Background—Echocardiographic contrast media have been used to assess myocardial perfusion and to enhance endocardial definition for improved assessment of left ventricular (LV) function. These methodologies, however, have been qualitative or have required extensive offline image analysis. Power modulation is a recently developed imaging technique that provides selective enhancement of microbubble-generated reflections. Our goal was to test the feasibility of using power modulation for combined quantitative assessment of myocardial perfusion and regional LV function in an animal model of acute ischemia.

Methods and Results—Coronary balloon occlusions were performed in 18 anesthetized pigs. Transthoracic power modulation images (Agilent 5500) were obtained during continuous intravenous infusion of the contrast agent Definity (DuPont) at baseline and during brief coronary occlusion and reperfusion and were analyzed with custom software. At each phase, myocardial perfusion was assessed by calculation, in 6 myocardial regions of interest, of mean pixel intensity and the rate of contrast replenishment after high-power ultrasound impulses. LV function was assessed by calculation of regional fractional area change from semiautomatically detected endocardial borders. All ischemic episodes caused detectable and reversible changes in perfusion and function. Perfusion defects, validated with fluorescent microspheres, were visualized in real time and confirmed by a significant decrease in pixel intensity in the left anterior descending coronary artery territory after balloon inflation and reduced rate of contrast replenishment. Fractional area change decreased significantly in ischemic segments and was restored with reperfusion.

Conclusions—Power modulation allows simultaneous online assessment of myocardial perfusion and regional LV wall motion, which may improve the echocardiographic diagnosis of myocardial ischemia. (Circulation. 2001;104:352-357.)

Key Words: ultrasonics imaging echocardiography ischemia perfusion ventricles

Although the potential use of ultrasound contrast media to assess myocardial perfusion has been under investigation for more than a decade,1–8 the mainstream implementation of echocardiographic contrast remains left ventricular (LV) opacification, which is used in patients with poorly visualized endocardium to improve the assessment of LV function.9 Despite creative approaches and promising results, quantification or even imaging of myocardial perfusion has required extensive offline analysis,10 remaining a goal of future research,4,6,11,12 and the assessment of LV function with contrast remains subjective. No established technique for automated endocardial border detection and quantification of either global or regional LV function is applicable to contrast-enhanced images. We hypothesized that a new technique, referred to as power modulation, would allow real-time visualization of perfusion defects and automated endocardial border detection. Our goal was to test the feasibility of combined quantitative assessment of myocardial perfusion and regional LV function in an animal model by directly correlating acute ischemia with changes in images that may indicate altered perfusion and compromised LV function. Specifically, we sought to determine (1) whether ischemia would cause variations in quantitative indices of myocardial perfusion and (2) whether contrast-enhanced power modulation images could be used for automated endocardial border detection and objective assessment of ischemic changes in regional LV function.

Methods

Power Modulation: Principles of Operation

The basic assumption underlying power modulation imaging (PMI) is that the reflective properties of cardiac structures, unlike those of
microbubbles, are mostly linear. Power modulation uses this assumption by transmitting repeated pulses of different intensities in the same direction. Two consecutive pulses of identical shape but 2-fold difference in amplitude would result in identical reflections from the heart, other than the expected 2-fold difference in amplitude. The smaller pulse is then multiplied by 2 and subtracted from the larger one, resulting in a zero signal (Figure 1, top). The same 2 pulses, when reflected by the nonlinear microbubbles, would differ from each other not only in amplitude but also in their shape. Amplifying the smaller pulse and subtracting it from the larger one would result in a nonzero signal (Figure 1, bottom). The amplitude of this signal is color-coded and displayed in an overlay over the gray-scale image. Thus, PMI uses the differences in acoustic properties to selectively enhance microbubble-generated reflections while suppressing reflections from cardiac structures and tissues.

Animal Preparation
Experiments were carried out in 18 male farm pigs (20 to 30 kg). Animals were pretreated with telazol (2.2 mg/kg IM) and atropine sulfate (0.05 mg/kg IM). After intubation, pigs were mechanically ventilated (Drager) at 13 to 20 strokes per minute, with a tidal volume of 12 to 18 mL/kg, and anesthetized with isoflurane (0.5% to 2.5% mixed with oxygen). Animals were restrained in a supine position. ECG, body temperature, noninvasive blood pressure, and expiratory gases were monitored with a Cardiocap monitoring system (Datex). Lidocaine was administered as a bolus (1 mg/kg IV) and then continuously infused (4 mg · kg⁻¹ · h⁻¹) to prevent ventricular arrhythmias.

