Fast High-Resolution Magnetic Resonance Imaging Demonstrates Fractality of Myocardial Perfusion in Microscopic Dimensions

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Abstract—The fractal nature of heterogeneity of myocardial blood flow and its implications for the healthy and diseased heart is not yet understood. The main hindrance for investigation of blood flow heterogeneity and its role in physiology and pathophysiology is that conventional methods for determination of myocardial perfusion have severe limitations concerning temporal and spatial resolution and invasiveness. In isolated rat hearts, we developed a nuclear magnetic resonance technique that does not depend on contrast agents and in which the apparent longitudinal relaxation time is made perfusion sensitive by selective preparation of the imaging slice. This perfusion-sensitive relaxation time is determined within 40 seconds as a map with a high spatial in-plane resolution of $140 \times 140 \mu m^2$ and a thickness of 1.5 mm. Perfusion imaging was validated with the established microsphere technique. Additionally, the congruence between perfusion-sensitive $T_1$ maps and first-pass perfusion imaging was demonstrated. As an application of high-resolution perfusion imaging, fractal analysis of the spatial distribution of perfusion was performed. We were able to demonstrate that the fractality of this distribution exists even in microscopic dimensions. Vasodilation by nitroglycerin modulated the fractal pattern of perfusion, and the decrease of the fractal dimension indicated a shift toward homogeneity. This implies that parameters of the fractal distribution depend on the microvascular tone rather than on anatomic preformations; ie, fractality is a functional characteristic of perfusion. (Circ Res. 2001;88:340-346.)

Key Words: magnetic resonance imaging ¡ perfusion ¡ microcirculation ¡ fractality

The understanding of the local regulation and coordination of myocardial blood flow is an important goal in cardiac and integrative physiology. It is well established that myocardial blood flow shows considerable spatial heterogeneity.¹² Quantitative analysis demonstrated¹ that after division of the heart into $N$ sections, the relative dispersion $RD = SD/\text{mean value of flow of these sections}$ follows a power law,

$RD \sim N^{0.1-1}$.

where $D$ is denoted as the fractal dimension, which takes values of $\approx 1.1$ to 1.3. A gaussian distribution of regional flow would reveal a value of 1.5 and a homogeneous flow of 1. This implies that heterogeneity of blood flow is neither organized in a purely random way, nor is it uniform, and that the spatial correlation of regional flow is long ranging. The origin of this pattern of flow heterogeneity is not understood. Some theories relate this to the branching of the vessel tree, ie, anatomic preformations; others postulate that this pattern of heterogeneity is a result of adaptive coordination of perfusion between myocardial segments; ie, fractality is a functional characteristic of perfusion.

Although the understanding of myocardial flow heterogeneity is of paramount interest in cardiac and integrative physiology, progress in this field of research is hampered by the limitations of available methods. The "gold standard" method is the application of microspheres,³ the deposition of which in tissue is proportional to perfusion. However, this is an invasive procedure, the microspheres itself affect perfusion, the spatial resolution is limited to pieces of $\approx 0.2$ g, the preparation of tissue implies systematic errors, and there are only a few states of perfusion assessable because of the restricted number of labels of microspheres. Quantitative magnetic resonance techniques that depend on contrast agent determine perfusion from signal-time curves⁴ during the first pass of the contrast agent; however, repetitive measurements that are necessary to increase precision or to investigate different perfusion states are hampered by residual contrast agent.

All methods mentioned have a rather low spatial resolution; ie, at the present it is not clear whether the fractal principle of flow heterogeneity also holds in microscopic dimensions. Therefore, our aim was to develop and validate a
fast high-resolution nuclear magnetic resonance (NMR) perfusion imaging technique that does not require contrast agents. With this technique, spatial heterogeneity of perfusion should become visible on high-resolution images, and our next aim was to perform quantitative analysis of this heterogeneity in pilot studies.

