Tracer kinetic model of regional pulmonary function using positron emission tomography

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Galletti, Gaetano G., and José G. Venegas. Tracer kinetic model of regional pulmonary function using positron emission tomography. J Appl Physiol 93: 1104–1114, 2002.—To determine the spatial distributions of pulmonary perfusion, shunt, and ventilation, we developed a compartmental model of regional 13N-labeled molecular nitrogen (13NN) kinetics measured from positron emission tomography (PET) images. The model features a compartment for right heart and pulmonary vasculature and two compartments for each region of interest: 1) aerated alveolar units and 2) alveolar units with no gas content (shunting). The model was tested on PET data from normal animals (dogs and sheep) and from animals with experimentally injured lungs simulating acute respiratory distress syndrome. The analysis yielded estimates of regional perfusion, shunt fraction, and specific ventilation with excellent goodness-of-fit to the data ($R^2 > 0.99$). Model parameters were estimated to within 10% accuracy in the presence of exaggerated levels of experimental noise by using a Monte Carlo sensitivity analysis. Main advantages of the present model are that it separates intraregional blood flow to aerated alveolar units from that shunting across nonaerated units and accounts and corrects for intraregional tracer removal by shunting blood when estimating ventilation from subsequent washout of tracer. The model was thus found to provide estimates of regional parameters of pulmonary function in sizes of lung regions that could potentially approach the intrinsic resolution for PET images of 13NN in lung (~7.0 mm for a multiring PET camera).

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Regional pulmonary perfusion and ventilation are often measured noninvasively by means of nuclear imaging techniques and analyzed by using models of tracer kinetics to represent tracer fate. Spatial distribution of pulmonary perfusion has been measured with positron emission tomography (PET) after deposition in lung during first transit of intravenously (IV) injected tracer-labeled particles ($^{68}$Ga), water ($H_2^{15}$O), and molecular nitrogen ($^{13}$NN) dissolved in saline (6–8). Ventilation has been measured from alveolar clearance (washout) of $^{13}$NN previously equilibrated in a closed (rebreathing) circuit (6, 8). Of these three isotopes, only the tracer $^{13}$NN lends itself to measurement of both perfusion and ventilation. The validity of the perfusion measurement after an IV injection of $^{13}$NN gas dissolved in saline rests on the assumption that at first pass all tracer diffuses from pulmonary capillaries into aerated alveoli. Because of the low solubility of nitrogen in water and tissues, in normal aerated lungs, the $^{13}$NN tracer remains in the alveoli during a breath hold and its intrapulmonary distribution measured by PET is directly proportional to local perfusion (6). During the breathing period after apnea, the dominant mechanism of tracer removal is alveolar ventilation, resulting in exponential clearance of the tracer. Regional specific ventilation is then derived from the clearance rate constant and represents ventilation per unit of perfused lung volume. The process is complicated in lungs with pulmonary pathology involving atelectatic or edematous lung units because the injected $^{13}$NN tracer is not retained in these units during breath hold and, instead, is reabsorbed by shunting blood. Therefore, in the presence of shunt, raw PET images of $^{13}$NN content collected during apnea cannot be directly used to quantify perfusion. Also, simple clearance analysis of $^{13}$NN during a washout period of ventilation cannot separate tracer removal by shunting blood from that by ventilation in regions including aerated and nonaerated alveolar units. In this paper, we present a three-compartment tracer kinetic model that was used to assess regional perfusion, shunt blood flow fraction, and specific alveolar ventilation of perfused and aerated alveoli. The model accounts for tracer transit from injection site to alveoli and alveolar removal of tracer by shunted blood flow and ventilation. The model was applied to PET images obtained from normal dog lungs and from dog and sheep lungs experimentally injured with IV oleic acid, smoke inhalation (14), and bilateral surfactant depletion simulating conditions in acute respiratory distress syndrome. The model, its relevant assumptions, and methods of parameter identification are described, and the sensitivity of derived parameters to expected and exaggerated levels of experimental noise is examined.

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METHODS

Experimental Data

Evaluation of the model was made by using experimental data previously obtained with protocols approved by the Institutional Animal Care and Use Committee of the Massachusetts General Hospital. Experimental data were collected as follows. Anesthetized and paralyzed animals were mechanically ventilated in steady-state conditions. Before imaging, cardiac output was measured by thermodilution, and a sample of 13NN-labeled saline was collected to assess its specific activity. At end exhalation, mechanical ventilation was interrupted, and intravenous infusion of 13NN in saline solution was started. Simultaneously, collection of a series of consecutive PET images was initiated. Depending on the specific protocol, acquisition time for each image was 5 or 10 s, yielding a series of 12 or 6 images, respectively, during 60 s of apnea. At the end of this period, mechanical ventilation was restarted, and four additional images were collected, each of 30-s acquisition time.

