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Imaging and Quantification of Myocardial Perfusion Using Real-Time Three-Dimensional Echocardiography

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OBJECTIVES We tested the feasibility of real-time three-dimensional echocardiographic (RT3DE) perfusion imaging and developed and validated an algorithm for volumetric analysis of myocardial contrast inflow. The study included three protocols wherein perfusion was measured: 1) in an ex-vivo model of controlled global coronary flow, 2) in an in-vivo model during regional perfusion variations, and 3) in humans during pharmacologically induced hyperemia.

BACKGROUND The RT3DE technology offers an opportunity for myocardial perfusion imaging without multi-slice reconstruction and repeated contrast maneuvers.

METHODS Electrocardiographically triggered harmonic RT3DE datasets were acquired (Philips 7500) while infusion of Definity was initiated and reached a steady state. Protocol 1 was performed in nine isolated rabbit hearts and included three coronary flow levels. In protocol 2, changes in regional perfusion caused by partial left anterior descending artery occlusion were measured in five pigs. In protocol 3, adenosine-induced changes in perfusion were measured in eight normal volunteers. Myocardial video-intensity (MVI) was measured over time in three-dimensional (3D) slices to calculate peak contrast inflow rate (PCIR). In pigs, PCIR was measured on a regional basis and validated against microspheres.

RESULTS The RT3DE imaging allowed selection of slices for perfusion analysis in rabbit hearts, pigs, and humans. Administration of contrast resulted in clearly visible and quantifiable changes in MVI. In rabbits, The PCIR progressively decreased with coronary flow ($p < 0.0001$). In pigs, coronary occlusion caused a 59 ± 26% decrease in PCIR exclusively in the left anterior descending artery territory ($p < 0.05$) in agreement with microspheres. In humans, adenosine increased PCIR to 198 ± 57% of baseline ($p < 0.05$).

CONCLUSIONS Contrast-enhanced RT3DE imaging provides the basis for volumetric imaging and quantification of myocardial perfusion. (J Am Coll Cardiol 2006;47:146–54) © 2006 by the American College of Cardiology Foundation

Multiple studies have shown the feasibility of contrast-enhanced, two-dimensional (2D) echocardiography for imaging myocardial perfusion (1). This methodology is limited because the extent of a perfusion defect cannot be accurately assessed from a single slice of the heart. Until recently, three-dimensional (3D) imaging had been mainly based on consecutive acquisition of multiple planes followed by off-line volume reconstruction (2,3). Furthermore, quantification of myocardial perfusion requires dynamic changes in myocardial contrast, such as boluses of contrast media (4,5). However, the use of contrast boluses is confounded by the need to guess a priori the imaging settings for optimal visualization of the short-lived enhancement. Microbubble destruction with high-energy ultrasound pulses during contrast infusion (6–8) has been used as an alternative maneuver with 2D imaging. Volumetric assessment of perfusion from 3D reconstructed data would require these maneuvers to be repeated for each imaging plane, which is impractical for clinical use.

The recently developed real-time three-dimensional echocardiographic (RT3DE) imaging technology offers an opportunity for online volumetric perfusion imaging without the need for volume reconstruction and repeated contrast maneuvers. Previous investigators reported visualizing perfusion defects as dark areas in contrast-enhanced RT3DE datasets (9). However, such use of reduced video-intensity as the sole indicator of a perfusion abnormality can be hindered by drop-out artifacts that are commonly seen with contrast and are even more difficult to explain in 3D. The optimal contrast maneuver for perfusion quantification from RT3DE datasets has yet to be determined. Although boluses are likely to suffer the same known limitations in 3D use, the alternative of using high-energy pulses is unresolved with RT3DE technology and may not become available because of the excessive energy required for microbubble destruction in the entire heart. Therefore, there is a need for an alternative contrast maneuver that would allow time to optimize imaging settings and that could be safely used to quantify perfusion from RT3DE images.

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Abbreviations and Acronyms

2D = two-dimensional
3D = three-dimensional
BL = baseline
LAD = left anterior descending
LV = left ventricular
MVI = myocardial video-intensity
PCIR = peak contrast inflow rate
RT3DE = real-time three-dimensional echocardiographic
TCI = transient contrast inflow

We hypothesized that the current RT3DE technology would allow live volumetric perfusion imaging and that quantification could be achieved by tracking changes in myocardial contrast during the transition from no enhancement to steady-state enhancement. The goals of this study were to: 1) develop and validate an algorithm for quantitative volumetric assessment of myocardial perfusion based on analysis of transient contrast inflow (TCI); 2) test and validate this technique in vivo; and 3) test the applicability of this technique to human data. Accordingly, this study included three protocols. The goal of protocol 1 was to validate perfusion measurements against direct flow measurements during controlled changes in global coronary flow in an isolated heart preparation. Protocol 2 was designed to detect regional perfusion variations using transthoracic RT3DE imaging and provide validation against the microsphere gold standard in pigs undergoing partial coronary occlusion. Protocol 3 was aimed at testing the applicability of our approach in human subjects, which was achieved by measuring pharmacologically induced changes in myocardial perfusion in a group of normal subjects.

