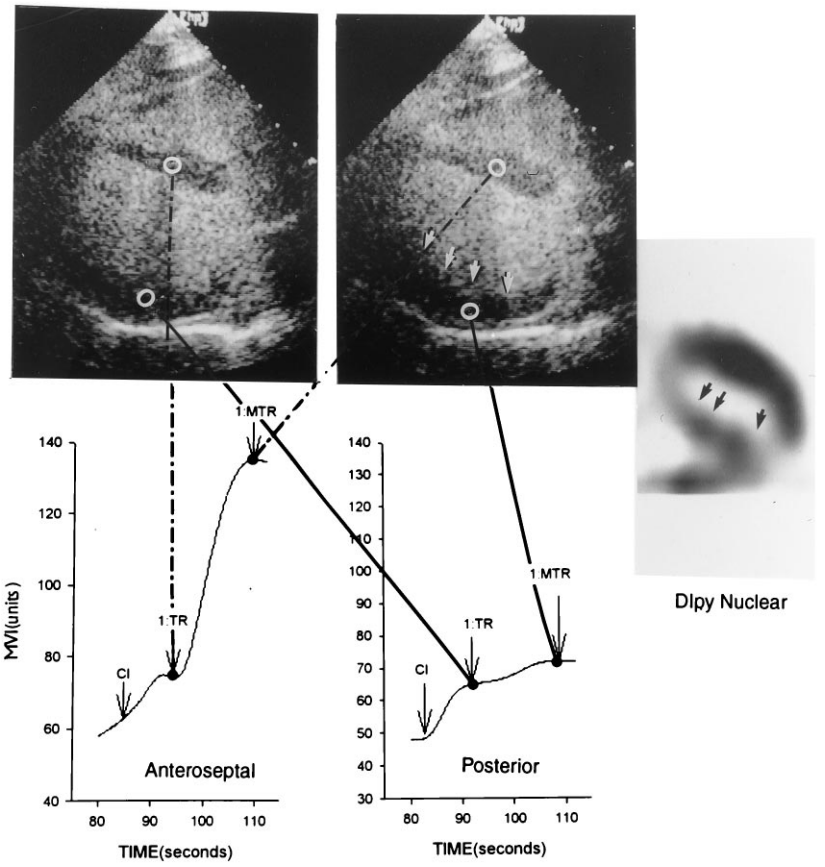

Figure 4. Example of a contrast defect in a secondary view (parasternal long axis) during dipyridamole stress in a patient who also exhibited quantitatively abnormal nuclear study results (Dipy Nuclear **image**). As can be seen, this defect was visually more evident when using an extended time interval between frame rates **(right panel)** because of lack of contrast enhancement in the posterior wall **(arrows)** when switching from triggering ultrasound frame rates to one point every cardiac cycle (1:TR) to once every multiple cardiac cycles (1:MTR). CI = conventional imaging; MVI = myocardial videointensity.

effect when perfusion was normal. This is exemplified in the normal regions shown graphically in Figures 4 and 5.

This increase in myocardial contrast when switching to one frame every multiple cardiac cycles did not occur in regions that had abnormal perfusion by dual-isotope imaging. In the 15 of the 25 quantitatively abnormal regions by dual-isotope imaging, the myocardial contrast defects were either visualized better or quantified in a secondary or tertiary view using this technique. The effect of extended time intervals between frame rates in quantifying these perfusion abnormalities is evident in the plots of PMVI versus time using different frame rates in Figures 4 and 5.

Measuring the peak myocardial contrast enhancement in each view was more sensitive than visual analysis in detecting tracer uptake abnormalities for at least two reasons: 1) we used only a gray scale to visually assess contrast enhancement. The human eye is incapable of accurately differentiating different shades of gray. Color-coded images have been shown (14) to produce more visually apparent regional differences in myocardial contrast enhancement, especially when using a map that reflects the color changes that occur in a progressively heated object. 2) Visual detection of myocardial contrast defects may have been improved had we used digital subtraction of the precontrast images, as has been done elsewhere (14). Since we could do this when measuring PMVI in both primary and secondary or tertiary views (by subtracting background videointensity), a more subtle difference in regional contrast enhancement was apparent with the quantitative analysis.

Differences in regional PMVI and regional tracer uptake during dipyridamole stress. PMVI changes during any intervention have been shown (15) to reflect changes in myocardial blood volume. However, changes in thallium or sestamibi uptake correlate with changes in myocardial blood flow. Myocardial blood volume changes correlate with flow changes only when autoregulation is preserved. During vasodilator stress, autoregulation may be abolished, leading to situations where flow changes may exceed volume changes (14). In this setting, the ratio of PMVI between a stenotic zone and a normally perfused zone has still been shown (16,17) to correlate with myocardial blood flow changes measured with radioactive microspheres. In our patients, we also observed good agreement between the PMVI ratios and regional thallium uptake at rest and during maximal vasodilator stress. Furthermore, there was good direct correlation in individual patients between PMVI obtained in the four different regions and tracer uptake in these same regions, both at rest and during vasodilator stress.

