Myocardial Perfusion Imaging Based on the Blood Oxygen Level–Dependent Effect Using T₂-Prepared Steady-State Free-Precession Magnetic Resonance Imaging

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Background—The decision to perform coronary revascularization procedures may hinge on assessment of myocardial perfusion reserve. Blood oxygen level–dependent (BOLD) MRI is a potential method to detect the effects of regional variations in myocardial blood flow during vasodilation.

Methods and Results—We imaged dogs (n = 13) on a 1.5-T whole-body MRI scanner using a new T₂-prepared steady-state free-precession (SSFP) MRI pulse sequence sensitive to BOLD contrast. Images (in-plane resolution = 1 mm²) of 5 short-axis and 2 long-axis slices of the heart were acquired during graded levels of adenosine infusion via a surgically placed left circumflex (LCx) catheter (n = 11) or via a right atrial catheter in animals with an LCx occluder (n = 2). Relative myocardial perfusion was measured with the use of fluorescent microspheres. Signal intensity changes in myocardium subtended by the left anterior descending coronary artery were compared with those in the LCx region. Unprocessed T₂-weighted images revealed changes in signal intensity corresponding to areas of regional vasodilation. At maximal vasodilation, the signal intensity ratio in the LCx versus left anterior descending territories increased by 33 ± 4% compared with baseline, corresponding to a 3.8 ± 0.3-fold increase in relative perfusion (P < 0.01). MR intensity at progressive levels of vasodilation demonstrated good agreement with microsphere flow (R² = 0.80, P < 0.01).

Conclusions—T₂-prepared SSFP BOLD imaging is a promising method to determine an index of myocardial perfusion reserve in this animal model. (Circulation. 2004;110:1284-1290.)

Key Words: coronary disease • magnetic resonance imaging • perfusion • vasodilation • myocardium

Myocardial perfusion and perfusion reserve are crucial physiological parameters in ischemic heart disease.¹ The decision to enhance myocardial blood flow by either angioplasty or surgery often is made on the assumption that regional flow is compromised by a coronary stenosis and can be partially or wholly reversed by treatment.² The decision to revascularize frequently is based on invasive coronary angiography with left ventriculography or on nuclear techniques and evaluation of contractile reserve by dobutamine echocardiography or MRI.¹–³ Recently, first-pass perfusion MRI with the use of gadolinium-based contrast has demonstrated utility for direct visualization of myocardial blood flow in animal models⁴,⁵ and in patients.⁶,⁷ Because first-pass techniques require high temporal resolution, image artifacts may occur, mimicking perfusion defects.

An alternative approach to MR first-pass perfusion imaging is to use the blood oxygen level–dependent (BOLD) effect. Whereas oxygenated hemoglobin is slightly diamagnetic, deoxygenated hemoglobin is paramagnetic and causes signal loss in T₂*- or T₂-weighted images. BOLD has been used to assess changes in myocardial venous blood oxygenation secondary to perfusion changes in both animals⁸–¹² and humans.¹³–¹⁶ Indeed, MR BOLD imaging detected attenuated myocardial vasodilator response in patients with concentric hypertrophy¹⁴ and poststenotic capillary recruitment dependent on coronary artery stenosis.¹⁵ The major problems with T₂*-weighted BOLD imaging and spoiled gradient-echo data acquisition are the relatively low signal-to-noise ratio and potential image artifacts caused by magnetic field inhomogeneity, blood flow, and motion in the heart. Recently, we developed a robust steady-state free-precession (SSFP) technique that generates T₂-weighted images in the heart and overcomes these problems.¹⁷ In the present study we sought to evaluate the feasibility of using this technique to detect changes in regional myocardial perfusion during vasodilation in a controlled animal model.
Methods
Surgical Preparation
Thirteen 15- to 25-kg mongrel dogs were studied in accordance with the “Position of the American Heart Association on Research Animal Use” adopted on November 15, 1984. Dogs were intubated and ventilated with 2% isoflurane. With the use of a sterile technique, a left lateral thoracotomy was performed at the fourth intercostal space. Right and left atrial catheters were inserted, secured, and routed for intravenous and intra-arterial injections, respectively. For 11 animals, a portion of the proximal left circumflex (LCx) coronary artery was isolated, and an upstream catheter was inserted, secured, and routed through the chest wall. For 2 animals, the LCx was fitted with a hydraulic occluder. A 3- to 4-mm Doppler flow probe was secured just distal to the catheter or occluder.

