Published in final edited form as:

Phys Med Biol. ; 63(1): 015032. doi:10.1088/1361-6560/aa9475.

A microvascular compartment model validated using ^{11}C -methylglucose liver PET in pigs

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Abstract

The standard compartment model (CM) is widely used to analyze dynamic PET data. The CM is fitted to time-activity curves to estimate rate constants that describe the transport of tracer between well-mixed compartments. The aim of this study was to develop and validate a more realistic microvascular compartment model (MCM) that includes capillary tracer concentration gradients, backflux from cells into the perfused capillaries, and multiple re-uptakes during the passage through a capillary.

The MCM incorporates only parameters with clear physiological meaning, it is easy to implement and it does not require numerical solution. We compared the MCM and CM for the analysis of 3-min dynamic PET data of pig livers (N=5) following injection of ^{11}C -methylglucose. During PET scans, the tracer concentrations in blood were measured in the hepatic artery, portal vein, and liver vein by manual sampling. We found that the MCM outperformed the CM and that dynamic PET data include information, which cannot be extracted using standard CM. The MCM fitted dynamic PET data better than CM (Akaike values were 46 ± 4 for best MCM fits, and 82 ± 8 for best CM fits; mean $\pm$ standard deviation) and extracted physiologically reasonable parameter estimates such as blood perfusion that were in agreement with independent measurements. The difference between model-independent perfusion estimates and the best MCM perfusion estimates was -0.01 ± 0.05 mL/mL/min, whereas the difference was 0.30 ± 0.13 mL/mL/min using CM. In addition, the MCM predicted the time course of concentrations in the liver vein, a prediction fundamentally unobtainable using the CM as it does not return tracer backflux from cells to capillary blood.

The results demonstrate the benefit of using models that include more physiology and that models including concentration gradients should be preferred when analyzing the blood-cell exchange of any tracer in any capillary bed.

1. Introduction

The standard approach for kinetic analysis of dynamic positron emission tomography (PET) studies is *compartment models* (CM), where the tracer and its metabolites are confined to a few well-mixed compartments (Maguire and Leenders 2007, Carson 2005). CMs have

simple analytical solutions that are easy to implement, but they are based on questionable assumptions (Zierler 1981, Larson *et al* 1987, Munk *et al* 2003a, Carson 2005). A different class of models is *distributed models* (DM) that include tracer concentration gradients along perfused capillaries to account for the fact that tracers are distributed in both time and space at the microvascular level. The tracer is transported through the capillaries by blood flow while accounting for diffusion and exchange between blood and cells across capillary walls (Bassingthwaite and Goresky 1984). DMs are physiologically more realistic and have mainly been used for whole-organ analyses using blood samples of the venous outflow.

Mixed results have been reported from studies comparing CMs and DMs for analyses of dynamic PET data. Larson KB *et al* (1987) found that the standard one-tissue CM (1T-CM) did not possess sufficient realism for adequately describing the movement of labeled water in brain, whereas Quarles RP *et al* (1993) found that the 1T-CM performed better than a DM for measurements of cerebral blood flow. Muzic RF *et al* (2003) found that a DM had no advantage over a CM for PET receptor quantification. To this point, the use of DMs seems hampered by greater computational complexity and additional parameters in combination with the statistical uncertainty and limited temporal resolution of dynamic PET data.

A realistic microvascular model of the blood-cell exchange is essential because it is the first step in the uptake of tracer from blood into cells. It has been shown that a microvascular theory led to a more informative analysis of dynamic liver PET data using an intravascular ^{15}O -carbonmonoxide including estimation of the hepatic blood perfusion (Munk *et al* 2003b). Furthermore, a study of ^{18}F FDG uptake in human livers showed that a microvascular theory led to prediction of a more physiologically realistic distribution of tracer than the standard CM (Baker *et al* 2015). Thus, we believe that many dynamic PET applications could benefit from being analyzed using models that include more physiological realism (Keiding *et al* 2012).