METHODS

Isolated heart preparation. Experiments were performed in nine male New Zealand White rabbits (2.2 to 2.8 kg). Animals were pre-anesthetized (xylazine, 5 mg/kg; glycopyrrolate, 0.01 mg/kg intramuscularly), anesthetized (ketamine, 45 mg/kg; acepromazine, 1 mg/kg intramuscularly), and mechanically ventilated. After mid-sternotomy, the heart was quickly removed and attached to a Langendorff apparatus, and perfused in a retrograde fashion via the aortic root with Krebs-Henseleit solution, continuously gassed with carbogen at 38°C. Global coronary flow was controlled using a mechanical regulator and continuously monitored using an ultrasonic in-line flowmeter. Perfusion pressure was kept constant at 86 mm Hg, and the heart was allowed to beat spontaneously. Left atrial tissue was dissected, and a latex balloon introduced into the left ventricular (LV) cavity via the mitral annulus and filled with fluid to allow physiologic pressures during isovolumic contractions. The LV pressure was measured continuously using a catheter-tip transducer. The heart was immersed in Krebs-Henseleit solution at 38°C in a plastic container with thin (<1 mm) walls.

Large animal preparation. Experiments were performed in five male farm pigs (20 to 28 kg). Animals were pre-treated with telazol (2.2 mg/kg, intramuscularly) and atropine sulfate (0.05 mg/kg, intramuscularly). Indomethacin (100 mg, per orogastric tube) was given to suppress allergic reactions. After intubation, pigs were mechanically ventilated (Drager, Drager Medical, Lubeck, Germany) and anesthetized with isoflurane (0.5% to 2.5% mixed with oxygen). Electrocardiogram, blood pressure, and expiratory gases were monitored (Cardiocap, Datex, Madison, Wisconsin). Lidocaine was administered as a bolus (1 mg/kg, intravenously), and then infused (4 mg/kg/h) to prevent arrhythmias. An intracoronary balloon catheter (2.0 to 2.5 mm in diameter) was positioned under fluoroscopic guidance near the origin of the left anterior descending (LAD) artery to maximize the affected perfusion territory. To ensure partial occlusion, distal coronary flow was visually assessed by intracoronary Renografin injections during brief balloon inflations, and balloon position adjusted if necessary. A 7-F catheter was placed in the left atrium for microsphere injections.

Human subjects. Eight normal volunteers (age 31 ± 6 years) with high-quality transthoracic images were studied in the left lateral decubitus position.

Ultrasound imaging. The RT3DE imaging was performed using a SONOS 7500 system (Philips Medical Systems, Andover, Massachusetts) equipped with a matrix-array transducer (X4) in the harmonic mode (1.6-MHz transmit frequency). The spatial aperture was set to be the widest available (58° × 29°) for triggered acquisition of series of end-systolic pyramidal datasets. Contrast enhancement was achieved by infusion of Definity (Bristol-Myers Squibb, North Billerica, Massachusetts) (1.3 ml in 25 ml saline). Infusion rate was determined by maximizing myocardial contrast without visible attenuation. To minimize bubble destruction, the minimal mechanical index necessary to visualize myocardial contrast was used (0.4 to 0.8).

The isolated heart was imaged through the container wall, with the transducer stabilized using a flexible arm. The pyramidal volume of acquisition contained the entire heart (Fig. 1A). Contrast solution was infused at 16 to 25 ml/h into the perfusion line just proximal to the heart. In pigs, imaging was performed from the left parasternal approach, resulting in volumetric short-axis datasets containing the mid portion of the left ventricle (Fig. 1B). Contrast solution was infused intravenously (150 to 260 ml/h). In humans, imaging was performed from apical windows, resulting in volumetric datasets containing mostly the lateral and septal walls and parts of the inferior and posterior walls near the base, whereas the more distal parts of these walls were not included (Fig. 1C). Contrast solution was infused intravenously (300 to 360 ml/h).

The TCI maneuver (Fig. 2) included: 1) optimization of contrast infusion rate and imaging settings during steady-

