Evaluation of the severity of a coronary artery stenosis is of paramount importance for therapy. A relevant stenosis provokes post-stenotic microvascular dilation with capillary recruitment. This autoregulatory response was investigated in the present study by use of susceptibility-sensitive magnetic resonance imaging (MRI) without contrast agents.

Functional alterations of the microvascular system may be studied noninvasively and without a contrast agent by susceptibility-sensitive MRI, which is based on the paramagnetic property of deoxyhemoglobin. This effect, also referred to as the "blood oxygenation level-dependent (BOLD) effect," is investigated by phase relaxation ($T_2^*$) measurements.

In patients (n = 16) with single-vessel coronary artery disease, no history of myocardial infarction, normal left ventricular function at rest, and a positive stress echocardiogram, the susceptibility-sensitive parameter $T_2^*$ was assessed in the myocardium. In regions associated with the stenotic artery, $T_2^*$ was significantly lower than in residual myocardium ($p < 0.01$). This difference in $T_2^*$ increased after application of the vasodilator dipyridamole ($p < 0.001$). In patients being re-investigated after therapeutic interventions, the microvascular dilation was partly removed.

For the first time, we could show that myocardial BOLD MRI detects post-stenotic capillary recruitment dependent on coronary artery stenosis. (J Am Coll Cardiol 2003;41:834–40) © 2003 by the American College of Cardiology Foundation
Abbreviations and Acronyms

- BOLD = blood oxygenation level-dependent
- CABG = coronary artery bypass graft surgery
- CAD = coronary artery disease
- ECG = electrocardiographic
- LAD = left anterior descending coronary artery
- MRI = magnetic resonance imaging
- PTCA = percutaneous transluminal coronary angioplasty
- ROI = region of interest
- T2* = phase relaxation

under rest conditions, capillary recruitment in regions supplied by a stenotic coronary artery should result in an increase of tissue concentration of deoxyhemoglobin and, hence, in reduced T2*. Additionally, measurements were done after vasodilation with dipyridamole, which normally shifts the blood supply/oxygen demand balance toward hyperperfusion. In myocardium supplied by a normal coronary artery, this decreases the tissue concentration of deoxyhemoglobin and T2* increases, as previously shown in our laboratory (14). In myocardium supplied by a stenotic coronary artery, post-stenotic capillary recruitment implies only a moderate dilatory reserve (i.e., one would expect only a moderate increase in T2*).

It is obvious that verification of the aforementioned hypothesis should have significant implications for BOLD imaging as a diagnostic tool for determination of capillary reserve and, hence, evaluation of the severity of coronary artery stenosis.

METHODS

Volunteers and patients. Before the patient study, 16 volunteers (20 to 59 years old; mean 31 ± 10 years) without a history of cardiovascular disease were studied by MRI.

Then, 16 patients were included (44 to 77 years old, mean 63 ± 9 years). Inclusion criteria included stable angina, single-vessel CAD on the coronary angiogram (degree of stenosis >70%; in the left anterior descending coronary artery [LAD; n = 12], right coronary artery [n = 2], and left circumflex branch of the coronary artery [n = 2]), wall motion abnormalities on the stress echocardiogram, and no wall motion abnormalities of the left ventricle at rest. A previous myocardial infarction was excluded by a medical history, evaluation of electrocardiographic (ECG) abnormalities, and past enzyme elevations. Stable angina implies that there was no ischemia under rest conditions, but that there was functional severity of the stenosis under stress conditions. The single-vessel condition assured a simple relationship between the culprit artery and supplied myocardium. The last criteria excluded that structural abnormalities (e.g., scar) were responsible for T2* alterations.

Patients underwent X-ray angiography, stress echocardiography, and MRI within 4 ± 2 days. Two patients with LAD stenosis were re-investigated—one after percutaneous transluminal coronary angioplasty (PTCA) with stent im-plantation and another after coronary artery bypass graft surgery (CABG). Written, informed consent was obtained from all participants, and the local ethics committee approved the study.

X-ray angiography. Cardiac catheterization was done after inguinal puncture of the right femoral artery with a 5F catheter. Coronary arteries were assessed in the typical left and right anterior oblique projections.

Stress echocardiography. Echocardiography was done at rest and after administration of dipyridamole (rate of 0.56 mg/kg body weight over 4 min). After the examination, patients were antagonized with 200 mg aminophylline.

Magnetic resonance examination. Magnetic resonance examinations were performed on a 1.5-T whole-body scanner (SIEMENS Vision, Erlangen, Germany) with gradient overdrive, using the integrated body coil for radiofrequency excitation and a four-element phased-array coil for signal reception. Fast, non–ECG-triggered scout images (i.e., fast low-angle shot [FLASH]) were used to position the imaging slice at the short-axis plane of the heart between the valve system and papillary muscle.