### Materials and Methods

#### Experimental Preparations

All experiments with animals were conducted in accordance with the European regulation on care and use of laboratory animals.

#### Isolated Heart Model

Isolated hearts of male Wistar rats (body weight 300 to 350 g) were perfused (Langendorff mode) with oxygenated Krebs-Henseleit buffer (37°C) in a perfusion system that was adapted to a NMR-microscopy system. Coronary flow was measured by an ultrasonic flowmeter (TI06, Transonic Systems Inc), and left ventricular pressure was measured by a balloon that was inserted in the left ventricle and connected to a Statham P23XL pressure transducer (Gould Instruments). Left ventricular end-diastolic pressure (LVEDP) was adjusted to 5 mm Hg by the balloon volume at the beginning of the experiment.

#### Induction of Coronary Artery Stenosis

To obtain hearts with a stenotic coronary artery, rats were intubated under ether anesthesia, and after thoracotomy the heart was exposed. A suture including a probe of 300 μm was positioned 2 to 3 mm from the origin of the left coronary artery. After ligation the probe was removed, the heart replaced, and the thorax closed. Hearts were excised after 2 weeks and treated as described above.

#### NMR Imaging

**Pulse Triggering**

Because the timing and the mode of NMR pulse triggering by the left ventricular pressure were described in detail elsewhere, only essentials are repeated. NMR imaging was triggered in early diastole. Cine NMR imaging had revealed a maximum transversal displacement during systole of 1.5 to 2 mm in the short-axis view. Motion was assumed in diastole; ie, NMR imaging triggered at the beginning of diastole was not affected by artifacts.

**Noncontrast Perfusion Imaging**

Non–contrast-agent techniques make the apparent $T_1$ of tissue perfusion sensitive by different labeling of inflowing arterial spins and stationary spins in the imaging slice. To achieve this, we performed, in a vertical high-field system (Bruker AMX 500, 11.75 T), a slice-selective spin inversion (thickness 3 mm, rectangular shaped) in the short-axis view 3 mm below the valvular plane of the heart. This was followed by a series of 16 2-dimensional FLASH images (TE = 2.5 ms, TR = 5.0 ms, flip angle = 30°, pixel size in plane = $140\times140\mu\text{m}^2$) within this slice. The imaging slice had a thickness of 1.5 mm (gaussian shaped) and was located symmetrically within the inversion slice; ie, >95% of the gaussian-shaped imaging profile was covered symmetrically by the inversion slice. It was verified in static fluid phantoms that the thickness of the inversion slice was the minimum value for which $T_1$ without slice-selective preparation and $T_1$ with slice-selective preparation were identical. This procedure provided high perfusion sensitivity and excluded systematic errors due to insufficient inversion of spins in the imaging slice. Imaging was performed in the short-axis view, because epicardial flow is directed from base to apex; ie, myocardium in the selected slice is mainly perfused by equilibration spins.

A $T_1$ map was obtained by a single exponential fit for each pixel from the time course of the 16 signal intensities. Four acquisitions were performed to gain the final $T_1$ map. The $T_1$ value of a pixel was rejected when the average relative deviation of the fitting curve and the time course of the signal intensities within this pixel were >5%.

Those pixels appeared black on the $T_1$ map. The high-resolution $T_1$ map was obtained in <40 seconds. In our setup, the magnetization of inflowing spins was that of thermomagnetic equilibrium; ie, the apparent $T_1$ relaxation in the imaging slice was accelerated. This acceleration depended on perfusion and mixing of intra- and extravascular spins, which were predominantly located in the capillary region. Estimation of the intracapillary-extracapillary water exchange rate and taking into account the volume distribution of coronary vessel segments and the geometry of the experimental setup implied a linear dependence between perfusion and relaxation rate.

$$
\Delta \rho = \lambda \frac{d(T_1^{-1})}{dL}
$$

where $\lambda$ is the tissue/perfusate partition coefficient of water.