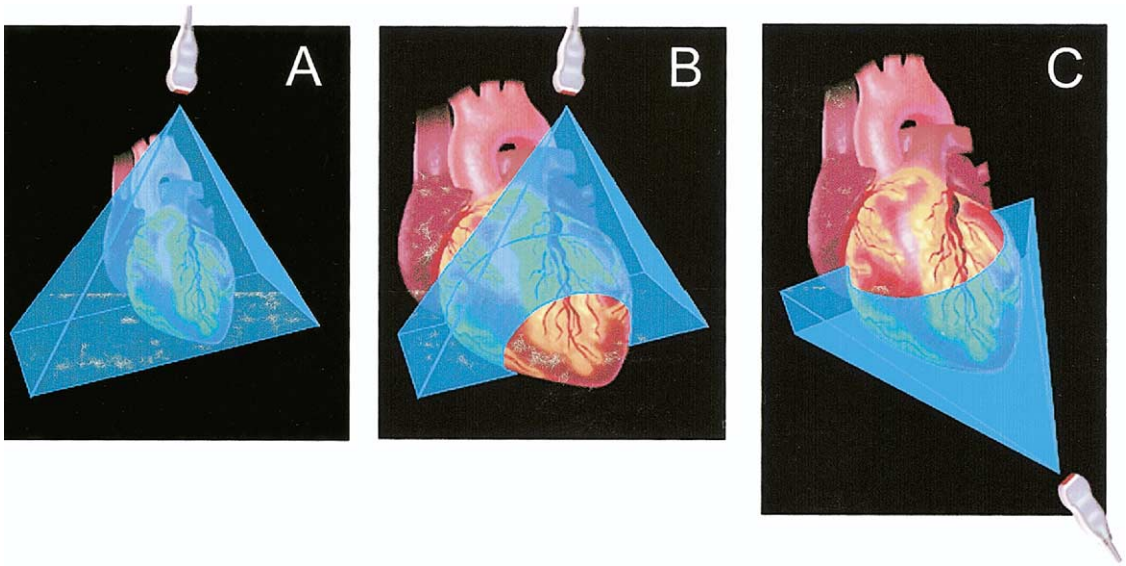

Figure 1. Schematic representation of volumetric acquisition in the three protocols: **(A)** isolated rabbit heart that fits entirely into the pyramidal scan volume; **(B)** volumetric parasternal short-axis imaging of the pig heart that allows acquisition of the mid portion of the left ventricle; **(C)** volumetric apical imaging of the human heart that allows acquisition of a partial left ventricular volume that includes mostly the septal and lateral walls.

state enhancement; 2) infusion interruption to allow contrast clearance, which was expedited by continuous 2D imaging at a high mechanical index; and 3) resumption of contrast infusion, resulting in contrast inflow. Image acquisition started approximately five seconds before the resumption of infusion to capture the entire transition from non-contrast to reinstated steady-state enhancement.

Protocol 1. In rabbit hearts, TCI maneuvers were initially performed under baseline (BL) conditions, and then at two levels of reduced coronary flow achieved by partially obstructing the perfusion line: 50% of BL flow (F1; 40% to 60% acceptable), and 15% of BL (F2; 10% to 20% acceptable). To minimize ischemic damage, flow reduction was limited to four minutes with at least five minutes of reperfusion. To assess the reproducibility of perfusion quantification, three consecutive sequences were performed at each experimental phase.

Protocol 2. In pigs, TCI maneuvers were initially performed under control conditions followed by reduced LAD artery flow and then reperfusion. Flow restriction was achieved by intracoronary balloon inflation immediately before image acquisition (Fig. 2) and lasted <4 min, allowing ultrasound imaging and microsphere measurements to be performed under stable conditions. The reperfusion sequence was initiated immediately after balloon deflation with image acquisition beginning one to two minutes after reflow. The respirator was stopped during image acquisition (<1 min).

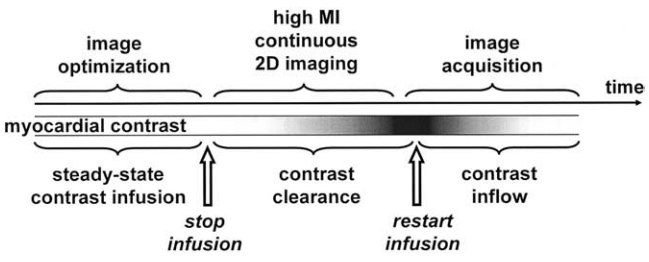

Figure 2. Schematic representation of the transient contrast inflow sequence. The upper portion of the diagram shows the time line of imaging steps that comprise the sequence. The bottom portion shows the timing of infusion interruption and resumption **(arrows)** and their effects on the myocardial contrast with the level of enhancement shown schematically in the **shaded band** below the time axis. 2D = two-dimensional; MI = mechanical index.

In three animals, NuFlow fluorescent microspheres (Interactive Medical Technologies, Irvine, California) were injected over 30 s, followed by a 30-s 10-ml saline flush. Reference blood was collected from the femoral artery at 5 ml/min over 2 min starting 5 s before microsphere injection. Microspheres with different emission spectra were used at each experimental phase.

At the end of experiment, a lethal dose of pentobarbital sodium (120 mg/kg, intravenously) was given. In the three animals injected with microspheres, the heart was excised and the LV myocardium was cut into 1-cm-thick horizontal slices. The slice containing the mid-ventricular portion was divided into six wedge-shaped segments and sent to a specialized laboratory for flow-cytometry analysis of tissue blood flow.

Protocol 3. In human subjects, a TCI maneuver was initially performed at rest, followed by a repeated sequence during infusion of adenosine (0.142 ml/kg/min). In the stress TCI sequence, adenosine infusion was initiated shortly after infusion interruption. A set of images was acquired two minutes later (Fig. 2). Image acquisition was performed during breath-hold. The total dose of Definity was limited to a single 1.3-ml vial.

Image analysis. Images were analyzed using custom software. Initially, each pyramidal dataset was reviewed to allow selection of parallel, 3.3-mm-thick slices. In rabbits, three

