Non-invasive characterization of the area-at-risk using magnetic resonance imaging in chronic ischaemia

Ming Wu, Jan D’hooge, Javier Ganame, Vesselina Ferferieva, Karin R. Sipido, Frederik Maes, Steven Dymarkowski, Jan Bogaert, Frank E. Rademakers, and Piet Claus*

1Cardiovascular Imaging and Dynamics, Department of Cardiovascular Diseases, Catholic University Leuven, Medical Imaging Research Center, University Hospitals Leuven, Campus Gasthuisberg, Herestraat 49, 3000 Leuven, Belgium; 2Department of Radiology, Catholic University Leuven, Leuven, Belgium; 3Experimental Cardiology, Department of Cardiovascular Diseases, Catholic University Leuven, Leuven, Belgium; and 4Department of Electrical Engineering, Catholic University Leuven, Leuven, Belgium

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Aims
We investigated the performance of quantitative stress perfusion magnetic resonance imaging (MRI) as a basis for identifying and characterizing the area-at-risk subtending a chronic coronary artery (CA) stenosis.

Methods and results
Pigs underwent a percutaneous copper-coated stent implantation in the circumflex CA (n = 11) or a sham operation (n = 5). After 6 weeks, angiography and MRI were performed including cine (rest, low- and high-dose dobutamine stress), dual-bolus first-pass perfusion (rest and adenosine stress), and contrast-enhanced imaging to quantify myocardial infarction (MI). Myocardial blood flow (MBF) was quantified based on Fermi-model deconvolution and compared with microsphere measurements. On the basis of Evan’s blue staining, MBF thresholds to define the area-at-risk were determined by receiver-operating characteristic (ROC) analysis. CA stenosis was 94 ± 7% and infarct size (IS) 7.3 ± 3.1% of left ventricular mass. Segmental thresholds of hyperaemic MBF yielded the best performance for detecting area-at-risk. There was a good correlation between MRI and microsphere perfusion (r² = 0.84, P < .0001). The area-at-risk presented a mixed substrate of non-infarcted (non-MI), <50% infarcted (MI+), and >50% infarcted (MI++) segments. MBF was reduced in at-risk vs. remote segments at rest (non-MI, 0.50 ± 0.21; MI+, 0.47 ± 0.14; MI++, 0.42 ± 0.14; remote, 0.84 ± 0.25 mL/min/g) and during stress (non-MI, 0.69 ± 0.09; MI+, 0.66 ± 0.14; MI++, 0.51 ± 0.11; remote, 1.70 ± 0.36 mL/min/g). Segmental wall thickening showed different responses to stress (remote, progressive increase during incremental stress; non-MI, increase at low-dose and discontinued at high-dose; MI+, initial increase and decrease at high-dose; MI++, progressive decrease).

Conclusion
Quantitative hyperaemic perfusion MRI accurately defines segments in the area-at-risk in chronic ischaemia, which present with different functional response to stress related to segmental IS.

Keywords
Ischaemia • Myocardial infarction • Perfusion • Myocardial contraction • Magnetic resonance imaging

1. Introduction
Despite successful treatment of acute coronary events, coronary artery (CA) disease still accounts for about two-thirds of the heart failure cases, which are frequently secondary to myocardial infarction (MI). The extent of the area-at-risk subtending a stenotic CA provides important diagnostic information. Recent investigations have shown that the myocardial ischaemic area-at-risk can be determined with magnetic resonance imaging (MRI) T2 STIR imaging by assessing oedema in acute infarction. However, in ‘chronic’ CA disease, the assessment of the area-at-risk relies on myocardial perfusion measurements. Myocardial perfusion has often been assessed using microspheres or nuclear techniques. For the detection of the area-at-risk, post-mortem staining has been the method of choice. First-pass perfusion MRI offers an alternative non-invasive method to assess myocardial blood flow (MBF). Dual-bolus first-pass...
myocardial perfusion can assess MBF quantitatively and has been validated against microsphere blood flow measurements in the acute setting.\textsuperscript{2,8}

Over the past years, a closed-chest preclinical porcine model for regional chronic ischaemia has been developed in our lab by the implantation of a copper-coated stent.\textsuperscript{5} This intervention results in a mixed model where the area-at-risk contains substrates ranging from transmural over non-transmural MI, to hibernating and stunned myocardium at the edges of the territory. As opposed to animal models for pure substrates,\textsuperscript{3–4} this model resembles the clinical correlate of chronic ischaemia due to CA stenosis. Moreover, the interaction of different substrates in a mixed model might result in other characteristics than those deduced from simple coexistence of pure substrates. However, the complete in vivo characterization of the different chronic ischaemic substrates in the area-at-risk remains challenging.

