Chronic coronary artery stenosis induces impaired function of remote myocardium: MRI and spectroscopy study in rat

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Our purpose was to study morphological, functional, and metabolic changes induced by chronic ischemia in myocardium supplied by the stenotic vessel and in the remote area by MR techniques. A new technique of image fusion is proposed for analysis of coronary artery stenosis involving coronary MR angiography and spectroscopic imaging. Cine-MRI was performed 2 wk after induction of coronary stenosis. Global heart function and regional wall thickening were determined in 11 Wistar rats with stenosis and compared with 7 control rats. Two weeks after stenosis was induced, spin-labeling MRI for measurement of perfusion was performed in 14 isolated hearts. In eight isolated hearts with coronary stenosis, MR spectroscopy was performed, followed by angiography. 31P metabolite maps were fused with three-dimensional coronary angiograms. Induction of stenosis led to reduced segmental wall thickening (control: 75 ± 9%, ischemic region: 9 ± 3%, P < 0.05 vs. control) but also to impaired function of the remote region and lower cardiac output. Perfusion was reduced by 74.9 ± 4.0% within ischemic segments compared with a septal control region. The phosphocreatine (PCr)/ATP ratio as a marker of ischemia was reduced in the region associated with stenosis (1.09 ± 0.09) compared with remote (1.27 ± 0.08) and control hearts (1.43 ± 0.08; P < 0.05). The histological fraction of fibrosis within the ischemic region (12.8 ± 1.4%) correlated to ATP signal reduction from remote to the ischemic region (r = 0.71, P < 0.05), but not to reduced wall thickening. Coronary narrowing caused declining function accompanied by diminished PCr/ATP, indicating impaired energy metabolism. Neither decline of function nor PCr signal decline correlated to fraction of fibrosis in histology. In contrast, reduction of ATP correlated to fibrosis and therefore to loss of viability. Impaired function within the ischemic region is associated with decreased PCr. Function of the remote region was affected as well. The fusion of PCr metabolite maps and the coronary angiogram may help to assess coronary morphology and resulting metabolic changes simultaneously.

Several mechanisms whereby ischemic heart disease leads to chronic global mechanical dysfunction of the left ventricle have been well investigated. One is remodeling of remote myocardium after infarction, which has been studied extensively in animal models and humans (11, 23, 26). Another is hibernation of chronically ischemic myocardium, which is essentially reversible after revascularization (14, 28). A third is microinfarction secondary to coronary microembolization (8, 33). However, the interplay between chronic ischemic myocardium and its adjacent normally supplied area is less well investigated but clinically important. The purpose of this study is to analyze how ischemic myocardium may influence function and metabolism of remote regions with normal perfusion values. After revascularization, global function of the left ventricle may improve to a great extent. So far, it is believed that this is caused by recovery of the ischemic region only. However, in this study we discuss whether impaired function of remote regions may add to global dysfunction of hearts, which suffer from regional hypoperfusion.

The rat model of left ventricular (LV) remodeling induced by myocardial infarction has proved to be particularly valuable for the investigation of the development of heart failure and its prevention by therapy (16, 21, 25). The application of MRI to this model has added new insights due to its exact and noninvasive nature (22, 23, 31). These techniques are now used to study a model of coronary stenosis originally proposed by Guski et al. (12) and Capasso et al. (6). In the present study we evaluate LV morphology and performance (resting conditions and stress) over a specified time course and compare it with the myocardial infarct...
(MI) model. Perfusion measurements were performed to confirm that induction of coronary stenosis led to hypoperfusion. To assess metabolic changes, localized $^{31}$P MR spectroscopy was utilized. A new concept of fusion of angiographic and metabolic images is proposed.

