Noninvasive Measurement of Myocardial Activity Concentrations and Perfusion Defect Sizes in Rats With a New Small-Animal Positron Emission Tomograph

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Background—We explored the feasibility of measuring regional tracer activity concentrations and flow defects in myocardium of rats with a high spatial resolution small-animal PET system (microPET).

Methods and Results—Myocardial images were obtained after intravenous $^{18}$F-fluorodeoxyglucose ($^{18}$FDG) in 11 normal rats (group 1) and assembled into polar maps. Regional $^{18}$F activity concentrations were measured in 9 regions of interest and compared with tissue activity concentrations measured by well counting. In another 9 rats (group 2), myocardial perfusion images were acquired with $^{13}$N-ammonia at baseline and during coronary occlusion. On the polar maps recorded during coronary occlusion, the size of perfusion defects was measured as the myocardium with $<50\%$ of maximum activity and expressed as percent total myocardium and was correlated with the area at risk defined by postmortem staining. The diagnostic quality of $^{18}$FDG and $^{13}$N-ammonia microPET images was good to excellent; the images were easily assembled into polar maps. In group 1, regional $^{18}$F concentrations by microPET and postmortem were correlated linearly ($r=0.99; P<0.01$ for average and $r=0.97; P<0.01$ for regional concentrations). In group 2, perfusion defect sizes by microPET and postmortem were correlated linearly ($P<0.01; r=0.93$).

Conclusions—The findings indicate the feasibility of noninvasive studies of the myocardium in rats with a dedicated small-animal PET-imaging device. (Circulation. 2002;106:118-123.)

Key Words: myocardium $\bullet$ imaging $\bullet$ perfusion

Cardiovascular research in animals increasingly relies on small animals like rats and mice. They allow the study of phenotypic expressions of genomic alterations and design and exploration of gene therapeutic approaches.¹ Phenotypic consequences on myocardial function, perfusion, and substrate metabolism in small animals can be evaluated with dedicated MR imaging, CT scan, and radionuclide imaging systems.²-⁶ A more recent approach entails a dedicated small-animal, high spatial resolution positron emission tomograph (microPET).⁷ Together with novel radiotracers, the device allows serial noninvasive monitoring of the location, magnitude, and time course of expression of transfected genes.⁸⁹ The device has been used only for studies of the liver and tumors. Examination of the heart remains challenging because of its small size, its thin wall, and motion and activity cross-contamination between blood and myocardium. Therefore, we explored the feasibility of cardiac imaging in small animals with this new small-animal PET. Specifically, we asked whether regional radiotracer concentrations and flow defect sizes in the myocardium of rats could be measured noninvasively.

Methods

Study Design

Two groups of animals were studied. In group 1, the possibility of measuring regional myocardial tracer activity concentrations was explored. Eight animals were studied after normal feeding and 3 after an overnight fast to vary myocardial glucose utilization and, thus, uptake of intravenous $^{18}$F-fluorodeoxyglucose ($^{18}$FDG). In vivo observed $^{3}$H-$^{18}$FDG myocardial concentrations were validated against true myocardial activity concentrations by postmortem well counting of myocardial tissue samples. In group 2, the possibility of measuring the size of myocardial perfusion defects was examined. The flow tracer $^{13}$N-ammonia was injected intravenously at baseline and after coronary occlusion. The size of flow defects on the images was compared with postmortem determined defect sizes by tissue staining. The University of Los Angeles Animal Research Committee approved the study.

Imaging Device

The UCLA microPET, a prototype dedicated small-animal PET, was used.¹⁰ It consists of a ring of 30 high-resolution detectors with lutetium oxyorthosilicate scintillator elements coupled via optical fibers into a multichannel photomultiplier tube.¹¹ Its transverse field of view (FOV) is 11.75 cm, and the axial FOV is 1.8 cm. It acquires...
data in three-dimensional mode, yielding images with an isotropic resolution of 1.8 mm full-width at half-maximum when data are reconstructed using filtered backprojection with a ramp filter cutoff at the maximum sampling frequency of 0.9 mm⁻¹.

Animal Preparations and Study Protocols

Of 20 male Sprague-Dawley rats (316±35 g; Harlan, San Diego, Calif), 11 were assigned to group 1 and 9 to group 2. All animals were anesthetized with intraperitoneal sodium pentobarbital (50 mg · kg⁻¹) and placed on a heat pad circulated with warm water. The tail vein was cannulated for injection of radiotracers and pharmaceuticals.

