

Noninvasive Quantification of Regional Myocardial Blood Flow in Coronary Artery Disease With Oxygen-15-Labeled Carbon Dioxide Inhalation and Positron Emission Tomography

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Background. Oxygen-15-labeled water is a diffusible, metabolically inert myocardial blood flow tracer with a short half-life (2 minutes) that can be used quantitatively with positron emission tomography (PET). The purpose of this study was to validate a new technique to quantify myocardial blood flow (MBF) in animals and to assess its application in patients.

Methods and Results. The technique involves the administration of ^{15}O -labeled carbon dioxide (C^{15}O_2) and rapid dynamic scanning. Arterial and myocardial time activity curves were fitted to a single tissue compartment tracer kinetic model to estimate MBF in each myocardial region. Validation studies consisted of 52 simultaneous measurements of MBF with PET and γ -labeled microspheres in nine closed-chest dogs over a flow range of 0.5–6.1 ml/g/min. A good correlation between the two methods was obtained ($y=0.36+1.0x$, $r=0.91$). Human studies consisted of 11 normal volunteers and eight patients with chronic stable angina and single-vessel disease, before and after intravenous dipyridamole infusion. In the normal group, MBF was homogeneous throughout the left ventricle both at rest and after administration of dipyridamole (0.88 ± 0.08 ml/g/min and 3.52 ± 1.12 ml/g/min, respectively; $p\le0.001$). In patients, resting MBF was similar in the distribution of the normal and stenotic arteries (1.03 ± 0.23 and 0.93 ± 0.21 ml/g/min, respectively). After dipyridamole infusion, MBF in normally perfused areas increased to 2.86 ± 0.83 ml/g/min, whereas in the regions supplied by stenotic arteries it increased to only 1.32 ± 0.27 ml/g/min ($p\le0.001$).

Conclusions. PET with C^{15}O_2 inhalation provides an accurate noninvasive quantitative method for measuring regional myocardial blood flow in patients. (*Circulation* 1991;83:875–885)

The noninvasive and quantitative measurement of regional myocardial perfusion has been the goal of many research efforts. However, this has been very difficult to accomplish because of limitations of the tracers and instrumentation previously used. The technique used most often in the clinical setting is the assessment of relative perfusion with the potassium analogue ^{201}Tl , which uses either planar or tomographic γ -camera imaging. Although widely used, this tracer has some disadvantages. It

has variable extraction at different flow rates¹; additionally, its retention in the myocardium is proportional to the intracellular potassium pool and is consequently related to cell metabolism, which can be severely disrupted in various pathological conditions.² These characteristics, together with limitations in the detection systems used (particularly artifacts related to tissue attenuation), have prevented this technique from being a quantitative tool for measuring myocardial perfusion.

The development of positron emission tomography (PET) allows the use of tracers of myocardial perfusion with a detection system that provides quantitative measurement of myocardial and blood tracer concentrations.³ Thus, several techniques are under development, which include ^{68}Ga - or ^{11}C -labeled albumin

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Received April 11, 1990; revision accepted October 23, 1990.

microspheres,^{4–5} ^{82}Rb ,^{6–9} and ^{13}N ammonia.¹⁰ Of these tracers, microspheres have the disadvantage of being invasive; ^{82}Rb and ^{13}N ammonia (like ^{201}Tl) are not pure flow tracers, and its extraction may depend on the metabolic state of the tissue.^{9,11,12} We, as well as others,^{13,14} have focused on the development of methods that use H_2^{15}O as a freely diffusible tracer for measuring myocardial perfusion.

H_2^{15}O features the following advantages: 1) It is almost entirely extracted from the blood over a wide range of flows during its first pass across the myocardium; 2) it is metabolically inert; and 3) the half-life of ^{15}O is only 123 seconds, which facilitates repeated or combined measurements with other tracers. Some of these characteristics are of great importance in clinical conditions involving severe myocardial injury, in which changes in flow and metabolism occur. These changes may be difficult to separate if tracers that are metabolically trapped in the myocardium are used to measure flow.

Intravenous bolus injection of H_2^{15}O and fast PET scanning have demonstrated usefulness in measuring myocardial blood flow (MBF). However, the accurate measurement of high blood concentrations of H_2^{15}O within a short time frame to determine the arterial input function is required, which may not be possible with all types of scanners; therefore, the invasive measurement of the arterial H_2^{15}O concentration is necessary.¹⁵

The slow and continuous delivery of the tracer as C^{15}O_2 gas, which is almost instantaneously transformed to H_2^{15}O in the lung capillaries, could be an alternative method of delivering the tracer, thus avoiding the scanning limitations related to the speed of tracer concentration changes of high levels of radioactivity in the cardiac chambers.

Therefore, in the present study a method was developed for measuring regional MBF by means of C^{15}O_2 gas inhalation and multiple-slice dynamic PET scanning. Validation measurements of the model in experimental animals are reported. In addition, we applied the technique to normal volunteers and patients with chronic stable angina and single-vessel coronary disease at rest and after the administration of dipyridamole.

Methods

Validation Studies in Dogs: Experimental Preparation

Nine greyhounds were included in this study. After overnight fasting, they were sedated with morphine 1 mg/kg s.c., and anesthesia was induced with intravenous thiopental. Animals were intubated and ventilated with a mixture of O_2 and room air. Anesthesia was maintained by the inhalation of 0.5–1% halothane throughout the study.

Catheters were placed in the femoral vein and artery and in the left ventricular cavity. The latter was used to inject γ -labeled microspheres. Injection of microspheres into the left ventricle was chosen to allow use of a closed-chest dog preparation to mini-

mize potential changes in chest geometry that could have occurred after thoracotomy and introduced additional errors in the measurements. Arterial pressure, an electrocardiogram, and arterial blood gases were monitored throughout the procedure.

To evaluate a wide range of flow values, intravenous dipyridamole infusion was performed after the resting measurement. In some dogs, plasma expanders were given to obtain higher myocardial blood flows by avoiding the drop of blood pressure observed after the vasodilator administration. In one dog, intravenous morphine was administered to reduce global MBF.

