

## Noninvasive quantification of regional myocardial blood flow and ammonia extraction fraction using Nitrogen-13 ammonia and positron emission tomography

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This report describes the theoretical basis and a method to quantitate regional myocardial blood flow (RMBF) and ammonia extraction fraction (E) in man, noninvasively, with N-13 ammonia and positron emission tomography (PET). Two patients with hypertrophic cardiomyopathy, whose left ventricular (LV) walls were markedly thick, were employed in this study to avoid partial volume effects and cross contamination between LV walls and blood pool. RMBF and E were calculated from time-activity curves of myocardial tissue and left atrium derived from serial 6-second PET images of the heart. The time-activity curve of left atrium was used as an arterial input function. The results were  $\text{RMBF} = 67 \pm 4 \text{ ml/min/100 g}$ ,  $\text{E} = 80 \pm 13\%$  and  $65 \pm 10 \text{ ml/min/100 g}$ ,  $81 \pm 16\%$  for each patient. The validity of the present method was discussed.

**Key words:** Dynamic positron emission tomography, Regional myocardial blood flow, N-13 ammonia, Hypertrophic cardiomyopathy

### 1. INTRODUCTION

NONINVASIVE QUANTIFICATION of regional myocardial blood flow (RMBF) is necessary to facilitate detection and evaluation of cardiac pathophysiology. In animal experiments the microsphere technique has been regarded as the "gold standard". However because of high invasiveness, it is not easily available for clinical use. A positron-emitting blood flow tracer, N-13 ammonia, exhibits properties which to some extent resemble those of the radioactive microsphere. Recently Shah et al<sup>1</sup> reported measurement of RMBF in dogs with N-13 ammonia and positron emission tomography (PET). Their method was much less invasive than the microsphere method, because it employed intravenous administration of N-13 ammonia and tissue radioactive concentration determined by PET scans.

But it still needed withdrawal of arterial blood to calibrate arterial input function. The newer generation positron tomographs, with much higher temporal resolution, make it possible to determine the arterial input function directly from regions of interest assigned to the cardiac cavity, and thus make this approach largely noninvasive.

N-13 ammonia is extracted and fixed in myocardium in proportion to blood flow. However, a fraction of N-13 ammonia effectively retained in myocardium (net extraction fraction) is less than 100%, decreases with higher flows, and may be influenced by blood pH, disturbance of glutamine synthesis in myocardial cells, etc.<sup>2,3</sup> These findings need simultaneous determination of the RMBF and the extraction fraction of ammonia in myocardial tissue. The extraction fraction itself, if obtained, may provide an important physiologic and diagnostic indicator of the cellular viability in myocardium. Mullani et al<sup>4</sup> and Goldstein et al<sup>5</sup> suggested this possibility with rubidium extraction in dog.

It is the purpose of our study to develop a method of quantitating the RMBF and the extraction fraction

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of ammonia in man with time-activity curves of cardiac cavity and myocardium, determined by fast dynamic PET scans after intravenous injection of N-13 ammonia. To avoid partial volume effects and cross contaminations between left ventricular (LV) walls and blood pool in reconstructed images, patients with markedly thick LV walls were employed in the present study.

## 2. THEORY AND METHODS

### 2.1. Compartmental model

A model which characterizes physiologically correct processes of N-13 ammonia kinetics in myocardium seems to consist of at least three compartments, which are vascular, extravascular and cellular (metabolic) phases. But because of limited information obtained noninvasively by PET scans, analysis with the complicated models is difficult and it is necessary to employ an alternative simple functional model.

Schelbert et al<sup>2</sup> made intensive experimental studies on N-13 ammonia kinetics in canine myocardium and obtained conclusions as follows:

- (1) N-13 ammonia freely crosses the capillary and is nearly 100% extracted during its initial capillary transit.
- (2) The rate of metabolic fixation appears to be the rate-limiting step.
- (3) N-13 ammonia is distributed in the extravascular space and back diffusion competes with metabolic trapping.