We used two PET cameras to collect experimental data analyzed in this paper: a single-slice prototype camera PCR1, described previously (12, 13), and a PC-4096 PET scanner (Scanditronix) that imaged 15 contiguous slices of 6.5-mm separation. Collected sinograms were reconstructed into tomographic images by using a convolution-back-projection algorithm yielding an effective spatial resolution of 7.0 mm for multilung PET camera images and 10 mm for single-ring PET camera images. Image reconstruction included corrections for nonuniform crystal sensitivity and gamma-ray energy attenuation in body tissue and PET camera supporting structures. Reconstructed images, in units of radioactivity per cubic centimeter of imaged volume, were used to calculate tracer-averaged kinetics data from regions of interest (ROI) defined as follows. First, a unity mask was defined manually over the imaged lung field with all pixels in extrapulmonary regions set equal to zero. Three ROI of equal height [nondependent (ND), middle (M), and dependent (D)] were defined by horizontal lines. The volume of each ROI was calculated from the corresponding number of voxels and were typically 25, 50, and 25% of the volume of the mask for the ND, M, and D regions, respectively.

Average specific activity in each ROI was then calculated from each sequential image. The resulting tracer kinetics data were decay corrected (13NN half-life = 9.96 min) to a reference time taken as the onset of intravenous injection. Infusate-specific activities were measured in a radiation counter previously cross-calibrated with the PET camera. This activity was also corrected to the same reference time as the tracer kinetics data. Tracer kinetics data were then plotted vs. time, with each data point plotted in the middle of its image-collection time interval.

Tracer Kinetic Model and General Assumptions

The model architecture is sketched in Fig. 1 (cf. APPENDIX A for definitions of symbols used). Time \( t = 0 \) marks the start of intravenous infusion of the \( ^{13}\text{NN} \) tracer in saline solution with concentration \( C_t \) at a flow rate \( Q_c \). The tracer mixes with venous blood as it transits a compartment of volume \( V_h \) lumping the right heart and the large arterial pulmonary vessels. This results in a total pulmonary arterial blood flow of \( Q_t \) with a tracer concentration \( C_{pa}(t) \). Transit time from infusion site to an ROI within the lung is accounted by a time delay \( \Delta_{tr} \).

The function \( C_{pa}(t + \Delta_{tr}) \) is then used as input to the D, M, and ND ROI, each with a net regional perfusion flow rate \( Q_R \). Alveolar units of each ROI are lumped into two independent parallel compartments. One compartment represents aerated units with regional tracer concentration \( C_{a}(t) \) and volume of distribution \( V_{a} \). This compartment is continuously perfused with a blood flow \( Q_A \) and is ventilated with specific alveolar ventilation \( s_{VA} \) starting at the time at which mechanical ventilation is restarted after apnea \( (t_v) \). The second compartment represents airless (fluid-filled or collapsed) alveolar units with regional tracer concentration \( C_{s}(t) \) and volume of distribution \( V_{s} \). This compartment is perfused with a shunt flow \( Q_S \) and is never ventilated. Regional perfusion \( Q_R \) is the sum of perfusion to these two compartments: \( Q_R = Q_A + Q_S \). Total tracer content of each region \( VA_{CA}(t) + VS_{CS}(t) \) is used as an input function to a PET camera module that calculates for each image, \( i \), the regional average tracer content \( C_i \). This model makes four general assumptions: 1) Tracer is distributed uniformly in each compartment. 2) Tracer is distributed in aerated alveoli in amount proportional to local perfusion. 3) Tracer transport between compartments or ROI caused by diffusion, cardiogenic motion, or rebreathing is negligible. 4) Perfusion and ventilation are invariant during the apneic and washout imaging periods.

Model Equations and Specific Assumptions

Mixing and transport of infused bolus. Tracer entering the right heart (and pulmonary blood) compartment has two sources: 1) tracer in saline solution infused, from \( t = 0 \) to length of time during which \( ^{13}\text{NN} \)-labeled saline is infused \( (t_{nd}) \), as a bolus at rate \( Q_c \) and specific activity \( C_t \), and 2) recirculating tracer with specific activity \( C_{R} \) returning to the heart at flow rate equal to the cardiac output \( Q_t \). The rate of change of tracer concentration in the right heart compartment is approximated by

\[
V_h \frac{dC_{pa}}{dt} = Q_tC_i + Q_tC_R - Q_tC_{pa} \tag{1}
\]

if we assume that cardiac output is much greater than tracer infusion rate.

Regional tracer kinetics. Tracer, at a concentration \( C_{pa}(t) \), enters the aerated alveoli compartment at perfusion rate \( Q_A \) and is removed simultaneously by the pulmonary venous blood flow at concentration \( C_v \) and by alveolar ventilation \( s_{VA} \) at concentration \( C_{va}(t) \). To account for intraregional heterogeneity in ventilation, when appropriate, the aerated compartment is subdivided into two compartments \( i = 1 \) for a “fast” compartment and \( i = 2 \) for a “slow” compartment, each with its respective perfusion and ventilation rates. The rate of change of tracer content in each aerated subcompartment is therefore

\[
V_A \frac{dC_{a}}{dt} = Q_A_{C_{pa}} - Q_A_{C_{va}} - V_A \cdot C_{a} \tag{2}
\]

To further simplify Eq. 2, we assume full equilibration of the tracer between end-capillary blood and alveolar gas with \( C_v \) determined by the product of \( C_A \) times the nitrogen gas-water partition coefficient \( (\lambda_{N_2} = 0.018) \). In that condition, removal of tracer by pulmonary blood may be assumed to be negligible. We also assume that the volumes of tracer distribution, \( V_{a} \), of each aerated compartment is equal to the regional volume of gas. [Regarding this latter assumption, as pointed out by Schuster (9), the volume of distribution of tracer depends on the number of perfused alveoli. Because some aerated alveoli may not be perfused by tracer-labeled blood, gas volume in a region may be larger than \( V_{a} \).] With these assumptions, and taking the total regional perfusion as
the sum of perfusion rates to aerated and to shunted compartments, Eq. 2 may be rewritten for each subcompartment $i$ as

$$\frac{d}{dt} [V_{Ai}CA_i] = Q_i \left( \frac{Q_R}{Q_T} \right) \left( 1 - \frac{Q_S}{Q_R} \right) C_{pa} - (sV_A) [V_{Ai}CA_i]$$

(3)

where total regional perfusion to the aerated region is $Q_1 + Q_2 = Q_A$. It is understood that for conditions in which a single compartment is appropriate, one of the regional perfusions, $Q_1$ or $Q_2$, is zero, whereas the other is equal to $Q_A$.