Measurement of Myocardial Blood Flow With Radiolabeled Microspheres

Results obtained with PET were compared with those obtained with ^{57}Co -, ^{113}Ti -, and ^{46}Sc -labeled 15- μm microspheres. These microspheres were injected into the left ventricular chamber, and a reference sample was taken at a known flow rate from the femoral artery with a calibrated Harvard pump (Harvard Apparatus, South Natick, Mass.). After the study, the dogs were killed with an overdose of barbiturates. The heart was then excised and cut in 6–8 one-centimeter-thick sections perpendicular to the long axis of the left ventricle, from which approximately 40–60 one gram samples were taken. Tissue and arterial blood samples were counted in an automatic γ -counter (Wallak Ultragamma 2, LKB, Stockholm, Sweden), and blood flow was calculated with the standard reference technique.¹⁶ Results were grouped corresponding to anterior, lateral, inferoposterior, and septal walls to compare with the results obtained with PET for similar regions.

Scanning Procedures

All PET scans were recorded with an ECAT 931-08/12 (CTI, Inc., Knoxville, Tenn.). Briefly, it consists of eight contiguous rings of bismuth germanate detectors, so that 15 cross-sectional images of the heart can be recorded simultaneously in an 10.5-cm axial field of view. All emission scans were reconstructed with a Hanning filter that had a cutoff frequency of 0.5 of maximum. This procedure resulted in a transaxial resolution of 8.4 FWHM (full width at half maximum) at the center of the field of view (i.e., the measured activity is equal to the true activity when the object size is approximately 16 mm thick).¹⁷ Thus, with this scanner it was possible to simultaneously record myocardial and blood tracer concentrations of the whole heart.

The dogs were placed in the scanner, and a 5-minute rectilinear transmission scan was recorded to aid in the positioning of the heart in the center of the field of view. A transmission scan was then recorded for a 20-minute period, which was subsequently used to correct all emission scans for tissue attenuation.

Blood pool scans were performed with ^{15}O -labeled carbon monoxide (C^{15}O). This tracer, which forms

[^{15}O]carboxyhemoglobin, was continuously delivered by way of the endotracheal tube (3 MBq/ml at a flow rate of 500 ml/min) for a 4-minute period, and a 6-minute single scan was recorded starting 1 minute after the end of the gas delivery. Seven arterial blood samples were taken during scanning, and the C^{15}O concentration was measured with a NaI well counter cross-calibrated with the scanner. After a 15-minute period to allow for decay, a continuous inhalation of C^{15}O_2 was given for a period of 3.5 minutes (3–5 MBq/ml at a flow rate of 500 ml/min). This gas is transformed almost instantaneously to ^{15}O water by the enzyme carbonic anhydrase in the lung capillaries, resulting in the delivery of radiolabeled water to arterial blood.¹⁸ A sequence of 24 scans was started at the beginning of the C^{15}O_2 delivery, consisting of six scans of 5, 10, 20, and 30 seconds. Thus, a total scanning time of 6.5 minutes allowed the collection of the buildup (3.5 minutes) and the washout (3 minutes) of activity from the heart. After recording the baseline MBF scan and allowing approximately 15–20 minutes for decay, dipyridamole infusion was begun at doses of 0.3–0.56 mg/kg for a 4-minute period, and the second C^{15}O_2 scan was initiated 2 minutes after the end of the dipyridamole infusion.

Microspheres were injected 2 minutes after the onset of the gas delivery for each of the perfusion measurements.

Data Analysis

Original coincidence data were stored as sinograms and then normalized, corrected for attenuation, and backprojected in a dedicated array processor to obtain the final images. Data collection and initial processing were performed under the control of a MicroVax 2 computer (Digital Equipment Corp., Marlboro, Mass.). The final images were transferred to SUN 3/60 workstations for further analysis by use of Analyze image analysis (Mayo Foundation, Rochester, Minn.) and Pro-Matlab (The MathWorks, Inc., South Natick, Mass.) mathematical software packages.

Blood Volume

Functional blood volume images were created by dividing the PET counts by the blood sample counts measured in the cross-calibrated well counter. This blood volume image was used to define regions of interest (ROI) in the left atrium, showing more than a 90% recovery of the true blood tracer concentration. This procedure was followed to avoid the underestimation of the arterial input function due to the spatial resolution of the PET scanner (partial volume effect).¹⁹ Irregular drawings of 3–5 cm³ ROIs were defined on three to five transaxial images, where the atrial chamber was clearly identified. These regions were then projected onto the dynamic ^{15}O water images to generate time–activity curves for each ROI. The average of these curves was used as the arterial input function.

ROIs for myocardial tissue were drawn on an integrated H_2^{15}O image obtained by subtracting the

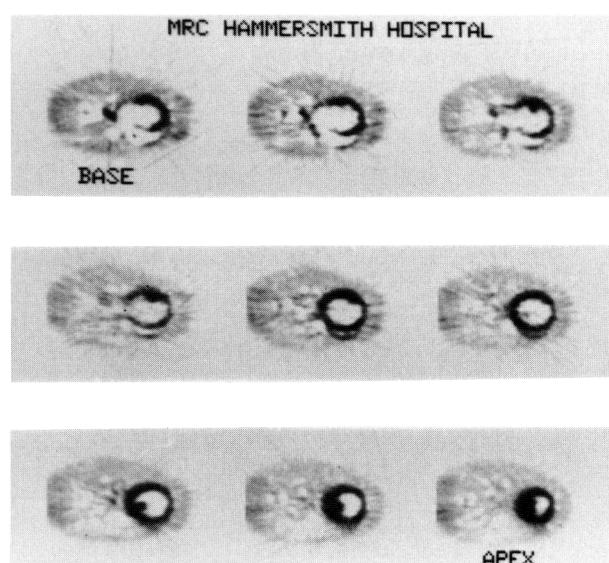


FIGURE 1. Transaxial images of ^{15}O myocardial distribution in a dog heart from the base (top left) toward the apex (bottom right). These images were obtained by subtracting the blood volume activity from an integrated H_2^{15}O water image. Note the anatomical delineation of the papillary muscles and the right ventricular wall.

blood from the sum of scans recorded during the C^{15}O_2 delivery (Figure 1). Twenty-five to 30 circular-tissue ROIs (20-mm diameter) were defined on six to eight transaxial images of the left ventricular wall and projected onto the original dynamic H_2^{15}O time frames. Time–activity curves were generated for each ROI and then averaged for the four anatomical regions as described for microsphere flow (Figure 2).

