Characterization of the functional significance of subcritical coronary stenoses with H$_2^{15}$O and positron-emission tomography*

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ABSTRACT We have previously developed a method employing cardiac positron-emission tomography (PET) with $^{15}$O (half-life 2.1 min)-labeled water ($H_2^{15}$O) and blood pool subtraction with $^{15}$O for assessment of myocardial perfusion. This study was performed to determine whether the method developed permits detection of the differences in blood flow, induced by vasodilator stress, indicative of functionally significant subcritical coronary stenosis despite normal perfusion at rest. Coronary stenoses were induced with a small Teflon cylinder placed in the left anterior descending coronary artery of the closed-chest dog. Regional myocardial blood flow was assessed tomographically with $H_2^{15}$O given intravenously and $^{15}$O given by inhalation. Blood flow distal to the stenoses was normal under conditions of rest. However, significant reductions in the hyperemic response to dipyridamole were detected consistently in regions distal to 50% to 70% diameter stenoses. Flow distal to stenoses more than doubled in absolute terms in response to dipyridamole but was only 43 ± 9% of the increased flow in normal regions in the same dogs or in corresponding anterior regions in normal dogs. Relative myocardial blood flow measured noninvasively with PET correlated closely with the distribution of radiolabeled microspheres measured in vitro ($r = .88$). Thus, assessment of myocardial blood flow with $H_2^{15}$O and PET in dogs at rest and during vasodilator-induced stress permits detection of physiologically significant coronary stenoses. The procedure should therefore prove useful diagnostically for the detection of coronary insufficiency in patients as well as for the assessment of clinical interventions designed to augment regional perfusion.


CORONARY ARTERIAL LESIONS that decrease cross-sectional diameter by as much as 70% to 75% (referred to here as subcritical stenosis for convenience) generally do not alter myocardial blood flow under resting conditions. However, augmentation of myocardial blood flow in response to increased oxygen demand in regions supplied by such vessels is limited. Thus, in patients with such lesions, symptoms typically first appear only during strenuous activity, when an imbalance occurs between myocardial blood flow and oxygen demand.

Conventional assessment of coronary arterial lesions relies heavily on cardiac catheterization, which is usually performed only after the onset of symptoms. Assessment of coronary flow reserve by direct measurement of the reactive hyperemic response in patients undergoing cardiac surgery has demonstrated that angiography often underestimates the physiologic severity of obstructive lesions. The purpose of the present study was to determine whether a method developed recently for assessment of perfusion with positron-emission tomography (PET) and $H_2^{15}$O (ref. 3) is sensitive enough to detect the reduction of coronary flow reserve by coronary stenoses that are insufficient to alter blood flow under resting conditions, and thus to detect and characterize the functional significance of subcritical coronary stenoses noninvasively.

Myocardial blood flow distal to stenoses depends on

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many factors, including collateral flow, the presence or absence of multiple lesions in series, the length of segments narrowed, and lesion geometry. The physiologic significance of stenoses may become apparent only when the demand for blood flow is increased—a response not necessarily demonstrable with conventional angiography. Although exercise stress testing with electrocardiographic criteria is used to characterize the severity of ischemia, it suffers from unavoidable limitations of sensitivity and specificity. Improved localization of ischemic zones may result from coupling radionuclide scintigraphy with exercise stress testing, but quantitative limitations are not obviated.

Pharmacologically induced vasodilation has been proposed as an alternative to exercise stress for the demonstration of coronary stenoses. Vasodilator stress facilitates assessment of coronary vascular reserve and its impairment by physiologically significant coronary stenoses under conditions that do not entail elevation of myocardial oxygen demands and concomitant ischemia and its associated risks. Previous studies have demonstrated the utility of the use of vasodilator stress with radionuclide scintigraphy. However, conventional scintigraphy is limited by spatial resolution, incomplete correction for attenuation, and spillover of radioactivity from sources overlying or underlying the myocardium. Furthermore, the extraction of tracers such as Tl by myocardium is influenced by metabolic activity as well as by flow, impairing quantification of perfusion.

PET has been developed in part to overcome many of these limitations. It permits quantification of the spatial distribution of tracers of physiologic substrates labeled with positron-emitting isotopes. Previous studies have used NH₃ to detect disparities of perfusion during vasodilator-induced stress. However, this tracer exhibits kinetics similar to those of Tl; both have a variable extraction fraction that is a function of flow. Thus, results with NH₃ are subject to some of the same limitations as those with Tl. Our laboratory has recently demonstrated that PET with H,¹⁵O permits estimation of relative myocardial blood flow in vivo. The extraction fraction of H,¹⁵O was found to be independent of blood flow. Furthermore, the short half-life of (2.1 min) was found to permit rapid sequential evaluation of flow with low exposure of the subject to radiation.