An intracoronary balloon catheter (2.5 to 3.5-mm balloon diameter; Guidant) was introduced via the right femoral artery into the left anterior descending coronary artery (LAD) under fluoroscopic guidance. The balloon was positioned near the origin of the artery to maximize the perfusion territory affected by balloon inflations. In a subset of 5 animals, a custom-made 7F retractable hollow-lumen catheter was introduced via the left femoral artery and placed in the left atrium for microsphere injections.

Imaging
Transthoracic images were obtained with an S3 transducer (SONOS 5500, Agilent Technologies). Parasternal short-axis views were obtained at the level of the papillary muscles. Power modulation was activated at mechanical indices of 0.1 to 0.2, gain 65 to 75, pulse repetition frequency 3.7, and low line density. Frame rate was maximized by minimizing the depth and narrowing the sector. Contrast enhancement was achieved with intravenous infusion of Definity (DuPont, 4 mL in 50 mL normal saline). Infusion rates were optimized for dense opacification of the LV cavity without attenuation and with clearly visualized intramyocardial contrast (typically 5 mL/min).

Protocol
Part 1 of the protocol was designed to track ischemic changes in myocardial perfusion. To prevent cardiac translation, the ventilator was stopped at end expiration during image acquisition. PMI images triggered every end systole were acquired over a period of 60 seconds, including 15 seconds baseline, 30 seconds complete coronary occlusion, and 15 seconds reperfusion. During each phase, a high-energy ultrasound pulse (25 frames, mechanical index 1.7) was transmitted to image contrast destruction and subsequent replenishment (Figure 2, top).

In a subset of 5 animals, 5 million NuFlow fluorescent microspheres (Interactive Medical Technologies) were injected into the left atrium over 30 seconds, followed by a 30-second flush with 10 mL saline. Reference blood was collected from the femoral artery at 5 mL/min over a period of 2 minutes starting 5 seconds before microsphere injection. This procedure was repeated during near-complete coronary occlusion and subsequent reperfusion using microspheres with different emission spectra to allow independent tissue blood flow measurements.
Part 2 of the protocol was aimed at quantifying changes in regional wall motion. Images were acquired continuously over 3 cardiac cycles under control conditions, 30 seconds after coronary occlusion, and again after balloon deflation and were stored on magneto-optical disk. This part of the protocol was performed in 13 experiments in which microspheres were not used.

At the end of the experiments, a lethal dose of pentobarbital sodium (120 mg/kg IV) was given. In the 5 animals injected with microspheres, the heart was excised, and the LV myocardium was cut into horizontal slices 1 cm thick. The slice containing the middle portion of the papillary muscles was divided into 6 wedge-shaped segments, which were used to measure tissue blood flow on the basis of flow-cytometric analysis by a specialized laboratory.

Image Analysis
Images were analyzed with custom-designed software. Initially, 6 myocardial regions of interest were manually drawn (Figure 2, bottom, right). In each region, mean pixel intensity was measured frame-by-frame throughout the entire image sequence. The position of each region was manually adjusted when necessary to compensate for translation. For each region, mean pixel intensity was plotted over time. To represent regional pixel intensity at a steady state during the baseline, ischemia, and reperfusion phases, 6 consecutive heartbeats were averaged, starting 5 beats after each high-energy pulse. The slope of the linear regression of the first 5 beats after each pulse was used as an index of contrast replenishment rate. To normalize for possible changes in heart rate, the slopes were expressed in gray scale units/s.

To evaluate regional LV function, the endocardial border was first detected frame-by-frame throughout the cardiac cycle by thresholding PMI intensity. A binary image was created with a depth-dependent threshold and further processed with standard morphological operators to extract the LV cavity. To verify the endocardial boundary position and adjust thresholding parameters when necessary, the resulting contour was superimposed on the original images. The LV was then divided into six 60° sectors, corresponding to the regions of interest used to assess perfusion (Figure 3). For each segment, cavity area was measured frame-by-frame during each experimental phase, expressed in percent of regional end-diastolic area, and plotted over time, and regional fractional area change was calculated (Figure 3, right).

Statistical Analysis
Regional PMI intensities, contrast replenishment rates, and fractional area changes, normalized by their control values, were averaged for all animals during baseline, coronary occlusion, and reperfusion. Data were displayed as mean±SD. Two-way ANOVA with repeated measures was used to test the differences between phases for all segments. Changes in tissue blood flow caused by ischemia and reperfusion and measured with microspheres were tested with t tests. Differences were considered significant for P<0.05 compared with control conditions.