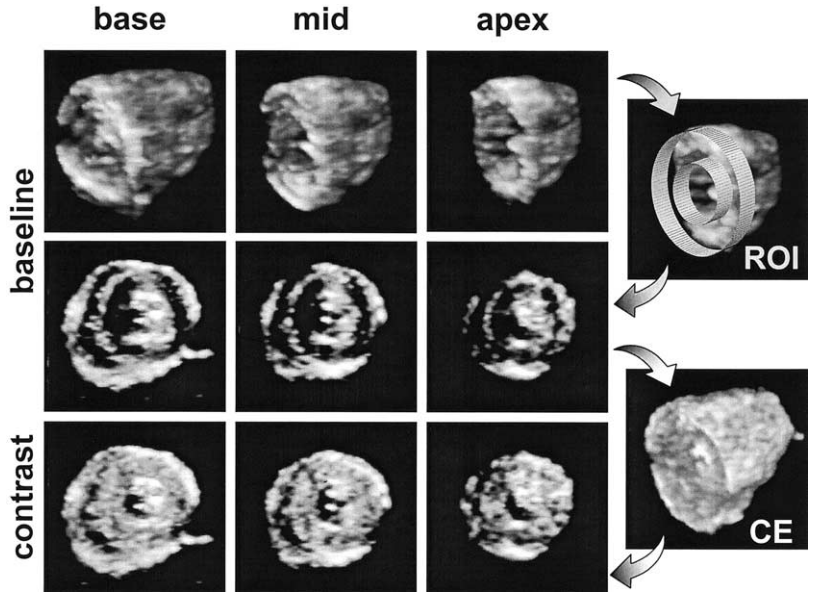

Figure 3. Three-dimensional rendering of the isolated rabbit heart showing how each dataset was sliced at different levels of the left ventricle **(top)**. At each level, a roughly ring-shaped region of interest (ROI) was defined **(top, right)** to allow video-intensity measurements in three 3.3-mm-thick slices **(middle)**. The effects of contrast enhancement (CE) on the different slices are shown in the bottom panels.

short-axis slices were selected, including one slice at the mid-papillary level and two slices 5 mm above and below (Fig. 3, top). In pigs, two adjacent short-axis slices containing proximal and distal portions of the papillary muscles were selected. In both animal models, a roughly ring-shaped, 3.3-mm-thick region of interest (ROI) that included most of the LV myocardium was manually drawn in each slice. In pigs, the ROI was subdivided into six wedge-shaped segments. In humans, three adjacent apical four-chamber slices were selected, including a mid-ventricular slice that contained the LV apex and two slices shifted toward the anterior wall. For each slice, a 3D horseshoe-shaped ROI was defined and its position was adjusted frame-by-frame, when necessary, to compensate for translation.

In each ROI, mean myocardial video-intensity (MVI) was calculated by averaging pixel intensity in each consecutive frame throughout the sequence to generate MVI over time curves. We assumed that myocardial contrast inflow followed the indicator dilution equation:

$MVI(t) = A\,[1 - e^{-\beta t}] + C$, where C is the initial MVI before contrast inflow, A is the maximum contrast-induced increase in MVI, and β is the characteristic constant related to tissue blood flow. The rate of change in MVI(t), expressed by its time derivative, would attain its peak value ($A \cdot \beta$) at the onset of contrast inflow (at $t \simeq 0$). Thus, the slope of MVI(t) near the onset of contrast inflow divided by A would represent β. Accordingly, linear regression slope of MVI(t) was calculated from the first 11 time points (based on preliminary experiments), starting where an increase above signal noise was detected. This slope was then normalized by the measured maximum contrast-induced increase in MVI (i.e., A), resulting in peak contrast inflow rate (PCIR, in units of 1/s). To compensate for recirculation and possible differences in transpulmonary contrast passage rates, myocardial PCIR values in protocols 2 and 3 were normalized by PCIR measured in the LV cavity and used as an index of myocardial perfusion (unitless after normalization).

Statistical analysis. To assess the effects of coronary flow restrictions in protocols 1 and 2, PCIR values obtained from repeated measurements at each experimental phase were compared using analysis of variance with repeated measures. For significant F-ratios, a Bonferroni post-hoc test was used to specify pair-wise differences. The reproducibility of PCIR was assessed by calculating the inter-measurement variability, defined as the standard deviation of repeated measurements in percent of their mean, for each animal, at each experimental phase. Inter-slice variability was defined as the standard deviation of PCIR measured over different slices selected from the same dataset in percent of their mean. In protocol 3, baseline and adenosine-stress PCIR values were compared using paired *t* test. All p values < 0.05 were considered significant.

RESULTS

Isolated heart experiments. At baseline, global coronary flow was 43 ± 8 ml/min and peak systolic LV pressure was 87 ± 11 mm Hg. A decrease in coronary flow to 50% and 15% of baseline resulted in reduced peak systolic pressures of 51 ± 10 mm Hg and 26 ± 10 mm Hg, respectively.

The RT3DE imaging allowed high-quality volumetric rendering of the entire heart (Fig. 3, top). Administration of contrast resulted in clearly visible and uniform dynamic changes in MVI in all LV slices (Fig. 3, middle vs. bottom). In all rabbits, contrast inflow reached steady-state enhance-

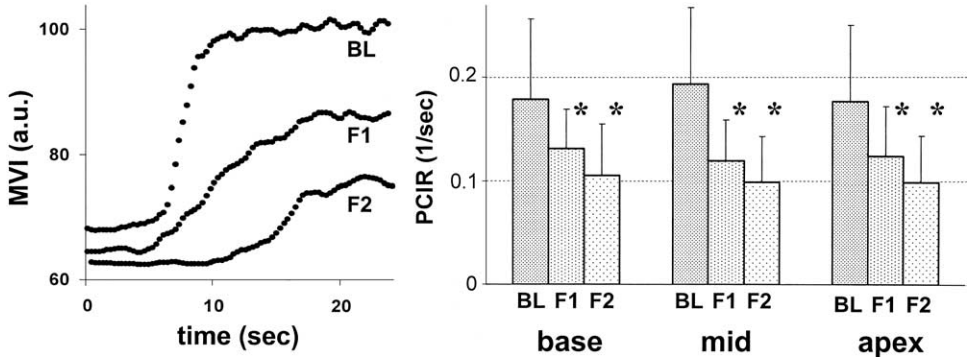

Figure 4. (Left) Example of myocardial video-intensity (MVI) time curves obtained from one three-dimensional slice of an isolated rabbit heart at three levels of coronary flow: BL, baseline; F1, 40% to 60% of baseline flow; F2, 10% to 20% of baseline flow. **(Right)** Average values of myocardial peak contrast inflow rate (PCIR) measured at the different levels of coronary flow (*$p < 0.0001$ compared with baseline) at each of the three levels of the ventricle.