On the day of imaging, animals were examined in the laboratory setting to determine responses to graded doses of adenosine. Doppler velocity was measured in the coronary artery at rest and during infusion of adenosine through the LCx catheter or systemically.

MRI Protocol
Before imaging, dogs were tranquilized and transported to a clinical MRI facility (1.5-T MAGNETOM Sonata, Siemens Medical Solutions). After induction of anesthesia, animals were maintained with the use of a mechanical respirator with 2% isoflurane and laid on the MRI scan table in the right lateral decubitus position. ECG leads were attached, and a 28×28-cm flexible, phased-array surface coil was secured around the thorax. An auxiliary catheter running into a right atrial catheter (n=2) or the right atrial catheter (n=11) or to the left atrial catheter (n=2). The animal was then positioned in the isocenter of the magnet, the long and short axes of the heart were identified with scout images, and cine images in short and long-axis orientations were acquired. Next, heart rate was noted, and 5 short-axis and 2 long-axis baseline BOLD images were acquired with the use of a novel, T2-prepared SSFP pulse sequence with the following parameters: repetition time/echo time 3.0/1.5 ms, T2 preparation time 40 ms, field of view 260×140 to 160 mm2, lines per heartbeat ~7, matrix 256×100 to 120, slice thickness 5 mm, slice spacing 5 mm, number of averages 3, breath-hold duration ~30 seconds.

After BOLD images were acquired at baseline, perfusion was measured at resting conditions by injecting 3×106 polystyrene fluorescent microspheres (Molecular Probes) through the left atrial catheter. For the animals with an LCx catheter, low-level adenosine infusion (typically 0.01 mg/min=0.5 μg/kg per minute) was then initiated with the use of the LCx catheter to selectively vasodilate the associated myocardium. After microspheres were injected for at least 5 minutes, another set of BOLD images was acquired at the same long- and short-axis slice locations acquired at rest. Microspheres were again injected to determine perfusion. The same imaging and microsphere protocols were then performed for medium-level (typically 0.05 mg/min=2.5 μg/kg per minute) and high-level (typically 0.30 mg/min=15 μg/kg per minute) vasodilation. For animals with a coronary occluder, Doppler flow in the LCx was noted at rest and maintained by inflating the occluder with a microtiter pipette during systemic adenosine administration (1 to 6 mg/min=50 to 300 μg/kg per minute) via a right atrial catheter. In similar fashion, BOLD images were acquired at each level of vasodilation with microsphere data to verify actual perfusion.

After the final BOLD images were acquired, multislice, short-axis saturation recovery SSFP first-pass myocardial perfusion images were collected during LCx injection of 0.05 mmol/kg gadoteridol (Gd-DPTA, ProHance, Bracco) at the same slice positions acquired for BOLD images in animals with an LCx catheter. Imaging parameters were as follows: repetition time/echo time 3.0/1.5 ms, field of view 260×140 to 160 mm2, matrix 128×60 to 80, slice thickness 5 mm, number of slices 4 to 6, breath-hold duration ~30 seconds. These images were acquired to determine the myocardial perfusion territory of the LCx artery. Delayed enhancement images were then acquired to ascertain whether irreversible injury had occurred. Animals were typically studied on 2 separate dates, on which at least 3 levels of pharmacological vasodilation were examined during each study.

Euthanasia and Tissue Sectioning
Animals were euthanized by overdose with sodium pentobarbital followed by KCl injection. The ventricle was dissected free and cut into circumferential rings in an attempt to replicate the same slice positions acquired during MR scanning. For each ring, the landmarks of the left ventricle were identified, and the ring was sectioned into 8 equal circumferential sectors for standard microsphere analysis. Each piece was weighed and dissolved for 96 hours in 4 mol/L KOH. After filtration and dissolution in 2-ethoxyethyl acetate, each test tube was analyzed spectrophotometrically (Perkin-Elmer) for fluorescence that reflected microsphere concentrations.