In the patient group, a surface-shaded three-dimensional reconstruction was performed with a threshold of 40% of the maximum activity. Although these images may demonstrate usefulness in assess-

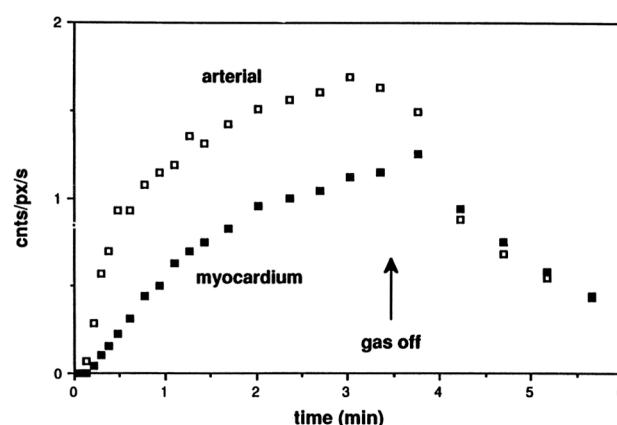


FIGURE 2. Representative time–activity curve obtained from ROIs in the left atrium (arterial input function) and over the left ventricular wall (myocardium). The data are recorded during the gas inhalation (0–3.5 minutes) and up to 3 minutes after the end of the tracer administration.

ing regional abnormalities, they were not used for the quantitative analysis.

Calculation of Myocardial Blood Flow From the Time-Activity Curves

MBF was calculated by fitting the arterial input and tissue time-activity curves to a single tissue compartment tracer kinetic model, which includes corrections for the underestimation of tissue activity due to the partial volume effect and the spillover of activity from the left ventricular chamber into the myocardial wall. This model is a modification of the Kety model for diffusible tracers, which has been changed to account for those artifacts related to the limited spatial resolution of the PET scanner. Briefly, the model estimates MBF, the fraction of exchangeable tissue (FET), and the arterial blood volume with the spillover fraction (V_a) within the ROI measured. The FET is the fraction of the ROI that consists of tissue capable of exchanging water within the time period of the study. The V_a contains information on the arterial blood within the myocardium and the cross-contamination of activity originating in the ventricular chamber. MBF is expressed in milliliters per minute per milliliter of exchangeable tissue and FET in milliliters of exchangeable tissue per milliliter of ROI. (For further details, see the Appendix.) For comparison with microsphere flow data, MBF PET results were multiplied by the density of myocardial tissue (1.04 ml/g) to express MBF in milliliters per minute per gram.

Studies in Normal Volunteers and Patients

All human studies were performed with the same PET scanner and procedures as described in the animal studies. For all subjects, radioactive gas was supplied with a regular oxygen-type open-face mask, which provides a mixture of 5–8% of radioactive gas in room air. Previous estimates have indicated that the effective dose for a single run of 2 minutes of CO_2 inhalation with a concentration of 6 MBq/ml at 500 ml/min was 1.2 mSv (10 mSv=1 rad).²⁰ For the present study, the equivalent effective dose would be 1–2 mSv per run. The radiation dose for the trachea has also been estimated showing that after inhalation of radioactive gas, the concentrations used in clinical studies were well within the safety standards.²¹

No caffeine-containing food or drink was allowed before the study.

All members of the normal group were male (aged 26–42 years) with no history, physical findings, or electrocardiographic signs of previous cardiac disease.

Of the eight patients with coronary disease (aged 47–67 years), 50% were female. All had a well-documented history of effort angina but no evidence of previous myocardial infarction. Coronary angiography revealed significant coronary stenoses ($\geq 75\%$ diameter reduction) in the left anterior descending artery in six patients, the right coronary artery in one patient, and the circumflex artery in one patient. Single-plane ventricular angiography was normal in all patients. At least

PET MBF and Microspheres MBF in Dogs

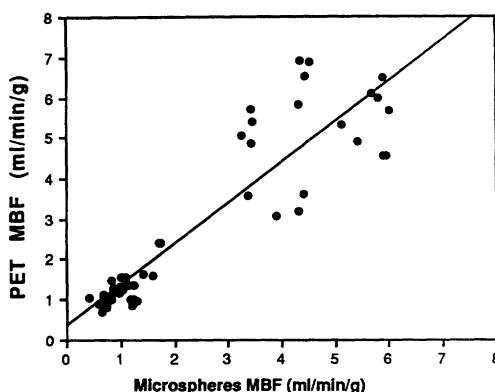


FIGURE 3. Scatterplot with regression line showing the correlation between myocardial blood flow (MBF) measured by positron emission tomography (PET) and γ -labeled microspheres performed in the validation studies.

72 hours before the study, all nitrate, β -blockers and calcium antagonists were discontinued except for sublingual nitroglycerin as needed.

Data Analysis in Humans

Data analysis procedures were identical to those used in animal studies. MBF was calculated in four anatomical regions (anterior, lateral, inferoposterior, and septal) for each normal participant and in myocardial regions supplied by normal and stenotic arteries for each patient. Studies in the normal group were analyzed by two independent observers to evaluate the operator-related variability.

This protocol was approved by the United Kingdom Administration of Radioactive Substances Advisory Committee and the Hammersmith Hospital Ethics Committee. Written consent was obtained from all the subjects enrolled in this study.

