Assessment of Regional Myocardial Blood Flow and Regional Fractional Oxygen Extraction in Dogs, Using $^{15}$O-Water and $^{15}$O-Hemoglobin

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SUMMARY  A new approach to the assessment of regional myocardial blood flow and fractional oxygen extraction has been developed using $^{15}$O-water ($H_{2}^{15}$O) and $^{15}$O-hemoglobin ($^{15}$O-Hb). Bolus doses (1 mCi) of $H_{2}^{15}$O and $^{15}$O-Hb were injected 10 minutes apart into the left main coronary artery of 12 normal dogs. Sequential images of regional myocardial tracer clearance were obtained over 5 minutes with a positron camera. Myocardial blood flow calculated from the monoexponential washout of $H_{2}^{15}$O after background correction was $78 \pm 6$ (SE) ml/100 g per min. Functional images of regional blood flow in which the image of peak activity was divided by the integrated image of $H_{2}^{15}$O washout were derived by computer processing. These images demonstrated homogeneous blood flow in the normal myocardium. Fractional myocardial O$_2$ extraction was determined from an image of initial distribution of O$_2$ used (obtained by extrapolating back to time zero the series of images obtained after $^{15}$O-Hb administration), divided by initial distribution of O$_2$ delivered (obtained by back extrapolating $H_{2}^{15}$O washout). These functional images showed uniform distribution of fractional O$_2$ extraction in the normal myocardium. Thus, these studies show that regional myocardial blood flow and regional oxygen extraction can be measured simultaneously by sequential imaging after serial intracoronary injections of $H_{2}^{15}$O and $^{15}$O-Hb.
attempt to obtain labeled blood with nearly physiological values of blood gases. A 4-ml sample of arterial blood was oxygenated in the tonometer, using 90% O₂ and 10% CO₂ for 10 minutes. During the last 4 minutes (two 15O half-lives), the target gas was recycled through the cyclotron to build up maximal 15O activity. At the end of this period, the tonometer chamber was included in the recycling path for 3 minutes to label the blood. A sample of the blood was used for determination of PO₂, PCO₂, and pH. In this series of experiments whole blood was obtained with specific activities of about 1 mCi/ml with PO₂ 106 ± 31, PCO₂ = 20 ± 6, and pH = 7.46 ± 0.07. There was no evidence of hemolysis as assessed by visual inspection of the plasma.

15O-Labeled Water

After irradiation, the target gas was passed over charcoal in a furnace at 600°C to transform the 15O16O to C15O16O. The 15O-labeled CO₂ was then exposed to heparinized whole blood in the tonometer for 4 minutes. CO₂ is in rapid equilibrium with bicarbonate, H₂CO₃, catalyzed by carbonic anhydrase:

$$\text{CO}_2 + H_2O \rightarrow H_2CO_3 \rightarrow HCO_3^- + H^+$$

When a bicarbonate molecule is labeled by the reaction: CO16O + H₂O → H₂CO₃ and dissociated by the back-reaction: H₂CO₃15O → CO₂ + H₂15O, the 15O is distributed to the CO₂ or the water molecule randomly. An equilibrium is established with the 15O distributed on the carbon dioxide, bicarbonate, and water molecules in proportion to their respective pool sizes. Since the water pool is much larger than the CO₂ or HCO₃⁻ pools, almost all of the 15O will be in the form of H₂15O.² By use of this procedure, whole blood labeled with H₂15O was produced with specific activity averaging 5 mCi/ml.