Group 1

The anesthetized rats were placed supine on the cradle of the microPET, and the heart was positioned within the axial FOV. Transmission images were acquired for 1 hour. ¹¹C-FDG (70 MBq) was injected intravenously; image data acquired from 44 to 104 minutes were reconstructed. The rats were then killed with saturated KCl IV, and the hearts were removed. The left ventricle (LV) was dissected free of atrial and right ventricular myocardium and sliced perpendicular to the long axis of the LV into 3 short-axis cuts; each was subdivided into 3 sectors that corresponded to the regions of interest (ROI) on the microPET images. All tissue samples were weighed and their activity concentrations determined by well counting. Average myocardial activity concentrations were determined from the mean of the regional tissue activity concentrations times the weight of the LV myocardium.

Group 2

The anesthetized rats were intubated and ventilated with a small-animal respirator (Model 683, Harvard Apparatus) at a rate of 70 minutes⁻¹ and a tidal volume adjusted to the weight of the rat. After a left thoracotomy, a suture (6 to 0 Ethicon prolene monofilament) was introduced through the myocardium surrounding the proximal left coronary artery. Both suture ends were threaded through a small polyester tubing to form a snare around the artery.

Each rat was imaged with ¹³N-ammonia twice, first at baseline and 1 hour later, during coronary occlusion. Each time, ¹³N-ammonia (~70 MBq) was injected intravenously; image data acquired from 2 to 19 minutes were used for image reconstruction. At baseline, the suture remained placed loosely around the coronary artery. During the occlusion study, the snare was tightened 5 minutes before and released 5 minutes after the ¹¹C-FDG injection. To prevent arrhythmias, lidocaine (10 mg · kg⁻¹ IV) was given 1 minute before perfusion. To shorten the total study time, transmission images were not acquired.

After image acquisition, the coronary artery was reoccluded. A blue pigment dye (0.5 mL 2% Bonney’s blue dye) was injected intravenously for staining the nonoccluded but not the occluded portion of the myocardium. The heart was then arrested with saturated KCl IV and removed. The LV was dissected free of right ventricular and atrial myocardium and cut into 3 slices perpendicular to its long axis. Each slice was weighed and photographed with a digital camera. Differences between stained and unstained myocardium were enhanced by converting the color into black and white images that were projected and traced onto sheets with a 1×1-mm grid. The number of 1-mm squares in unstained to that of the entire myocardial slice represented the percent of at-risk myocardium. To account for differences in thickness between slices and, thus, in weight, the fraction of at-risk myocardium in each slice was expressed in weight. The sum of the weights of at-risk myocardium in each of the 3 slices represented the total weight of at-risk myocardium and was expressed in percent LV weight.

Image Reconstruction and Image Analysis

The image data were reconstructed on a SPARC Ultra 1 workstation (Sun Microsystems) into 128×128 image matrices with a pixel size of 0.29×0.29 mm using a Ramp filter with a cutoff frequency of 0.9 mm⁻¹ and, in the group 1 animals, corrected for photon attenuation. The transaxial images were reoriented into short-axis images using standard clinical software. For estimating regional myocardial ¹⁸F concentrations, polar maps were constructed from maximum activity profiles of the 15 contiguous short-axis cuts including the apex.11 The polar maps were subdivided into 9 ROIs (Figure 1). Myocardial activity concentrations (MBq/g) were determined for each ROI and for the entire LV myocardium. Volume units were converted into mass units, assuming a specific gravity of 1.055 for myocardium.

Correction for Partial Volume

Each circumferential activity profile was corrected for effects of partial volume that cause an underestimation of true activity concentrations; its magnitude depends on the performance characteristics of the imaging device and on LV wall thickness.¹²,¹³ Myocardial wall thickness was measured with M-mode echocardiography (Echoanalyzer 51 DMK 100, Advanced Technology Laboratories, Inc) in 6 additional anesthetized rats, comparable in weight with group 1 and 2 animals. The recovery coefficient (RC) was determined from the end-diastolic wall thickness based on a one-dimensional bar phantom approximation and the estimated effective spatial resolution of the microPET. The thickness of the LV myocardium was assumed to be uniform.