The objective of the current study was to determine whether quantitative stress perfusion MRI could be used as an imaging tool to identify segments in the hypo-perfused area-at-risk subtending a chronic CA stenosis. This quantitative perfusion MRI was compared with coloured microsphere measurements. It was also hypothesized that segments in this area-at-risk could be further classified into hibernating myocardium, and non-transmurally and/or transmurally infarcted myocardium by combining contrast-enhanced and functional stress MRI.

2. Methods

2.1 Instrumentation

This investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by a local Ethics Committee (Ethische Commissie Dierproeven, K.U. Leuven, Leuven, Belgium).

Seventeen crossbred domestic pigs of either gender (weight 25–30 kg, Animalium K.U. Leuven, Leuven, Belgium) were loaded with 300 mg aspirin (ASA) (Dispiril, Reckitt Benckiser, Brussels, Belgium) and 300 mg clopidogrel (Plavix, Sanofi-aventis, Paris, France) 1 day before the intervention. After intramuscular pre-medication with Telazol (tiletamine 4 mg/kg and zolazepam 4 mg/kg) (Zoletil100, Virbac Animal Health, Carros, France) and xylazine (2.5 mg/kg) (Vexylan, CEVA Sante Animale, Brussels, Belgium), anaesthesia was induced with intravenous propofol (10 mg/kg/h) and remifentanil (18 g/kg/min) (Ultiva, GSK, Overijse, Belgium) stress. Dobutamine was intravenously infused at a same rate. Adenosine (140 μg/kg/min) (Adenosin-Item Carinopharm, Gronau, Germany) was infused during 6 min for the stress study. Stress images were acquired after 4 min. Rest perfusion was assessed 20 min later to minimize contamination of the rest signal intensities by the residual GdDTPA of the stress boluses. The mean half-life time of GdDTPA is 12 ± 7.8 min.

Three slice locations (basal, mid, and apical) were acquired every R-R interval with a TurboFLASH sequence during 80 heartbeats. Typical imaging parameters included repetition time 241.61 ms, echo time 5.0 mm. The inversion time was modified iteratively to obtain maximal nulling of normal myocardium.

2.2 Data acquisition

In the sixth week after stent placement or sham operation, an angio- graphic examination and a cardiac MRI were performed. HR and BP were monitored during the entire protocol. Pre-medication, anaesthesia, and ventilation followed the same protocol as described above. After the study, animals were euthanized with an overdose of saturated potassium chloride under deep anaesthesia and hearts were rapidly excised for further microsphere analysis and pathological examination.

2.2.1 Coronary angiography

Coronary angiography was performed with a 6 F Judkins left coronary catheter. Contrast medium (Visipaque 320, Amersham Health, Wommel, Belgium) was injected selectively in the left and right CA.

2.2.2 MRI protocol

All animals were scanned in the supine position in a 3.0 T MRI scanner (Magnetom, Trio Tim, Siemens Medical Solutions, Erlangen, Germany) with a phased array body coil wrapped over the heart to enhance the signal-to-noise ratio. Images were acquired with ECG gating and during suspended respiration.

2.2.2.1 Perfusion imaging

Dual-bolus first-pass perfusion imaging was performed\textsuperscript{7,8} Gadolinium diethylenetriaminepentaacetic acid bis(methylene) (GdDTPA-BMA) was used as a contrast medium (Omniscan, GE Healthcare, Diegem, Belgium). Two GdDTPA-BMA doses of 0.00 125 and 0.05 mmol/kg in equal volumes of 8 mL were injected intravenously with an MRI compatible contrast injector (Sonic Shot, Nipomo Kyorindo Co., Tokyo, Japan) at 3.5 mL/s consecutively, each followed by an injection of 20 mL of saline at the same rate. Adenosine (140 μg/kg/min) (Adenosin-Item Carinopharm, Gronau, Germany) was infused during 6 min for the stress study. Stress images were acquired after 4 min. Rest perfusion was assessed 20 min later to minimize contamination of the rest signal intensities by the residual GdDTPA of the stress boluses. The mean half-life time of GdDTPA is 12 ± 7.8 min.