Results
The effects of a high-energy ultrasound pulse on PMI images are depicted in Figure 2 (top). The pulse resulted in a saturated image and in destruction of microbubble reflective properties, which were then gradually restored to baseline level. The destructive effects of high-energy pulses were found to depend on imaging settings (primarily pulse intensity and duration), which at times required stepwise adjustments. The effects of coronary occlusion and reperfusion on the intramyocardial PMI intensity are shown in Figure 2.
The different experimental phases. Both perfusion indices, ie, perfusion and function indices obtained from all animals during ischemia. Measurements showed a slight increase in fractional area changes reperfusion. In this example, the inferior and posterior segments, coronary occlusion resulted in an increase in cycle under control conditions and during coronary occlusion. Regional fractional area curves obtained throughout 1 cardiac period followed similar patterns in all animals (Figure 4). Note decrease in amplitude of time curves during coronary occlusion in LAD-related segments (top) vs nonischemic segments (bottom).

In all animals, the loss of pixel intensity in the LAD territory during coronary occlusion was followed by an increase during reperfusion. No concurrent changes were noted outside the LAD territory.

PMI intensity measured in the myocardium in part 1 of the protocol followed similar patterns in all animals (Figure 4). The 3 sharp peaks in each curve reflect the high-energy pulses that caused image saturation (Figure 2, top). The intensity drop after each peak reflects the loss of microbubble reflectivity. Contrast replenishment rate is reflected by the slope of intensity increase after the pulse. Balloon inflation caused a gradual decrease in pixel intensity in the LAD territory, reflecting lack of blood flow, where little or no replenishment was noted after the second pulse. Balloon deflation resulted in rapid contrast replenishment, confirming the reversal of the perfusion defect. The third pulse demonstrated rapid contrast replenishment consistent with reperfusion. In contrast, inferior, posterior, and lateral segments were not affected by coronary occlusion.

In all animals, coronary occlusions resulted in visible wall-motion abnormalities in the LAD territory, which were reversed during reperfusion. Endocardial border detection worked accurately throughout the cardiac cycle in 11 of 13 animals (Figure 3), which were included in statistical analysis. In the remaining 2 animals, it was difficult to consistently differentiate between the LV cavity and the lateral wall. Regional fractional area curves obtained throughout 1 cardiac cycle under control conditions and during coronary occlusion and reperfusion are shown in Figure 5. In the LAD-related segments, coronary occlusion resulted in an increase in end-systolic fractional area, with a consequent decrease in fractional area changes. These indices were restored during reperfusion. In this example, the inferior and posterior segments showed a slight increase in fractional area changes during ischemia.

Figure 6 presents the summary of control-normalized perfusion and function indices obtained from all animals at the different experimental phases. Both perfusion indices, ie, pixel intensity and contrast replenishment rate, decreased significantly as a result of coronary occlusion in the anterior and anteroseptal segments, whereas other segments showed no significant changes. Reperfusion caused an increase in these indices to levels higher than those of the corresponding controls in all segments. These ischemic changes in perfusion coincided with a significant decrease in regional fractional area change in the anterior and anteroseptal regions, with no significant evidence of hyperdynamic motion during reperfusion.

Microsphere measurements confirmed a significant occlusion-induced decrease in tissue blood flow in the anterior and anteroseptal segments, followed by a rebound increase above the baseline levels in the anterior, anteroseptal, septal, and lateral segments during reperfusion (Figure 7), in agreement with the echocardiographic indices (Figure 6).

Discussion

Although echocardiography is one of the major noninvasive tools used in the diagnosis of coronary artery disease, the detection of wall motion abnormalities is based on subjective visual interpretation.14,15 The sensitivity of this methodology is limited, because even severe stenosis may not cause a wall motion abnormality at rest. Accordingly, there is a strong need for techniques capable of extracting more information from ultrasound images that may provide additional insights into myocardial physiology.