ment within 15 to 25 cardiac cycles at baseline and was longer at lower coronary flows. Flow reduction resulted in a slower intensity increase and eventually lower levels of steady-state enhancement (Fig. 4, left). The PCIR followed the changes in coronary flow at both levels of reduced flow, F1 and F2 ($p < 0.0001$, Bonferroni-adjusted for two comparisons; Fig. 4, right). Inter-slice variability was 9 ± 6% and 9 ± 4% for BL and F1, respectively, but was higher for F2 (29 ± 23%, $p < 0.05$ vs. both BL and F1). Inter-measurement variability was 18 ± 15% and was similar at all flow levels.

Pig experiments. Baseline systolic and diastolic blood pressures were 123 ± 12 mm Hg and 60 ± 10 mm Hg, respectively, with no significant changes during partial coronary occlusion. Heart rates ranged from 95 to 125 beats/min between animals, with no ischemia-related changes or wall-motion abnormalities. In all animals, visible myocardial contrast clearance occurred within 90 s.

Figure 5 shows an example of a dataset that contains the mid portion of the left ventricle and en-face short-axis views of a mid-papillary 3D slice obtained at different phases of the TCI sequences at baseline and during LAD artery occlusion. Although no differences were visible between the pre-contrast images (left), the LAD artery territory appeared darker during steady-state enhancement, and contrast inflow was slower during restricted coronary flow compared with baseline (right). Regional MVI curves obtained in the LAD artery territory during occlusion showed a slower rate of inflow to a lower steady-state level, compared with baseline (top right). Curves obtained in segments outside the LAD artery territory were not affected.

Figure 6 shows the summary of regional perfusion measured in two myocardial slices in five pigs at baseline, during partial coronary occlusion, and during reperfusion. Coronary occlusion resulted in a significant decrease in PCIR in the anterior and anteroseptal segments (56 ± 29% and 62 ± 26% decrease, respectively; $p < 0.05$, Bonferroni-adjusted for two comparisons) in both slices, with no concomitant decrease in other segments. These changes were reversed with reperfusion, during which PCIR values were not different from baseline. Microsphere measurements performed during coronary occlusion averaged 43 ± 12% of baseline values in the anterior and anteroseptal segments, compared with 84 ± 35% in the rest of the myocardium. The inter-slice variability in regional PCIR at baseline was 22 ± 18%.

Human studies. In all subjects, dynamic changes in MVI were visible and quantifiable in all slices, and visible contrast clearance occurred within 90 s. The transition from no enhancement to steady-state enhancement occurred in all subjects within <45 s, and was captured in a single acquisition. Figure 7 (top) shows an example of pyramidal datasets that show an apical four-chamber view at different phases of a resting TCI sequence. Figure 7 (bottom left) shows MVI curves obtained at rest and during adenosine infusion. Adenosine resulted in increased contrast inflow rate in all three myocardial slices in all subjects. Consequently, PCIR with adenosine was 198 ± 57% of the resting value ($p < 0.0001$) when averaged over the three slices (bottom right), and was similar in all slices. The inter-slice variability was 10 ± 4% with no significant difference between rest and adenosine.

DISCUSSION

The ability of 2D contrast echocardiography to image myocardial perfusion has been shown in multiple publications (10,11). This technique has lately been improved by stable contrast agents suitable for intravenous administration (6,7,12) and contrast-targeted imaging technology (e.g., power modulation and pulse inversion) that provides higher sensitivity for detecting intramyocardial contrast (7,13–15). These improvements have made myocardial perfusion imaging feasible, and importantly, reproducible in the majority of patients.

Nevertheless, the ability of conventional perfusion imaging to accurately determine the extent of perfusion defects is limited by its 2D nature, which mandates repeated multiplane acquisition. Despite the obvious appeal of 3D imaging in this context, this methodology has not been possible until recently because it has relied on volume reconstruction from multiple planes, essentially precluding the possibility of