#### Error Analysis and Accuracy of $T_1$ Measurement

Spatial deviations of $T_1$ in myocardium arise as a superposition of spatial heterogeneity of (1) morphological and physiological parameters, (2) the random error, and (3) systematic spatial deviations due to the NMR system. The latter 2 were estimated by measuring $T_1$ and its SD for stationary buffer, which was added contrast agent (Gd–diethylenetriamine penta-acetic acid [DTPA]), to cover a range of $T_1 = 1$ to $T_1 = 3.5$ seconds. There was no systematic spatial deviation of $T_1$. The relative spatial SDs of the $T_1$ maps were <2%.

It was demonstrated previously in detail that almost no motion of the heart was present in diastole in which 2-dimensional FLASH imaging was performed. This implies that $T_1$ measurements were not affected by cardiac motion.

#### Validation of Perfusion Imaging

**Experimental Protocols**

The partition coefficient $\lambda$ of Equation 2 was determined from a group of 8 isolated, buffer-perfused hearts. After beating 40 minutes at a perfusion pressure of 100 mm Hg, hearts were removed and the wet weight of left and right ventricular wall was measured. Hearts were placed in an oven (55°C) for 48 hours, and the partition coefficient was determined according to the following:

$$
\lambda \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}}.
$$

In 2 groups, the NMR perfusion imaging technique was validated with the established microspheres technique. Maps of the perfusion-sensitive relaxation time $T_1$ were obtained in the short-axis view at different perfusion levels, and microspheres were injected simultaneously. In one group (n = 5), perfusion was varied by reduction of perfusion pressure (to 100, 80, and 70 mm Hg in 4 hearts and to 100, 80, and 50 mm Hg in 1 heart). To induce more heterogeneous variations of perfusion, we elevated in another group (n = 12) left LVEDP from 5 to 60 and thereafter to 80 mm Hg at a constant coronary perfusion pressure of 100 mm Hg.

Local hypoperfusion was investigated in hearts (n = 5) with a stenotic coronary artery. Because the spatial resolution of the microsphere technique is lower than the hypoperfused area in the imaging slice, we compared perfusion-sensitive $T_1$ mapping with first-pass imaging after application of contrast agent. First, 3-dimensional NMR angiography documented that the affected coronary artery was not occluded. After $T_1$ mapping without contrast agent, a bolus of 0.2 mL Gd-DTPA (1.5 mmol/l) was injected into the Windkessel of the perfusion system, which was located just above the aorta of the heart. A series of $T_1$-weighted diastolically triggered FLASH images (TE = 1 ms, TR = 2.5 ms, spatial resolution = $140\times140\mu\text{m}^2$) followed.

**Perfusion Measurement by Colored Microspheres**

The microsphere technique and its adaptation to the NMR microscope were recently described by us. The number of microspheres (Dye-Trak; Triton Technology Inc; mean diameter 15 μm) of 1 color was ~30 000. Microspheres were flushed into the aorta with Krebs-Henseleit buffer containing 0.002% Tween 80 at 0.5 mL per minute over 10 minutes. After the experiments, hearts were frozen in
Fractal Analysis of Perfusion Heterogeneity

Equation 2 shows that the analysis of heterogeneity of perfusion according to relation 1 is equivalent with that of the relaxation rate \(1/T_1\). For fractal analysis, we therefore considered 4 nonoverlapping squares containing 16×16 pixels that were inserted into \(T_1\) maps of left ventricular myocardium. In each square, the relative dispersion \(RD\) of \(1/T_1\) was obtained for \(N=16\times16=256\) pixels and thereafter for \(N=128, 64, 32, 16, 8\), and 4 blocks of neighboring pixels (aggregates). Linear regression analysis of log\(RD(N)\) versus log\(N\) followed (Figure 4). This procedure was performed for each square under various flow conditions. We investigated 5 hearts, in each of which coronary flow was passively altered by adjustment of perfusion pressure at 100, 80, and 50 mm Hg. To investigate the effect of active vasodynamics on the heterogeneity of perfusion, we investigated another group of 5 hearts before, during, and after continuous infusion of nitroglycerin (0.5 mg/min) at constant perfusion pressure (100 mm Hg).