For T2* measurements, a segmented gradient echo pulse sequence was used, which acquired 10 successive gradient echoes per radiofrequency excitation in a single breathhold, as recently proposed by our laboratory (14).

Volunteers and patients were informed about the possible side effects of dipyridamole, such as headaches, nausea, and general feelings of warmth. All participants avoided the consumption of tea and coffee, as well as medications containing aminophylline at least 12 h before the dipyridamole test protocol. Heart rate was continuously monitored, and blood pressure was measured before and after the examinations. Images were acquired repeatedly at rest and under dipyridamole-induced stress (rate of 0.56 mg/kg, infused over 4 min through an antecubital vein). Measurements were done in 1-min repetitions until the initial heart rate was re-established. Infusion was controlled by an infusion system (CAI 626P, DOLTRON AG, Uster, Switzerland), which allowed precise adjustment of the infusion rate relative to the patient’s weight.

Data evaluation. For data evaluation, magnetic resonance images were transferred to an off-line workstation and processed using a home-written software package. In a first step, a region of interest (ROI) was placed around the complete myocardium in the first-amplitude image (which was the brightest) of the 10 echo image series. Superimposing susceptibility artifacts, mainly at the inferolateral border of the heart, could be clearly identified in the corresponding amplitude images and led to exclusion of these pixels from evaluation. To minimize the effect of ROI positioning on the final results, the ROI placement was repeated 2 to 3 times and compared by the same observer.

Maps of the apparent transverse T2* were calculated by applying a linear fit to the logarithm of the signal intensities in the ROI. The noise amplitude was first determined in a region outside the body. In the following fitting procedure,
only those signal intensities that exceeded the noise amplitude threshold by a factor of 2 to 3 were used. Average $T_2^*$ values of the myocardium were calculated from the mean value of the signal intensities in the ROI. To localize myocardial areas with lower $T_2^*$ values, a series of $T_2^*$ maps acquired before, during, and after dipyridamole administration were displayed in a fast cine loop. In this display mode, areas of reduced or no $T_2^*$ increase (i.e., $T_2^{*r}$) were identified visually, and ROIs were placed manually. For these regions, average $T_2^{*r}$ values were determined and compared with the remaining myocardium ($T_2^{*n}$).

**Statistical analysis.** To test the statistical significance of the $T_2^*$ increase with dipyridamole, $T_2^*$ values (i.e., $T_2^{*r}$ and $T_2^{*n}$) were grouped into two sets. Baseline values of $T_2^*$ were defined from the data acquired before dipyridamole administration and before there was an increase in heart rate. The second data set included all those measurements where the heart rate exceeded the baseline value by 10%. The relative change in $T_2^*$ was defined as the mean $T_2^{*r} - \text{mean } T_2^{*n}$ normalized by $T_2^{*n}$. An assumption of normality was demonstrated using the Kolmogorov-Smirnov test. The significance (p value) of the difference of mean values was calculated using the paired $t$ test. Results are expressed as the mean value ± SD.

**RESULTS**

**Volunteers.** After dipyridamole infusion, $T_2^*$ increased significantly from $35 \pm 3$ ms (range 28 to 40 ms) to $40 \pm 4$ ms (range 29 to 48 ms) (i.e., by $10 \pm 5$ %; $p = 0.01$). The $T_2^*$ maps appeared homogeneous under baseline and stress conditions, and no areas with reduced $T_2^*$ values could be delineated (Fig. 1). The average heart rate increased from $62 \pm 9$ beats/min to $84 \pm 11$ beats/min (i.e., by $35 \pm 15$ %). The mean duration of the increased heart rate was $9 \pm 6$ min.

**Patients.** Under rest conditions, regions with reduced $T_2^*$ values (i.e., $T_2^{*r}$) were clearly detectable (Fig. 2). These areas (covering 25% to 50% of the area of the left ventricle in the imaged short-axis view) corresponded well to the regions with wall motion abnormalities. Reduced $T_2^*$ values due to superimposing susceptibility artifacts at phrenicome-diastinal recess (15) were clearly detectable in a few patients and led to exclusion of these myocardial areas from evaluation. The relative change in $T_2^*$ was $31 \pm 9$% ($p < 0.01$), and this difference increased to $43 \pm 21$% ($p = 0.0001$) under stress conditions (Fig. 3).