These findings suggest that the physiological complicated model can be simplified to a functional two-compartment model shown in Fig. 1, where the two compartments are free and trapped (metabolized) ammonia space. The present model is essentially the same as the one proposed by Mullani et al<sup>4</sup> on Rb-82 kinetics in myocardium. The release of ammonia metabolites from the trapped compartment can be neglected in the present study, because it is a very slow process (its half-time averaged 273 minutes at control flows<sup>2</sup>) and our observation time is only two minutes after injection of ammonia.

If amounts of N-13 ammonia in the free and trapped compartments for a unit volume of tissue are defined as  $Q_1$  and  $Q_2$  respectively, their relationships are given by,

$$\begin{aligned} dQ_1/dt &= F \cdot Ca(t) - k \cdot Q_1 - F \cdot Q_1/Vd \\ dQ_2/dt &= k \cdot Q_1 \end{aligned} \quad (1)$$

where we assume N-13 ammonia is 100% extracted during its initial capillary transit.  $k$  is a rate constant for the transport from the free to the trapped space.  $F$  is RMBF or perfusion and  $Ca(t)$  is an input func-

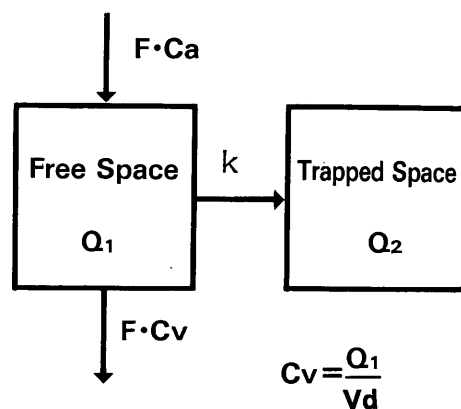


Fig. 1 Functional two-compartment model for N-13 ammonia kinetics in myocardium.  $F$  is regional myocardial blood flow (RMBF) or perfusion,  $Ca(t)$  arterial concentration of N-13 ammonia,  $Cv(t)$  venous concentration of N-13 ammonia.  $Q_1$  and  $Q_2$  are amounts of N-13 ammonia in the free and trapped compartments for a unit volume of tissue, respectively.  $Cv(t)$  is approximated by  $Q_1/Vd$ , where  $Vd$  is a distribution volume of N-13 ammonia in the free compartment.

tion, which is arterial activity concentration as a function of time.  $Vd$  is a distribution volume of N-13 ammonia in the free compartment, which is the volume of tissue space in which the tracer in the free compartment would have been distributed with the same concentration as in venous blood at equilibrium.

A time-activity curve of myocardium is given by,

$$Q(t) = (1-fa)(Q_1(t) + Q_2(t)) + fa \cdot Ca(t) \quad (2)$$

where  $fa$  is a fractional volume of arteries in the region of interest. From the solutions of eq. (1),  $Q(t)$  can be expressed as,

$$Q(t) = (1-fa)(Ae^{-at} + B) \otimes Ca(t) + fa \cdot Ca(t) \quad (3)$$

where,

$$\begin{aligned} A &= F \frac{F/Vd}{k + F/Vd} \\ B &= F \frac{F/Vd}{k + F/Vd} \\ \alpha &= k + F/Vd \end{aligned} \quad (4)$$

and  $\otimes$  denotes a convolution operation. From the eq. (4), RMBF is given by,

$$F = A + B \quad (5)$$

Net extraction fraction of ammonia ( $E$ ) is a ratio of myocardial uptake to the product of RMBF and the integral of input function. The myocardial uptake is

$$Q_2(\infty) = \frac{kF}{k + F/Vd} \int_0^\infty Ca(t) dt \quad (6)$$

Therefore the net extraction fraction is given by,

$$E = Q2(\infty)/F \int_0^{\infty} Ca(t)dt = F/(k+Vd) = B/(A+B) \quad (7)$$

For simplicity the term "extraction fraction" is used instead of "net extraction fraction" in the rest of this paper.