Canine Experimental Model

In 12 mongrel dogs, the left main coronary artery was cannulated and autoperfused via a shunt from the left subclavian artery. The dogs were anesthetized with pentobarbital (30 mg/kg, iv) and artificially ventilated with 100% oxygen. A femoral artery was cannulated and used to monitor systemic pressure. The heart was exposed through a midline sternotomy. The left main coronary artery was dissected and a ligature placed loosely around it. A cannula was placed through the right atrium into the coronary sinus for blood samples. A large bore cannula, connected to the perfusion apparatus, was placed in the left subclavian artery and the dog was heparinized. The perfusion apparatus consisted of Tygon tubing with a variable speed roller pump and a pressure transducer located distal to the pump. The distal end of the perfusion apparatus was a preformed stainless steel cannula with a flared end. The cannula was introduced through the common carotid artery into the aorta. The perfusion pump was turned on so that the saline in the tubing would be replaced with arterial blood from the subclavian artery. With the pump on, the flared end of the cannula was positioned in the left main coronary artery, and the tie was secured around the cannula so that the only blood entering the left main coronary artery came from the perfusion apparatus. The perfusion pump was adjusted so that the perfusion pressure approximated systemic arterial pressure. Flow was not adjusted during the series of radionuclide injections. Injections were made upstream from the cannula with a 3-ml volume separating the injection site from the left main coronary artery. The injection site was selected to allow good mixing of the isotope prior to delivery into the coronary artery, although it necessitated some loss of sharpness in the peak of activity as discussed in the Results section. The radiotracers were injected as a bolus into both the left main coronary artery (LCA) and right atrium via a cannula introduced through the right atrial appendage. Of the 12 dogs studied, the hemodynamic status of six animals remained sufficiently stable for them to undergo a full sequence of radionuclide injections.

Positron Camera

The MGH positron camera is a multicrystal device designed for the detection of coincident gamma photons emitted upon the annihilation of a positron.³ The camera used in these studies has two parallel detector arrays, each of which contains 127 NaI(Tl) crystals 2.0 cm in diameter, 3.8 cm deep, with 2.8-cm center-to-center distance giving a sensing area 27 cm by 30 cm. The parallel detector arrays are movable from a separation of 30-90 cm. In these studies, the two detector arrays were moved as close together as the animal preparation would permit (about 45 cm) to maximize sensitivity. Seventy-two (8 rows by 9 columns) photomultiplier tubes view the 127 NaI crystals at a 45° angle. Each photomultiplier tube detects scintillations in four crystals, and each crystal is viewed by two photomultiplier tubes. Each crystal thus can be uniquely identified by scintillations in two photomultiplier tubes.

Collimation is provided by coincidence detection. An event is recorded when simultaneous scintillations are recorded in a single crystal in each array within the 20-nsec resolving time of the camera. This event results from the detection of photons from a positron annihilation event that has occurred on a straight line between this crystal pair. Of the 127² (16,129) possible crystal pairs, 2,549 are identified; each crystal in one array is tested for coincidence with the 25 nearest crystals (5 × 5) in the other array. Without the use of scanning motion, the spatial resolution of the camera is 1.2 cm.

As the activity in the field of view increases, the probability of two unrelated photons being detected with the resolving time (i.e., a random coincidence) increases as the square of the single channel count rate. The MGH positron camera can detect random coincidence data by delaying signals from one array so that no coincidence events are detected. Because of the inherent low spatial resolution of random coincident data, random coincident images can be broadly smoothed and therefore have reduced statistical requirements. The MGH positron camera can be used to collect random coincident data one-eighth of the time in the upper byte of each crystal pair.
word and total coincidence data seven-eights of the time in the lower byte of each crystal pair word. The smoothed random data multiplied by 8 (determined experimentally) can be subtracted from the total coincident data to approximate the true coincident events.

Dynamic data collection, image synthesis, data processing, and data storage were accomplished by NUMEDICS, a PDP 9 (Digital Equipment Corp.)-based operating system developed specifically for this purpose. Crystal pair data were synthesized into images at five depths between the detector arrays. At these depths, the lines between the crystal pairs are evenly spaced. In these studies the heart was always positioned on the 0.4 plane, i.e., four-tenths of the distance between the detector arrays. The images were viewed on an oscilloscope with a 128 x 128 raster with 64 gray levels. Various types of data processing, as described subsequently, were performed with this computer system.