There are five independent parameters in Eq. 3: $Q_R/Q_T$, regional perfusion expressed as a fraction of total cardiac output; $Q_S/Q_R$, regional shunt blood flow expressed as a fraction of regional perfusion; $Q_1/Q_A$, regional perfusion to the fast subcompartment expressed as a fraction of total regional perfusion to nonshunting (aerated) alveoli; and $sV_A_1$ and $sV_A_2$, the regional specific ventilation of the fast and slow subcompartments, respectively. Fractional perfusion to the slow compartment is calculated from the identity $Q_1 + Q_2 = Q_A$.

**Regional index of specific ventilation.** To provide an index of ventilation for the aerated compartment in the presence of a two-compartment model of ventilation, we defined $sV_A$ as a perfusion-weighted average of the specific ventilation of each subcompartment, i.e.,

$$sV_A = \left( \frac{Q_1}{Q_A} \right) sV_{A_1} + \left( \frac{Q_2}{Q_A} \right) sV_{A_2}$$

(4)

**Regional shunt compartment.** Intrapulmonary shunt refers to deoxygenated blood that enters the pulmonary venous circulation without passing through aerated alveolar units. In these nonaerated units, tracer removal occurs only by back-diffusion into pulmonary blood at a tracer concentration $CS(t)$. By analogy to Eq. 3, the rate of change of regional tracer content of a shunt region is

$$\frac{d}{dt} (VsCs) = QT \left( \frac{Q_S}{Q_T} \right) \left( \frac{Q_S}{Q_R} \right) C_{pa} - \frac{[VsCs]}{\tau_s}$$

(5)

where the model parameter $\tau_s = Vs/Q_S$ is a transport time constant. An implicit assumption in Eq. 5 is that the nitrogen partition coefficient between blood and atelectatic, edematous, and/or interstitial fluid is 1.0.

**PET camera module.** Total regional tracer content $V_{A_1}CA_1(t) + V_{A_2}CA_2(t) + VsCs(t)$, the input function to the
PET camera module, is normalized by the image collection time $\Delta t_{\text{img}}$ and by regional organ volume to be expressed in terms of instantaneous tracer radioactivity per voxel. Given that the PET camera effectively averages the radioactivity originating from each voxel, a tracer kinetics data point $S_i$ for a given ROI from an image collected between times $t_i$ and $t_i + \Delta t_{\text{img}}$ is equivalent to

$$S_i = \frac{1}{\Delta t_{\text{img}}} \int_{t_i}^{t_i + \Delta t_{\text{img}}} (V \frac{\partial C}{\partial t} + V \frac{\partial V_{\text{SCS}}}{\partial t}) dt$$

(6)

**Tracer recirculation.** From our studies, we have observed that, even in severely injured lungs, tracer content from nondependent regions does not decrease during the post-infusion apneic period. This observation suggests the absence of shunt in these nondependent regions even when substantial shunt is evident in the rest of the lung. Also, in lungs with high levels of shunt in dependent and middle regions, we observed a slow but progressive increase in tracer concentration in nondependent ROI starting after the first 20–30 s of apnea (see Fig. 5). Given that during that time no tracer was infused into the animal, we attributed this increase in activity to recirculating tracer that had bypassed nonaerated alveoli. In cases in which such tracer content increase was observed in the nondependent ROI, the specific activity of recirculating blood, $C_R$, was defined as a step function starting at time $\Delta t$, with height equal to $C_R$ and ending $\Delta t$ seconds after the initiation of the washout period. Such a tracer concentration was assumed to be the same for all ROI, and tracer leaving the lung after the initiation of ventilation was neglected.

**Nonlinear Parameter Identification**

Nonlinear system identification was used to find the set of $n$ parameters ($p_1, p_2, ..., p_n$) of the model such that its output $M(t_i)$ matched the experimental data $S(t_i)$ sampled at discrete times ($t_1, t_2, ..., t_n$). A gradient-descent search algorithm was implemented to minimize a multidimensional cost function, defined as the sum-of-squared errors between model output and experimental data, $E(p_1, p_2, ..., p_n) = \sum_{i=1}^{n} [M(t_i) - S(t_i)]^2$. A systematic search for model parameters was conducted by use of numerical minimization algorithms from the Nonlinear Identification Toolkit (NLID) (Cambridge Control, Cambridge, UK) (1) in combination with the numerical integration toolbox SIMULINK from MATLAB (The Mathworks, Natick, MA). NLID assumes that the experimental data come in the form of a time series with time intervals equal to those used by SIMULINK to solve the model’s differential equations. However, experimental data derived from PET images do not correspond to instantaneous values of regional activity, but rather to a mean tracer activity, obtained during discrete imaging intervals much longer than the short time intervals needed to integrate the model differential equations. The error function calculation in NLID was therefore modified to accept a reduced data series compatible with the experimental PET data. The parameter identification was conducted by running a model simulation at a fine time resolution (time interval = 0.1 s) to calculate the time average tracer content for the period corresponding to each PET image collection to obtain values $M(t_i)$ equivalent to the regional tracer kinetics series obtained from the PET images.