In the present study we used H,¹⁵O and PET to characterize regional myocardial blood flow distribution at rest and in response to pharmacologically induced vasodilation in dogs with subcritical coronary stenoses. The results obtained indicate that PET with intravenously administered H,¹⁵O permits noninvasive regional localization of impaired coronary flow reserve distal to subcritical stenoses. The approach should be useful diagnostically in patients with coronary insufficiency caused by subcritical coronary stenoses and for assessment of the efficacy of interventions designed to augment perfusion of myocardium supplied by vessels affected by such lesions.

Methods

Animal preparations. Eighteen conditioned mongrel dogs (20 to 30 kg) were anesthetized with intravenously administered thiopental (12.5 mg/kg) and a-chloralose (60 mg/kg) after premedication with morphine sulfate (1 mg/kg subcutaneously). A cuffed endotracheal tube was inserted in each. Ventilation was maintained with room air with a Harvard respirator. Catheters were placed in the abdominal aorta and vena cava via the left femoral artery and vein. Retrograde catheterization of the left atrium for administration of radiolabeled microspheres was accomplished via the right femoral artery with a left atrial catheter (Cordis Corporation) under fluoroscopic guidance. The left common carotid artery was isolated for introduction of coronary catheters and for intracoronary insertion of an indwelling Teflon cylinder that partially occluded coronary flow, simulating stenosis. Arterial pressure and the electrocardiogram were monitored continuously.

Each of 15 dogs was instrumented with an intracoronary Teflon cylinder to simulate atherosclerotic stenosis and reduce the cross-sectional area of the lumen. Three dogs served as controls (i.e., no stenosis was induced despite coronary and left atrial catheterization).

For production of intracoronary stenosis, we modified the method of Gewirtz and Most. After obtaining a control angiogram of the left anterior descending coronary artery (LAD) with a modified Amplatz catheter, as previously described, we advanced a 0.018 inch guidewire through the catheter under fluoroscopy into this artery. The catheter was then carefully withdrawn with the wire still in place. A small Teflon cylinder (approximately 5 mm long) with an outside diameter of 2.5 to 4.0 mm and an inside diameter of 0.6 to 1.1 mm was advanced over the guidewire with the aid of a small flexible catheter (outside diameter 1.2 mm) until the cylinder lodged in the proximal half of the LAD. The catheter was left inside the LAD but pulled back approximately 1 cm from the proximal site of the stenosis, and the guidewire was removed. Injections of contrast medium through the intracoronary catheter permitted angiographic documentation of the stenosis before and after cardiac PET. Preparations were deemed to be unacceptable if the position of the stenosis changed between the two angiograms or if the Teflon cylinder occluded a branch of the LAD. Heparin (500 units/kg) was administered intravenously before catheterization to prevent thrombosis. Cross-sectional diameter narrowing was determined as the difference between internal diameter of the LAD and internal diameter of the Teflon cylinder divided by the internal diameter of the LAD and expressed as a percentage. The exact internal diameter of the Teflon cylinder was known and internal diameter of the LAD at the site of the stenosis was determined from measurements on the control angiograms, with correction for magnification error by use of a reference object.

Determination of regional myocardial blood flow. Animals were secured in a Plexiglass shell and positioned within the PET T VI (Positron Emission Transaxial Tomograph) system such that the entire left ventricle was within the field of view.

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All scans were performed in the high-resolution mode (full width at half maximum 0.7 cm).

A transmission scan was acquired with an external ring source of $^{68}$Ge for correction of attenuation in each of the emission scans. Correction for intravascular tracer in perfusion images was performed with the use of blood pool images obtained after administration, by inhalation, of 10 to 25 mCi $^{13}$O (half-life 2.1 min), a tracer that binds avidly to red blood cells, as described previously. In each pixel, the $H_2^{15}$O activity attributable to tracer in the vascular pool was determined as the product of $^{15}$O counts in that pixel and the ratio of $H_2^{15}$O/$^{15}$O counts in the left ventricular blood pool. These values were subtracted pixel by pixel from total $H_2^{15}$O counts. To measure the distribution of myocardial blood flow with PET, we used the diffusable tracer $H_2^{15}$O and a method recently validated in our laboratory.