**METHODS**

**Experimental protocol.** In 30 female Wistar rats, left coronary artery stenosis was induced, as described by Guski et al. (12) and Capasso et al. (6). Seven control animals were sham operated. In 10 rats, coronary artery ligation was performed to induce MI. Cine-MRI investigations of 11 rats with coronary stenosis, 7 sham rats, and 6 rats with MI were done 1 and 2 wk after surgery at rest and under intravenous dobutamine stress ($10 \mu l/kg \times min$) in a 7-T Biospec. This dosage of pharmacological stress was determined in pilot experiments as the dose with maximal response in systolic wall thickening. After completion of in vivo experiments, coronary angiograms were acquired in isolated hearts of all rats in a 12-T wide-bore magnet (model AMX, Bruker; Karlsruhe, Germany) to confirm stenosis or complete occlusion of the coronary artery. In additional experiments, localized $^{31}$P MR spectroscopy and angiography were performed in 8 isolated hearts of rats 2 wk after induction of stenosis and in 4 sham-operated rats, and perfusion MRI in 14 isolated hearts of rats 2 wk after induction of coronary stenosis.

The investigation conforms to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1996).

**Cine MRI.** Cine MRI experiments were performed on a 7.05 T Biospec 70/21 (Bruker) under 5 µl/min $^{-1}kg^{-1}$ disoprovanine anesthesia applied via tail vein, as described before (22, 30). An ECG-triggered fast gradient echo sequence (FLASH) (13) was used with the following parameters: flip angle $30^\circ$ to $40^\circ$, echo time 1.1 ms, repetition time (TR) 3.2 ms and 12 frames per heart cycle. With a field of view of 30–40 mm and an image matrix of 128 × 128, in-plane resolution was 230 to 310 µm. MI size was determined for every slice as the myocardial portion with significant thinning and akinesia or dyskinesia during systole, as described in (23). Myocardial thickening of the left ventricle was determined in the end-diastolic (EDW) and end-systolic (ESW) frame to calculate systolic wall thickening (ESW − EDW)/EDW in percent.

**Three-dimensional MRA.** After completion of in vivo MRI, angiography was performed in isolated hearts of all rats. The rats were anesthetized by pentobarbital sodium (160 mg/kg ip; Narcoren, Rhone Merieux; Laupheim, Germany). Hearts were rapidly excised, immersed in ice-cold buffer, and perfused within 2 min [Langendorff mode (17)] with nonrecirculating 37°C Krebs-Henseleit buffer equilibrated with 95% O$_2$-5% CO$_2$ (pH 7.4) at constant perfusion pressure (100 mmHg). A water-filled latex balloon was inserted in the left ventricle and connected to a pressure transducer (model P23 XL, Statham) to measure LV pressure, which was recorded by a personal computer to trigger MRI pulse sequences. MR coronary angiography was performed as described by Roder et al. (29) in a 12-T wide-bore magnet (model AMX 500, Bruker; Karlsruhe, Germany). High-resolution images were obtained by flow-weighted pulse sequences. Coronary vessels were imaged by a middiastolic triggered flow-weighted three-dimensional (3D) gradient echo pulse sequence with an echo time (TE) of 1.0 ms and a repetition time (TR) of one heart cycle (~200 ms). Complex data points (96$^2 \times 128$) were acquired. Data were zero filled before Fourier transformation to 128$^3$ data points.

**MR spectroscopy.** In an additional set of experiments, in eight rats 2 wk after induction of coronary stenosis and four sham-operated rats, hearts were excised and first MRA as described above and then localized $^{31}$P MR spectroscopy was performed in the Langendorff perfusion mode on a Bruker 12-T AMX spectrometer (36). The custom-built double-resonant cross-cage probe (18) enabled the detection of $^1$H and $^{31}$P signals in one setup. An accumulation-weighted (AW) 3D phase-encoded $^{31}$P chemical shift imaging (CSI) experiment (19, 27) was performed. The number of accumulations at each phase-encoding step was calculated according to a Hanning window in $k$ space, which resulted in a significantly lower signal contamination of neighboring voxels at preserved spatial resolution and sensitivity. The field of view of the 3D AW CSI experiment was $40 \times 40 \times 60 \, mm^3$ and the nominal voxel size was $4 \times 4 \times 6 \, mm^3$. In an overall experiment time of ~60 min, the average signal-to-noise ratio for phosphocreatine (PCr) in the local spectra at the nominal voxel size of 96 µl, determined from the fitted initial signal amplitudes of the single metabolites and the standard deviation of the noise in the last 1/5 of the acquisition window was 7.6 in the sham hearts, 6.5 in the ischemic regions of hearts with coronary stenosis, and 9.4 in the remote regions. Anatomic multislice $^1$H gradient echo (GE) images were acquired to correlate the position of the stenotic region in the $^{31}$P CSI metabolite maps and the coronary angiography data. By segmentation of these images, a mask was generated and used for volume correction of spectroscopic data during postprocessing. Because the voxel size in the CSI experiment was in the range of the LV wall thickness, every AW CSI voxel is only partially filled with myocardium. Therefore, the signal levels in the local $^{31}$P spectra are attenuated by a spatially dependent factor $K_P$PVE a vector indicating the position of the vessel under consider-