$$M(t_1, t_2, ..., t_n) = \frac{1}{\Delta t_{\text{img}}} \int_{0}^{\Delta t_{\text{img}}} (V \frac{\partial C}{\partial t} + V \frac{\partial V_{\text{SCS}}}{\partial t}) dt$$

$$+ \frac{1}{\Delta t_{\text{img}}} \int_{t_1}^{t_2} (V \frac{\partial C}{\partial t} + V \frac{\partial V_{\text{SCS}}}{\partial t}) dt$$

$$+ \frac{1}{\Delta t_{\text{img}}} \int_{t_2}^{t_3} (V \frac{\partial C}{\partial t} + V \frac{\partial V_{\text{SCS}}}{\partial t}) dt$$

where $n$ is equal to the number of images collected in the imaging protocol. For the two-compartment model of aerated units, the integrands in the above equation are replaced with $V_{\text{SCS}}$ and $V_{\text{SCS}} + V_{\text{CA}}$, respectively.

PET imaging data for each ROI was normalized by total injected activity. This normalization allowed us to use a unit area pulse of height equal to the reciprocal of the injection time as input to the model. Because the time required for the algorithm to converge roughly increases exponentially with the number of model parameters, to shorten computation time each ROI was analyzed individually and its parameters were identified in two phases. First, parameters related to pulmonary perfusion, shunt fraction, and shunt compartment transport rate constant were identified by analyzing exclusively the PET data obtained during the apneic period. Then, parameters related to ventilation were identified by running the NLID model with perfusion-related parameters kept constant at the previously identified values. Details of the parameter identification scheme and the selection of initial parameter guesses are presented in APPENDIX B.

**Parameter Sensitivity Analysis**

Sensitivity of the identification of parameters $Q_{TB}/Q_T$, $Q_{CA}/Q_R$, $T_1$, $T_2$, and $s_{VA}$ to experimental noise was investigated by using a Monte Carlo simulation approach. Two tracer kinetics data sets were analyzed from a study documented in an accompanying paper (14). One set was taken from a normal sheep lung with minimal shunt and another from lungs after 4 h of 100-breath exposure to cotton smoke. Because the success of the Monte Carlo approach relies on proper knowledge of the measurement noise statistics, we used data sets obtained from a single-ring PET camera whose noise characteristics have been previously defined (13).

Each data set was analyzed with the NLID model in the manner described above, for which values of the parameters $\Delta T_{TB}$, $Q_{TB}/Q_T$, $Q_{CA}/Q_R$, and $s_{VA}$ were obtained. Identified parameters were recorded, and the model run with these parameters to define noise-free data sets. These noise-free data sets were perturbed in proportion to expected and exaggerated noise levels in the following way. The coefficient of variation (cov) of expected noise corresponding to each ROI in each image was assumed to be inversely proportional to the number of radioactive decay events detected in that ROI and directly proportional to a constant reported previously for this camera (13). A pseudorandom number generator was used to define a series of 10 elements from a normal distribution with a unity variance and mean value equal to zero. The expected cov array was multiplied element by element to this random series and unity was added before multiplying element by element this resulting array to the noise-free data set. This method produced noise-perturbed tracer kinetics data with a cov in each ROI and image equal to that of the expected corresponding noise. Each of the two noise-free data sets (corresponding to a normal and an injured lung) was perturbed 10 times by use of this method, and the process was repeated for 8 and 16 times the expected noise levels.
Each of the perturbed data sets was reidentified with the NLID method described above to estimate the variability of the parameters caused by the three levels of noise. Mean deviations from the noiseless parameters and the corresponding standard deviations (SD) of the reidentified parameters were calculated.

RESULTS

The model had excellent fit to experimental data from normal and acute respiratory distress syndrome (ARDS) lungs, allowing quantification of $ΔT_{TD}$, $Q_{R}/Q_{T}$, $Q_{S}/Q_{R}$, $\tau_{S}$, and $sV_{A}$. Examples of model simulations fitted to experimental data under different physiological conditions are presented in Figs. 2–4. In those examples, and in all the data reported in the accompanying paper (14), the model fitted the data with regression coefficients $R^2 > 0.99$. Thus the model accounted for more than 99% of the total variance in the experimental PET data.

Figure 5 shows tracer kinetics data from a surfactant-depleted sheep lung.

Parameter Sensitivity Analysis

As expected, the increase of noise from expected to exaggerated levels resulted in a progressive increase in the standard deviations of the reidentified parameters around the original noise-free data sets (Figs. 6–9).