The $T_2^*$ increase started $3 \pm 1$ min after the onset of dipyridamole infusion. The average heart rate increased from $62 \pm 8$ beats/min to $73 \pm 9$ beats/min (i.e., by $19 \pm$ 10% for volunteers and $31 \pm 9$% for patients).
The mean duration of the increased heart rate was 15\%\ 15/11006\ 6\ min. The observed side effects of dipyridamole were angina (n = 5), dyspnea (n = 1), and dizziness (n = 2). In two cases, the examination had to be interrupted early due to severe angina. One patient was antagonized with intravenous aminophylline (200 mg), and two patients took nitroglycerin to overcome angina.

Two patients with stenosis of the proximal LAD were re-investigated after therapeutic interventions (10 weeks after PTCA with stenting [Fig. 4] and 20 weeks after CABG [Fig. 5]). When observing the obtained T2* maps, differences between regions of reduced T2* and regions of normal myocardium were now less pronounced than they were before the interventions.

**DISCUSSION**

The BOLD effect. The BOLD MRI makes use of the paramagnetic property of intravascular deoxyhemoglobin. In the presence of an external magnetic field, deoxyhemoglobin reduces the transverse spin relaxation time T2* as a function of its tissue concentration (i.e., intravascular oxygenation state) and its distribution in tissue (i.e., the arrangement and the filling state of the vessel tree in tissue).

**What affects T2* in myocardium?** In contrast to that of patients, the myocardium of healthy volunteers did not show areas with reduced T2*. In normal hearts, an increase of myocardial perfusion by dipyridamole led to a significant increase in T2*, and calculated T2* maps were homogeneous under rest and stress conditions. This result is related to the fact that dipyridamole enhances coronary flow without much alteration of myocardial performance (16). This imbalance toward higher oxygen supply than oxygen demand decreases the deoxyhemoglobin concentration and, hence, the amplitude of the field inhomogeneities. The reduced T2* in myocardial segments supplied by a stenotic coronary artery and its modest response to vasodilation could, in principle, be due to structural tissue alterations and to factors related to functional changes in the coronary circulation. However, when considering the first, a different tissue composition of myocardium supplied by the stenotic artery is unlikely, as there was no history of previous myocardial infarctions in these patients, and the normal left ventricular function at rest ruled out structurally altered, viable (e.g., stunned or hibernating) myocardium. The best explanation for the decreased T2* in regions associated with a stenotic artery is that there is a higher concentration of paramagnetic deoxyhemoglobin. In principle, this could be caused by either an increase of intravascular deoxyhemoglobin or an increase of the vessel compartments containing deoxyhemoglobin. In nonischemic myocardium, however, coronary autoregulation maintains the arteriovenous difference of oxygen saturation of hemoglobin at an almost constant rate of \(\approx 70\%\) (17). According to the clinical selection of the patients and normal left ventricular function, the myocardium supplied by the stenotic artery was not ischemic under rest conditions (i.e., an increase of the intravascular concentration of deoxyhemoglobin can be ruled out as responsible for the reduced T2* at rest). Hence, the explanation left is that the relative intravascular volume of vessels containing deoxyhemoglobin must increase.

**Evidence for capillary recruitment?** Only vessels containing a significant amount of deoxyhemoglobin are responsible for the BOLD effect. These are capillaries and veins, when arterial oxygenation is normal. Cardiac blood vessels

![Figure 3. (a) Individual T2* values of stenotic and nonstenotic myocardial areas (examination at rest). (b) Relative changes in T2* before and after administration of dipyridamole (DIP). Results of all patients. Error bars represent the standard deviation.](image)
can be divided in epicardial and intramyocardial vessels. The intracapillary volume is a fraction of the intramyocardial blood volume. Based on the anatomic data of Kassab et al. (18), Kaul and Jayaweera (4) state that this fraction is >90% of the intramyocardial blood volume. Thus, the fraction of the intramyocardial venous compartment is smaller than 10%. This implies that of all intramyocardial vessels, mainly capillaries contribute to the BOLD effect. Theoretical considerations from our group demonstrated that this capillary contribution is sufficient to be responsible for the myocardial BOLD effect, as observed in animal studies and humans (1,2). The main portion of the venous volume resides in larger epicardial veins, which, in principle, may also contribute to the BOLD effect. However, one would have expected a spotted texture of myocardium visible in amplitude images and calculated $T_2^*$ maps, which was not the case. In our study, visible veins were excluded from segmentation.