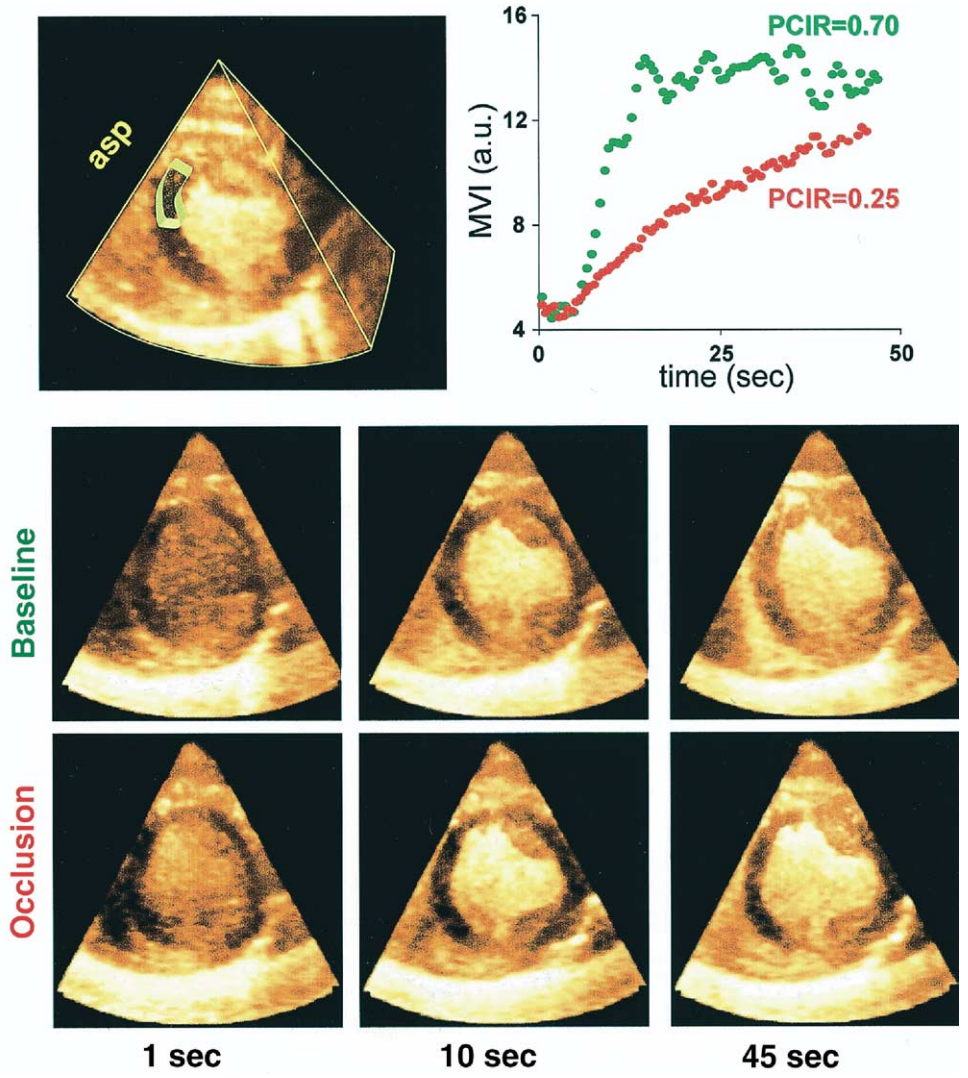

Figure 5. (Top left) Dataset that contains the mid portion of the left ventricle, obtained using transthoracic imaging in a pig (anteroseptal region [asp] of interest shown in yellow). Example of en-face short-axis views of a mid-papillary three-dimensional slice obtained at baseline **(middle row)** and during partial left anterior descending artery occlusion **(bottom row)** before the onset of **(left)** and during **(center)** myocardial contrast inflow, and **(right)** after reinstatement of steady-state contrast enhancement. **(Top right)** Regional myocardial video-intensity (MVI) curves obtained in the mid-papillary anteroseptal region of interest: baseline **(green)** and partial left anterior descending artery occlusion **(red)**. PCIR = peak contrast inflow rate.

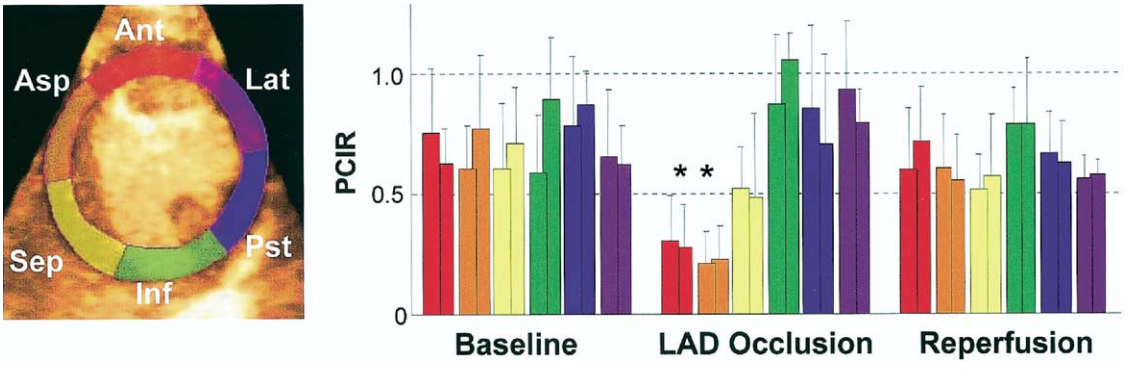

Figure 6. (Left) En-face view of a three-dimensional short-axis slice of a pig heart with the myocardial region of interest divided into six segments. **(Right)** Regional peak contrast inflow rate (PCIR) (unitless after normalization) calculated from transient contrast inflow (TCI) sequences at baseline, during partial left anterior descending (LAD) artery occlusion, and during reperfusion, averaged over all animals (*$p < 0.05$ vs. baseline). Different bar colors correspond to specific myocardial segments **(left)**. The two bars of each color represent regions of interest in adjacent slices. Ant = anterior; Asp = anteroseptal; Inf = inferior; Lat = lateral; Pst = posterior; Sep = septal.