The shunt transport time constant, $\tau_{S}$, for a normal lung ROI deviated from a noiseless value of 6.3 s by 0.52, 0.93, and 1.16 s in average with cov ($\pm$SD/mean) of 0.094, 0.018, and 0.19 for noise levels of 1, 8, and 16 times the expected experimental levels, respectively. For the ARDS lung data, average deviations of $\tau_{S}$ from a noiseless value of 6.8 s were 0.09, 0.35, and 1.05 s with respective cov of 0.001, 0.07, and 0.16. The parameter $Q_{S}/Q_{T}$ had average deviations from normal data of 0.4, 1.8, and 2.9% for the respective increasing levels of noise and of 0.02, 1.1, and 2.3% in the ARDS data for the respective levels of noise.

For the normal lung data, the parameter $Q_{R}/Q_{T}$ deviated from the noise-free value by only 0.14, 0.43, and 0.52% for 1-, 8-, and 16-fold levels of noise above...
expected values. For the ARDS data, the mean deviations were 0.06, 2.17, and 5.6% for the corresponding levels of noise.

Finally, for the same increasing levels of noise, the parameter estimates of sVA in the normal lung data deviated in average by 0.85, 2.98, and 2.97% from the noiseless value, and in the ARDS lung data the parameter estimates deviated by 0.19, 4.81, and 9.96%, respectively.

**DISCUSSION**

We developed a multicompartmental model for analysis of 13NN tracer kinetics data from PET images collected after intravenous bolus injection of 13NN-labeled saline solution during a transient period of breath hold (apnea) and during subsequent ventilatory clearance of tracer (washout). The model was successfully used to analyze PET data obtained from animals with experimentally altered pulmonary physiology including sheep lungs imaged 1, 2, and 4 h after exposure to cotton smoke inhalation, as presented in the accompanying paper (14). Using a nonlinear parameter identification routine, we fitted the model predictions to experimental data to yield regional parameters of perfusion fraction Qo/Qr, shunt fraction Qs/Qr, and specific alveolar ventilation sVA. Model-simulated data with these parameters accounted for more than 99% of the variance in the experimental data. The most significant improvements of this model over existing methodologies using radiolabeled microspheres or 15O-labeled water are 1) the ability to separate regional perfusion into shunt and gas-exchanging fractions and 2) the ability to account for two competing mechanisms of tracer removal during the washout: transport by shunt flow from nonaerated spaces and by ventilation from aerated spaces.

Pulmonary blood flow determined from distribution of tracer-labeled microspheres has the advantage that
measured tracer content is linearly related to regional blood flow over a wide physiological range. However, the typically long half-life of tracer-labeled microspheres restricts the ability to conduct multiple studies in the same subject. Also, microspheres may be subject to artifacts that include clustering and streaming in the pulmonary vasculature. Furthermore, microspheres lodge in small precapillary arterioles of the lung, irrespective of whether those pulmonary blood vessels feed aerated or nonaerated shunting alveolar units. Regional perfusion data evaluated from the distribution of microspheres therefore cannot distinguish between flow to gas-exchanging or shunting regions. More importantly, ventilation cannot be assessed from microsphere data.

Measurements of pulmonary blood flow using water labeled with $H_2^{15}O$ water (half-life ~2 min) are repeatable, and the data have been shown to correlate with data obtained from microspheres over a wide range (7). That method has the additional advantage that measured tissue activity reflects local tissue water content because $H_2^{15}O$ diffuses freely into and out of vascular and extravascular spaces. However, the short residence time of this tracer in the lung (~20 s) necessitates the collection of short-duration images, limiting the signal-to-noise ratio and/or the spatial resolution of the resulting PET images. Furthermore, the single-compartment model used to analyze $H_2^{15}O$ data requires the assumptions that tracer is fully extracted during a single pass through the lung and that the partition coefficient for the tracer describes the equilibrium $^{15}O$ distribution volume in the tissue divided by its distribution volume in blood (7). Because the density of lung tissue varies throughout the thorax depending on local transpulmonary pressures, the partition coefficient for $H_2^{15}O$ is expected to vary within the lung, thereby requiring its independent measurement, before assessment of the regional distribution of blood flow. Finally, the measurement of blood flow with $H_2^{15}O$ cannot quantify regional shunt fraction or regional ventilation.

Use of $^{13}NN$ gas as a tracer is nearly ideal for the assessment of lung function. Nitrogen gas is biologically inert, and $^{13}NN$-labeled molecular nitrogen can be dissolved in aqueous saline to obtain sufficiently practical specific activity for imaging during intravenous infusion. Rhodes et al. (8) pioneered tomographic measurements of regional ventilation-perfusion ratio during constant infusion of $^{13}NN$-labeled saline solution. Mijailovich et al. (6) added to that technique the measurement of regional perfusion after injecting a single bolus injection of the labeled saline during apnea. However, the primary assumption of these techniques, namely that $^{13}NN$ gas resides only in aerated spaces, breaks down in lungs with atelectatic or edematous alveolar units.
Our analysis of $^{13}$NN tracer kinetic imaging expands the previous techniques for use in pathological lungs and makes it possible to identify the fractions of blood flow reaching gas-filled and fluid-filled or collapsed alveolar units. Because of the low solubility of nitrogen in water and blood, as the bolus of $^{13}$NN reaches aerated alveolar units most of the tracer ($\geq 98\%$) diffuses from the capillary bed into alveolar airspace during the first pass and remains there for the duration of a short apneic period of imaging (6). Although interregional mixing, by diffusion or cardiogenic oscillations, may alter the local distribution of $^{13}$NN during apnea, this effect has not been found to be important at spatial length scales equal to or greater than the resolution of our PET images (7.0 mm for images collected with the multiring PET camera and 1.0 cm for images collected with the single-ring PET camera). Thus, in healthy and fully aerated lung units, local tracer content is directly proportional to local perfusion, and PET images collected during the postinfusion apneic period yield a direct measurement of regional perfusion distribution. Subsequent ventilatory clearance rate of $^{13}$NN (washout) is then representative of regional ventilation of perfused and aerated lung units.