Experimental Protocol

After preparation, the dog was placed between the detector arrays of the positron camera in the posteroanterior projection. Lead II of the electrocardiogram, central aortic pressure, and coronary perfusion pressure were continuously recorded on a Hewlett-Packard physiologic recorder. In 12 dogs, 0.4-ml bolus doses of 15O-Hb and H218O were sequentially injected into the LCA via the perfusion line. A period of at least 4 physical half-lives (8 minutes) separated sequential injections to allow for radioactive decay. Between these two injections, systemic arterial and coronary sinus blood samples were obtained for determination of Po2, Pco2, pH, and O2 content by the Van Slyke technique. In addition, a separate injection of H218O was made into the right atrium to get an impression, qualitatively, of the time course of tracer recirculation. The manner in which background correction was accomplished quantitatively is described in a subsequent portion of this section. After each isotope was administered, serial images were obtained during tracer washout with the positron camera as described below.

Data Collection

Data were collected over a 5-minute period after isotope injection, using the positron camera-computer system. These data were collected by means of a variable frame rate. Fifteen sequential frames were collected lasting 2, 2, 2, 2, 4, 6, 9, 11, 25, 25, 25, 43, 43, 55, and 103 seconds. Initial studies using more rapid framing rates at the beginning of the study suggested that this timing interval was adequate for the injection model used, since intervals chosen were small with respect to the time required for significant changes in activity to occur. The times used in later frames were relatively long in duration compared to the physical half-life of 15O. We chose to correct for the effect of long time intervals by assigning the frame data to a point prior to the midpoint.

Consider a time interval (o, T) during which the time-activity curve is monoexponential with a decay constant, α. The time, T, to which the frame collected during this interval should be assigned is the time at which the value of the exponential function \[ e^{-\alpha T} \] is equal to the average value of the function

\[
e^{-\alpha t} = \frac{1}{T} \int_0^T e^{-\alpha t} \, dt
\]

\[
T = \frac{-\ln \left(1 - \frac{1}{e^{-\alpha T}}\right)}{\alpha}
\]

From the first two terms in the power series expansion:

\[
T = \frac{1}{2\alpha} \left(1 - \frac{\alpha T}{12}\right)
\]

Thus, the time to which the frame is assigned is the midpoint time, less a correction factor.

In these studies, an exponential function governed by a physical decay of 122 seconds and a biological decay of 100 seconds was chosen. The times to which the frames are assigned were therefore 1, 3, 5, 7, 10, 15, 22.5, 32.5, 50, 75, 100, 135, 175, 225, and 300 seconds. Since the initial correction in the assignment of these times is relatively small, interactive readjustment after collection of the data was not considered necessary.

Data Analysis

Using the NUMEDICS computer system, the rate of washout of H218O was used to obtain the myocardial blood flow in units of ml/min per 100 g of tissue. This measurement was obtained after injection of both 15O-Hb and H218O. A rectangular region of interest was selected to include the entire myocardium. A value proportional to the activity in the region of interest was obtained for both the total coincidence counts and the random coincidence counts in each frame of a study. The random coincidence counts were subtracted from the total coincidence counts to give the "true" coincidence counts. These data were then plotted as time-activity curves.

After washout from the myocardium, water recirculates through the cardiovascular system. This recirculating labeled water resulted in a significant background contribution to the activity measured in the region of interest over the myocardium and, therefore, was corrected for to avoid underestimation of blood flow. To evaluate recirculating activity quantitatively, a separate injection of H218O was made into the right atrium. After right atrial injection, activity passed to the lungs, then through the left ventricular blood pool, and then to the body where the radioactive water mixed with the body water pool. Recirculation of activity representing washout from the body water pool from the body was observed at about 100 seconds and probably reflected recirculation from the more highly perfused tissues, such as kidney and brain.

To correct quantitatively for background activity due to recirculation, it was assumed that, at 5 minutes, the background activity was much greater than the activity still in the myocardium, i.e., that essentially all the activity was due to background. A statistically adequate number of counts was available at this time. It was then assumed that this activity was relatively constant throughout the study, save for physical decay, and it was used to correct for background.
Image Processing