## 2.2 Methods

N-13 was produced in the National Institute of Radiological Sciences medical cyclotron by the bombardment of pure water with proton beams by the  $^{16}\text{O}(p,\alpha)^{13}\text{N}$  reaction. The product was reduced to ammonia and collected in physiological saline. Radiochemical purity of N-13 ammonia was greater than 99.5%.

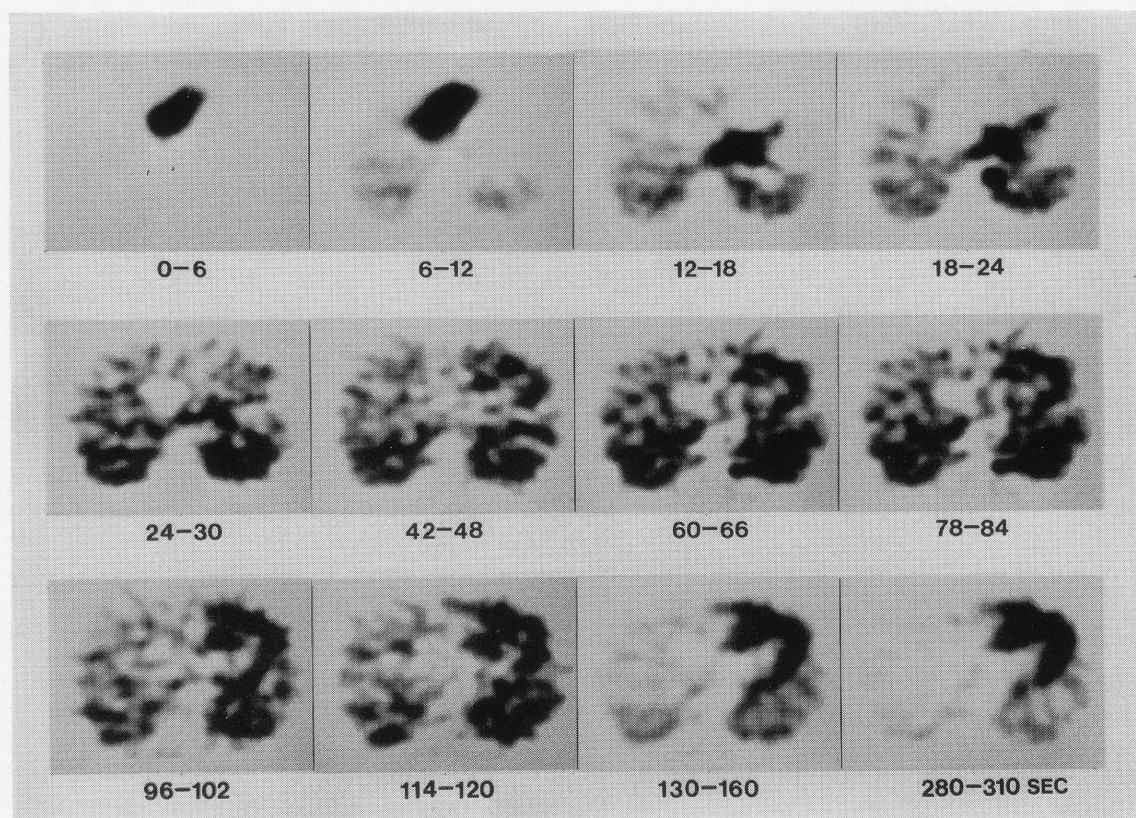
Tomography was performed with POSITOLIGICA-II<sup>6</sup>, which permitted serial acquisition of data in 6-second intervals and provided five transaxial sections simultaneously. Midpoints of the sections are separated by 18mm. Sensitivities for a

20 cm diameter phantom are 22.5 and 33.6 kcps/ $\mu\text{Ci}/\text{ml}$  for in-plane and cross-plane, respectively.