Collection of data in consecutive 10 sec frames permitted use of greater amounts of activity (45 to 60 mCi) and hence improved counting statistics. Only the data obtained within 40 sec after activity had reached the left side of the heart were used for analysis of relative myocardial blood flow to provide conditions consistent with the constraints of the method previously developed. In addition, this approach permitted qualitative comparison of changes in successive tomograms by the construction of time-activity curves. For regions of interest in myocardial tissue and blood, the average counts per pixel for each 10 sec frame were decay-corrected to the time of injection and summed with counts from preceding time frames. Corrected results were plotted as a function of time after injection.

To determine relative myocardial blood flow (anterior/normal ratio), images of the distribution of myocardial $H_2^{15}$O were analyzed by selecting transmural regions of interest (approximately 1 cm$^2$) in the center of the anterior or posteriorstotic region as well as in normal zones (defined as the average of values in the septal, posterior, and posterolateral ventricle) and determining the ratio of counts attributable to tissue $H_2^{15}$O, as previously described. Corresponding regions of interest in control and postdipyridamole tomograms were compared.

Relative myocardial blood flow determined with PET was compared with that determined in vitro by analysis of the distribution of 15 $\mu$m radiolabeled microspheres. Within 30 sec after each collection of $H_2^{15}$O data, approximately 3 to $4 \times 10^6$ carbonized microspheres (3M Corporation) labeled with $^{46}$Sc, $^{51}$Cr, $^{85}$Sr, or $^{141}$Ce were injected into the left atrium. Absolute myocardial blood flow was determined by the standard reference withdrawal method$^{29}$ with the use of blood collected via the femoral artery. Relative myocardial blood flow determined with microspheres was computed from 0.5 to 1 g tissue samples obtained at the end of each experiment from regions corresponding to those studied by PET.

**Experimental protocol.** A 45 min equilibration period followed instrumentation of the animals. During this interval all animals were hemodynamically stable. After the transmission scan had been acquired, the external ring of $^{68}$Ge was removed. A 5 min data collection interval began 1 to 2 min after administration by inhalation of 10 to 25 mCi $^{13}$O for blood pool imaging. When activity had decayed to near background, approximately 5 min after data collection, a bolus injection of 45 to 60 mCi $H_2^{15}$O in 8 to 12 ml saline was given via the femoral vein catheter. Collection of data in six 10 sec frames was initiated when activity reached the field of view, as monitored with a digital strobe meter connected to an oscilloscope. Radiolabeled microspheres were administered immediately after the $H_2^{15}$O tomograms had been obtained.

Ten to fifteen minutes later, dipyridamole (Parsantine, supplied by Boehringer Ingelheim, Ridgefield, CT) was administered intravenously in a dose of 0.2 mg/kg/min over 5 min. Five minutes after infusion of dipyridamole, imaging was repeated after another intravenous bolus of $H_2^{15}$O, followed immediately by administration of radiolabeled microspheres. The blood pool was labeled with $^{13}$O when the $H_2^{15}$O activity had declined to near background. After the postdipyridamole $^{13}$O tomograms were obtained, each animal was restudied angiographically. Cardiac arrest was induced with KCl and the heart of each dog was removed. The location of the stenosis was confirmed, the heart and stenotic site were examined for any signs of trauma or thrombus, and the heart was sectioned for analysis of the distribution of gamma-emitting microspheres in vitro.

**Results**

Three dogs were sham-operated controls. Fifteen had induced intracoronary stenoses, but five of these did not meet the criteria for inclusion in the study. In three, the stenotic cylinder had moved during the tomographic study and in two others the Teflon cylinder occluded the origin of a small branch of the LAD, resulting in flow defects under resting conditions detectable on tomograms as well as with microspheres. Five dogs had stenoses that produced 50% to 55% reductions in diameter of the LAD, corresponding to 75% to 80% reductions in cross-sectional area. The other five had stenoses of 65% to 70% diameter resulting in 88% to 91% reductions in cross-sectional area. Figure 1 illustrates representative coronary angiograms obtained before and after placement of an intracoronary stenotic cylinder that resulted, in this case, in a 70% diameter reduction.