Cross-Calibration of Well Counter and microPET Measurements

Quantitation of activity concentrations by PET requires cross-calibration of image based with true activity concentrations. This is normally accomplished by imaging a large phantom containing a solution with a known radioactivity concentration. Susceptibility of the three-dimensional imaging mode of microPET to scatter from sources outside the heart rendered the calibration approach more complex. The relatively high ¹⁸F dose caused significant deadtime losses of coincidence counts so that the calibration factor became a nonlinear function of the activity concentration. Consequently, we constructed a phantom that mimics the activity distribution throughout the body of the rat and that yielded the calibration factor and a means for deadtime correction based on singles count rates.

Estimating the Extent of At-Risk Myocardium in Group 2 Animals

On each polar map, myocardial N-13 concentrations were normalized to maximum counts. On the polar maps of the coronary occlusion study, the area with <50% of the maximum activity concentration was defined as at risk area and expressed as percent total polar map area. Geometric distortions attributable to mapping of the three-dimensional myocardial tracer distributions onto two-dimensional polar map displays were corrected for as validated previously.¹⁴
Statistical Analysis
Mean values are given with SDs. Regional radiotracer concentrations estimated in vivo and in vitro and at risk areas determined by microPET and ex-vivo by tissue staining were correlated by linear least-square regression analysis. Interregional and interanimal differences in tissue activity concentrations were submitted to ANOVA. \( P < 0.05 \) was considered statistically significant.

Results
Image Quality
Images obtained with \(^{18}\)FDG or \(^{13}\)N-ammonia were of good to excellent diagnostic quality. Total true counts per image averaged \(41\pm15 \times 10^6\) counts for \(^{18}\)FDG and \(11\pm1 \times 10^6\) counts for \(^{13}\)N-ammonia. Figure 2 depicts transaxial images obtained with \(^{13}\)N-ammonia at baseline and during coronary occlusion and with \(^{18}\)F-deoxyglucose. Reorientation of transaxial images into short- and long-axis slices and construction of polar maps was accomplished with a software program used for clinical PET studies.

Regional Myocardial \(^{18}\)F Activity Concentrations
The internal LV diameter averaged in the 6 rats \(6.0\pm0.7\) mm in end-diastole and \(3.8\pm0.9\) mm in end-systole. The average LV wall thickness was \(1.4\pm0.2\) mm in end-diastole and \(2.2\pm0.6\) mm in end-systole. The RC derived from the end-diastolic wall thickness was 0.59.

Average true myocardial \(^{18}\)F activity concentrations by well counting of tissue samples ranged from 1.13 to 9.72 MBq/g and averaged 5.01±2.80 MBq/g. In vivo observed myocardial activity concentrations, corrected for partial volume, radioisotope decay, and deadtime losses and adjusted with the calibration factor, ranged in the 11 animals from 1.30 to 9.88 MBq/g. They averaged 5.24±2.84 MBq/g and, thus, were similar to those measured by well counting. Finally, average myocardial \(^{18}\)F concentrations measured in vivo correlated to those determined by in vitro counting (Figure 3).

Regional myocardial \(^{18}\)F activity concentrations derived from the 9 polar map ROIs ranged from 1.18 to 10.58 MBq/g, whereas the corresponding in vitro measured tissue concentrations ranged from 0.89 to 10.46 MBq/g (\( P = 0.31 \)). In vitro and in vivo determined regional activity concentrations were correlated linearly (Figure 4); the scatter of the data about the regression line was greater than that for the correlation of the average myocardial activity concentrations. When images were not corrected for photon attenuation, observed \(^{18}\)FDG tissue concentrations ranged from 0.88 to 8.10 MBq/g (mean, 3.96 MBq/g) and systematically underestimated the true tissue concentrations but continued to correlate significantly and linearly with the true regional concentrations (\( P < 0.0001 \); Figure 4). \(^{18}\)FDG was relatively homogeneously distributed throughout the rat myocardium. The coefficient of variation (COV) as a measure of the homogeneity averaged 7.5±2.5% for the true regional concentrations and was 8.7±4.2% (NS) for in vivo observed concentrations.

To examine effects of correction for photon attenuation, the COV was also determined for regional myocardial F-18

![Figure 2](https://example.com/image2.png)

**Figure 2.** Examples of transaxial myocardial images in rats. A, Four contiguous \(^{18}\)FDG image slices in a group 1 animal, spaced 1.4 mm apart and arranged from superior to inferior (left to right). B, Four contiguous myocardial slices in a group 2 animal; top row shows \(^{13}\)N-ammonia images at baseline, and the bottom row, after coronary occlusion. Note the left and right ventricular myocardia and the flow defect in the anterior wall after coronary occlusion.