Statistics

Group data are expressed as mean±SD and were analyzed according to the Wilcoxon-Mann-Whitney \(U\) test or Scheffe \(F\) test when \(>2\) groups were compared. Differences were considered as significant when \(P<0.05\).

Results

Validation of Perfusion Imaging

The partition coefficient was determined as \(\lambda=0.83±0.003\), and there was no difference between left and right ventricular wall.

Variations of myocardial perfusion were directly reflected by myocardial \(T_1\) (Figure 1). When coronary flow was varied by reduction of perfusion pressure, microspheres measured initial perfusion of the imaged myocardium as 13±1.3 mL/g per minute, and almost equal decrements were obtained in the right and left ventricular wall. When variations of perfusion of the imaged region were obtained from \(T_1\) mapping according to Equation 2, a very close correlation was found with...
microsphere data (Figure 2), which was also present when left and right ventricular wall were analyzed separately as linear regression analysis demonstrated, as follows: \( r = 0.96 \), slope = 1.1, and offset = 0.15 mL/g per minute for the left, and \( r = 0.91 \), slope = 0.92, and offset = -0.2 mL/g per minute for the right ventricular wall.

In hearts in which LVEDP was elevated, microspheres demonstrated significant different decrements of perfusion in the right and left ventricular wall at each pressure step, 2.2 ± 1.8 versus 3.3 ± 1.3 mL/g per minute and 1.0 ± 0.36 versus 1.6 ± 0.7 mL/g per minute. Again, a close correlation was found between perfusion obtained from \( T_1 \) mapping according to Equation 2 and microsphere data (Figure 2). At LVEDP = 5 mm Hg, perfusion was higher in the endocardial layer than in the epicardial one (endocardial-epicardial difference = 2.3 ± 0.3 mL/g per minute [NMR] and 2.5 ± 0.2 mL/g per minute [microspheres]). The increments of LVEDP induced significant different decrements (Figure 1) of endo- and epicardial perfusion (endocardium/epicardium), as follows: 5 → 60 mm Hg, 3.8 ± 0.8/2.5 ± 0.4 mL/g per minute (NMR) and 4.0 ± 0.9/2.7 ± 0.5 mL/g per minute (microspheres), and 60 → 80 mm Hg, 3.5 ± 0.6/1.8 ± 0.3 mL/g per minute (NMR) and 3.4 ± 0.5/1.9 ± 0.6 mL/g per minute (microspheres). A close correlation was found between variations of perfusion determined by \( T_1 \) mapping and microspheres, as follows: \( r = 0.90 \), slope = 0.94, and offset = 0.2 mL/g per minute for the epicardium, and \( r = 0.88 \), slope = 0.09, and offset = 0.13 mL/g per minute for the endocardium.

Evident congruence was found between the hypoperfused regions obtained from \( T_1 \) mapping and first-pass imaging in the hearts with coronary artery stenosis (Figure 3). This was...
therefore the fractal dimension \( D \) (Equation 1) can be determined from linear regression analysis of \( \log \) RD vs \( \log N \), where \( N \) is the number of blocks of neighboring pixels (aggregates). Note that the power law of relation 1 holds up to microscopic dimensions (lower panel; \( r \) indicates correlation coefficient).

**Analysis of Heterogeneity of Perfusion**

The perfusion-sensitive \( T_1 \) maps appeared very heterogeneous (Figure 1), and the relative dispersion of the maps was in the range of 10% to 34%, which was far above the relative error of \( T_1 \) determination of 2%. Hence, the heterogeneity of \( T_1 \) reflected that of perfusion.