Three slice locations (basal, mid, and apical) were acquired every R-R interval with a TurboFLASH sequence during 80 heartbeats. Typical imaging parameters included repetition time 241.61 ms, echo time 1.6 ms, inversion time 130 ms, 10° flip angle, field of view 360 mm, voxel size 2.5 × 1.9 × 8.0 mm, acquisition window 738 ms, and bandwidth 299 Hz/Px.

2.2.2.2 Late enhancement imaging

Following perfusion imaging, an additional contrast bolus of 0.1 mmol/kg GdDTPA-BMA was injected. After an additional 10 min, contrast-enhanced images were obtained using a three-dimensional inversion-recovery TurboFLASH sequence: repetition time 2.19 ms, echo time 0.78 ms, 15° flip angle, field of view 350 mm, voxel size 2.0 × 1.4 × 5.0 mm. The inversion time was modified iteratively to obtain maximal nulling of normal myocardium.

2.2.2.3 Functional imaging

Cine images at rest were acquired in a vertical long-axis (VLA) plane, a horizontal long-axis (HLA) plane, and a stack of short-axis (SA) planes using a two-dimensional FLASH (fast low-angle shot) sequence with the following imaging parameters: repetition time 35.35 ms, echo time 2.47 ms, flip angle 12°, field of view 330 mm, voxel size 2.1 × 1.7 × 6.0 mm, 40 cardiac phases, and bandwidth 449 Hz/Px. Contiguous SA slices covered the entire left ventricle (LV) along its long axis from base to apex.

Subsequently, VLA, HLA, and SA cine images at three levels were assessed during a two-stage incremental dobutamine (Dobutrex, Merck, Overijse, Belgium) stress. Dobutamine was intravenously infused at a
low dose (5 μg/kg/min) and a high dose (30 or 40 μg/kg/min) to archive an HR of 80% above baseline. The doses were increased in steps of 5 μg/kg/min, which lasted for 5 min.

2.2.3 Anatomical pathology studies
After removal of the heart, the LCx was ligated over the stent location and 2% Evan’s blue (Evan’s blue, Acros Organics, Geel, Belgium) was perfused through the right and left main CA at a pressure of 100 mmHg for 5 min in five stented animals. The hearts were embedded in a 20% gelatine solution and stored over night in a refrigerator. Corresponding to the MRI SA slices, the hearts were sectioned into 7 mm thick slices parallel to the atrioventricular groove with a commercial rotating meat slicer. All slices were scanned with a commercial colour flatbed scanner at 300 dpi. The scanned images were combined to SA stacks and stored using the MRI data format.

2.2.4 Regional MBF measurement with microspheres
In four stented pigs, regional MBF obtained by MRI was compared with microsphere measurements. Hereto, 2 million coloured microspheres with a diameter of 15 μm (Triton Technologies, San Diego, CA, USA) were diluted in 10 mL of saline. Different colours were injected into the LV for rest perfusion (red) and during adenosine stress (yellow), during the withdrawal of a 20 mL reference blood sample at a constant rate from the carotid artery. For the stress study, microspheres were injected during the same stress as the MRI perfusion imaging, whereas the rest microsphere injection was performed within 15 min of the rest MRI perfusion imaging. After prelevation, the heart was embedded and sectioned as described above.

2.3 Data analysis
2.3.1 Coronary angiography
Coronary angiography was assessed quantitatively using commercial software (ACOM, Siemens Medical Solutions) and relative luminal diameter reduction (in %) is reported. Animals with a stenosis larger than 75% were considered as appropriate models for chronic ischaemia.

All MRI and pathological data sets were analysed with dedicated software (CardioViewer, K.U. Leuven).

2.3.2 Segmentation model for regional analysis
To evaluate myocardial perfusion, function, and late enhancement (LE) in identical anatomical regions, corresponding SA slices of all MRI modalities and the pathological sections were aligned. Starting from the basal, mid-, and apical perfusion slices, corresponding slices were selected from the cine and LE stacks based on global scanner coordinates (Figure 1). Corresponding pathological sections were selected visually based on anatomical landmarks (Figure 2A). On these slices, the LV epicardial and endocardial borders were delineated and divided into 12 equiangular segments (Figures 1 and 2A).