About 10 mCi N-13 ammonia was injected intravenously as a bolus from the antecubital vein. Serial PET imaging was initiated at the time of tracer injection, and twenty 6-second PET scans were performed without gating of cardiac cycle. 44 to 180 k counts were obtained per slice. Tomographic data were collected and reconstructed in  $128 \times 128$  matrix.

In the present study, the spatial resolution for reconstructed image was 13 mm FWHM at the center, and slice thicknesses were 13 mm and 10 mm for in-plane and cross-plane, respectively. To avoid partial volume effects and cross contaminations between left ventricular (LV) walls and blood pool, two patients with hypertrophic cardiomyopathy (HCM), whose LV wall thicknesses were more than twice the FWHM, were employed.

A time-activity curve of the myocardial tissue  $Q(t)$  was determined by assigning a region of interest (ROI) over the myocardium. The number of slices was three for each patient, and the number of ROIs

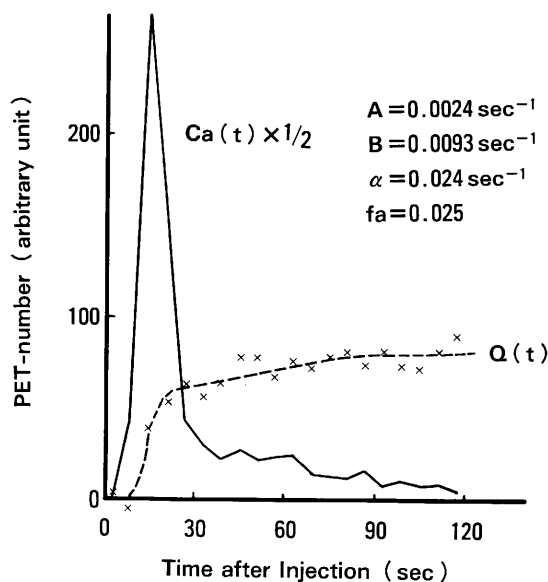


**Fig. 2** 6-sec serial PET imaging with N-13 ammonia at the midventricular level in a patient with hypertrophic cardiomyopathy (HCM). The numbers below the images show time (in sec) after intravenous injection of N-13 ammonia. The transit of N-13 activity through right ventricle, both lungs and left ventricle is visualized at the earlier images. Then occurs clearance of N-13 activity in the blood pools and lungs, and finally the myocardial image is delineated. The last two images represent 30-sec PET scans taken just after the initial twenty 6-sec PET were completed.

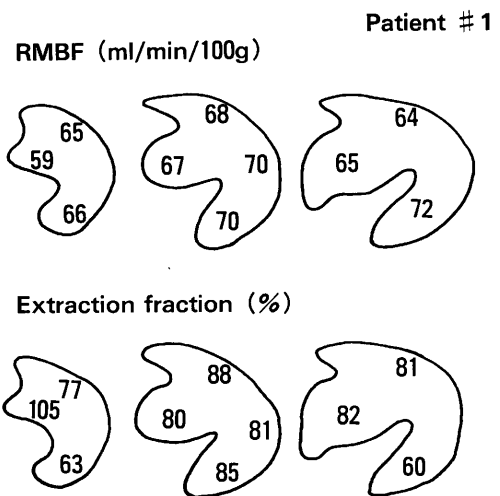
was three to five for each slice. An arterial input function  $Ca(t)$  was determined noninvasively by assigning a ROI over the left atrium, which was large enough to avoid the partial volume effects and cross contaminations. The sizes of ROIs were about  $10 \times 10$  mm. Count losses in high count rate were corrected with an object-independent method using a single count rate.<sup>7</sup> Although the measured values were integrals of 6 second intervals, they showed enough temporal resolution for the present analysis. The parameters  $A$ ,  $B$ ,  $\alpha$  and  $fa$  were estimated by fitting eq. (3) to the measured time-activity curve using a modified Gauss-Newton least-squares algorithm. The convolution in eq. (3) was calculated by a numerical integration. The curve-fitting algorithm was reproducible given the appropriate starting values for  $A$ ,  $B$ ,  $\alpha$  and  $fa$ . Finally RMBF and extraction fraction of ammonia were calculated by the eqs. (5) and (7). Because RMBF thus obtained was in the unit volume of myocardium, it was corrected by a myocardial density (1.05 g/ml) and expressed as ml/min/100g.