### Table 1. Results of Cine-MRI at rest

<table>
<thead>
<tr>
<th></th>
<th>Week 1</th>
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<th>Week 2</th>
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<tr>
<td></td>
<td>Sham</td>
<td>MI</td>
<td>Stenosis</td>
<td>Sham</td>
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<td></td>
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<tr>
<td>Heart rate, beats/min</td>
<td>364 ± 11</td>
<td>329 ± 15</td>
<td>348 ± 13</td>
<td>325 ± 14</td>
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<tr>
<td></td>
<td>283 ± 8</td>
<td>290 ± 6</td>
<td>276.1 ± 4.9</td>
<td>286.8 ± 6.8</td>
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<td>BW, g</td>
<td>485.7 ± 15.1</td>
<td>553.3 ± 28.8</td>
<td>532.1 ± 14</td>
<td>509.1 ± 26.6</td>
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<td>LV mass, mg</td>
<td>175.2 ± 30.1</td>
<td>175.2 ± 30.1</td>
<td>104.4 ± 17.4</td>
<td>48.3 ± 7.3</td>
</tr>
<tr>
<td>ESW, µl</td>
<td>50.2 ± 5.4</td>
<td>96.4 ± 12.2</td>
<td>85.2 ± 7.1</td>
<td>146.9 ± 20</td>
</tr>
<tr>
<td>SV, µl</td>
<td>126.8 ± 8</td>
<td>96.4 ± 12.2</td>
<td>112.1 ± 11</td>
<td>106.5 ± 7.4</td>
</tr>
<tr>
<td>EDWT, mm</td>
<td>1.93 ± 0.07</td>
<td>1.92 ± 0.09</td>
<td>2.1 ± 0.07</td>
<td>1.8 ± 0.06</td>
</tr>
<tr>
<td>Area T, mm</td>
<td>0.91 ± 0.09†</td>
<td>1.01 ± 0.1</td>
<td>1.8 ± 0.09†</td>
<td>1.4 ± 0.09†</td>
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Values are means ± SE. MRI, magnetic resonance imaging; MI, myocardial infarction; BW, body weight; LV, left ventricle; ESW, end-systolic volume; SV, stroke volume; EDWT, end-diastolic wall thickness; Area T, end-diastolic wall thickness of regions associated with stenosis or MI. *P < 0.05 vs. sham; †P < 0.05 vs. stenosis.
ation in space \((r)\) reflecting to the so-called “partial volume effect” (PVE). As a consequence, the absolute signal amplitudes of the metabolite resonances in the local spectra from different positions in the same heart cannot be compared. To correct for the PVE, the 3D segment mask derived from the \(^1\text{H} \text{ GE image data was convolved with the simulated 3D point spread function of the spectroscopic experiment. The resulting matrix was used to calculate local correction factors } k_{\text{corPVE}}(r) = 1/k_{\text{PVE}}(r) \text{ for the } ^{31}\text{P CSI data. Only voxels that had a correction factor } k_{\text{corPVE}}(r) < 2.5 \text{ were included. All other positions were set to zero. With this correction, local signal levels in the same heart could be compared in a quantitative manner. Local spectra from four different positions in a midventricular transversal slice, one in the ischemic and three equally spaced in remote regions, were extracted from the 3D AW CSI dataset. All spectra were fitted with the AMARES time domain fitting routine (36). To determine a correction factor for T\(_1\) saturation, heartbeat-triggered (TR \(\sim 200 \text{ ms}\)) and fully relaxed (TR \(\sim 10 \text{ s}\)) global \(^{31}\text{P} \text{ spectra were acquired. The peak area ratios were used to calculate the saturation correction factors for each individual metabolite. The signal amplitudes and PCr-to-ATP ratios (PCr/ATP) were derived from the peak areas in the local spectra. After MR spectroscopy, 3D MR angiography was