Figure 1  SA MR perfusion (A), LE (B), and cine images (C) were aligned based on their long-axis position (D). The myocardium of each slice was divided into 12 segments.
2.3.3 MRI image analysis

On each of the three slices of the perfusion images, a region of interest (ROI) in the LV cavity was delineated at the first time point and propagated together with the epicardial and endocardial borders throughout the sequence with manual adjustment to compensate for overall heart motion.

Time–signal intensity curves for the LV cavity and the myocardial segments were generated (Figure 3). In the low (input)- and high (output)-concentration contrast dose curves, several time frames were selected. Baseline frames (input and output) were used to adjust the zero level of the signal intensity curves. The timing of the first pass of contrast was manually determined as the time period between the initial onset of contrast in the cavity and the onset of the recirculation of contrast. A y-variate function was fitted to the signal intensities during this first pass. This fit (scaled with the ratio of the high bolus over the low bolus concentration) was used as an input for a Fermi-constrained deconvolution to calculate MBF. The onset of the myocardial contrast was fitted as an independent parameter. MBF was derived from the limit (t = 0) (corresponding to the onset of myocardial contrast) of the impulse response function (modelled as a Fermi function).

For the complete SA cine stack, endo- and epicardial boundaries were delineated at end-diastole and end-systole to extract global parameters, such as myocardial mass, LV end-systolic volume (LVESV), LV end-diastolic volume, and ejection fraction (EF). For the regional analysis,
wall thickening was defined as: (end-systolic wall thickness − end-diastolic wall thickness)/end-diastolic wall thickness in each of the 36 segments. After delineation of the LE regions, infarct size (IS) was calculated as the percentage of total LE volume (as LE area × slice thickness) over LV myocardial volume. For each segment, infarct extent was quantified as the percentage of LE area over segment area.

2.3.4 Microsphere analysis
Three heart slices were matched to the 8 mm-thick MRI perfusion slices at the basal, mid-, and apical levels. Myocardial samples were obtained in 12 segments, digested in 4 mol/L KOH with 1% Tween 80, and filtered. Microspheres were eluted using di-(ethylene glycol) ethyl ether acetate and analysed using a luminescence spectrophotometer (8453E UV-visible spectroscopy system, Agilent, Santa Clara, CA, USA).

2.3.5 Area-at-risk determined with MRI vs. pathology
On the pathology sections, the area-at-risk was defined as the segments belonging to the same ROI had been averaged for each animal. If segments were further classified into three substrates: MI+++ (>50% of the segment area shows LE), MI+ (<50% of the segment area shows LE), and non-MI segments (no LE). One remote segment per slice was selected from the opposing anterior wall.

2.3.6 Substrate definition
On the basis of contrast-enhanced imaging, segments in the area-at-risk were further classified into three substrates: MI+++ (>50% of the segment area shows LE), MI+ (<50% of the segment area shows LE), and non-MI segments (no LE). One remote segment per slice was selected from the opposing anterior wall.

2.4 Statistical methods
Data were analysed with Statistica (Statistica 8.0, Statsoft Inc., Tulsa, OK, USA). Repeated-measures analysis of variance was used to test the levels of significance between the different ROIs at rest and during stress after all segments belonging to the same ROI had been averaged for each animal. If significance was indicated, a Duncan test correction was used as a post hoc test for multiple comparisons. Data are expressed as mean ± SD. Linear correlation was used to compare MRI perfusion-derived MBF with microsphere measurements. ROC curves for different approaches to define segments in the area-at-risk were compared following Hanley and McNeil. Statistical significance was inferred for a value of P < 0.05.

3. Results
In total, 11 stented and five sham pigs were studied. One of the originally 12 stented animal was excluded from further analysis because of a CA stenosis <75%.

3.1 Global characteristics
Global characteristics and haemodynamic findings at rest and during stress are listed in Table 1. The degree of CA stenosis was 94 ± 7% ranging from 81 to 100%. The IS was 7.3 ± 3.1%, ranging from 3.5 to 13.4% of LV mass. Functionally, stented pigs showed significantly higher LVEDV than sham pigs (P = 0.02), resulting in a significantly lower LVEF (P = 0.002). Neither BP nor HR was significantly different between groups at rest and during stress. In both groups, adenosine-induced hyperaemia resulted in a significant decrease in BP, whereas high-dose dobutamine stress induced an increase in BP.