The need for the more sophisticated model presented in this paper arises when aerated and nonaerated alveolar units coexist within an ROI. In contrast to what happens when $^{13}$NN reaches aerated units, when it reaches atelectatic or edematous alveolar units, tracer content rises to a peak value and then decreases exponentially toward an asymptote. This drop in tracer content is caused by reabsorption of the $^{13}$NN gas into the pulmonary circulation because there is no preferential solubility between blood and collapsed, or fluid-filled, alveolar spaces. If peak tracer content value is taken as an index of total regional perfusion, the asymptotic value would represent the fraction of blood flow reaching aerated alveolar units, and the relative drop in activity provides direct assessment of regional shunt fraction. Unfortunately, the peak and asymptote values are difficult to measure or extrapolate from PET data, plus there are two different mechanisms removing regional tracer during the ventilation-washout period: shunt in atelectatic or edematous units and ventilation in aerated units.

As a first approximation, we described a method to quantify regional perfusion and shunt flow by back-extrapolating regional tracer concentration to the time of arrival and then estimating the shunt fraction by curve fitting the tracer kinetics data (10). Application of that method to unilaterally surfactant-depleted dog lungs after lavage with Tween-80 yielded values of $Q_s/Q_R$ ranging from 80 to 95% in dependent lung regions with a tissue-to-water content ratio of 70–95%. A shortcoming of that method became evident when long infusion times were used. In those cases, tracer reaching nonaerated units clearly shunted away before the end of the injection, leading to an underestimation of total perfusion and shunt. For example, data for a dependent ROI in a bilaterally lavaged lung analyzed with that method yielded a regional perfusion of 16.5% of the total cardiac output and a regional shunt fraction of 68%. This result underestimates the regional perfusion of 23.79% and the regional shunt fraction of 81.07% obtained by using our new method.

The general approach that we adopted to formulate the model was to use the minimum number of compartments capable of simulating the injected $^{13}$NN tracer kinetics yielding a good fit ($R \geq 0.99$) to our experimental data. To accomplish this, we lumped regional blood flow into nonaerated and aerated subcompartments and assumed that regional washout from aerated units in a compartment followed a single or double exponential model. These are oversimplifications of a system that has been shown to have regional heterogeneity at length scales much smaller than those of the ROI used in this study (12). The model, however, is able to estimate an average regional behavior and with greater computing power may be used to analyze data from substantially smaller ROI, as discussed below. We also made simplifying assumptions to model the effect of tracer recirculation by assuming a constant tracer concentration in the venous return during a period equal to the duration of the breath hold. To qualify these assumptions, we stipulated that unless extremely high levels of shunt and low levels of ventilation are present, the amount of recirculation should rapidly decrease as the tracer is washed out from the lungs. Although tracer recirculation is minimal in normal lungs, it is not insignificant in injured lungs and can have a measurable effect on the measured tracer kinetics of lungs with substantial amounts of shunt. We observed that effect on nondependent ROI of ARDS lungs with large shunt levels by noting that, after 20–30 s, regional tracer content began to monotonically increase in that region. Given that no tracer was being injected during that time, we concluded that such an increase had to be caused by recirculation of tracer bypassing the lungs via shunting regions. We acknowledge that tracer recirculation could have been modeled with lumped compartments to represent the tracer kinetics along the systemic circulation. However, adding these compartments would have enlarged the model with new parameters that are difficult to assess independently. In future studies, we plan to refine the model to include the systemic circulation and extend our experimental measurements to assess the tracer concentration of the mixed venous blood during the PET imaging period. Finally, we acknowledge that mechanisms other than tracer recirculation (a second-order effect) could have been responsible for the slow increase in tracer content during apnea in the nondependent ROI of injured lungs. One could, for example, theorize that, owing to the interregional gradients in blood flow, regional gradients in tracer content could have been responsible for diffusive intraregional transport during the apneic period. This mechanism, however, is unlikely to be responsible for the increase in ND activity seen in shunting lungs because in normal supine lungs no increase in tracer content was observed in ND ROI despite the large ventro-dorsal gradients in tracer concentration.
A parameter-identification scheme was designed to minimize the computation time of the identification algorithm. The scheme consisted of analyzing the tracer kinetics data in two parts: first the data collected during apnea to identify \( q_1/q_T, q_2/Q_R, \) and \( \tau_S \), and second the data collected during the washout period to obtain regional \( SVA \). By identifying the perfusion-related parameters independently, the number of identified parameters was reduced from four to three for the single-compartment model or from six to three for the dual-compartment washout model. In addition, we used objective methods to define initial parameter guesses and realistic boundaries to further reduce the computation time.