Data Analysis
BOLD Images
Images were analyzed on an offline viewing station for changes in signal intensity at each level of vasodilation. Image intensity in the anterior, left anterior descending (LAD)–supplied and in the inferior, LCx-supplied myocardium was determined simultaneously in the same region of interest at baseline and during vasodilation for each study date. The region chosen for analysis in animals with a coronary catheter was based on the center of the gadolinium-enhanced region visible in the selective LCx first-pass perfusion images, as shown in Figure 1. For these animals, a ratio of image intensity in the LCx region divided by that in the LAD region was computed. For the animals with coronary occluders, a ratio of LAD to LCx myocardium was computed because this was consistent with the vasodilated to remote territories. Image intensities at different levels of adenosine were normalized by the image intensity at rest to remove any potential errors due to coil sensitivity. First-pass images acquired during LCx infusion of gadolinium were examined qualitatively for whether the LCx-perfused myocardium matched the area of increased signal in BOLD images.

Microspheres
Each microsphere fluorescence was normalized to the mass of the myocardium and grouped according to slice and position. Fluorescence values in the inferior myocardial segments, corresponding to
the regions of interest analyzed in the MR images, were divided by fluorescence from the remote, anterior territory. This provided a reference index of relative myocardial blood flow in the 2 territories. The ratio was computed for each level of vasodilation.

**Statistical Analysis**

All results are expressed as mean±SE. Repeated-measures ANOVA was used to determine whether changes in signal intensity and microsphere flow at each level of pharmacological vasodilation were significant. Linear regression with ANOVA and 95% CIs was used to compare BOLD image intensity and microsphere-derived relative myocardial perfusion. All statistical tests were 2 tailed, and values of P<0.05 were considered significant.

**Results**

A total of 21 MRI studies were performed on 13 animals. Heart rate remained virtually unchanged during intracoronary adenosine injection for animals instrumented with an LCx catheter (baseline, 84±13; low, 86±10; medium, 88±12; high, 87±13; P=NS), whereas heart rate increased for animals with an occluder that received systemic adenosine (baseline, 87±11; stress, 113±5; P<0.05). It should be noted that these measurements do not rigorously demonstrate the status of myocardial work at each phase of the experiment because blood pressure was not recorded during imaging. Of all animals, one had a significant wall motion abnormality at baseline; this same animal had irreversible myocardial injury on the basis of delayed gadolinium enhancement and was the only subject with this finding. This animal and 2 others did not demonstrate a significant increase in microsphere flow (regional flow ratio normalized to baseline was 1.1±0.1; P=NS) or BOLD image intensity (increase in regional signal ratio normalized to baseline was 4±3%; P=NS) during adenosine vasodilation. Presumably, these subjects may not have vasodilated because of obstruction of the coronary artery secondary to catheter placement. The remaining 10 animals showed increases in regional myocardial blood flow and BOLD signal intensity during adenosine infusion and did not have wall motion abnormalities or demonstrate delayed gadolinium hyperenhancement.

Figure 2 shows example BOLD images at rest and during graded adenosine vasodilation. With an increase in perfusion to the circumflex-supplied myocardial wall, a clear increase in signal intensity in this region was apparent in the BOLD image.
images. These changes corresponded to regional perfusion in the circumflex territory based on the microsphere maps that were computed at each adenosine dosage. These microsphere images revealed a gradual increase in perfusion to the inferior myocardium. The graph at the bottom of Figure 2 demonstrates that the increase in myocardial signal intensity observed in the BOLD images followed the increase in myocardial blood flow at each level of pharmacological vasodilation.