3.2 Relation between measures of MBF
Figure 4 shows a good correlation (r² = 0.84, P < .0001) between quantitative MBF measurements by microspheres and dual-bolus perfusion MRI.

3.3 Performance of quantitative MRI perfusion to determine the area-at-risk
In the comparison of staining and perfusion images, nine of the 180 segments (5%) had to be excluded due to image artefacts. On the basis of Evan’s blue staining, 40 (23%) of the 171 segments belonged to the area-at-risk. Thresholds were determined from MBF values in sham pigs (Figure 2B), in which average resting MBF

<p>| Table 1 The characteristics and haemodynamic parameters at rest, during adenosine stress, and during low- and high-dose dobutamine stress (mean and range or mean ± SD) |</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>Sham</th>
<th>Stented</th>
</tr>
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<tbody>
<tr>
<td>IS (%)</td>
<td>7.25 (3.51–13.41)</td>
<td>13.4% (81–100)</td>
</tr>
<tr>
<td>LVEDV (mL)</td>
<td>158.60 ± 31.19</td>
<td>171.87 ± 24.24</td>
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<tr>
<td>LVESV (mL)</td>
<td>74.23 ± 11.10</td>
<td>91.80 ± 12.90</td>
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<tr>
<td>LV mass (g)</td>
<td>83.70 ± 10.90</td>
<td>86.42 ± 11.06</td>
</tr>
<tr>
<td>EF (%)</td>
<td>52.81 ± 2.50</td>
<td>46.49 ± 3.27</td>
</tr>
<tr>
<td>At rest</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BPsys (mmHg)</td>
<td>114.20 ± 7.95</td>
<td>115.09 ± 9.12</td>
</tr>
<tr>
<td>BPdias (mmHg)</td>
<td>63.60 ± 7.83</td>
<td>58.45 ± 6.67</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>84.20 ± 9.26</td>
<td>80.45 ± 6.44</td>
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<tr>
<td>HR (b.p.m.)</td>
<td>104 ± 21</td>
<td>98 ± 16</td>
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<tr>
<td>During adenosine-induced hyperaemia</td>
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<td></td>
</tr>
<tr>
<td>BPsys (mmHg)</td>
<td>102.20 ± 7.95</td>
<td>101.28 ± 10.71</td>
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<td>BPdias (mmHg)</td>
<td>45.00 ± 7.31</td>
<td>41.18 ± 5.65</td>
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<tr>
<td>MAP (mmHg)</td>
<td>66.80 ± 4.97</td>
<td>62.18 ± 8.33</td>
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<tr>
<td>HR (b.p.m.)</td>
<td>116 ± 13</td>
<td>99 ± 13</td>
</tr>
<tr>
<td>During low-dose dobutamine stress</td>
<td></td>
<td></td>
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<tr>
<td>BPsys (mmHg)</td>
<td>122.20 ± 9.07</td>
<td>111.09 ± 8.97</td>
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<tr>
<td>BPdias (mmHg)</td>
<td>56.60 ± 15.11</td>
<td>55.45 ± 8.73</td>
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<tr>
<td>MAP (mmHg)</td>
<td>83.00 ± 9.72</td>
<td>76.73 ± 8.78</td>
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<tr>
<td>HR (b.p.m.)</td>
<td>112 ± 15</td>
<td>95 ± 17</td>
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<tr>
<td>During high-dose dobutamine stress</td>
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<td></td>
</tr>
<tr>
<td>BPsys (mmHg)</td>
<td>167.33 ± 36.12</td>
<td>154.09 ± 27.79</td>
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<td>BPdias (mmHg)</td>
<td>60.67 ± 18.15</td>
<td>50.27 ± 7.58</td>
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<tr>
<td>MAP (mmHg)</td>
<td>91.00 ± 20.07</td>
<td>80.09 ± 11.89</td>
</tr>
<tr>
<td>HR (b.p.m.)</td>
<td>178 ± 7</td>
<td>173 ± 17</td>
</tr>
</tbody>
</table>

*IS, infarct size; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; EF, ejection fraction; BPsys, systolic blood pressure; BPdias, diastolic blood pressure; MAP, mean anteropressure; HR, heart rate.
†P < 0.05 vs. sham.
‡P < 0.05 vs. rest.
§P < 0.0001 vs. low-dose dobutamine stress.
formed better than a global approach (threshold, mean 2$\\bar{\text{mean}}$ MBF values. Moreover, a segmental approach (threshold, mean) showed the best performance for determining the area-at-risk using AUC, 0.66).