Data taken during washout of $^{15}$O-Hb and $^{2}$H$^{15}$O were used to extrapolate back to time zero in order to produce functional images proportional to total oxygen extracted by and the total oxygen delivered to the myocardium. Pixel-by-pixel back extrapolation had two main advantages over attempting to image peak activity directly: first, all of the data from washout could be used to fit the function, thereby improving the statistical accuracy of the image; and second, rapidly changing events around time zero (passage of isotope through the coronary arteries) which obscure the image of initial isotope distribution could be isolated. If the function is monoexponential within each pixel, with an initial activity $A_0$, at time $t$ the activity is $A_0 \exp \left[-\lambda t\right]$ and at time $2T$, the activity is $A_0 \exp \left[-2\lambda t\right]$. Squaring the first value and dividing by the second yields

$$\frac{[A_0 \exp (-\lambda T)]^2}{A_0 \exp (-2\lambda T)} = A_0$$

With these assumptions, a back-extrapolated image at time zero, $t_0$, was obtained by squaring the image at time $t$ and dividing it by an image at time $2t$. Small deviations from the monoexponential assumption will produce small errors. For example, images 8 and 9 were added to give an image centered at time 44 seconds; images 10 and 11 were added to give an image centered at time 87 seconds. Although this method of back extrapolation tended to accentuate statistical fluctuations, this method was chosen because it simplified computer operations. Whenever image division was performed, a mask region over the heart was defined. Outside of this region, all elements were set to zero; this prevented division artifacts due to low statistics. The mask region was selected as those elements which were greater than a defined percentage of the maximum value.

A functional image of regional fractional myocardial oxygen extraction was obtained by dividing the functional image of extracted oxygen by the functional image of delivered oxygen. Finally, a functional image of regional myocardial blood flow was obtained by the height-over-area method; an image of the initial distribution of labeled $^{2}$H$^{15}$O was divided by the sum of the images during water washout. Using the data after either $^{2}$H$^{15}$O or $^{15}$O-Hb injection, the back-extrapolated image at $t = 0$ was proportional to the initial distribution of labeled water. Dividing this image by the image during washout after correction for background gave an image proportional to regional myocardial blood flow.

Results

Oxygen-15-Labeled Hemoglobin

Time-Activity Curves and Measurement of Myocardial Oxygen Extraction

A 0.4-ml bolus of $^{15}$O-Hb was the first radioisotope injected into the LCA via the perfusion line. Serial images of the heart during washout were then obtained with the positron camera. Figure 1 shows anterior images of the chest after intracoronary injection of $^{15}$O-Hb in a representative dog. Early images in this sequence demonstrated appearance of activity in myocardium supplied by the left anterior descending and left circumflex coronary arteries. In the first image of this sequence, entry of the isotope through the perfusion cannula was observed. As the isotope was washed out of the myocardium, there was a
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80% Fractional Oxygen Extraction

50 100 150 200 250 SECONDS AFTER INTRACORONARY $^{15}$O-Hb

Figure 2 Idealized time-activity curve from the region of the myocardium after bolus injection of $^{15}$O-Hb into the left main coronary artery. In this semilog plot, it is assumed that the oxygen-15 is delivered as an instantaneous bolus and that there is no background activity. At the time of injection there is a rapid upslope as all of the injected activity comes into the field of view. The fraction of oxygen-15 not used in metabolism washes out of the myocardium, rapidly producing the initial peak in activity. The oxygen-15 used in metabolism is rapidly converted to H$_2$15O and washes out with a slower half-time, producing a bi-exponential curve. Back extrapolating from a portion of the curve where the water washout predominates to the time of injection yields a value proportional to the amount of oxygen-15 extracted by metabolism.

Relative increase of tracer activity in the surrounding lung tissue. The H$_2$15O equilibrated with the lung water and was washed out of the lungs in much the same fashion as it washed out of the myocardium. However, since the tissue volume of the lungs was small and blood flow was great, washout was rapid in comparison with myocardial washout. Later images, from 10 to 100 seconds, showed uniform distribution of oxygen-15 in the left ventricular myocardium. Cardiac activity in later images of this sequence was due largely to H$_2$15O in the cardiac blood pool.

Rapid disappearance of activity was observed after H$_2$15O was injected into the right atrium. The activity rapidly traversed the lungs to the left heart and became distributed in the total body water.