Statistical Analysis

Results are expressed as mean \pm SD. Linear regression analysis was used to compare PET MBF and microsphere MBF and the results in the normal group obtained by two observers. Student's *t* test for paired and independent variables was used to establish statistical differences.

Results

Estimates of Myocardial Blood Flow in Animals

A total of 52 paired measurements were obtained in the nine dogs included in this study, with flow values ranging from 0.6 to 6.1 ml/g/min. Figure 2 depicts time-activity curves obtained from the left atrium and a myocardial region of a dog during baseline and after dipyridamole infusion. The correlation of PET MBF and microsphere MBF was not different from the identity line ($y=0.36+1.0x$, $r=0.91$) (Figure 3). Regional analysis for each individual dog showed that MBF was homogeneous throughout the myocardium both at rest and at high

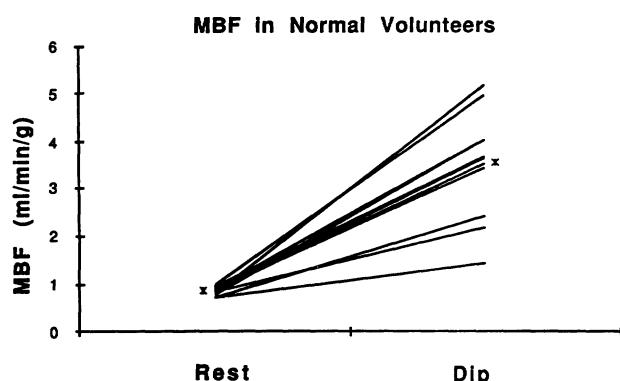


FIGURE 4. Line graph showing average myocardial blood flow (MBF) results for the normal group during baseline and after dipyridamole. The individual variability agrees with data obtained by invasive procedures.

flows. The mean coefficient of variation (COV) at rest was 6% for microsphere MBF and 11% for PET. This finding remained similar after dipyridamole administration (5% and 13%, respectively).

The mean FET was 0.87 ± 0.14 ml/ml ROI, and the mean Va was 0.07 ± 0.07 during resting conditions and remained unchanged after dipyridamole infusion (0.86 ± 0.14 and 0.06 ± 0.07 , respectively).

Normal Volunteers

Hemodynamics. The mean heart rate at rest was 63 ± 10 beats/min and increased to 95 ± 13 beats/min after dipyridamole administration. Blood pressure was 112 ± 10 mm Hg at rest and 116 ± 12 mm Hg after dipyridamole infusion. The heart rate blood pressure product increased from $7,056 \pm 1,369$ at rest to $10,920 \pm 1,959$ after dipyridamole administration ($p \leq 0.005$).

Estimates of myocardial blood flow in normal volunteers. The mean MBF during resting conditions was 0.88 ± 0.08 ml/g/min and increased to an average of 3.52 ± 1.12 ml/g/min after dipyridamole infusion (Fig-

ure 4 and Table 1). The mean coronary vasodilatory response to dipyridamole administration (MBF dipyridamole/MBF rest) was 4.19 ± 1.25 (range, 2.05 – 6.65). Analysis of the four anatomical regions showed homogeneous flow throughout the myocardium during both rest and vasodilation (mean COV, 15.24% and 10.54%, respectively) (Figure 5). Analysis of the data by two independent observers showed a very good agreement between the two estimates ($y = 0.2 + 0.9x$, $r = 0.93$). The mean FET was 0.61 ± 0.08 and 0.66 ± 0.12 ml exchangeable tissue/ml ROI at rest and after dipyridamole infusion, respectively. Va was 0.24 ± 0.04 at rest and 0.20 ± 0.08 after dipyridamole administration. Regional analysis of the FET showed that the septum had an 18% higher value than other regions.

Patients With Coronary Disease

Clinical findings and hemodynamics. The mean resting heart rate was 69 ± 7 beats/min, which increased to 89 ± 7 beats/min after dipyridamole infusion. Blood pressure was 139 ± 11 mm Hg at rest and 144 ± 14 mm Hg after dipyridamole administration. The heart rate blood pressure product increased from $9,508 \pm 1,220$ at rest to $12,775 \pm 1,223$ after dipyridamole infusion ($p \leq 0.005$). Five patients had chest pain, and three of them showed ST depression on the electrocardiogram.

Estimates of myocardial blood flow. Figure 6 shows a representative example of transaxial H_2^{15}O integrated images, and Figure 7 shows a three-dimensional surface shaded image at rest and after dipyridamole infusion of a patient with a lesion on the left anterior descending artery.

The average MBF at rest was 1.03 ± 0.23 ml/g/min in the normal regions and 0.93 ± 0.21 ml/g/min in areas supplied by the stenotic artery ($p = \text{NS}$). After dipyridamole infusion, MBF increased to 2.86 ± 0.83 ml/g/min in the normally perfused areas and to only 1.32 ± 0.27 ml/g/min in the distribution areas of the stenotic arteries ($p \leq 0.001$) (Table 2). The FET at

TABLE 1. Regional Myocardial Blood Flow Measurements at Rest and After Vasodilation in Normal Volunteers