![Images of rats with various heart conditions](attachment)

**Fig. 1.** Systolic frames of cine-MRI at week 2. **Top,** sham-operated rats. **Middle,** rats with coronary stenosis. Associated segments exhibit lower end-systolic wall thickness (arrows). **Left,** short axis view of a rat with a small region affected by coronary stenosis. **Middle,** short-axis view of a rat with a large affected region. **Bottom,** rat with large myocardial infarction (MI).
performed as described above to verify coronary stenosis. The metabolite maps of the PCr and the γ-ATP resonance were fused with the coronary angiogram using the Amira software on a SGI workstation (see http://www.amiravis.com).

**Spin-labeling perfusion MRI.** To assess the impact of coronary artery narrowing on myocardial perfusion, a separate group of 14 rats was studied 2 wk after surgery. In the retrogradely perfused isolated hearts, a high spatial resolution spin-labeling MRI experiment was performed in a 12-T wide-bore magnet with an experimental set up as described above. The spin-labeling technique has previously been validated against microspheres and first-pass MR perfusion measurements (3, 4). The spins of a selected slice in the short-axis view were inverted by an adiabatic 180° pulse with a slice thickness of 3 mm. After the slice-selective inversion, a series of 16 diastolic-triggered Snapshot FLASH images were acquired to observe the T1 relaxation (TE = 1 ms, TR = 3.6 ms, α = 3°, in plane resolution 140 μm², slice thickness 1.5 mm, acquisition time <1 min). T1 maps were used to determine differences of perfusion between a control region within the septum and hypoperfused regions in the LV free wall within the same heart. After perfusion measurements, coronary angiography was performed to confirm coronary stenosis.

**Histology.** The hearts were excised and fixed in distended form in 3.4% buffered formalin. They were dehydrated in alcohol, washed in xylene, and embedded in paraffin. Transverse serial sections of 3-μm thickness were obtained from the midventricular region. The sections were mounted and stained with hematoxylin and eosin. The slices were scanned with a Sony video scanner and the following areas were determined planimetrically: the region associated to the stenosed vessel containing fibrotic islets (region A + B in Fig. 8), islets of fibrotic tissue within this region (region B in Fig. 8), and the entire area of the myocardial ring (Scan Pro, Jandel Software).

**Statistical data analysis.** If not indicated otherwise, all data are given as means ± SE. Two-tailed Student’s t-test for paired group was performed with Duncan’s posttest. P < 0.05 was considered statistically significant.

**RESULTS**

Fourteen of 16 rats survived the induction of coronary stenosis and 6 of 10 survived the MI procedure. In MR angiography (MRA), three rats that were originally subjected to coronary stenosis developed coronary occlusion and were removed from the stenosis group. The mean infarct size of rats in the MI group was 32 ± 4%. There were no differences in LV mass between groups or timepoints.

**Segmental morphology and function.** Induction of coronary stenosis led to significant thinning of the ischemic segment (Table 1 and MR images in Fig. 1). However, thinning was more pronounced in the infarcted region in the MI group. End-diastolic remote wall thickness was stable from week 1 to week 2 in the stenosis as well as in the MI group (Table 1). As shown in Fig. 2, systolic function of the segment associated with stenosis was decreased and deteriorated from week 1 to week 2 (Fig. 2B), but was better than in the MI group. The infarcted region of the MI group was akinetic or dyskinetic (Fig. 2A).

**Global LV performance.** Remote systolic wall thickening was impaired in the stenosis as well as in the MI group (Fig. 2A). As shown in Fig. 3, end-diastolic volume increased significantly only in the MI group. In rats with coronary stenosis, there was a trend toward larger ventricles. Global LV function as characterized by ejection fraction and cardiac output was affected in rats with MI as well as in rats with coronary stenosis (Figs. 4 and 5).