Rest (threshold, mean 0.52; specificity, 0.64; AUC, 0.73) and segmental approach at 10 MI based on the substrate definition by using LE image data. We obtained animals, the segments in the area-at-risk were further classified segmental thresholds during stress perfusion. In all 11 stented animals, the ROC analysis, the area-at-risk was defined using the area-at-risk, irrespective of the approach used, i.e. global MBF, and myocardial perfusion reserve were significantly lower in the MI area than in the remote (57 + 4%; P < 0.0001) and non-MI (51 ± 3%; P < 0.0005) myocardium. The severity of functional impairment increased with increasing infarct transmurality, i.e. MI+, 31 ± 7%; MI++, 20 ± 10%. The remote myocardium exhibited a linear increase in wall thickening during incremental dobutamine stress similar to the response in the sham animals. In contrast, the area-at-risk showed different patterns according to the underlying substrates. In the non-MI area, wall thickening increased during low-dose stress without further increase during high-dose stress showing a plateau phase. In the MI+ segments, a biphasic pattern was found, whereas in the MI++ segments, significant deterioration in wall thickening was found during low- and high-dose dobutamine. However, no differences in myocardial perfusion could be detected between the different substrates of the area-at-risk neither at rest nor during stress (Figure 5).

4. Discussion
In the present study, in chronic ischaemia, we showed that dual-bolus first-pass MRI perfusion enables accurate measurement of absolute MBF at rest and during stress in a 3.0 T magnet. Furthermore, quantitative hyperaemic perfusion MRI can be used as an imaging tool to identifying segments in the hypo-perfused area-at-risk subtending a chronic CA stenosis. Segments with differential degree of LE within this at-risk area define than further substrates with a differential myocardial function response to low- and high-dose stress. This ranges from a ‘plateau’ response (wall thickening increased during low-dose stress without further increase during high-dose stress) to a continuously deteriorative response in segments with a large amount of infarction. Therefore, the chronic area-at-risk consists of a combination of reversible and irreversible injured myocardium that can be associated with hibernating, non-transmurally, and transmurally infarcted myocardium.

3.4 Perfusion and function in the area-at-risk
On the basis of ROC analysis, the area-at-risk was defined using the segmental thresholds during stress perfusion. In all 11 stented animals, the segments in the area-at-risk were further classified based on the substrate definition by using LE image data. We obtained 10 MI++, 11 MI+, and six non-MI areas, and compared with 11 remote regions.

Perfusion MRI-derived MBF data are presented in Figure 5 and Table 2. In the remote region, the MBF at rest and during stress was 0.84 ± 0.25 and 1.70 ± 0.36 mL/min/g, respectively, and the myocardial perfusion reserve was 2.10 ± 0.37. Resting MBF, hyperaemic MBF, and myocardial perfusion reserve were significantly lower in all segments within the area-at-risk compared with the remote myocardium.

In the sham group, regional wall thickening at rest was 60 ± 2%, showing a progressive and significant increase during incremental dobutamine infusion (low dose: 74 ± 2%, P = 0.0002 vs. rest; high dose: 88 ± 2%, P < 0.0001 vs. rest, P = 0.0002 vs. low dose). In the stented animals (Figure 6), wall thickening at rest was significantly lower in the MI area than in the remote (57 ± 4%, P < 0.0001) and

**Figure 4** The MBF measured by microspheres correlated with MRI perfusion ($r^2 = 0.84; P < 0.0001$).

**Figure 5** MRI perfusion measurements at rest (grey bar) and during adenosine-induced hyperaemia (black bar) in the stented group. *P < 0.005 vs. remote regions at rest perfusion; †P < 0.0005 vs. remote regions during hyperaemia; ‡P = 0.0001 vs. rest perfusion.