$T_2$-prepared SSFP BOLD images were acquired in all animals at rest and under varying conditions of vasodilation. Example images acquired in the long-axis orientation at rest and during adenosine vasodilation of the LCx myocardium are shown in Figure 3. Unprocessed BOLD images revealed clear changes in signal intensity in the inferior myocardium. In addition, the high resolution possible using a segmented MR pulse sequence allowed for delineation of endocardial and epicardial heart borders, the myocardial wall itself, and anatomic features such as papillary muscles (eg, Figure 2) and insertion of the right ventricle (eg, Figure 3). Image signal-to-noise ratio in the myocardium was on average $12 \pm 3$.

Figure 4 shows SSFP BOLD images at rest and at maximum LCx vasodilation. As shown, an increase in the MR signal intensity of the myocardial territory that corresponds to the circumflex distribution is apparent in all slices,
and this region matches the territory that was enhanced during selective gadolinium infusion through the LCx catheter. Figure 5 shows another example in which rest images are acquired with graded doses of adenosine through the LCx catheter. As shown in the figure, the inferior myocardium demonstrated a gradual increase in signal intensity as adenosine dose was increased. In addition, the region that increased in image intensity corresponded visually to the myocardial territory that was enhanced during selective LCx gadolinium administration.

Figure 6 shows example images at rest and during vasodilation in a stenosis model. The increase in image intensity in the anterior myocardium during vasodilation is seen to be different from the LCx-supplied myocardium; these changes matched corresponding perfusion ratios calculated with the use of microspheres. When data from all animals were pooled and increases in SSFP BOLD signal were compared with increases in relative myocardial blood flow measured by microspheres, good correlation was observed ($R=0.80$, $R^2=0.643$, $P<0.01$), as shown in Figure 7. At maximal vasodilation for the 10 animals that responded to adenosine infusion, signal intensity in the LCx territory increased by an average of 28±3% compared with baseline ($P<0.01$), whereas image intensity in remote myocardium remained unchanged (5±3%, $P=NS$); these together resulted in an increase in signal intensity ratio in vasodilated to remote myocardium.
imaging does not rely on accurate timing of an exogenous transmural perfusion gradients. In addition, SSFP BOLD study) that, in principle, may allow for the assessment of in plane, respectively, for first-pass versus BOLD in this acquisition across several cardiac cycles (2.6 versus 1.0 mm proved spatial resolution made possible with steady-state data assessment. For example, BOLD imaging may allow for im-

Figure 7. Relationship between microsphere flow (LCx divided by LAD myocardium) vs bold image intensity (same ratio) normalized to baseline with 95% CIs shown. Open symbols indicate animals with an LCx catheter; solid symbols, animals with an LCx occluder.

regions of 33±4% (P<0.01). Microsphere analysis demonstrated a 3.8±0.3-fold increase in relative flow ratio between the vasodilated and remote regions in these animals (P<0.01).

For the same animal studied on different dates, relative perfusion during the first study increased by a factor of 3.2±0.6, whereas the mean increase during the second study was 3.6±0.7-fold (P=NS); similarly, the mean increase in image intensity ratio during the first study was 18±7%, whereas the mean increase during the second study was 25±7% (P=NS). For animals that did not vasodilate, regions remained isointense at various adenosine doses, and micro-
sphere data showed no increase in flow in that area of myocardium. These data suggest reproducibility of the technique, ie, that different scans of myocardium in the same flow state appear similar on T2-weighted SSFP BOLD images acquired at different times.

Discussion

Results of the present study indicate that, in this animal model, regional changes in T2-prepared SSFP BOLD image intensity were directly related to relative blood flow. BOLD images of the heart were acquired in the steady state without the use of exogenous contrast media, and changes in regional signal intensity corresponding to relative myocardial blood flow changes were clearly visible in unprocessed images. This was possible largely because of the improved signal, contrast, and image quality achieved with the use of the new SSFP technique with T2 preparation.

Regional Cardiac Perfusion Assessment Using BOLD Imaging

BOLD imaging has potential advantages over contrast-enhanced first-pass techniques for myocardial perfusion as-

several cardiac cycles (2.6 versus 1.0 mm in plane, respectively, for first-pass versus BOLD in this study) that, in principle, may allow for the assessment of transmural perfusion gradients. In addition, SSFP BOLD imaging does not rely on accurate timing of an exogenous contrast agent bolus but rather can be performed in the steady state and repeated if necessary.