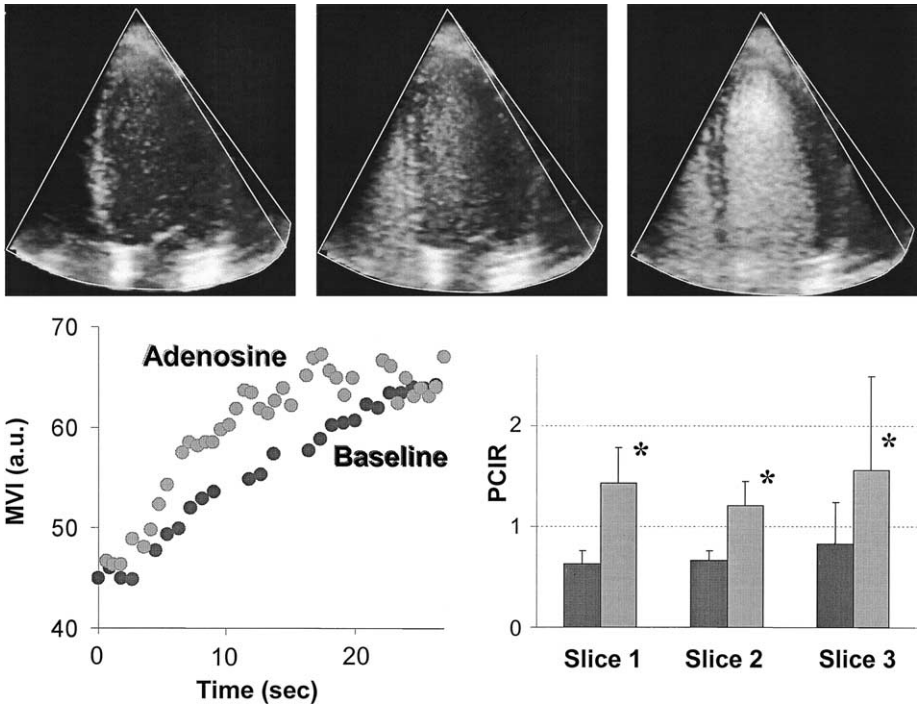

Figure 7. **(Top)** Near en-face apical four-chamber view of the left ventricle obtained in a normal volunteer at different phases of a transient contrast inflow sequence (left to right). **(Bottom left)** Corresponding myocardial video-intensity (MVI) curves obtained at rest and during adenosine infusion in one myocardial slice. **(Bottom right)** Summary of peak contrast inflow rate (peak contrast inflow rate [PCIR], unitless after normalization) measured in three myocardial slices and averaged over all study subjects (*$p < 0.01$ vs. baseline).

quantification that would require repeating contrast maneuvers for each plane. This study was designed to test the hypothesis that the current RT3DE technology can provide consecutive scans of the entire volume during a single contrast maneuver and thus allow live volumetric perfusion imaging and quantification.

This is the first study to show the feasibility of real-time, high-resolution, contrast-enhanced 3D perfusion imaging using widely available equipment. To quantify perfusion, we used transient contrast inflow as an alternative to high-energy ultrasound pulses, which are not applicable with RT3DE imaging. An important advantage of the TCI maneuver over bolus injections is the ample time it provides to optimize gain settings before image acquisition. To evaluate this approach, we developed software for volumetric analysis of TCI sequences aimed at quantification of global and regional myocardial perfusion in multiple 3D slices and tested it under various conditions.

The isolated heart preparation proved to be a valuable tool because it provided near-ideal conditions for contrast-enhanced imaging (5). This protocol provided the scientific basis for further evaluation in pigs and humans. Our results in this experimental setup proved the feasibility of both RT3DE imaging and quantitative volumetric assessment of myocardial perfusion. Our approach to the analysis of MVI time curves provided reproducible values of PCIR, which directly reflected the changes in coronary flow. Moreover, the low inter-slice variability of PCIR indicates that this parameter is a reliable index of myocardial perfusion. The increase in inter-slice variability between non-adjacent slices during 85% flow restriction is likely attributable to the non-uniform flow distribution in severely underperfused myocardium. Importantly, the inter-measurement variability was lower than the inter-slice variability, indicating that the former is unlikely to be related to a measurement error, but rather represents physiologic non-uniformity in perfusion.

Another noteworthy finding is that although PCIR followed the changes in coronary flow, it was not proportional to flow (50% flow reduction caused a smaller decrease in PCIR). This could be explained by the fact that the contrast infusion rate was kept constant and was not adjusted for reduced coronary flow, resulting in higher contrast concentrations at lower flows. Although our technique includes normalization by contrast-induced change in video-intensity, the non-linear relationship between video-intensity and bubble concentration may explain this finding.