Figure 2 shows an idealized semilog plot of the time-activity curve obtained after an intracoronary injection of $^{15}$O-Hb. In this plot, it was assumed that the activity enters the heart as a discrete bolus and that there is no background. A fraction of the injected oxygen-15, namely, that fraction not used in metabolism, is rapidly washed out of the myocardium with the coronary venous blood. The fraction of oxygen-15 used in metabolism is rapidly converted to water and washes out at a slower rate as H$_2$15O. By isolating a region of the curve where the water washout predominates and extrapolating back to the time of isotope delivery, a value proportional to the

FIGURE 3 Experimental time-activity curve from the region of the myocardium after bolus injection of $^{15}$O-Hb. Compare the experimental curve to the idealized curve. In the experimental curve, the effect of recirculation of activity can be seen. Assuming that the background is equal to the value at 5 minutes and subtracting this value from the earlier points the background corrected curve is obtained. Because of the injection method used (see text), the initial peak cannot be identified. From the half-time of water washout, blood flow in ml/min per g was calculated.

Figure 4 Posterior-anterior unsmoothed functional image of regional myocardial oxygen extraction. After intracoronary administration of $^{15}$O-Hb, images during the time when water washout predominates were back extrapolated to the time of injection. This image, which is proportional to the total amount of oxygen extracted in each region, is proportional to the mass of the myocardium. There is relatively less activity in the ventricular cavity as compared with the septum, free wall, and apex. In all of the functional images, the noncardiac region has been masked to eliminate artifacts caused by division by small numbers.
amount of oxygen extracted by the myocardium is obtained. Figure 3 shows the actual time-activity curve obtained after the intracoronary administration of $^{15}$O-Hb in the representative dog whose images were shown in Figure 1. The semilog plot of $H_2^{15}$O clearance was similar to the idealized plot shown in Figure 2, except that the peak has been rounded out. In our experiment, the fact that isotopes were injected upstream in the coronary perfusion apparatus to ensure complete mixing limited the sharpness of the activity peak at the time the radioisotope bolus was injected.

Images of Regional Myocardial Oxygen Extraction

By matrix algebra, the images obtained during washout of oxygen-15 from the myocardium, after intracoronary injection of $^{15}$O-Hb, were back-extrapolated element by element to yield a functional image proportional to the total oxygen extracted in each region of the myocardium. Note that this image is not proportional to the initial distribution of $^{15}$O-Hb but, rather, is proportional to only that amount of oxygen which is transformed to water (i.e., that portion extracted by the myocardium). Figure 4 shows such a functional image of regional myocardial oxygen extraction. Because of the relatively low count rate, this image shows some statistical mottling. As predicted, the total amount of oxygen extracted is proportional to the mass of myocardium in each region. As can be seen, the ventricular cavity area has less activity than apical, septal, and free wall areas. Similar functional images were obtained in the remainder of the dogs in this series.

Oxygen-15-Labeled Water

After the intracoronary injection of $H_2^{15}$O, the initial distribution of activity was similar to that observed after injection of $^{15}$O-Hb. The initial distribution of activity is proportional to regional blood flow in ml/min and therefore is proportional to total oxygen delivery. Figure 5 shows the series of positron images acquired after administration of $H_2^{15}$O. These images were back extrapolated to time zero in the same manner as described for $^{15}$O-Hb. This yielded a functional myocardial image proportional to delivered oxygen. Figure 6 shows such a functional image for the dog whose serial images are shown in Figure 5. Note again that, because of a relatively low count rate, this image appears somewhat mottled.

Regional Fractional Oxygen Extraction

By dividing the functional image of extracted oxygen (obtained after $^{15}$O-Hb injection) by the functional image

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**Figure 6 Posterior-anterior unsmoothed functional image of regional myocardial oxygen delivery. After intracoronary administration of $H_2^{15}$O, images during washout were back extrapolated to the time of injection, producing an initial image of the distribution of $H_2^{15}$O. Since oxygen will be initially distributed in the same manner as $H_2^{15}$O, this functional image is proportional to the total amount of oxygen delivered to each region.**
of delivered oxygen (obtained after \(^{18}\)O-Hb injection), an image proportional to regional fractional oxygen extraction was obtained (Fig. 7). This image has been smoothed by performing a 5-point weighted average. Although smoothing the data results in some loss of spatial resolution, it is necessary in order to maintain the statistical accuracy in this quotient image. This functional image of fractional oxygen extraction is not proportional to the mass of myocardium in each region but, rather, represents the fraction of total delivered oxygen extracted in each region. In all six dogs that remained hemodynamically stable during the injections of both \(^{18}\)O-Hb and \(^{18}\)O-H\(_{2}\), functional images demonstrated uniform fractional oxygen extraction.