| No. | Rest | | | | | | Dipyridamole | | | | | |
|------|------|------|------|------|------|-------|--------------|------|------|------|------|-------|
| | Ant | Lat | Inf | Sep | Mean | COV | Ant | Lat | Sep | Inf | Mean | COV |
| 1 | 0.92 | 1.10 | 0.91 | 0.85 | 0.93 | 14.64 | 3.98 | 4.00 | 3.79 | 3.44 | 3.66 | 9.04 |
| 2 | 1.11 | 0.82 | 0.61 | 0.87 | 0.77 | 26.10 | 4.50 | 4.45 | 3.57 | 3.67 | 4.05 | 9.04 |
| 3 | 0.75 | 0.97 | 0.90 | 0.89 | 0.89 | 8.38 | 3.66 | 3.73 | 3.76 | 3.06 | 3.45 | 15.15 |
| 4 | 0.99 | 0.97 | 0.86 | 0.90 | 0.91 | 9.21 | 3.74 | 3.97 | 3.32 | 3.40 | 3.69 | 6.94 |
| 5 | 0.88 | 0.80 | 0.86 | 0.68 | 0.82 | 11.38 | 2.16 | 2.53 | 2.13 | 2.08 | 2.18 | 7.75 |
| 6 | 0.70 | 0.70 | 0.77 | 0.98 | 0.78 | 13.79 | 4.87 | 6.67 | 5.65 | 4.42 | 5.18 | 21.06 |
| 7 | 1.04 | 0.93 | 0.79 | 0.81 | 0.86 | 11.79 | 3.44 | 3.74 | 3.59 | 3.69 | 3.64 | 3.06 |
| 8 | 0.53 | 0.72 | 0.77 | 0.67 | 0.71 | 15.63 | 2.94 | 2.21 | 2.83 | 2.37 | 2.44 | 14.36 |
| 9 | 1.04 | 1.16 | 0.94 | 0.90 | 0.99 | 10.26 | 4.85 | 5.45 | 5.04 | 4.83 | 4.95 | 5.31 |
| 10 | 0.89 | 0.86 | 0.66 | 0.55 | 0.71 | 22.94 | 1.47 | 2.00 | 1.32 | 1.28 | 1.47 | 18.08 |
| 11 | 0.83 | 1.13 | 0.91 | 0.54 | 0.87 | 23.52 | 4.49 | 3.75 | 3.91 | 3.88 | 4.02 | 6.13 |
| Mean | 0.88 | 0.92 | 0.82 | 0.79 | 0.84 | 15.24 | 3.64 | 3.86 | 3.54 | 3.28 | 3.52 | 10.54 |
| SD | 0.17 | 0.16 | 0.11 | 0.15 | 0.09 | 6.19 | 1.10 | 1.37 | 1.20 | 1.04 | 1.12 | 5.76 |

Ant, anterior; Lat, lateral; Inf, inferoposterior; COV, coefficient of variation between regions; SD, standard deviation.

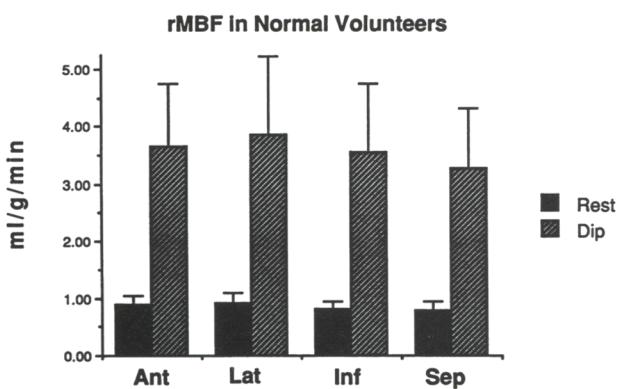


FIGURE 5. Bar graph showing regional myocardial blood flow (rMBF) in the normal group at rest and after dipyridamole.

control in the normal area was 0.63 ± 0.05 and in the stenotic area 0.69 ± 0.11 ml/ml ROI. After dipyridamole administration, it remained similar in the

normal area (0.66 ± 0.34) but decreased slightly in the stenotic area (0.61 ± 0.11 ; $p \leq 0.05$ comparison between normal and stenotic areas after dipyridamole infusion). Va was 0.20 ± 0.05 during resting conditions in the normal and 0.16 ± 0.06 in the stenotic areas. After dipyridamole administration, Va in the normal area remained similar (0.18 ± 0.07) but was slightly higher in the stenotic area (0.22 ± 0.09 , $p \leq 0.05$).

Discussion

In the present study, we report the data of the validation and clinical application of a method for measuring regional MBF noninvasively and quantitatively. This method involves the inhalation of C^{15}O_2 as a means of delivering H_2^{15}O into the left cardiac chambers and rapid dynamic PET scanning of the whole heart. By use of a mathematical tracer kinetic model that describes the behavior of a diffusible tracer in the myocardium, we estimated regional MBF, the FET, and the Va. The last two estimates