(0.67 ± 0.11 mL/min/g) increased significantly during hyperaemia (1.87 ± 0.25 mL/min/g) (P < 0.001) The ROC analysis (Figure 2C) showed the best performance for determining the area-at-risk using stress MBF values. Moreover, a segmental approach (threshold, mean $-2.95$ SD; sensitivity, 0.95; specificity, 0.94; AUC, 0.97) performed better than a global approach (threshold, mean $-1.65$ SD or $1.46$ mL/min/g; sensitivity, 0.84; specificity, 0.86; AUC, 0.96). In contrast, resting MBF was unreliable to classify segments in the area-at-risk, irrespective of the approach used, i.e. global MBF at rest (threshold, mean $-0.2$ SD or 0.65 mL/min/g; sensitivity, 0.52; specificity, 0.64; AUC, 0.73) and segmental approach at rest (threshold, mean $-0.1$ SD; sensitivity, 0.59; specificity, 0.60; AUC, 0.66).
setting against microspheres. To our knowledge, this is the first time that this modality is validated in the setting of chronic ischaemia and shows acceptable quantification of absolute MBF. Our normal values appear lower than previously reported, especially during stress. For stress, this could be mainly due to a different protocol. Fallavollita et al. performed hyperaemic perfusion using 0.9 mg/kg/min adenosine vasodilatation with phényléphrine infusion instead of our 0.14 mg/kg/min adenosine only. However, our values are in range with recently published studies validating positron emission tomography (PET) perfusion against radioactive microspheres in healthy pigs and in a chronic reperfused infarction model.

Furthermore, we investigated the performance of quantitative MRI perfusion to detect the area-at-risk in the presence of a high-grade chronic CA stenosis. Previously and in most clinical studies, the non-invasive definition of the area-at-risk is based on the anatomical position of a myocardial segment in relation to the location of the CA stenosis. A key feature of the current analysis is that it accounts for the inter-subject variability by defining the area-at-risk using the regional microvascular dysfunction or relate to myocardial dysfunction and/or insufficient collateralization.

In non-MI segments, resting MBF was not different from MI+ or MI++ segments, suggesting that the stenotic lesion is the main cause for the MBF reduction in the area-at-risk. This hypo-perfusion was accompanied by a lower wall thickening at rest. This is in agreement with the study of Fallavollita et al. who studied wall thickening in pure hibernating myocardium. During low-dose dobutamine stress, wall thickening increased and maintained a similar level at high-dose stress. This pattern showing a plateau phase is typical of pure.

Table 2 The MBF at rest, during stress, and myocardial perfusion reserve values in different myocardial substrates

<table>
<thead>
<tr>
<th>Regions</th>
<th>Resting MBF (mL/min/g)</th>
<th>P-value*</th>
<th>Stress MBF (mL/min/g)</th>
<th>P-value*</th>
<th>Myocardial perfusion reserve</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remote</td>
<td>0.84 ± 0.25</td>
<td></td>
<td>1.70 ± 0.36</td>
<td></td>
<td>2.10 ± 0.37</td>
<td></td>
</tr>
<tr>
<td>Non-MI</td>
<td>0.50 ± 0.21</td>
<td>0.0022</td>
<td>0.69 ± 0.09</td>
<td>0.0001</td>
<td>1.59 ± 0.61</td>
<td>0.018</td>
</tr>
<tr>
<td>MI+</td>
<td>0.47 ± 0.14</td>
<td>0.0011</td>
<td>0.66 ± 0.14</td>
<td>0.0001</td>
<td>1.50 ± 0.46</td>
<td>0.008</td>
</tr>
<tr>
<td>MI++</td>
<td>0.42 ± 0.14</td>
<td>0.0003</td>
<td>0.51 ± 0.11</td>
<td>0.0000</td>
<td>1.26 ± 0.33</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

*vs. remote.

4.2 Functional and perfusion response to stress in the area-at-risk

The at-risk segments were further classified using the presence and severity of LE in three different ischaemic substrates (i.e. non-MI, MI+, and MI++). Noteworthy, no LE was detected in segments outside the area-at-risk as defined above. Quantitative perfusion MRI was unable to detect significant differences between these different substrates and showed a rather uniformly reduced perfusion and perfusion reserve in the area-at-risk. This indicates that functional differences within the area-at-risk could be mostly attributed to the presence of scar tissue.

In MI++ segments (LE present in >50% of the segment area), LE indicates the presence of a considerable amount of myocardial fibrosis and scar formation. In these regions, resting contractile function and MBF are most profoundly reduced and neither perfusion reserve nor contractile reserve was present during stress. Low systolic wall thickening, due to extensive scar formation, either at rest or during a graded dobutamine infusion, is consistent with the previous studies.