Studying the sensitivity of the model parameters to different levels of imaging noise was important to test the robustness of the modeling approach and to extrapolate the usefulness of the model to analyze experimental data with higher levels of noise. High noise levels can occur when the injected activity of the \( ^{131} \)I-labeled saline is low, the sizes of the ROI are small, or short-duration images are required. We evaluated parameter sensitivity to experimental noise by use of the standard Monte Carlo approach. As discussed by Edelman et al. (3), the Monte Carlo approach can be used to determine confidence on any model parameter regardless of its relation to the dependent variable of the model. This was the only viable approach to test the sensitivity to noise of our model, given the complexity of its structure and the iterative process of the parameter identification procedure. To conduct the Monte Carlo simulations with data from realistic distributions of these parameters, we started by fitting experimental data, collected with a single-ring PET camera whose noise characteristics had been well documented (13), in two representative conditions (normal and ARDS lung). These data were then used to re-create noise-free data sets that were perturbed 10 times each for 1, 8, and 16 times the expected noise level. For each noise-perturbed data set, all four parameters were reidentified and then compared with their respective original values. As expected, variability of the identified parameters systematically increased as noise levels were increased. For example, as the level of imaging noise was increased by 16 times from the expected value, the identified values of \( Q_2/Q_T \) had an increased deviation around the noiseless values from 0.14 to 0.5% for the normal lung data and from 0.06 to 5.6% for the ARDS lung data. Likewise, the 16-fold increase in noise increased the mean deviation of identified \( Q_1 \) around the noiseless values from 0.4 to 2.9% and from 0.02 to 2.3% in the normal and surfactant-depleted lung data, respectively. The shunt compartment time constant, \( \tau_S \), was relatively the most sensitive parameter to noise, with a cov that changed from 0.094 to 0.19 and from 0.001 to 0.16 for the normal and ARDS lung data sets, respectively. All together, the low deviations of these parameters demonstrate the robustness of the model and the parameter-identification scheme against exaggerated levels of experimental noise. We conclude that these parameters could be calculated to within 10% accuracy in ROI that would be reduced in volume by a factor of 256. For \( \tau_S \), accuracy under the same conditions is within 20%.

In summary, we have developed a model to analyze regional tracer kinetics obtained with PET after an apneic IV bolus infusion of \( ^{131} \)I-labeled saline and a subsequent washout period. The model quantifies regional perfusion, shunt fraction, and specific ventilation and overcomes two important limitations of previous methods of analysis: 1) it can separate intra-regional perfusion into gas-exchanging and shunt flow and 2) it can account for parallel mechanisms of regional tracer removal by ventilation from aerated spaces and by shunting blood flow in nonaerated spaces. Model-fitted data accounted for >99% of experimental ROI data for both normal and injured lungs presented in the accompanying paper (14).

**APPENDIX A**

**Definitions of Symbols Used**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_A(t) )</td>
<td>Tracer concentration in alveoli</td>
</tr>
<tr>
<td>( C_R )</td>
<td>Tracer concentration in recirculating blood</td>
</tr>
<tr>
<td>( C_V )</td>
<td>Tracer concentration in pulmonary venous blood</td>
</tr>
<tr>
<td>( C_i )</td>
<td>Tracer concentration initially infused</td>
</tr>
<tr>
<td>( C_{pa}(t) )</td>
<td>Tracer concentration in pulmonary arterial blood</td>
</tr>
<tr>
<td>( C_S(t) )</td>
<td>Tracer concentration in shunting (edematous or atelectatic) alveoli</td>
</tr>
<tr>
<td>( \bar{C}_i )</td>
<td>Simulated average tracer concentration in images</td>
</tr>
<tr>
<td>( E )</td>
<td>Sum-of-squares error</td>
</tr>
<tr>
<td>( F_R )</td>
<td>Fraction of tracer concentration per unit time in recirculating blood</td>
</tr>
<tr>
<td>( M )</td>
<td>Model output</td>
</tr>
<tr>
<td>( R^2 )</td>
<td>Goodness of fit coefficient</td>
</tr>
<tr>
<td>( S )</td>
<td>Sampled output (experimental PET tracer kinetics data)</td>
</tr>
<tr>
<td>( sVA )</td>
<td>Specific alveolar ventilation</td>
</tr>
<tr>
<td>( t )</td>
<td>Time</td>
</tr>
<tr>
<td>( t_{inf} )</td>
<td>Length of time during which ( ^{131} )I-labeled saline is infused</td>
</tr>
<tr>
<td>( t_v )</td>
<td>Time at which mechanical ventilation is restarted after apnea</td>
</tr>
<tr>
<td>( V_A )</td>
<td>Volume of distribution of tracer in aerated alveoli</td>
</tr>
<tr>
<td>( V_H )</td>
<td>Volume of right heart and pulmonary blood</td>
</tr>
<tr>
<td>( V_S )</td>
<td>Volume of distribution of tracer in shunting alveoli (edematous or atelectatic) that shunt blood flow</td>
</tr>
<tr>
<td>( \dot{Q}_1 )</td>
<td>Rate of initially infused tracer</td>
</tr>
<tr>
<td>( Q_T )</td>
<td>Cardiac output</td>
</tr>
<tr>
<td>( Q_{R}/Q_T )</td>
<td>Regional perfusion expressed as a fraction of total cardiac output</td>
</tr>
<tr>
<td>( Q_{S}/Q_R )</td>
<td>Regional shunt blood flow expressed as a fraction of regional perfusion</td>
</tr>
</tbody>
</table>
During that period, regional perfusion fraction (Q_R/Q_A) was defined as the ratio of perfusion to aerated alveoli. The value of Q_R/Q_A in the fast compartment was equal to the PET-measured value. Keeping the tracer concentration, CV, was evaluated from the rate of change in activity during that period. Regional perfusion fraction (Q_R/Q_T) and local transport delay (ΔT_TD) were then identified for this ND region by running the NLID model up to the end of the apneic period. In a few rare cases of extreme lung injury (1 of 6 cases analyzed), the ND ROI exhibited a decrease in tracer content during apnea, CR could not be determined, and recirculation was therefore neglected.