When the BOLD effect is mainly related to intracapillary deoxyhemoglobin, it is evident that the reduced $T_2^*$ in myocardium supplied by a stenotic coronary artery reflects the elevated relative intracapillary blood volume. This in-

**Figure 4.** Patient with high-grade stenosis of the proximal left anterior descending coronary artery (LAD). Coronary angiograms in typical (a) right anterior oblique (RAO) and (b) left anterior oblique (LAO) projections. The $T_2^*$ maps (short-axis view of left ventricle) before (c) and after (d) administration of dipyridamole. In the latter case, the magnetic resonance examination had to be interrupted during dipyridamole infusion owing to severe angina of the patient. Note the reduced $T_2^*$ in the anteroseptal area (the region which is associated with the diseased vessel). The dark region at the inferolateral zone (d) is due to susceptibility artifacts arising from phrenicomediastinal recess. Ten weeks after dilation of the LAD (e), the measurement (also with dipyridamole) was finished without complications. When observing the obtained $T_2^*$ maps, differences between regions with reduced $T_2^*$ values and regions of normal myocardium were less pronounced than they were before percutaneous transluminal coronary angioplasty. Colors from black to white reflect $T_2^*$ values from 15 to 50 ms. LCx = circumflex branch of left coronary artery.
Figure 5. The $T_2^*$ maps of a patient with high-grade stenosis of the proximal left anterior descending coronary artery. (a) Before coronary artery bypass graft surgery (CABG), areas with reduced $T_2^*$ values in the septal region are clearly detectable. (b) Twenty weeks after CABG of the left internal mammary artery, differences in $T_2^*$ were less pronounced. Note the homogeneity in the septal region after the intervention. Colors from black to white reflect $T_2^*$ values from 15 to 50 ms.

Figure 6. Concept of the blood oxygenation level-dependent (BOLD)-related effect of capillary recruitment on $T_2^*$. (a) Under normal perfusion conditions at rest, only a fraction of capillaries was open, contributing to the BOLD effect (the large arteriovenous difference along the capillary is represented by the dark end of the capillary). (b) Dipyridamole (DIP) produced enhanced coronary vasodilation without increasing heart work (light end of the capillary represents a decreased arteriovenous difference). (c) In the presence of a coronary artery stenosis, autoregulation induced compensatory relaxation of coronary resistance vessels and precapillary sphincters, which maintain sufficient blood supply under rest conditions. (d) Hence, DIP cannot produce further vasodilation. The green curve symbolizes the magnetic field inhomogeneities.
crease of the relative intracapillary blood volume is most likely a result of coronary autoregulation, which maintains sufficient blood supply by post-stenotic dilation of coronary resistance vessels and precapillary sphincters, leading to capillary recruitment. This vasodynamic response was also found by echocardiographic studies in animal models with an induced coronary stenosis (3). In summary, there is evidence that the decrease of $T_2^*$ reflects the autoregulatory response to a coronary stenosis on the microvascular level. This vasodynamic response also explains the diminished increase of $T_2^*$ after dipyridamole application in regions supplied by a stenotic artery. Because dilation of resistance vessels and precapillary sphincters already occurs under rest conditions in these regions, dipyridamole cannot considerably dilate these vessels further (i.e., there is no significant increase of local blood flow and, hence, no increase in $T_2^*$) (Fig. 6).

**Artifacts.** A reduction in $T_2^*$ can also arise due to artifacts, as recently described (11,14,15). In our study, in a few patients, reproducible susceptibility artifacts occurred in phrenicomedial sternal recess, which superimposed inferolateral sections of myocardium. This phenomenon is caused by through-plane perturbations of veins (11) and the heart-lung interface along the inferior segments of the heart (15). We found that in humans, these artifacts could be reduced by performing measurements in end-expiratory breathhold when phrenicomedial sternal recess was diminished (14).

**Study limitations.** One might argue that patients undergoing MRI were selected according to their clinical and angiographic presentation and a positive stress echocardiogram. However, this was a pilot study to demonstrate that a noncontrast agent MRI technique may detect alterations of microcirculation in myocardium supplied by a stenotic coronary artery. The ischemia-related potential of this stenosis was proven by stress echocardiography, which is a well-established method in this field (19). It was not our intention to compare established techniques with the MRI technique to gain specificity or sensitivity of the latter, which requires a much higher number of patients. In this pilot study, we did not perform scintigraphy for the following practical reasons: in order to find well-defined regions with potential altered microcirculation, only patients with single-vessel disease were included. In normal clinical routine, angiographic diagnosis and therapy are performed in one session in these patients. However, MRI interrupted this procedure, and the interval between diagnosis and therapy would have been prolonged if scintigraphy were performed, which would have been in conflict with ethical and cost considerations. Two patients were re-examined after therapeutic interventions, and the obtained $T_2^*$ maps were more homogeneous than they were before therapy. Of course, stenting and CABG induce susceptibility artifacts, which can affect image quality. In the present study, however, this could reliably be excluded by choosing a suitable imaging plane (i.e., below the stent), and in the future, nonmagnetic stents and clips might overcome this problem.

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