Transverse, processed, $H_2^{15}$O tomographic reconstructions from a plane through the middle of the left ventricle of a dog with a 70% stenosis are shown in figure 2. Processed $H_2^{15}$O tomograms typically contained 700,000 to 1.3 million counts/slice. Images were summed from data collected over 40 sec after $H_2^{15}$O had first reached the left ventricle and were corrected for vascular $H_2^{15}$O with the use of blood pool tomograms obtained with $^{13}$O. Tomograms obtained from dogs at rest depicted relatively homogeneous distributions of $H_2^{15}$O and were qualitatively similar to tomograms from sham-operated controls or normal dogs. In the 10 dogs with LAD stenoses, there was no significant difference between tomographically delineated resting regional myocardial blood flow in the anterior (poststenotic) region and that in the normal region (table 1). These results corresponded with results with microspheres in which relative (anterior/normal) myocardial blood flow was 0.94 ± 0.15.

Tomographic analysis of the distribution of $H_2^{15}$O yielded a relative myocardial blood flow of less than 1 under resting conditions. A corresponding ratio was evident in control dogs as well.

Administration of dipyridamole reduced mean arterial pressure from a control value of 135 ± 22 to 119
± 21 mm Hg (mean ± SD, p = NS). Heart rate increased reflexly from 110 ± 33 to 147 ± 23 beats/min (p < .05). The electrocardiogram was otherwise unaltered.

In dogs with induced LAD stenoses, tomograms obtained after administration of dipyridamole exhibited a markedly decreased ratio of anterior to normal zone H215O. Figure 2 depicts the tomograms obtained before (baseline) and after administration of dipyridamole in one of the dogs. Results were qualitatively similar in dogs with 50% to 70% stenoses. In addition, hyperemia induced by atrial pacing or infusion of isoproterenol in other experiments (data not shown) resulted in similar relative flow differences. In contrast, tomograms from control animals exhibited homogeneity after dipyridamole, with distributions of H215O similar to those under baseline conditions but with a greater absolute accumulation of tracer, which is consistent with the global increase in flow (figure 3).

Results with microspheres demonstrated that despite the presence of a severe stenosis in the LAD, absolute anterior wall myocardial blood flow more than doubled in response to dipyridamole (table 1). Regional myocardial blood flow in the normal region increased to an even greater extent (table 2), resulting in a decline in the relative (anterior/normal) myocardial blood flow ratio to 0.43 and 0.47 as measured with H215O and microspheres, respectively (table 1). The hyperemia in control dogs was of similar magnitude in anterior and normal regions, resulting in a relative myocardial blood flow ratio that was unchanged with dipyridamole compared with that at baseline (table 1).

Comparison of relative myocardial blood flow determined by PET with H215O and that determined with microspheres in vitro demonstrated a close correlation, as shown in figure 4, which depicts data from dogs with LAD stenoses and the three control dogs. The least squares regression line for all dogs is described by y = 0.17 + 0.63x, r = .87 and the line for dogs with stenosis by y = 0.15 + 0.64x, r = .88. Both regressions were highly significant (p < .01).

Although determination of absolute myocardial blood flow with PET requires correction for spillover, partial volume effects, and cardiac motion, as well as a defined tracer input function,3, 26-29 differences in flow detectable tomographically after dipyridamole com-
of dipyridamole was much more striking in normal regions than in zones distal to stenoses (2.4 and 1.4, respectively).

**Discussion**

PET with H$_{2}^{15}$O permits noninvasive characterization of myocardial perfusion to an extent not possible with conventional single-photon scintigraphy, in part because of the greater quantitative power of the method resulting from its physical attributes. Unlike $^{30}$T1, $^{13}$NH$_{3}$, and other monovalent cations, H$_{2}^{15}$O is not extracted differentially as a result of changes in myocardial metabolism associated with altered flow. Thus, it better fulfills assumptions inherent in the mathematical models underlying quantification of perfusion. This study demonstrates that, together with pharmacologically induced vasodilation, PET with H$_{2}^{15}$O is capable of noninvasively characterizing the functional significance of subcritical coronary stenoses in terms of coronary flow reserve. Tomograms obtained under resting conditions in animals with 50% to 70% reductions in LAD diameter were not different from those in control dogs. In contrast, under conditions in which dipyridamole induced vasodilation, the stenoses limited the magnitude of the hyperemic response — a phenomenon that was easily detectable by PET.

With the use of fast-scanning tomographs, the method developed should be directly applicable to patients for determination of the extent to which occult or clinically overt coronary stenoses limit the augmentation of coronary flow. Assessment of coronary flow reserve in localized regions of myocardium should permit noninvasive delineation of the location and functional importance of coronary lesions, including functionally significant left main coronary disease.