The correlation analysis of relative dispersion of perfusion versus sample size (\( \log \) RD versus \( \log N \)) demonstrated a linear logarithmic dependence of both parameters (Figures 4 and 5) with correlation coefficients ranging between 0.90 and 0.98 (mean 0.96). In the hearts subjected to varying perfusion pressure, the mean of the fractal dimension \( D \) was 1.118±0.007 (100 mm Hg), 1.119±0.017 (80 mm Hg), and 1.120±0.016 (50 mm Hg).

Nitroglycerin infusion increased coronary flow from 16.2±4.3 mL/min to 19.0±4.1 mL/min, and after cessation of the drug, coronary flow declined to 15.3±3.9 mL/min. The corresponding time course of the mean fractal dimension revealed a decrease from 1.124±0.02 to 1.117±0.013 and thereafter a significant increase to 1.154±0.01 (Figure 5). The \( 1/T_1 \) maps in Figure 5 reveal that onset and cessation of nitroglycerin do not simply shift local perfusion to higher or lower values, but rearrange spatial heterogeneity of perfusion.

**Discussion**

**Development of Perfusion Imaging**

There exist qualitative \(^{11,12}\) and quantitative \(^{13–17}\) non–contrast–agent perfusion imaging techniques. Most of the latter tech-

niques work with active labeling of the inflowing spins. This requires spatial separation of tissue and its associated supplying artery; ie, this approach is suitable for the brain, the kidney, or the isolated perfused heart. However, in the intact heart the complex topography of coronary arteries, left ventricle, and myocardium hampers the active spin labeling. Another obstacle is that many sensitive parameters of spin labeling have to be considered.

In contrast to active spin labeling, we \(^6\) and others \(^{18}\) exploited the inflow of equilibrium spins to accelerate longitudinal relaxation by perfusion after selective inversion. This acceleration is due to inflow and subsequent transcapillary mixing of equilibrium spins with stationary spins. The dynamics of the latter are much faster than \( T_1 \), which implies the linear dependence of relaxation rate on perfusion (Equation 2). In contrast to us, Reeders et al \(^{18}\) performed \( T_1 \)-weighted spin-echo imaging after selective inversion. A repetition time of \( \approx 12 \) seconds and 64 phase-encoding steps imply an acquisition time of 13 minutes, whereas we performed direct \( T_1 \) mapping from 16 complete FLASH images during one relaxation course (snapshot FLASH); ie, 4 acquisitions provided a \( T_1 \) map in <40 seconds. This technique combines high accuracy concerning determination of \( T_1 \) with a high speed of the imaging procedure.

Potential delay of the inflow effect and, hence, underestimation of perfusion due to arterial spins in the inversion slice was minimized by short-axis imaging, because epicardial flow is directed from base to apex. The contribution of intramyocardial arteries in the inversion slice such as vessels directing blood from epicardium to endocardium is negligible as well, given that their volume fraction is <1% of tissue volume. This implies that perfusate inside these arteries is exchanged in <200 ms for perfusion values >3 mL/g per
minute. Because we determine relaxation time from an observation period of (16×1 image per heart cycle) >3 seconds, the initial delay is negligible.

The validation of perfusion imaging with the microsphere technique is hampered in part by the fact that the imaging slice is not quite identical with the slice prepared for microsphere measurements. This is due to obvious technical restrictions of the preparation and to the fact that the microsphere technique demands more myocardial mass than that of the imaging slice to provide reliable data. On the other hand, the close correlation of microsphere and NMR perfusion data suggests that the difference of the slices is of minor importance. Furthermore, the elevated endocardial perfusion obtained from NMR experiments under baseline conditions (LVEDP=5 mm Hg, perfusion pressure=100 mm Hg) is in agreement with our microsphere data and data from literature.19,20

It is obvious that the maximum spatial resolution on which the perfusion imaging technique could be validated was that of the microsphere technique, which we already reached in our preparations. The fact that our mathematical model of determining perfusion from $T_1$ is independent from the sample size,6 and the accuracy of the $T_1$ measurements on the scale of 140×140×1500 μm$^3$ makes it evident that perfusion imaging is also valid in these dimensions. The congruence between the perfusion-sensitive $T_1$ map and signal enhancement in the first-pass experiments in hearts with coronary artery stenosis confirm the validity of the non–contrast-agent technique.