Our pig experiments were designed to test this approach in vivo using transthoracic imaging and to validate regional volumetric perfusion measurements against microspheres. The use of partial coronary occlusion in a closed-chest pig provided an experimental model of reduced coronary flow without ischemia. This model was chosen to test this technique under conditions of a moderate decrease in perfusion, which is more difficult to detect visually than the near-complete lack of perfusion caused by critical coronary stenosis. The results of this protocol showed that our technique could be used to detect a moderate reduction in

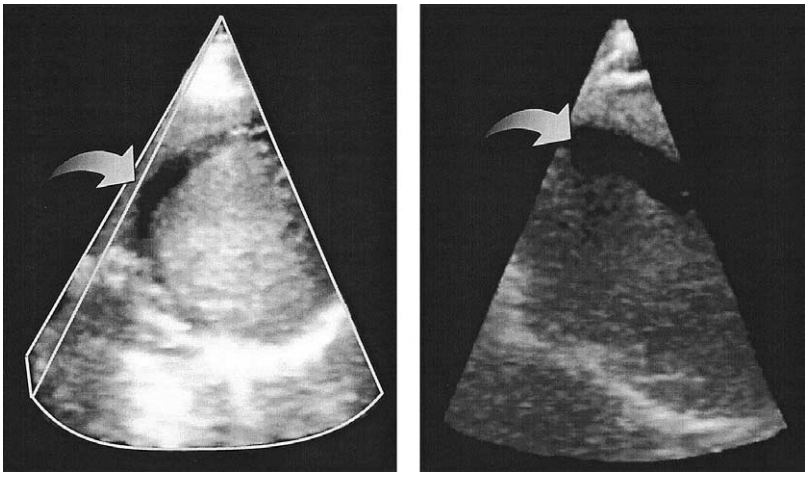

Figure 8. Pyramidal dataset obtained in a patient with severe discrete left anterior descending artery stenosis **(left)**. A part of the interventricular septum shows clear lack of contrast enhancement, indicating a perfusion defect that was supported by abnormal wall motion. This defect was visible in multiple cross-sections **(right)**, allowing easy estimation of its extent.

myocardial perfusion, which was confirmed by microspheres, on a regional basis. The relatively low baseline inter-slice variability indicates that PCIR is a reliable perfusion index even on a regional basis. Inter-slice variability was not tested during occlusion because uniform distribution of blood flow was not expected under these conditions. In addition, our attempted solution for the issues of recirculation and differences in transpulmonary contrast passage proved effective because it resulted in a relatively small standard deviation in PCIR (Fig. 6), reflecting the low inter-animal variability of this index.

Protocol 3 was aimed at initial testing of clinical applicability of RT3DE perfusion imaging with TCI as the contrast maneuver for quantification. This was achieved by measuring pharmacologically induced changes in myocardial perfusion in a small group of normal subjects. This strategy was chosen because the clinical application of this approach is the detection of stress-induced perfusion abnormalities (Fig. 8). The use of normal subjects allowed us to validate our technique against the anticipated normal response to adenosine, which is a marked increase in myocardial perfusion in the absence of coronary artery disease. Our results indeed showed a two-fold increase in the measured perfusion index. Although one might expect a larger increase (three- to five-fold) in response to adenosine, the non-linear relationship between contrast concentration and video-intensity may explain this dampened response.

The results of this protocol also showed that contrast clearance and inflow occur within a time frame applicable in the clinical setting. Importantly, the TCI maneuver does not require complete contrast clearance before resuming infusion, but instead relies on the temporal aspects of the relative change in contrast levels during inflow. Of note, the low inter-slice variability in global PCIR essentially did not increase in the transition from the isolated heart preparation to humans. These results indicate that RT3DE perfusion quantification can be reliably performed in humans and establish the basis for future clinical studies geared toward the validation of this technique in different cardiac disease states.

Study limitations. Our measurements were not directly validated against microspheres in every experiment. This is because PCIR is an index related to perfusion rather than an absolute perfusion measurement and therefore cannot be directly compared with microsphere data. In the isolated heart experiments, changes in global myocardial perfusion were validated using direct measurements of coronary flow. Also, no regional variations in myocardial perfusion were induced in this setup because of the known high prevalence of collateral circulation in rabbits (16). In the pig experiments, which were designed to study changes in regional perfusion, the reduction in LAD artery flow was confirmed by angiography in every animal. Microspheres were only used in a subset of animals to confirm the effectiveness of coronary occlusion using a widely accepted gold standard technique.

The initial feasibility in humans was tested in normal subjects selected based on quality of images. The RT3DE perfusion imaging needs to be evaluated in larger groups of unselected patients, which will also allow determining the impact of suboptimal images such as those frequently encountered in clinical practice. Also, this study was performed using harmonic imaging rather than a contrast-targeted mode, which might further improve the sensitivity to intramyocardial contrast. Another limitation of the RT3DE technology is the limited spatial aperture angle, which does not allow imaging the entire ventricle. This limitation mostly stems from insufficient computational resources and does not represent a fundamental constraint of this technology. Therefore, while anticipating a technological solution to this issue, we focused on testing the feasibility of volumetric perfusion imaging and quantification in a partial volume of the heart that can be scanned using the current RT3DE equipment.

CONCLUSIONS

We found that RT3DE imaging and quantification of myocardial perfusion within a single contrast maneuver is feasible, and that PCIR is a sensitive, accurate, and reproducible index that can be used to track global and regional changes in myocardial perfusion. This approach can potentially allow more accurate assessment of the extent of perfusion defects than 2D myocardial contrast echocardiography. The potential clinical use of this technique for the diagnosis of ischemic heart disease may have important implications for the future of myocardial contrast echocardiography.

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