Perfusion-related parameters of M and D ROI were then identified by using the value of CR determined from the ND ROI. Tracer transport delay, ΔT_TD, identified from the ND ROI data was used as an initial estimate to obtain initial guesses of the regional parameters Q_R/Q_T, Q_S/Q_R, and τ_S. Model simulation was then run with these parameters fixed, and ΔT_TD was refined interactively until the first simulated data point was equal to the PET-measured value. Keeping this value of ΔT_TD fixed, the three regional parameters were reidentified by NLID.

Recruitment parameter(s) sV_A, sV_A1, sV_A2, and Q_R/Q_A1 were identified by running the NLID model from the start of tracer infusion to the end of washout imaging, keeping the parameters ΔT_TD, Q_R/Q_T, Q_S/Q_R, and τ_S fixed at the values identified earlier.

### APPENDIX B

#### Parameter Identification Scheme

Model parameter identification was conducted independently for each ROI defined from the PET data in the following specific order. Perfusion-related parameters of ND ROI were identified first. Tracer kinetics data from this region typically exhibited no drop in regional tracer content during the apneic period and could therefore be described quite well by Eqs. 1 and 3 that excluded the shunt compartment. If a systematic increase in regional tracer content was observed during the last 30 s of apnea, this was taken as an indication of tracer recirculation. For those cases, recirculation concentration, C_R, was evaluated from the rate of change in activity during that period. Regional perfusion fraction (Q_R/Q_T) and local transport delay (ΔT_TD) were then identified for this ND region by running the NLID model up to the end of the apneic period. In a few rare cases of extreme lung injury (1 of 6 cases analyzed), the ND ROI exhibited a decrease in tracer content during apnea, CR could not be determined, and recirculation was therefore neglected.

Perfusion-related parameters of M and D ROI were then identified by using the value of CR determined from the ND ROI. Tracer transport delay, ΔT_TD, identified from the ND ROI data was used as an initial estimate to obtain initial guesses of the regional parameters Q_R/Q_T, Q_S/Q_R, and τ_S. Model simulation was then run with these parameters fixed, and ΔT_TD was refined interactively until the first simulated data point was equal to the PET-measured value. Keeping this value of ΔT_TD fixed, the three regional parameters were reidentified by NLID.

#### Parameter Bounds and Initial Estimates

Selection of an appropriate mixing blood volume (V_B) and tracer transit delay (ΔT_TD) affected the fit of the model to the data from the first two PET images. Adequate fit of the model to these data points improved identification accuracy of the parameters associated with the following portion of the data. The first image was primarily affected by ΔT_TD, whereas the second image was mostly affected by V_B. For our sheep and dog data, we found excellent model performance by fixing V_B at 50 ml and adjusting ΔT_TD as mentioned above. This yielded values of ΔT_TD on the order of 3 s.

On the basis of the assumption that all 13NN diffused into the alveolar airspace and remained there for the duration of apnea for normal lungs, the normalized data should reach a plateau level proportional to Q_R/Q_T and Q_S/Q_R should be zero. Thus an initial estimate of Q_R/Q_T was obtained from the initial plateau of ROI data normalized by total injected radioactivity. In atelectatic or edematous regions, regional 13NN content reached a peak, C_F, and then declined toward an asymptote, C_F (Figs. 3–5). An initial estimate of Q_S/Q_R was taken as 1 – (C_F/C_P). An initial estimate of τ_S was obtained by fitting a monoeponential decay function to the tracer content data between C_F and C_P.

In the absence of regional shunt, Q_R/Q_R = 0, an initial guess was taken as the reciprocal of a washout time constant τ_WO derived from fitting a single exponential function to the first and last data points of the washout. In the presence of shunt (Q_S/Q_R > 0), initial guesses were defined in the following manner: first, fixing the perfusion-related parameters identified before (ΔT_TD, Q_R/Q_T, Q_S/Q_R, and τ_S), the model was run and tracer content in the subcompartment was estimated from the model simulation. These estimated shunt compartment tracer content values were subtracted from the corresponding PET-measured values during the washout to obtain an estimate of tracer content in the subcompartment of aerated alveolar units. From this estimate, a single exponential decay function was then calculated from the first two data points of the washout to give an initial estimate of the time constant τ_1 for the fast-ventilated subcompartment. A lower parameter bound for sV_A was taken as the reciprocal of τ_1. An upper parameter bound for sV_A was fixed as 1 s⁻¹, on the basis of typical values of tidal volume, anatomic dead space, lung volume at functional residual capacity, and breathing frequency in our experimental animals.

An upper parameter bound for sV_A was taken as the reciprocal of the time constant τ_2 estimated by a single exponential connecting the last two data points of the aerated units subcompartment washout. A lower bound for sV_A was taken as zero.

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### REFERENCES