### 3. RESULTS

Figure 2 shows a 6-sec serial PET imaging with N-13 ammonia at the midventricular level in one of the HCM patients (patient #1) employed in this study. The numbers below the images show time (in sec) after intravenous injection of N-13 ammonia. The transit of N-13 activity through right ventricle, both



**Fig. 3** Time-activity curves of septum ( $Q(t)$ ) and left atrium ( $Ca(t)$ ). The solid line shows  $Ca(t)$ , the cross marks ( $\times$ ) a measured time-activity curve of septum and the dotted line an estimated myocardial response with the parameter values listed above right.  $Ca(t)$  is illustrated in a half of real PET-number.



**Fig. 4** Estimated values of RMBF (ml/min/100 g) and extraction fraction (%) of ammonia on the anatomical cross sections. The sections correspond to high ventricular (atrial), mid-ventricular and low ventricular levels from left to right.

lungs and left ventricle is visualized at the earlier images. Then clearance of N-13 activity occurs in the blood pools and lungs, and finally the myocardial image is delineated. The last two images represent 30-second PET scans taken just after the initial twenty 6-second PET scans were completed.

Fig. 3 shows time-activity curves of septum and left atrium for patient #1. The solid line in the figure shows a time-activity curve of the left atrium which was used as an input function in the present analysis. The cross marks ( $\times$ ) show a measured time-activity curve of the myocardium, while the dotted line shows an estimated myocardial response.

Fig. 4 shows estimated values of RMBF and extraction fraction of ammonia on the anatomical cross sections in patient #1. The sections correspond to high ventricular (atrial), mid-ventricular and low ventricular levels from left to right. The upper panel shows RMBF and the lower shows extraction fraction. Table 1 summarizes the estimated results.

### 4. DISCUSSION

In this paper we estimated RMBF and extraction fraction in man with intravenous injection of N-13 ammonia and PET. To our knowledge this is the first report describing noninvasive measurements of such physiologic parameters in man. Although we have no direct evidence to judge whether the results obtained here are true or not, they seem consistent with values obtained in animal experiments<sup>2</sup> or by a much more invasive method in man.<sup>8</sup>

Schelbert et al<sup>2</sup> measured extraction fraction and

**Table 1** Summary of parameter estimation

	$F$ (ml/min/100 g)	$E$ (%)	$\alpha$ (sec <sup>-1</sup> )	$fa$ (%)
PT#1	67 ± 4 <sup>a</sup> (10) <sup>b</sup>	80 ± 13 (10)	0.0231 ± 0.0040 (9) <sup>c</sup>	3.5 ± 2.0 (10)
PT#2	65 ± 10 (12)	81 ± 16 (12)	0.0205 ± 0.0040 (9)	2.5 ± 1.8 (12)

<sup>a</sup>Mean ± Standard Deviation (SD)<sup>b</sup>The number of ROIs<sup>c</sup>The number of ROIs used for calculating means and SDs of  $\alpha$  were smaller than others because ROIs in which  $E$  was greater than 90% were omitted.

clearance half-time of ammonia after coronary injection of N-13 ammonia in dog. Their results were summarized from our point of view as  $E=82\%$  and  $\alpha=0.027\text{ sec}^{-1}$  at the control flow of  $F=100\text{ ml/min/100g}$ . Selwyn et al<sup>8</sup> measured RMBF in man with human albumin microspheres labeled with C-11 and PET. Their results showed that RMBF was  $82.0 \pm 32.0\text{ ml/min/100g}$  in the normal myocardium. Although our results in Table 1 were obtained from the HCM patients, they seem to agree well with these values. The interpretation of this agreement needs further study. Fractional volume of vascular space in myocardium is usually about 10%.<sup>9</sup>  $fa$  in Table 1 is much smaller than this value. However  $fa$  is a fractional volume of arterial space in the field of view. Because it does not include capillaries and veins, the values in Table 1 seem reasonable.