In contrast to techniques with active spin labeling, the method of perfusion mapping by selective preparation of the imaging slice is not restricted to the isolated rat heart but can be adopted to the intact rat20,21 and with some modifications also to humans, as pilot studies demonstrate.22 Another advantage compared with active spin labeling is that our technique is independent from the $T_1$ of perfusate; ie, the strong field dependence of this parameter is excluded. The $T_1$ of myocardium in low field systems is in the range of 1 second, ie, only a factor of 2 to 2.5 smaller than at 11.7 T. These facts imply that the field dependence of $T_1$ should not hamper the transfer to low field systems. A real challenge for the transfer to the intact animal is that baseline perfusion (perfusion pressure ≈100 mm Hg) is much lower than in the isolated saline perfused heart (in rats, 3.5 to 4 versus 12 to 15 mL/g per minute), which implies that variations of regional blood flow are much smaller as well. However, preliminary data in intact rats at 7 T with coronary artery stenosis demonstrate that under resting conditions hypoperfused regions can be detected with a spatial in-plane resolution of ≈0.5×0.5 mm$^2$.23 In humans, perfusion is even lower, and an increase of perfusion from baseline to maximum (0.75 versus 3.5 to 4 mL/g per minute) results in a 5% decrease of $T_1$. This implies that the accuracy of $T_1$ determination should be 2% in the segments under consideration to detect perfusion abnormalities.

Analysis of Heterogeneity of Perfusion

Our correlation analysis of relative dispersion versus sample size demonstrates that heterogeneity of perfusion can be described by the power law of Equation 1 (Figures 4 and 5). This implies that heterogeneity of perfusion reveals a fractal behavior in microscopic dimensions that has not been demonstrated yet. The origin of this fractality still remains a matter of discussion. The branching of the vessel tree, which itself follows a self-similar pattern,24 may be responsible as was mentioned by Van Beek et al.25 Our experiments demonstrate that this fractality is not a static characteristic of perfusion. Instead, the data obtained from hearts subjected to nitroglycerin infusion (Figure 5) demonstrate that vasodilation modulates the pattern of flow heterogeneity. After cessation of infusion, this pattern differs from the initial state. The fractal dimensions in these experiments demonstrate a shift toward a more uniform distribution of flow during nitroglycerin infusion. In contrast, when perfusion was varied by adjustment of perfusion pressure, almost no variations of the fractal dimension were observed. These observations speak in favor of the dependence of flow heterogeneity on the microvascular tone rather than on anatomic preformations.

The fractal analysis as indicated above and the physiological experiments we performed have to be considered as the first steps for our future goals. The way data are obtained from our method allows the investigation of more sophisticated problems and the application of complex mathematical methods, eg, multifractal analysis. Because the perfusion-sensitive $T_1$ map is obtained in <40 seconds, we achieve a high temporal resolution that allows us to study the dynamics of heterogeneity in future projects. The perfusion imaging technique can be combined with metabolic26 and oxygenation-dependent27 sensitive imaging, which will provide further insight into the origin of heterogeneity of myocardial blood flow.

Conclusion

We have demonstrated that fast high-resolution NMR imaging of cardiac perfusion is feasible without contrast agent. The basis for this technique is the selective labeling of spins in the imaging slice, which made $T_1$ perfusion sensitive. We have demonstrated that the fractal behavior of perfusion is maintained in microscopic dimensions and that this fractality is most likely a result of the microvascular tone.

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