**Quantitation of Total Myocardial Blood Flow**

Myocardial blood flow in the 12 dogs studied was quantitated from the \(^{18}\)O-H\(_{2}\) washout after background correction. In these 12 dogs, whole heart blood flows were 0.78 ± 0.06 (se) ml/min per g of tissue. This value is similar to that obtained in anesthetized dogs by other reported methods.\(^{14}\) Figure 3 shows a typical \(^{18}\)O washout curve from which a blood flow of 1.09 ml/min per g was calculated.

**Functional Image of Regional Myocardial Blood Flow**

In these experiments a functional image of regional myocardial blood flow was derived by the stochastic analysis method described in the Methods section. Figure 8 shows such a functional image for a representative dog. In this example, there is uniform blood flow in the myocardium as reflected by the uniform intensity of \(^{18}\)O activity in the image.

**Discussion**

Several radionuclide methods presently are available for the quantitative, semiquantitative, and qualitative assessment of regional myocardial blood flow. Descriptions of these methodologies are presented in several recent reviews on the subject.\(^{15,16}\) The majority of the radionuclide techniques that give quantitative values for regional myocardial blood flow are invasive and require intracoronary administration of tracer.

Cannon and co-workers\(^{16-19}\) have developed a method for quantitative estimates of regional myocardial blood flow (ml/min per 100 g) in patients at the time of coronary arteriography, using xenon-133 and a scintillation camera as the external detector to monitor the washout of this tracer. Xenon-133, dissolved in saline, is injected into the coronary artery, and external measurement of multiple tracer washout curves in different regions of the myocardium is made, using a multocrystal scintillation camera. Rate constants of regional clearance of \(^{133}\)Xe from heart muscle are calculated by computer analysis of the data recorded by each of the multiple crystals, and a monoeponential analysis of the initial portion of each washout curve is made. Myocardial blood flow rates are computed by the Kety formula, using an assumed blood-myocardial partition coefficient. A similar approach has been used by Maseri et al.\(^{20}\) and Holman et al.\(^{21}\) To date, the xenon washout technique is the most accurate method for determining the absolute changes in flow in regions of ischemia. This technique can be applied to assess coronary reserve in man during exercise,\(^{22}\) rapid atrial pacing,\(^{23}\) and the induction of contrast hyperemia.\(^{24}\) Disadvantages of this technique include the differing solubility of xenon in heart muscle, scar, and fat.

While radionuclide techniques for measuring regional myocardial blood flow are available, regional myocardial metabolism previously has not been assessed directly. In this paper, we report the feasibility of simultaneously assessing regional myocardial blood flow and regional fractional oxygen extraction in an animal model. By
positional myocardial oxygen metabolism can be repeated at intervals as short as 8 minutes because of the short half-life of oxygen-15. In this study, uniform regional myocardial blood flow and uniform fractional oxygen extraction were demonstrated in the normal canine myocardium.

Although this method is technically demanding, requiring a cyclotron and a positron scintillation camera, it provides the unique capability for simultaneously assessing myocardial blood flow and myocardial metabolism. Thus, potential discrepancies between regional blood flow and oxygen utilization can, for the first time, be assessed directly. This methodology might be used, for example, to evaluate myocardial metabolism and regional blood flow patterns in focally ischemic myocardium. With atrial pacing or exercise stress-induced increase in myocardial oxygen demand, heterogeneous alterations in fractional oxygen extraction could be quantified. Because of the short half-life of oxygen-15, sequential studies can be carried out before and after selected interventions. Thus, serial imaging after the intracoronary administrations of 15O-Hb and H215O may afford a more sensitive technique for the evaluation of the behavior of the ischemic myocardium rather than radionuclide techniques, which merely provide information relative to flow alterations.

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