Dipyridamole increases coronary blood flow without elevating myocardial oxygen demands. Thus, coronary vascular reserve can be assessed after administration of this agent without eliciting tissue damage.
that may occur with ischemia and without incurring the small but real risks associated with exercise stress in the face of critical coronary stenosis that precludes hyperemia.

Previous studies have demonstrated the usefulness of dipyridamole-induced vasodilation for detecting coronary stenoses. Most have employed conventional single photon-emitting radionuclides such as $^{201}$Tl. Accurate quantification of the distribution of single-photon tracers in tissue is limited by inaccurate correction for attenuation and the contribution of activity from multiple over- and underlying sources within the field of view. These limitations can be overcome with PET. Previous tomographic studies with $^{13}$NH$_3$ and dipyridamole-induced vasodilation have detected the functional impact of stenoses of slightly less than 50% diameter. However, with $^{13}$NH$_3$ as with $^{201}$Tl, extraction fraction is variable as a function of flow and is in part dependent on metabolic activity of the myocardium, which confounds interpretation.

We used $^{3}$H$_2$O to determine relative myocardial blood flow noninvasively and simultaneously administered radiolabeled microspheres to determine myocardial blood flow in absolute terms. The method developed for the use of $^{3}$H$_2$O and C$^{15}$O with PET would also allow determination of myocardial blood flow in absolute terms if tracer input function and myocardial tracer content could be measured. The tracer input function can be defined by sampling arterial blood sequentially or by estimating left ventricular cavitary blood pool radioactivity tomographically as a function of time. However, certain technical limitations must be overcome, including effects of cardiac motion, spillover of activity from adjacent structures, and the incomplete recovery of counts that occurs when imaging objects that are smaller than twice the resolution of

![FIGURE 3. Tomograms from a sham-operated dog that was catheterized but not subjected to LAD stenosis. A. Baseline tomogram obtained after injection of 53 mCi H$^{15}$O under resting conditions. B. Tomogram obtained with 48 mCi H$^{15}$O after infusion of dipyridamole. The increased accumulation of tracer is evident with reference to the scale on the right.](image)

![FIGURE 4. Relation between relative myocardial blood flow (MBF) determined with H$_2$O and PET and that determined with microspheres measured in vitro MBF$^{15}$O = 0.15 + 0.63 MBF$_{microspheres}$ ($r$ = .87).](image)
the instrument (partial volume effect).26,28 Although PETT VI, the instrument used for these studies, is not fast or sensitive enough to permit gating to correct for cardiac motion, an instrument now undergoing testing for parallel clinical studies has this capability.32

The results of the present study were affected only modestly by the lack of gating and partial volume corrections because tomograms were obtained at baseline and after dipyridamole under the same conditions and geometric constraints. However, the relative myocardial blood flow values determined with PET were somewhat lower than those determined with microspheres. We have observed a similar anterior/normal ratio in normal dogs studied with H\textsubscript{2}\textsuperscript{15}O and in those studied with \textsuperscript{13}C-palmitate.\textsuperscript{9} This results from the lower recovery of counts from the anterior wall compared with the lateral, septal, and posterior walls. A transverse section through the chest of a dog provides a slightly oblique section through the posterior left ventricle, resulting in a thicker cross section than in anterior or tissue and thus a greater partial volume effect in anterior regions. The systematic disparity accounts for the lower ratio obtained with tomography than with the microsphere technique (approximately 1). Nevertheless, the hyperemia that occurs with dipyridamole was readily detectable because the tissue extraction of H\textsubscript{2}\textsuperscript{15}O increased more than H\textsubscript{2}\textsuperscript{18}O activity in blood (figure 5).

The results of this study indicate that PET with H\textsubscript{2}\textsuperscript{15}O and C\textsuperscript{18}O permits detection of reduced coronary flow reserve caused by subcritical coronary stenoses that are not severe enough to alter blood flow under resting conditions. With the application of fast-scanning time-of-flight tomographs and algorithms designed to compensate for effects of partial volume and spillover, quantification of myocardial perfusion should be possible in patients. The approach developed and tested in this study in experimental animals should not only facilitate detection of functionally significant coronary stenoses, but also characterization of their effects on perfusion and coronary flow reserve before and after corrective procedures such as thrombolysis, angioplasty, and coronary artery bypass surgery.

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References


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