As described before, the present model is essentially the same as the one proposed by Mullani et al,<sup>4</sup> and its validity might be further supported by the following discussion. From the eqs. (4), (5) and (7),  $k$  and  $Vd$  can be expressed as,

$$k = E\alpha \quad (8)$$

$$Vd = F/(1-E)\alpha$$

If the average value of patient #1 is substituted in eq. (8),  $k=0.019\text{ sec}^{-1}$  ( $1.1\text{ min}^{-1}$ ) and  $Vd=2.5$ .

Because  $Vd$  is the distribution volume of N-13 ammonia in the free compartment, the result ( $Vd=2.5$ ) means that at equilibrium N-13 ammonia in the free compartment would occupy 2.5 times of actual tissue space if its concentration were the same as the venous concentration. Frank and Langer<sup>10</sup> measured extracellular space of perfused rabbit heart using radioactive  $\text{La}^{+++}$ , and found that if  $\text{La}$  were distributed in free solution it would occupy 194% of total tissue water, discussing that it was due to extensive binding of  $\text{La}^{+++}$  to polyanionic extracellular structures of myocardium. In extracellular space N-13 ammonia exists in the chemical equilibrium of  $\text{NH}_3$  and  $\text{NH}_4^+$ , in which the prominent form is  $\text{NH}_4^+$ . The possibility of extracellular trapping of  $\text{NH}_4^+$  just as  $\text{La}^{+++}$  suggests that N-13 ammonia concentration in the extracellular space may be greater than

in veins, which supports our result of  $Vd=2.5$  and the validity of the model. Further studies are required to interpret the values of  $k$  obtained here.

The present method employed a time-activity curve of left atrium measured with PET as an arterial input function. Iida et al<sup>11</sup> compared radial arterial curves with time-activity curves of left ventricle following intravenous injection of O-15 water, and concluded that the radial curve was significantly dispersed from the time-activity of left ventricle measured with PET. Their results suggest that the use of time-activity curve measured with PET as an input function not only is noninvasive but also may be more accurate, especially for arteries near the heart such as coronary artery.

Another problem on the input function is whether N-13 activity in the arterial blood is all due to ammonia or not. N-13 ammonia in the blood is rapidly uptaken and metabolized mainly by the liver, and its metabolites labeled with N-13 come into the blood. Lockwood et al<sup>12</sup> examined contents of N-13 activity in the arterial blood after intravenous injection of N-13 ammonia in man, and concluded that the labeled metabolites were not detectable until after 3 min of injection. Because our study utilized the activity changes up to 2 min after injection, N-13 activity observed in the arterial blood seems substantially due to ammonia.

In the present study to avoid partial volume effects and cross contamination of activity, we employed the HCM patients with a marked increase in LV mass and showed the reliable results as discussed so far. However we did not analyze normal cases and patients with ischemic heart diseases, which are the most important in clinical practice.

In order to apply the present method to these cases, we must correct the partial volume effects and cross contamination. Henze et al<sup>9</sup> proposed a deconvolution technique that permitted calculation of spillover fractions from geometric measurement of the imaged cross section and the spatial resolution of tomograph. Recently Carson<sup>13</sup> applied the EM (estimation maximum) algorithm to emission tomographic estimation of ROI activity and showed excellent results almost free from the partial volume

effects. As the next step we will combine these techniques and the present method to analyze normal cases and patients with ischemic heart disease.

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