Limitations of the study. Although extended time intervals between frame rates produced prolonged myocardial contrast after very low doses of intravenous PESDA in our patients, it may be impractical in some patients to hold a transducer in one

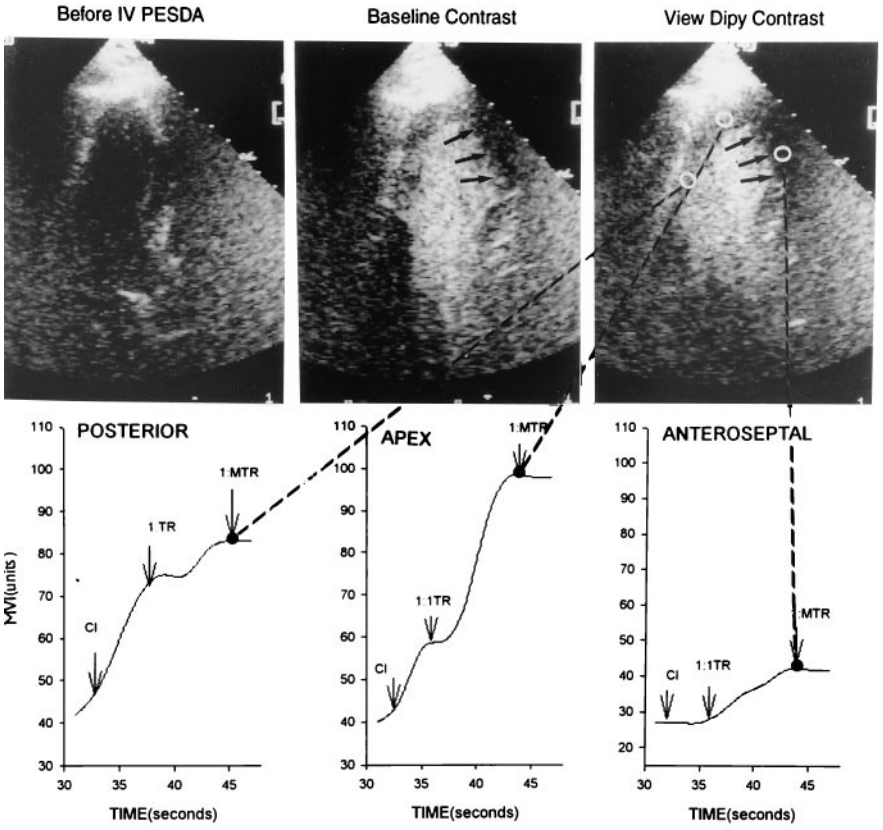

Figure 5. Apical long-axis view (tertiary view) in a patient who had both visual and quantitative evidence of an anteroseptal contrast defect **(arrows)** at rest and during dipyridamole (Dipy) stress when an extended time interval between frame rates was used. Note the abnormal enhancement in myocardial videointensity in the anteroseptal region when switching triggering ultrasound frame rates to one point every multiple cardiac cycles compared with that in the apical and posterior regions. However, the nuclear defect in this region was abnormal only during stress. Abbreviations as in as Figure 4.

position for this period of time. Another limitation with triggering frame rates to produce better myocardial contrast is that wall motion throughout the cardiac cycle cannot be observed while assessing perfusion. Modifications in transducer design may be possible that would improve microbubble survival and hence produce increased contrast without requiring the long time interval between frame rates. This may involve altering the pulse duration as well as acoustic output because both affect how a given ultrasound pulse interacts with microbubbles (18). Another method that would increase the number of views in which myocardial contrast could be observed with triggered imaging would be to give repeated smaller boluses of contrast intravenously when switching to different views. The bolus effect of intravenous contrast that was typically seen in the primary view after injection in our study could then be observed in other views as well. However, repeated boluses may increase the degree of attenuation of more distal regions in the basal and inferoposterior myocardium.

Attenuation due to left ventricular cavity contrast may make it difficult to distinguish between truly abnormal perfusion and attenuation in the inferior and posterior regions even with triggered imaging and low doses of intravenous contrast. However, the present study emphasizes that the best way to overcome this problem is by using extended time intervals between frame rates after the initial bolus of contrast agent in the left ventricular cavity. Even with a very small intravenous injection of PESDA, there is some attenuation of more distal myocardium with the initial bolus entry of contrast agent into the left ventricular cavity. However, once this bolus has resolved, we found that failure of inferoposterior myocardium to enhance with one frame every 10 cardiac cycles (as demonstrated in Fig. 4) was a much more reliable way of detecting abnormal perfusion compared with dual-isotope imaging. The normal posterior myocardium enhances with this technique, as demonstrated in Figure 5.

Clinical implications. To our knowledge, this is the first study to demonstrate that rest and stress-induced myocardial perfusion abnormalities can be detected using transient response imaging in multiple views with one dose of intravenous microbubbles in humans. This study also demonstrated that regional PMVI produced with transient response imaging both at rest and during dipyridamole stress correlates closely with regional rest thallium and dipyridamole sestamibi uptake. Because triggered imaging can be rapidly performed at the patient's bedside at rest and during stress, transient response imaging may be a cost-effective and accurate method of detecting perfusion abnormalities in humans. This imaging technique should be applicable to other microbubbles as well, especially the fluorocarbon-encapsulated microbubbles (19–21). Larger studies are now needed with these different agents to evaluate the incremental benefit of this technique over currently available ultrasound techniques, such as wall motion analysis, in improving the detection and quantification of myocardial ischemia.

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