SSFP generates higher signal-to-noise ratio than does spoiled gradient-echo acquisition.9,20 T2-weighted imaging minimizes potential field inhomogeneities and motion-related image artifacts. Previous BOLD studies in the heart with T2*-weighted imaging were plagued with low signal and poor overall quality.11,21 Images have required significant image processing to visualize changes. In the present study, in-

creases in image intensity were apparent in unprocessed images. An alternative method for T2-weighted imaging is fast spin echo.22 Because there is no need for blood suppres-
sion and each k-space line is acquired after 1 excitation pulse, T2-prepared SSFP is less sensitive to artifacts related to flow. However, SSFP is also more sensitive to off-resonance effects, and further studies are required.

The relationship between flow reserve measured by micro-
spheres and BOLD image intensity showed good agreement in the animal models studied. It can be seen from Figure 7 that BOLD data compared well with true myocardial perfu-
sion (R=0.80, P<0.01). Figure 7 also reveals some scatter around the regression line that may represent animal variabil-

ity in BOLD image intensity and/or may be related to registration of in vivo MR images to ex vivo tissue sections. Image intensity changes seen in BOLD images were consist-

ent with the perfusion territories outlined by direct injection of gadolinium through the left circumflex. In addition, the regression line of Figure 7 suggests that, in the present study, relative BOLD image intensity increased ~8% for every 1-fold increase in relative myocardial perfusion. The 95% CIs of Figure 7 also suggest that a 2.5:1 flow difference could be reliably detected with the present methodology.

Study Limitations

In the present study, an animal model of vasodilation of only the myocardium supplied by the LCx artery was studied. Because adenosine is nearly completely taken up by the myocardium on the first pass through the tissue, it was possible to study controlled changes in myocardial blood flow to that region without significantly affecting heart rate. It should be noted that, by themselves, measurements of heart rate do not rigorously demonstrate that myocardial work was identical at each phase of the experiment; rather, a measure-

ment of the rate-pressure double product at each stage of the experiment would have been useful in this regard. In clinical practice, significant changes in heart rate and blood pressure may be expected during systemic adenosine vasodilation. Furthermore, despite the correlation between perfusion and image intensity indices, the actual changes in image intensi-

ties observed in the present study were relatively small (~30%), and whether this is adequate to be reliably detected in clinical studies has not been determined. In addition, although data from 2 animals with occluders were included in this study, a systematic study with a more clinically relevant stenosis model is required before the technique is applied to patients with potentially serious coronary artery disease that causes reductions in myocardial perfusion reserve. In addi-

tion, the present study design did not address whether changes in BOLD image intensity have the potential to detect
smaller changes in flow, even slightly below baseline, secondary to proximal coronary occlusions, which would be clinically relevant. Additionally, a limitation of the present study was the relatively low dose of gadolinium (0.05 mmol/kg) used to characterize delayed enhancement. Finally, although our goal in the present study was to demonstrate the correlation between the changes in BOLD MR image intensity versus relative regional myocardial perfusion, it would have been instructive to measure absolute myocardial perfusion (in milliliters per gram per minute of cardiac tissue) with the use of radiolabeled microspheres and to investigate the relationship between venous oxygen saturation and myocardial MR signal intensity at each level of vasodilation.

**Application to Clinical Setting**

BOLD MRI may be applicable to patients with reduced myocardial perfusion reserve. In principle, BOLD imaging may be used not only to assess relative myocardial blood flow but also to determine absolute oxygen extraction from myocardial tissue, and hence it may provide information beyond the relative perfusion index examined in the present study. The ultimate utility of MR BOLD imaging in patients with decreased myocardial perfusion remains to be examined.

**Conclusion**

We conclude that this new T2-prepared SSFP BOLD technique provides an accurate index of myocardial blood flow in this animal model and has potential to be a sensitive marker of myocardial perfusion reserve.

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