**Dobutamine stress.** Dobutamine stress increased heart rate in all groups (Table 2). This was the main cause for the increase of cardiac output during dobutamine infusion (Fig. 5). End-diastolic and end-systolic volumes were smaller (Table 2). Smaller end-diastolic volumes with little change in stroke volumes led to higher ejection fraction in all groups (Fig. 4).

In sham rats, wall thickening increased by ~30% due to dobutamine stress. This contractile reserve was reduced to values well below 20% in rats with stenosis and MI in the remote zone. In the segments associated
Fig. 3. End-diastolic volumes at week 1 (a) and 2 (b), shown for rest (A) and during intravenous dobutamine infusion (B). Only rats with MI exhibit significant enlargement of the left ventricle compared with sham-operated rats. Rats with MI and sham animals show significantly reduced end-diastolic volumes in stress testing, but rats with coronary stenosis do not.

Fig. 4. Ejection fraction at week 1 (a) and 2 (b), shown for rest (A) and during intravenous dobutamine infusion (B). Both MI and stenosis show significantly reduced ejection fraction at rest and during stress, but ejection fraction improved during stress.

Fig. 5. Cardiac output at week 1 (a) and 2 (b), shown for rest (A) and during intravenous dobutamine infusion (B). Cardiac output was reduced in the MI and in the stenosis group in a similar fashion. Dobutamine stress led to a significant increase, mainly caused by higher heart rates.
with stenosis, it was reduced to 3% at week 1 and 10% at week 2. In the infarcted zone, dobutamine stress turned akinesia into dyskinesia (−9%).

MRA. Figure 6 displays two examples of coronary angiograms. The 3D dataset can be fully rotated around the z-axis. Vessels with a cross-sectional diameter of >140 μm were clearly detectable. Ligation was performed on a main branch or directly on the left coronary artery; therefore, the effect was clearly within the diagnostic range of imaging. In hearts with MI, the signal ceased completely at the site of ligation. In opposition to that, a short signal void with returning signal distal from the location of the stenosis was found in the hearts with coronary narrowing. With the used imaging sequence, the signal depends on flow. Within the stenotic vessel segment, turbulent flow leads to a lower signal. Because stenosis was induced by ligation, the result is a rather short stenotic segment.

MR spectroscopy. A significant reduction in the PCr/ATP (P < 0.05) was found in the region associated to stenosis (1.09 ± 0.09), compared with the average of the remote positions (1.27 ± 0.08) and to the control hearts (1.43 ± 0.08, n = 4), whereas the reduction of the PCr/ATP in remote areas of hearts with stenosis (n = 8) did not reach significance compared with values of control hearts. The signal of PCr in the ischemic region was reduced by 19.1 ± 5.7% (P < 0.05) and the signal of ATP by 5.8 ± 2.9% (P = not significant) compared with the mean value of three remote regions of the same heart.

Figure 7 shows the metabolite distribution for PCr in a midventricular transversal slice of an isolated heart with coronary stenosis. The two-dimensional 31P metabolite map was fused with the maximum intensity projection of the angiography data set. There is a PCr signal reduction in the ischemic region that corresponds to the stenosis seen in the culprit vessel.

Perfusion MRI. As shown in Fig. 8, regions with diminished perfusion were clearly detectable in perfusion T1 maps. On average, the difference between the control region within the septum and hypoperfused regions was −15.52 ± 1.18 ml·min⁻¹·g⁻¹, and this equals a reduction of 74.9 ± 4.0% compared with the control region.

Histology. The fibrotic islets (region B in Fig. 9) were a fraction of 12.8 ± 1.4% within the area associated to coronary stenosis (region A + B in Fig. 9). This area, consisting of fibrotic islets and ischemic myocardium (region A + B in Fig. 9) was 28.3 ± 4% of the whole myocardial ring. The fraction of fibrosis within the region associated to the narrowed coronary was correlated to the ATP signal reduction from control region to the ischemic region in spectroscopy (Fig. 10; r = 0.71, P < 0.05), whereas there was no correlation between PCr signal change and this area (r = −0.1, P = 0.4). No correlation was found between fraction of fibrosis and wall thickening in the ischemic segment.