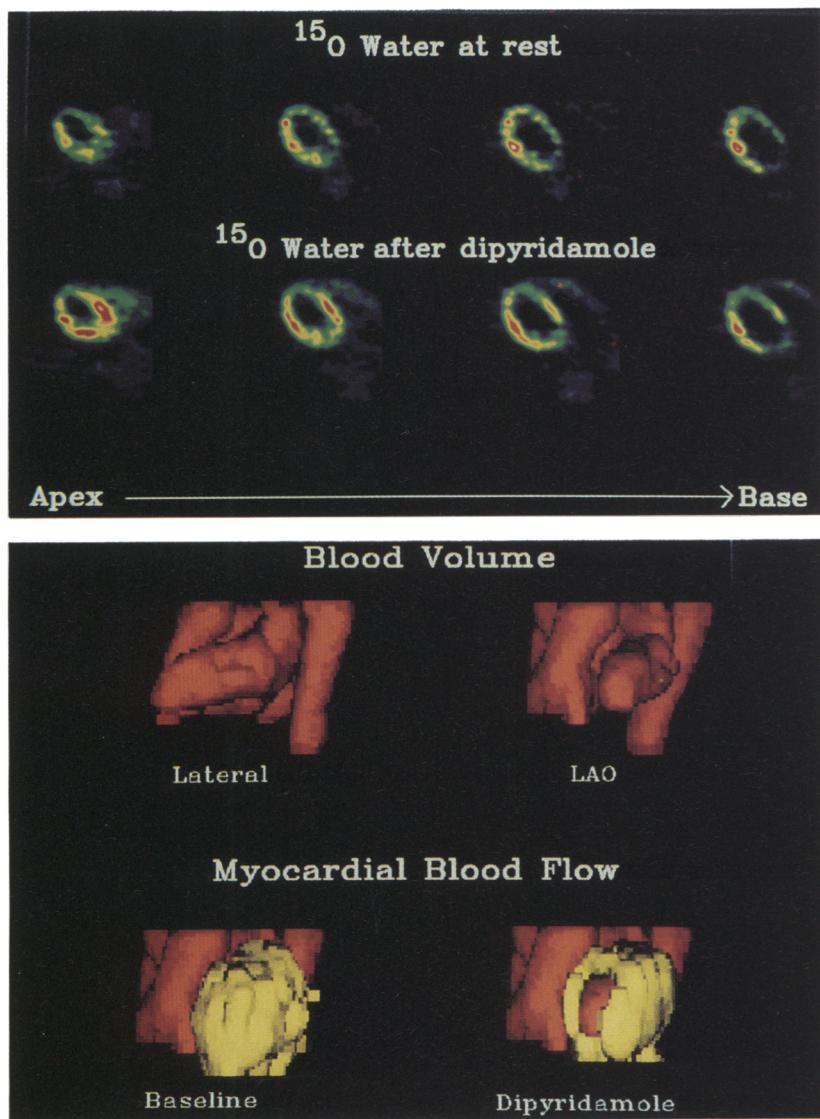


FIGURE 6. Positron emission tomographic (PET) scan showing transaxial images of the myocardial ^{15}O water distribution of a patient with a single lesion in the left anterior descending artery. These images depict the heart seen from the head of the patient, and the left of the image corresponds to the left of the patient. A perfusion defect in the anterior wall is detected in the images recorded after dipyridamole. During vasodilation, myocardial blood flow (MBF) was 2.87 and 1.15 ml/min/g in the normal and stenotic areas, respectively.

FIGURE 7. Positron emission tomographic (PET) scan showing different views of the three-dimensional surface-shaded images of the same study depicted in Figure 6. Blood volume (red) was overlapped by the myocardial ^{15}O water distribution (yellow). A perfusion defect is observed in the territory of distribution of the left anterior descending (LAD) artery.

TABLE 2. Myocardial Blood Flow in Patients with Single-Vessel Coronary Artery Disease

| No. | ST | CP | Myocardial Blood Flow | | | | | | | |
|------|------|----|-----------------------|--------|------|------|------|------|----------|------|
| | | | RPP | | Rest | | Dip | | Dip/Rest | |
| | | | Rest | Dip | Norm | Sten | Norm | Sten | Norm | Sten |
| 1 | - | + | 8,450 | 10,875 | 0.85 | 0.80 | 2.26 | 1.55 | 2.64 | 1.95 |
| 2 | 1 mm | + | 11,200 | 13,000 | 1.16 | 1.07 | 3.37 | 1.64 | 2.91 | 1.54 |
| 3 | 1 mm | + | 9,100 | 12,600 | 1.08 | 0.91 | 2.69 | 0.95 | 2.49 | 1.04 |
| 4 | - | - | 10,540 | 14,025 | 1.28 | 1.08 | 3.86 | 1.55 | 3.00 | 1.44 |
| 5 | - | - | 7,800 | 11,250 | 0.80 | 0.78 | 2.51 | 1.32 | 3.14 | 1.69 |
| 6 | - | - | 9,000 | 13,600 | 0.97 | 0.72 | 4.10 | 1.40 | 4.25 | 1.94 |
| 7 | - | + | 10,875 | 14,250 | 1.36 | 1.34 | 2.40 | 1.16 | 1.77 | 0.87 |
| 8 | 1 mm | + | 9,200 | 12,300 | 0.74 | 0.78 | 1.72 | 0.96 | 2.32 | 1.23 |
| Mean | | | 9,520 | 12,737 | 1.03 | 0.93 | 2.86 | 1.32 | 2.82 | 1.46 |
| SD | | | 1,216 | 1,233 | 0.23 | 0.21 | 0.83 | 0.27 | 0.73 | 0.40 |

ST, ST segment shifts; CP, chest pain; RPP, heart rate and blood pressure product; Dip, dipyridamole; Norm, normal; Sten, stenotic; -, no; +, yes.

account for the errors of the spatial resolution of the PET scanner and the size of the myocardial wall (partial volume and spillover effects).¹⁵

The results in dogs show that the noninvasive estimation of MBF closely correlate with those results simultaneously obtained with γ -labeled microspheres over a wide range of flow values. Furthermore, results in normal participants and patients are comparable to those obtained in other laboratories with the use of either noninvasive¹²⁻¹⁵ or invasive²² techniques.

Technical Considerations

The method we have used involves the slow delivery of radioactive tracer by gas inhalation, which is unlike other methods of $H_2^{15}O$ administration by intravenous bolus injection.¹³⁻¹⁶ The $C^{15}O_2$ inhalation method for administering $H_2^{15}O$ was chosen for the following considerations: 1) as $C^{15}O_2$ it is easy to produce and requires no direct handling by the radiochemist, 2) the delivery is switched on and off directly from the scanning room by the scanner operator, and 3) the slow (3.5-minute) inhalation provides a slow input function, thereby reducing artifacts that result from the dead time of the scanner that is associated with the high count rates resulting from bolus injections.

However, the main disadvantage of $H_2^{15}O$ for measuring MBF is the high level of activity in the cardiac chambers, which, because of the limited resolution of the PET scanners, interferes with the signal from the myocardium. For this purpose, the component V_a was introduced in the model and estimated in each measurement. This estimate carries information of both the true arterial blood volume of the myocardium and the spillover activity from the left ventricular chamber into the myocardial ROI. Thus, a thinner wall causes a higher V_a . Conversely, a thinner myocardial wall should provide a lower FET. The data show that the average FET in dogs is 0.87 and for V_a is 0.07, indicating that the myocardial wall was thick enough to allow close to 100% recovery and had a small spillover fraction. Measurements of recovery coefficients per-