![Figure 3](https://example.com/image3.png)

**Figure 3.** Average myocardial F-18 activity concentrations in vivo (A, corrected; B, not corrected for photon attenuation) by microPET and postmortem well counting. Regression equations for A: \( y_{corr} = 1.0x + 0.23; r = 0.99; P < 0.01; SE = 0.06 \); B: \( y_{uncor} = 0.77x + 0.10; r = 0.99; P < 0.01; SE = 0.04 \).
concentrations derived from uncorrected images. It averaged 8.5 ± 2.8%, which was comparable with that found for the true and for the attenuation corrected images (NS). Finally, correction for photon attenuation had no systematic effect on activity concentrations in any myocardial region. No significant differences in regional tissue concentrations between images with and without correction for photon attenuation were found on 2-way ANOVA.

Measurement of the Myocardial Area at Risk
On the baseline images, 13N-ammonia was distributed homogeneously throughout the LV myocardium. Although the images had not been corrected for photon attenuation, the COV for regional N-13 activity concentrations averaged 5.1 ± 0.7%, which was similar to the COV for the attenuation-corrected F-18 images in the group 1 animals. None of the baseline images demonstrated >50% reductions in regional N-13 concentrations. By contrast, all 13N-ammonia images recorded during the coronary occlusion revealed >50% uptake defects, although their size varied considerably between animals. Figure 5 depicts an example and compares the stained postmortem tissue samples to short-axis slices and the polar map display of the perfusion defect. Defects ranged in size from 3.0% to 49.4% (average, 34.9 ± 14.7%). Risk areas on postmortem examination of nonstained myocardium were similarly variable. In the animal with the smallest defect on the microPET images, unstained myocardium could not be identified so that the postmortem determined defect sizes ranged from 0% to 60.3% (average, 39.5 ± 18.5%). Figure 6 demonstrates the linear correlation between in vivo and postmortem determined defect sizes.

Discussion
Our results indicate that regional myocardial tissue concentrations of positron tracers and the extent of defects in regional radiotracer uptake and, thus, the size of myocardial flow defects can be determined accurately and noninvasively in rats with a dedicated small-animal PET imaging device.

Recent instrumentation-related improvements in ultra-high resolution imaging afford the noninvasive study of the cardiovascular anatomy and function in small-animal experimental models. Present radionuclide imaging devices, including dedicated SPECT systems with pinhole collimators, yield images of good diagnostic quality but evaluate only the relative distribution of radiotracer in tissue. With dedicated small-animal PET systems like microPET, myocardial tracer activity concentrations can, as our study shows, be measured noninvasively in absolute units. The measurements applied to a wide range of myocardial 18F concentrations (from 0.65 to 9.7 MBq) that probably resulted from differences in the 18FDG activity doses, differences in dietary states, such as fasting in 3 and normal feeding in 8 animals, and effects of anesthetics on plasma substrate levels.

The transaxial and reoriented images were consistently of good diagnostic quality. They depicted the normal homogeneous distribution of 13N-ammonia as tracer of blood flow and of 18FDG as tracer of glucose utilization throughout the normal LV myocardium in rats. The 13N-ammonia images were visually of lower quality probably because of lower total true counts per image (~25% of those for 18FDG); effects of the greater positron range of N-13 causing some spatial blurring were probably minimal. Nevertheless, the 13N-ammonia coronary occlusion images consistently identified

Figure 4. Regional myocardial F-18 activity concentrations determined in vivo by microPET (A, corrected; B, not corrected for photon attenuation) and postmortem by well counting. Regression equations for A: ycorr = 0.98x + 0.333; r = 0.97; P < 0.01; SEE = 0.03; B: yuncorr = 0.75x + 0.18; r = 0.97; P < 0.01; SEE = 0.02.