DISCUSSION

Combining diagnostic measures for in vivo assessment of anatomy, perfusion, function, and metabolism, the present study characterizes a potentially very useful animal model of coronary heart disease. Induction

![Fig. 6. Three-dimensional coronary angiograms were used to confirm status of coronary arteries. A: MR angiography (MRA) displays occlusion of the coronary, resulting in myocardial infarction. There is a complete loss of signal at the site of ligation. B: A short signal void at the place of coronary stenosis is followed by poststenotic signal within the coronary artery excluding complete closure of the coronary.](http://ajpheart.physiology.org/DownloadableFrom/10.1152/ajpheart.00533.2003.on/April_13_2017)
of coronary stenosis led to a significant impairment of regional wall thickening and reduced perfusion. Unexpectedly, the remote regions of hearts with coronary stenosis exhibited impaired contractility leading to a depressed global LV function comparable to hearts with MI, although the loss of viable myocardium was very small in hearts with stenosis (area of fibrotic islets is 3.7 ± 0.8% of the midventricular myocardial ring in histology, whereas in the MI group 32 ± 4% of the whole left ventricle was determined to be infarcted by multislice cine MRI).

Cine MRI: baseline and stress. The impairment of thickening of the remote region in the stenosis group in a similar fashion to the MI group (Fig. 2A) has not been reported so far. However, the resulting decline in global LV function with reduced EF and cardiac output is in line with previous reports about hemodynamics in this model. Capasso et al. (6) found a significant rise of the LV end-diastolic pressure and a drop of systolic pressure immediately after induction of stenosis and 5 days thereafter. Furthermore, in vitro measured peak shortening of left and right ventricular myocytes was impaired, accompanied by impaired function of α1-adrenergic receptors (7). The reason for reduced function in the remote zone needs to be determined in future studies. Humoral factors described to be effective in hibernation at the low-flow zone (2, 9) and remodeling of the extracellular matrix as described for remod-

![Fig. 7. Results of spectroscopy. A: phosphocreatine (PCr)-to-ATP ratios (PCr/ATP) for three regions: sham, sham-operated rats, stenosis, stenosis associated region; and stenosis remote, region not associated with stenosis. PCr/ATP was diminished within the ischemic region. The reduction in the remote regions was not significant compared with sham-operated rats. B: fusion image of coronary angiogram and PCr metabolite map. Arrow points at coronary stenosis. The culprit vessel feeds a region with diminished PCr signal caused by ischemia. In the future, this kind of fusion image may aid in the estimation of severity of stenosis.](image)

![Fig. 8. MR perfusion map of a heart 2 wk after induction of coronary stenosis, displaying a region with reduced perfusion (higher T1 values, arrow). T1 is measured in seconds.](image)

![Fig. 9. Short-axis histology of a heart with coronary stenosis. In this particular heart, fraction of fibrosis (region B) of the ischemic area (region A + B) was 18% and therefore above the mean value of all hearts.](image)
cause the difference in wall thickening between the ischemic and the nonaffected myocardium is enhanced. For example, differences of wall thickening between control and segments associated to the stenosis at 2 wk were 66% at rest but 91% during dobutamine stress. Furthermore, small differences between treatment groups in a study to test a therapeutic measure may be unmasked under dobutamine stress.

Coronary angiography. Coronary stenosis was confirmed by MR coronary angiography in the isolated hearts; however, the degree of narrowing could not be determined. Capasso et al. (6) demonstrated that using a wire with a diameter of 275 μm caused coronary artery narrowing of 62% in rats 5 days after induction of stenosis. Guski et al. (12) described a progressive narrowing of a stenosis induced by this technique due to proliferation of intimal layers of the vessel (90% narrowing after 3 wk). This may explain the decline of wall thickening from week 1 to week 2 in the ischemic segment and transgression of three animals with coronary narrowing toward total occlusion, which were identified by MRA.

MR spectroscopy. To assess whether the impaired function of the remote segment of hearts with stenosis is associated with altered cardiac high-energy phosphate metabolism, localized MR spectroscopy was employed in the isolated, beating hearts. In accordance with reports of decreased PCr/ATP in remote regions of infarcted hearts (10, 24), we found a trend for a reduction of that ratio between sham rats and the remote zone of rats with coronary stenosis (not significant).