formed with a stationary cardiac phantom in our PET scanner indicated that 100% recovery is achieved when the myocardial wall is over 18 mm thick.¹⁷ In fact, the greyhounds used in this study had a hypertrophic myocardium with a thickness of about 20 mm. Conversely, the wall thickness of normal human myocardium ranges from 10 to 12 mm.²³ Therefore, for the same ROI size a lower FET and higher spillover than that observed in greyhounds would be expected. Results show that the FET in normal volunteers was 0.61 and V_a was 0.20, which is consistent with the thinner myocardial wall. Measurements of recovery coefficients performed with a stationary cardiac phantom in our positron scanner indicated that for a wall thickness of 10 mm, the recovery coefficient is 55%.¹⁷ Interestingly, those areas supplied by stenotic arteries in patients showed a slight decrease in the FET and a slight increase of V_a , which may indicate transient mechanical abnormalities (thinning) of the ischemic segments contributing to the partial volume effect. These findings suggest that the use of the FET in each myocardial region enables us to account for different wall thicknesses, thus correcting for the myocardial tracer underestimation resulting from the partial volume effect. This avoids the need for an independent measurement of the myocardial thickness in conjunction with PET (i.e., magnetic resonance imaging or two-dimensional echocardiography).

An additional disadvantage of this technique is the relatively poor statistics that lead to a noisier image compared with other flow tracers (^{13}N ammonia and ^{82}Rb), which results from the kinetic characteristics of the tracer and the rapid dynamic scanning involved in the data collection.

The noninvasive measurement of the arterial input function obtained directly from the scans has been previously demonstrated.²⁴ However, its measurement is also affected by the partial volume and spillover effects, particularly those from the left ventricular wall into the left cardiac chambers. To minimize these effects, we have chosen ROIs in the left atrial chamber with a recovery larger than 90%,

as measured on the calibrated blood volume scan. Very good agreement was observed in dog studies when the arterial blood concentration measured from the scans was compared with the measurement obtained with a β -probe sampling directly from the femoral artery.²⁰

Myocardial Blood Flow Measurements in Dogs

MBF determined from this model correlated very closely with MBF calculated from γ -labeled microspheres. Similar results were recently reported by other laboratories.¹² In our study, we investigated flows as high as 6.1 ml/g/min to investigate the linearity of the PET estimates at high flows to determine its clinical usefulness in evaluating coronary flow reserve with vasodilators. Although the relation is consistent, a larger scatter in the data was observed at high flows. This may be the consequence of a lower sensitivity of the $H_2^{15}O$ model to high flow values as demonstrated by mathematical simulations.

Myocardial Blood Flow Measurements in Normal Volunteers and Patients

Results obtained in normal participants at rest agree with the results reported by Bergman¹² and Iida,¹⁵ both of whom used $H_2^{15}O$. Slightly lower values in normal participants (0.75 ± 0.43) were recently reported by Krivokapich et al²⁵ with ^{13}N ammonia; however, their data show considerable scatter.

MBF increased by 419% after dipyridamole infusion, which agrees with results recently reported with intravenous $H_2^{15}O$ ¹² and measurements of coronary flow velocity with a Doppler catheter.²² However, there was considerable intersubject variability. This finding could have resulted from the relative insensitivity of the PET method at very high flows or variable individual response to dipyridamole. Similar variability was observed in normal participants, in which Doppler measurements were used,²² indicating variable individual response to the administered dose of dipyridamole, and may partially explain the broad range of flows observed in our normal group.

Despite the less favorable scanning conditions of humans (higher tissue attenuation, lower effective radioactive dose, and higher probability of movement during scanning), MBF was homogeneous throughout the left ventricular myocardium during both baseline and vasodilation studies. These results were similar when analyzed by two independent observers.

Patients with single-vessel disease were selected to have a clinical model where a normally perfused area may be used as a control in each individual study, and no attempt was made to assess the diagnostic accuracy of the method. MBF in myocardial areas supplied by normal and stenotic arteries was not different from that measured in normal participants. This finding is consistent with the fact that these patients with chronic stable angina had no history of myocardial infarction and had normal left ventriculography. However, in all patients the MBF increase in the stenotic areas after dipyridamole administration was signifi-

cantly lower than that observed in the normal areas. This condition occurred even in the absence of clinical or electrocardiographic signs of ischemia observed in three patients. The increase of MBF in the area supplied by the stenotic artery in those patients with signs of ischemia must be interpreted in terms of the present measurement reflecting the average transmural MBF, and dipyridamole may cause transmural redistribution of flow with an absolute increase in the subepicardial layers but an absolute flow decrease in the subendocardial layers, which may be responsible for the ischemic signs.²⁶

In conclusion, in normal participants and patients we have presented the validation and clinical application of a method for quantitatively and noninvasively measuring regional MBF. ^{15}O -labeled water is a freely diffusible tracer and is metabolically inert. It is relatively easy to produce and has a short half-life, which allows repeat measurements or combined studies with other tracers of physiological processes. Together with PET scanners that allow simultaneous recording of the whole heart, it may be a useful research tool to investigate not only syndromes in the context of epicardial coronary disease but also areas such as small-vessel disease and cardiomyopathies, in which the value of animal models and angiographic data is less relevant.

Acknowledgments

We thank the Cyclotron and Radiochemistry section for providing us with isotopes and Claire Taylor and Graham Lewington for their technical assistance.

Appendix

^{15}O -labeled water is a metabolically inert, freely diffusible tracer. Its behavior in tissue can be described by the following differential equation for a single tissue compartment model:

$$dC_t(t)/dt = F.C_a(t) - (F/V_d + \lambda).C_t(t) \quad (1)$$

where $C_t(t)$ is the regional tissue concentration of $H_2^{15}O$, $C_a(t)$ the arterial whole blood concentration of $H_2^{15}O$, F the regional flow in milliliters per milliliter per minute, V_d the volume of distribution of water, and λ the decay constant of ^{15}O .

The solution to this differential equation is as follows:

$$C_t(t) = F.C_a(t) * \exp(-[F/V_d + \lambda].t) \quad (2)$$

where * denotes the operation of convolution. $C_t(t)$ represents the tissue response to arterial input function $C_a(t)$.

In Equation 1, two assumptions are made: 1) Both F and V_d are constant during the measurement period, 2) water is freely diffusible (i.e., the extraction fraction of water is unity and no binding of water in tissue occurs).