Mi+ segments (LE present in <50% of the segment area) show hypo-kinesia accompanied by hypo-perfusion at rest. These findings are in agreement with the results from our laboratory and from models using fixed stenosis and hydraulic occluders. Stress dobutamine MRI shows a biphasic response, with initially improved contractility during low-dose dobutamine followed by deterioration with high-dose dobutamine. A similar stress-related response has been reported previously in segments with limited MI (8 ± 2% of the area-at-risk). The absence of maximum inotropic reserve in these segments containing a majority of viable myocardium could be attributed to the development of further acute ischaemia. Besides the flow-limiting stenosis, the absent perfusion reserve could also be due to microvascular dysfunction or relate to myocardial dysfunction and/or insufficient collateralization.

In non-MI segments, resting MBF was not different from MI+ or MI++ segments, suggesting that the stenotic lesion is the main cause for the MBF reduction in the area-at-risk. This hypo-perfusion was accompanied by a lower wall thickening at rest. This is in agreement with the study of Fallavollita et al. who studied wall thickening in pure hibernating myocardium. During low-dose dobutamine stress, wall thickening increased and maintained a similar level at high-dose stress. This pattern showing a plateau phase is typical of pure...
hibernation (without any infarct). Several factors, e.g. metabolic factors and tethering, can influence the contractile response at maximal dobutamine stress. It has been shown that hibernating myocardium retains a limited ability to increase myocardial oxygen consumption in response to stress, which is attributed to a reduction in high-affinity binding and regional desensitization of receptor signalling, despite normal β-adrenergic receptor density and subtype distribution.

However, the major difference between the pure and the mixed animal model is the presence of scar tissue in or adjacent to the segment. Therefore, mechanical tethering with the infarcted tissue resulting in different loading within the area-at-risk could as well explain this response.

4.3 Limitations
The lower LV coverage of perfusion MRI (35%, 3 × 8 mm thick slices) remains a technical limitation of the current technique. Although it hampers the exact determination of the extent and size of the area-at-risk, MRI perfusion has better in-plane spatial resolution compared with nuclear imaging which is often used clinically and is free of radiation. However, it does allow for identification of ischaemic or at-risk segments, such that exact anatomically correlated regional data can be extracted. Further sequence development and optimization will enable larger regions to be covered in the future.

Although transmural heterogeneity of MBF in normal or hibernating myocardium has been shown, we only studied transmural myocardial perfusion. Whereas a subdivision into endo- and epicardial segments would have been feasible in segments with normal wall thicknesses, MI++ segments were too thin to allow an accurate intramural perfusion analysis. Moreover, since 12 segments per slice were used to analyse the substrates, further subdivision would impede reliable analysis, due to too small analysis regions.

We have applied different stressors to assess perfusion (adenosine) and functional (dobutamine) reserve. The reason is that increased HR during high-dose dobutamine deteriorates image quality of the first-pass perfusion and quantification becomes very difficult. However, it has been shown that perfusion reserve is similar with the two stressors.

4.4 Clinical implications
This closed-chest, chronic ischaemic/infarct animal model with mixed substrates in the area-at-risk mimics a group of patients with persistent regional hypo-perfusion and contractile dysfunction. The methods described in this paper can be readily translated to the clinical setting to accurately detect the area-at-risk, where the MBF threshold values would need to be defined with normal MBF in humans. The possibility of a patient-specific determination of the area-at-risk is expected to refine the assessment of the regional impact of mechanical or therapeutic interventions on perfusion, function, and IS in the setting of chronic ischaemia.

5. Conclusion
A single MRI study can fully characterize ischaemic substrates in the area-at-risk subtending a chronic CA stenosis. Perfusion MRI enables quantification of absolute MBF as validated against microsphere measurements and a classification model based on hyperaemic MBF can accurately define segments in the area-at-risk. Co-existing hypo-perfused substrates with differential amounts of MI in the area-at-risk showed different functional reserve, suggesting the presence of hibernating, non-transmurally, and transmurally infarcted myocardium. This characterization of ischaemic substrates with regional myocardial function and perfusion may contribute to the interpretation of pathophysiological mechanisms on cellular and molecular level and to the evaluation therapeutic interventions.

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