We found a correlation of histologically determined fraction of fibrosis with ATP signal reduction in line with a study by Friedrich et al. (10), who reported a direct relation between viable tissue mass and the ATP signal in chronically infarcted rat hearts. In a clinical study, Yabe et al. (38) reported diminished concentrations of ATP in patients with a fixed, but not in patients with a reversible thallium defect. In accordance with these studies, reduction of ATP is therefore likely to represent reduced viability in our study.

In line with the reduction of wall thickening and the decrease of perfusion, the PCr signal and PCr/ATP in the region associated with coronary stenosis were significantly reduced. This alteration in energy metabolism is comparable to the extent reported by Zhang et al. (39, 40) in acute ischemia. There are two possible explanations for the diminished PCr/ATP and the PCr signal reduction in conjunction with impaired wall thickening. First, viable myocardial portions may be in a chronic ischemic state, such as hibernation (39). This is supported by the lack of correlation between fibrosis in histology and PCr signal reduction and with reduced wall thickening, suggesting that the signal reduction of PCr can be attributed to the impaired metabolic situation of ischemic myocardium (Fig. 9, region A) rather than to fibrosis (Fig. 9, region B).

A second possible explanation of our findings may be that in fact there is no hibernation in the segment associated with coronary stenosis, but dysfunction may be rather introduced by microinfarcts, which interfere with the normal contraction pattern. However, we found no correlation between the fraction of fibrosis and decline of wall thickening, therefore the loss of function cannot be explained by the amount of loss in viable myocardium volume directly.

An additional explanation for the existence of microinfarcts and their lack of correlation to loss of function may be coronary microembolization, which has been described to cause progressive contractile dysfunction not related to the extent of myocardial necrosis or apoptosis. In microembolization, dysfunction is a consequence of inflammation and mediated by nitric oxide (NO), tumor necrosis factor, and sphingosine (8, 33). In that case, a decreased β-adrenergic responsiveness of the region associated to coronary stenosis might be a consequence of an increased NO concentration secondary to inducible NO synthase expression (8). However, microembolization has been induced by microspheres in the experimental setting and has been described in the clinical situation of plaque rupture. In our study, neither microspheres were used, nor did we collect evidence for the presence of intracoronary plaque.

An explanation for the reduced response of the remote region to dobutamine in our study (Fig. 2A) may be the finding of Meggs et al. (20), who reported a decrease of β-adrenoceptor density 5 mo after induction of stenosis.

Stress testing proved to be useful in this model to delineate areas associated to the stenotic vessel, because the difference in wall thickening between the ischemic and the nonaffected myocardium is enhanced. For example, differences of wall thickening between control and segments associated to the stenosis at 2 wk are 66% at rest but 91% during dobutamine stress. Furthermore, small differences between treatment groups in a study to test a therapeutic measure may be unmasked under dobutamine stress.

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An explanation for the reduced response of the remote region to dobutamine in our study (Fig. 2A) may be the finding of Meggs et al. (20), who reported a decrease of β-adrenoceptor density 5 mo after induction of stenosis.

Stress testing proved to be useful in this model to delineate areas associated to the stenotic vessel, because the difference in wall thickening between the ischemic and the nonaffected myocardium is enhanced. For example, differences of wall thickening between control and segments associated to the stenosis at 2 wk are 66% at rest but 91% during dobutamine stress. Furthermore, small differences between treatment groups in a study to test a therapeutic measure may be unmasked under dobutamine stress.

Coronary angiography. Coronary stenosis was confirmed by MR coronary angiography in the isolated hearts; however, the degree of narrowing could not be determined. Capasso et al. (6) demonstrated that using a wire with a diameter of 275 μm caused coronary artery narrowing of 62% in rats 5 days after induction of stenosis. Guski et al. (12) described a progressive narrowing of a stenosis induced by this technique due to proliferation of intimal layers of the vessel (90% narrowing after 3 wk). This may explain the decline of wall thickening from week 1 to week 2 in the ischemic segment and transgression of three animals with coronary narrowing toward total occlusion, which were identified by MRA.