It is widely accepted that the ability to assess myocardial perfusion would be diagnostically invaluable.1,7 Contrast echocardiography is increasingly referred to as a technique that could potentially allow quantification4–6 or at least imaging of myocardial perfusion.2,3,8,16,17 This goal, however, still remains a great challenge.18 Although LV opacification for improved endocardial visualization is better established than perfusion imaging, it is qualitative, because there is no technique to automatically detect the endocardial border from contrast-enhanced images. Recent relevant technological developments include pulse inversion and power modulation, which use multiple pulses combined with real-time manipulation of the reflections to improve the visualization of ultrasound contrast.19 Our goal was to test the feasibility of using PMI for combined quantitative assessment of regional myocardial perfusion and LV function.20,21

Study Design

Contrast-enhanced PMI images may be prone to artifacts, including false-positive perfusion defects.22 Therefore, a clear connection had to be established between ischemia and regional changes in PMI images, presumably reflecting abnormal perfusion. We used a pig model, in which regional myocardial perfusion and LV function were assessed during acute myocardial ischemia. To minimize the effects of changes in gain settings and/or imaging plane, all perfusion data were obtained in a single image sequence that included control conditions, ischemia, and reperfusion. To minimize bubble destruction and allow the uniform LV cavity opacification essential for border detection, low mechanical indices were used. Images were obtained digitally to avoid loss of data quality inherent to analog recording and to allow the...
analysis of PMI color overlays separately from the underlying gray-scale information. Microspheres were used to confirm the changes in tissue blood flow occurring in response to coronary occlusion and reperfusion.

**Analysis Techniques**

Power modulation images were analyzed with custom software to quantify changes in both myocardial perfusion and regional function by several indices. Perfusion was quantified with mean pixel intensity and contrast replenishment rate. Although the former parameter has been used previously, the latter had required repeated image acquisitions at variable pulsing intervals, which made both data acquisition and analysis complicated and time-consuming. In contrast, the use of PMI with high-energy ultrasound pulses allowed this index to be measured from a single image acquisition.

Our preliminary studies showed that contrast-enhanced PMI images provided more uniform LV cavity enhancement than gray-scale imaging. This observation led us to the assumption that PMI could be used for automated endocardial border detection, which was achieved by applying standard border-detection techniques. Although the current version of our analysis software is semiautomated and somewhat tedious (5 minutes per cardiac cycle), future implementations may be optimized to meet acceptable clinical standards.

**Interpretation of Results**

We found that acute ischemia resulted in reproducible, gradual, and reversible perfusion defects and reproducible and reversible wall motion abnormalities that were visualized by PMI and confirmed quantitatively. In addition to control data obtained from ischemic segments at baseline, perfusion territories of other coronary arteries provided a simultaneously obtained reference for comparison. The comparisons with these controls and microsphere data established the connection between ischemia and changes in contrast-enhanced PMI images.

Data obtained from regions of interest bordering the LAD territory showed a trend toward a decrease in some perfusion and function indices during coronary occlusion. This could be explained by the fact that the manually traced regions might have partially overlapped with the LAD perfusion territory. The increase in perfusion indices after balloon deflation is consistent with the well-documented reperfusion hyperemia. A trend of increased function in the inferior and posterior walls during coronary occlusion may reflect ischemia-induced cardiac translation and tethering.

**Limitations**

The major limitation of PMI is its low temporal resolution (~15 frames/s in this study), an intrinsic feature of this technique, which relies on transmitting repeated pulses and real-time processing to create a single scan line. Like other ultrasound imaging techniques, PMI may be prone to pitfalls and artifacts, the extent of which cannot be determined on the basis of the limited data in this feasibility study. Another methodological limitation is the need for initial adjustment of gain settings. Although the assessment of perfusion and function in this study was not truly simultaneous, simple software modifications could resolve this issue.

Microsphere measurements of tissue blood flow were performed in a subset of 5 animals during near-complete coronary occlusions. Microspheres were not used in all animals to demonstrate the lack of perfusion in the territory of a fully occluded coronary artery because of their high cost and because the absence of significant collaterals in pigs is well established. In all pigs, however, the lack of LAD flow was confirmed by angiography.

A major limitation of a closed-chest pig model is that it does not allow imaging from the apical views, which provide important information in humans. Therefore, the accuracy of the ischemic markers described here remains to be determined in future clinical studies in all standard views. Also, the ability to detect ischemia induced by partial coronary occlusions needs to be tested. Nevertheless, our findings reinforce the basis for the diagnostic use of this technique.
Summary
This experimental study was designed to investigate the effects of acute myocardial ischemia on contrast-enhanced power modulation images of the LV. This goal was achieved by acquiring and analyzing data in pigs undergoing coronary occlusion. Our findings demonstrated that ischemia results in noninvasively detectable and quantifiable changes in myocardial perfusion and regional LV function. Therefore, current echocardiographic techniques provide the basis for online, objective, and simultaneous assessment of regional myocardial perfusion and wall motion. The availability of this information promises to improve the accuracy of echocardiographic diagnosis of coronary artery disease.

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