In practice, not the actual tissue concentration is measured but the concentration within a macroscopic ROI. Such an ROI contains not only tissue but

also blood. It is assumed that for freely diffusible tracers, venous and tissue concentrations can be treated as a single compartment. In addition, for most tissues it is assumed that the contribution of arterial activity is negligible because of its small volume. Therefore, Equations 1 and 2 still hold for a macroscopic ROI under the following two additional assumptions: Venous and tissue concentrations are negligibly different (i.e., the effect of $V_d \neq 1$ is negligible), and 2) The contribution to the signal in an ROI arising from arterial activity is negligible.

The first assumption is a standard requirement for all practical PET techniques. It is a reasonable assumption for baseline studies, but some inaccuracies might occur in challenge studies such as after dipyridamole injection, where physiological steady state may not be achieved.

Although it has been shown that there are some diffusion limitations for water in the brain,²⁷⁻³⁰ this effect should be negligible in the myocardium because of the much larger permeability surface area(s) of myocardial tissue. For the myocardium, the permeability (P) has been estimated to be 6×10^{-4} cm/sec,³¹ and the estimated values of the surface area for the myocardium range from 730 to 1,260 cm²/ml.³² Therefore, the PS product will range from 3,091 to 5,336 ml/dl/min. Using these products, we calculated that even for a flow as high as 8 ml/ml/min, the extraction fraction for water will be at least 98%.

For a freely diffusible tracer, venous and tissue concentrations can be considered similar if the volume of distribution of the tracer in tissue is unity. From data of in vitro water content for blood and myocardial tissue,^{32,33} a partition coefficient of water of 0.96 ml/ml can be calculated for the myocardium. The resulting error in flow for such a value is negligible.³⁴

The main problem for the application of Equations 1 and 2 to the myocardium is the invalidity of the fourth assumption. The arterial fraction of the blood vessels within the myocardium itself is probably higher than in most other organs. More importantly, however, because of the limited spatial resolution of PET scanners, the measured regional tissue concentration will be contaminated by a signal originating from the high level of activity present within the cardiac chambers (spillover effect). If the size of the ROI sampled is not too small, this spillover signal will be much higher than the signal from the local arterial vessels. Consequently, Equation 2 can be expanded to include the following spillover term:

$$C_{tot}(t) = C_t(t) + V_a \cdot C_a(t) = F \cdot C_a(t) \cdot \exp(-[F/V_d + \lambda] \cdot t) + V_a \cdot C_a(t) \quad (3)$$

where $C_{tot}(t)$ is the measured tissue concentration in an ROI and V_a the arterial spillover fraction.

However, the limited resolution of PET scanners not only results in the inclusion of spillover activity but also in an underestimation of the $C_t(t)$ (partial volume effect; 19). The degree of underestimation

will be identical for F and V_d . One method to deal with this partial volume effect is to include a tissue fraction component as originally suggested by Iida¹⁵ as follows:

$$F = \alpha MBF \quad (4)$$

$$V_d = \alpha p \quad (5)$$

where α is the fraction of exchangeable tissue (milliliter of exchangeable tissue per milliliter of ROI), p the partition coefficient of water (milliliter of blood per milliliter of tissue), and MBF the flow for the exchangeable tissue (milliliter of blood per milliliter of tissue per minute).

In Equation 5, it was assumed that the volume of distribution of water for the exchangeable tissue is the same as the partition coefficient of water for normal myocardial tissue. Substituting Equations 4 and 5 into Equation 3 yields the following:

$$C_{tot}(t) = \alpha MBF C_a(t) \cdot \exp(-[MBF/p + \lambda]t) + V_a C_a(t) \quad (6)$$

C_a (left atrium) and C_{tot} (myocardium) can be measured noninvasively as a function of time with dynamic PET scanning. By standard nonlinear regression analysis, Equation 6 can then be fitted for an MBF, with V_a assuming a value for p . In the present study, a value of 0.96 ml/ml was used based on in vitro water content data.^{32,33} The main disadvantage of Equation 6 compared with Equation 3 concerns the assumption that p (i.e., the water content) of myocardial tissue is constant. Further studies will be required to determine if this assumption holds in various pathological conditions. However, at least in predominantly normal tissue, this potential drawback is compensated for by the major advantage that the MBF obtained with Equation 6 is independent of limitations of spatial resolution. This discovery was confirmed in observer variability studies. Results in normal volunteers obtained by independent observers were more closely matched when Equation 6 was used than Equation 3. Because Equation 6 is less sensitive to resolution effects, it is also less sensitive to slight variation in the placement of the myocardial ROIs.

It is clear from Equation 6 and also Equation 3 that if blood volume is measured separately, in principle it could be subtracted from the tissue data before fitting. Alternatively, it could be fixed to the measured value in the fitting process. In both cases, the number of parameters to be estimated in the regression analysis would be reduced to two. In theory this should provide better precision. However, by so doing the venous blood volume would be treated as being at arterial concentration. In addition, blood volume might change in dipyridamole studies, thus requiring a second blood volume scan. Actually, the validation studies in dogs showed poorer correlation between MBF and microsphere flow if blood volume was fixed to the measured value,

even in baseline studies. This finding was especially the case in the septum, where some spillover from the right ventricle occurs.

Although Equation 6 provides MBF results that are independent of both partial volume and spillover effects, care should be taken in the interpretation of clinical data. For example, in pure ischemic tissue the fraction of exchangeable tissue should theoretically be the same as that for normal tissue. Therefore, ischemic tissue is expected to show a reduced MBF combined with a normal FET. Contrastively, in infarcted tissue the fraction of exchangeable tissue will probably be reduced, and the remaining exchangeable tissue could have normal flow. Therefore, infarcted tissue demonstrates normal or reduced MBF but reduced FET. However, further studies will be required to evaluate and test this hypothesis.

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KEY WORDS • myocardial blood flow • positron emission tomography • ^{15}O -labeled water