Figure 5. Postmortem myocardial slices (left), contiguous short-axis images (middle), and the corresponding polar map (right) depicting an extensive flow defect in the anterior, lateral, and inferolateral myocardium in a group 2 animal.
regional defects. Their extent correlated with the size of defects on postmortem staining and varied considerably between animals. Interanimal differences in the occlusion site likely accounted for the range of flow defects. The in vivo observed defect size was validated by postmortem tissue analysis after coronary reocclusion and injection of blue dye immediately before arresting the heart. Although care was taken to exactly reproduce the initial coronary occlusion, variations may have occurred. This might explain the absence of the defect on postmortem staining that was seen on the microPET images in one animal but also the scatter of the data about the regression line. For estimating defect sizes, standard clinical software for whole-body PET systems for image reorientation and assembly of polar maps was used. Use of the 50% threshold for regional reductions in tracer uptake for delineating flow defects is somewhat arbitrary but supported by findings in animal and in clinical investigations with SPECT and PET.14,16,17

Measurements of regional myocardial activity concentrations in units of MBq/g from the images required correction for photon attenuation. However, this was accomplished through transmission images that required acquisition times of 1 hour to be of high-count density and of low statistical noise. This requirement substantially prolongs study times. The prototype imaging system in this study used a cylindrical transmission source. The three-dimensional image acquisition mode is susceptible to scatter and random coincidences. To minimize both, the transmission source was of low activity, which in turn required long periods for transmission imaging. Next-generation small-animal scanners are equipped with rotating point sources for transmission images as used in clinical PET systems so that transmission images can be recorded within 10 minutes.

As the present findings indicate, microPET studies are possible without attenuation correction. Observed regional and global myocardial activity concentrations obtained without such correction continued to correlate linearly with the true activity concentrations, although, as the regression lines between true and observed concentrations indicate, they systematically underestimated true concentrations by \( \approx 25\% \). Failure to correct for photon attenuation did not adversely affect the observed homogeneity of regional activity concentrations, as evidenced by a COV that remained similar to that for the corrected images and for the true, in vitro measured activity concentrations. Also, lack of correction for photon attenuation had no systematic effect on the observed activity concentration in any given myocardial segment. Because the COV for the uncorrected baseline \( ^{13} \text{N-} \text{ammonia} \) images was comparable to that for the \( ^{18} \text{FDG} \) images, lack of attenuation correction was unlikely to have adversely altered the observed tracer activity distributions or the estimates of defect size.

A property common to all imaging approaches is the partial volume effect.\ref{13} The resulting underestimation of tracer concentrations requires correction with a RC that depends on the performance characteristics of the microPET and the object size, that is, the thickness of the LV wall.\ref{12,13} This value was derived by M-mode echocardiography in 6 additional rats. Because of their similar weight and type of anesthesia, it was assumed to be valid for the animals in group 1. Assumption of a homogeneous wall thickness in addition to possible misalignments between the ROIs on the polar maps and the in vitro counted tissue samples may have accounted for the greater scatter of the data about the regression line for the regional myocardial activity concentrations.

Differences in counting efficiency require a calibration factor so that tissue activity concentrations derived from microPET images can be related to those determined by well counting. For conventional PET systems, the calibration factor is obtained by imaging a phantom with a known activity concentration. Because of its three-dimensional acquisition mode, the microPET system is more sensitive to singles counts originating from adjacent organs, such as the bladder in the present study. It necessitated a specific rat calibration phantom for determining variations of the calibration factor. In addition to a short cylindrical component of the heart, the phantom included additional more distally placed radiation sources that mimicked the bladder activity. With this rat calibration phantom, a more robust deadtime correction algorithm was developed, and adequate calibration of in vivo to in vitro determined activity concentrations became possible. The calibration approach has been standardized to consistently and accurately account for system deadtime with activity originating from adjacent organs.

**Conclusion**

The good correlations for estimates of myocardial \( ^{18} \text{FDG} \) concentrations and of flow defect sizes determined by microPET and by postmortem tissue analysis support the feasibility of noninvasive quantitative imaging studies in the myocardium of rats. It affords the serial evaluation of functional processes like blood flow and substrate metabolism in the myocardium of small animals and also offers an opportunity for longitudinal monitoring of the expression of transfected reporter or therapeutic genes and their location, magnitude, and time course as already performed in tumors or in the liver.\ref{18,19} Other opportunities include evaluation of novel radiotracers or noninvasive delineation of phenotypic responses to gene transfection. Furthermore, the ability to
measure regional radiotracer tissue concentrations might prove especially useful in transgenic animal models where the genetic modification may affect the myocardium homogeneously rather than regionally. It is acknowledged that most of these animal models consists of mice. Although the findings of the present study cannot be directly extrapolated to the even smaller-sized heart in mice, the images obtained with $^{15}$N-ammonia and $^{18}$F-deoxyglucose (FDG) in mice, as shown in Figure 7, suggest that similar studies may become feasible in mice. However, the accuracy of measurements of regional and global myocardial activity concentrations in these even smaller animals awaits additional determination.

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