MR spectroscopy. To assess whether the impaired function of the remote segment of hearts with stenosis is associated with altered cardiac high-energy phosphate metabolism, localized MR spectroscopy was employed in the isolated, beating hearts. In accordance with reports of decreased PCr/ATP in remote regions of infarcted hearts (10, 24), we found a trend for a reduction of that ratio between sham rats and the remote zone of rats with coronary stenosis (not significant).

We found a correlation of histologically determined fraction of fibrosis with ATP signal reduction in line with a study by Friedrich et al. (10), who reported a direct relation between viable tissue mass and the ATP signal in chronically infarcted rat hearts. In a clinical study, Yabe et al. (38) reported diminished concentrations of ATP in patients with a fixed, but not in patients with a reversible thallium defect. In accordance with these studies, reduction of ATP is therefore likely to represent reduced viability in our study.

In line with the reduction of wall thickening and the decrease of perfusion, the PCr signal and PCr/ATP in the region associated with coronary stenosis were significantly reduced. This alteration in energy metabolism is comparable to the extent reported by Zhang et al. (39, 40) in acute ischemia. There are two possible explanations for the diminished PCr/ATP and the PCr signal reduction in conjunction with impaired wall thickening. First, viable myocardial portions may be in a chronic ischemic state, such as hibernation (39). This is supported by the lack of correlation between fibrosis in histology and PCr signal reduction and with reduced wall thickening, suggesting that the signal reduction of PCr can be attributed to the impaired metabolic situation of ischemic myocardium (Fig. 9, region A) rather than to fibrosis (Fig. 9, region B).

A second possible explanation of our findings may be that in fact there is no hibernation in the segment associated with coronary stenosis, but dysfunction may be rather introduced by microinfarcts, which interfere
with the normal contraction pattern. In that case, decreased PCr and PCr/ATP may simply reflect the higher workload, which is imposed on the surviving myocytes by the disrupted fiber geometry caused by fibrotic islets. This theory should be verified in future studies through correlation of the decrease of perfusion with the fraction of fibrosis.

For the first time, MR coronary angiography and metabolite maps acquired by MR spectroscopy were fused (Fig. 7). In the future, this concept of image fusion may be useful in characterization of the severity of a stenosis and may aid decision making concerning revascularization because not only anatomy, but also metabolic information can be given simultaneously. However, in our study, data for image fusion was acquired from isolated hearts, and substantial technical progress in MR techniques is mandatory to perform a similar imaging procedure in a patient.

Limitations. Despite of the good quality of the coronary angiogram with visualization of even small side branches of the vascular bed, the degree of coronary narrowing could not be quantitated. It would be desirable to have a more exact morphological characterisation of the lesion.

Spatial resolution of spectroscopy was limited. This led to an underestimation of viability loss compared with histology (5.8% decline in ATP signal vs. 12.8% of fibrosis within area associated with stenosis). However, reducing the slice thickness in MR spectroscopy (6 mm in our study) with the current technique would mean to prolong the acquisition time beyond acceptable limits for valid metabolic studies. It would have been desirable to investigate regional function and metabolism after revascularization; however, this was not feasible in the given experimental setting.

In conclusion, a combination of Cine-MRI with 3D coronary angiography and phosphorous spectroscopy proved to be the appropriate diagnostic tool to characterize the rat model of coronary artery stenosis. Coronary narrowing led not only to local but also to global decline of function with reduced contractility in remote regions. Contrary to MI, no LV dilatation was detected. The effects of ischemia were rather severe: MRS and histology suggest that reduced wall thickening is not caused by reduced viability (ATP signal reduction and correlating fibrosis).

Application of MRI and MRS to this model is the future opportunity to assess the impact of pharmacological treatment of coronary artery disease or novel treatment options like induction of neoangiogenesis. Although some measurements were performed in the isolated heart, this study provides further arguments for the value of a single cardiac MR investigation integrating different modalities to thoroughly assess a patient’s heart problem. The proposal to fuse the results of different modalities into one image may further add to the advantages of a “one-stop shop” MR examination for cardiac diagnostics.

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