The aim of the present study was to develop and validate a new *microvascular compartment model* (MCM) and to examine whether the theory including more physiological realism can advance the interpretation of dynamic PET data compared to the standard CM. We have previously suggested to account for tracer backflux by an interpolation between no backflux at early times and the steady-state solution at late times (Munk *et al* 2003a) and we now propose a MCM that accounts for backflux at all time scales. The MCM unifies capillary tracer concentration gradients with the standard CM scheme, which leads to benefits including: perfusion and membrane transport are separate parameters (CM uses a lumped influx rate constant, k_1); tracer may be exchanged multiple times between cells and capillary plasma (CM has a backflux rate constant, k_2 , to the systemic circulation); and the blood volume parameter accounts for capillary concentration gradients (CM has a vascular volume parameter, V_0 , that relates only to the arterial tracer concentration). The presented MCM is generally applicable and we validate the model using dynamic PET data of pig livers following *i.v.* injection of ^{11}C -methylglucose (^{11}C -MG). ^{11}C -MG is a suitable glucose analog for testing kinetic models of non-metabolizable tracers because it has been biopsy-verified that ^{11}C -MG is not phosphorylated in contrast to ^{18}F FDG (Bender *et al* 2001). Furthermore, ^{11}C -MG enters and leaves hepatocytes through the same fast transporter

(GLUT-2) as glucose with similar transport rates as 2-deoxy-D-glucose and with an extraction fraction that depend on the blood glucose concentration (Sweet *et al* 1996).

Materials and methods

2.1. Theory

2.1.1 Model-independent analysis using arterial and venous measurements—

The time course of tracer concentration in tissue, $C_t(t)$, can be calculated using mass conservation (the change in the amount of tracer inside the tissue is equal to the difference between what enters the tissue and what leaves the tissue) (Lassen and Perl 1979) and the time course of tracer concentrations in arterial and venous blood, $C_a(t)$ and $C_v(t)$

$$C_t(t) = F \left(\int_0^t C_a(t) dt - \int_0^t C_v(t) dt \right), \quad (1a)$$

where F is the tissue blood perfusion (see list of symbols). The liver receives blood supply through the hepatic artery (HA) and portal vein (PV), which mix before entry to the liver sinusoids. Thus, the dual-input to the sinusoids is the flow-weighted average of the tracer concentrations in HA and PV, $C_{dual}(t) = f_{HA} C_a(t) + (1-f_{HA}) C_{pv}(t)$, so that

$$C_t(t) = F \left(f_{HA} \int_0^t C_a(t) dt + (1 - f_{HA}) \int_0^t C_{pv}(t) dt - \int_0^t C_v(t) dt \right), \quad (1b)$$

where f_{HA} is the hepatic arterial flow fraction (Munk *et al* 2001). Eqs.1 allow the estimation of blood perfusion without a specific tissue model but require venous blood sampling, which is rarely available in human studies.

2.1.2 The standard one-tissue compartment model (1T-CM)—We briefly outline the standard 1T-CM, which is widely used to analyze dynamic PET data and is discussed in many text books (Maguire and Leenders 2007, Carson 2005). The 1T-CM describes a bidirectional flux of tracer between arterial input function $C_a(t)$ ($C_{dual}(t)$ for the liver) and a tissue compartment $C_I(t)$, and is characterized by two rate constants K_1 and k_2

$$\frac{dC_I(t)}{dt} = K_1 C_a(t) - k_2 C_I(t). \quad (2)$$

The equation is solved to obtain an expression for C_I

$$C_I(t) = K_1 e^{-k_2 t} \otimes C_a(t), \quad (3)$$

where the symbol $\otimes$ denotes convolution. Any region in a PET image contains a fraction of blood vessels, which is accounted for by adding a vascular volume term $V_0 C_a(t)$.

$$C_t(t) = K_1 e^{-k_2 t} \otimes C_a(t) + V_0 C_a(t) \quad (4)$$

Thus, the 1T-CM that is fitted to dynamic PET data includes three parameters K_1 , k_2 and V_0 .

We note three limitations of the 1T-CM:

1. The influx rate constant, K_1 , relates only to the arterial input function (for the liver, the dual-input function). k_1 is interpreted as the product of perfusion F and unidirectional extraction fraction E , using the Renkin-Crone equation [Renkin 1959, Crone 1964] that relates F , E , and PS describing the capillary as a cylindrical tube

$$E = \left(1 - e^{-\frac{PS}{F}} \right) \quad (5)$$

where P is the permeability and S is the capillary surface area. Thus, $K_1 = FE$ is a lumped parameter from which F , E and PS cannot be separated.

2. Tracer leaves the cells by the backflux rate constant k_2 , to the systemic circulation and is not available for reuptake by K_1 (see Eq.2). Thus, the 1T-CM only allows a single uptake of each tracer molecule during its passage through the perfused capillary.
3. In the 1T-CM, the vascular tracer activity is accounted for by a vascular volume parameter, V_0 , which is multiplied by the arterial input function (or dual-input function for liver studies). While the $V_0 C_a(t)$ term correctly accounts for tracer in arteries and arterioles, there are complex concentration gradients caused by the bolus injection [Munk *et al* 2003a, Munk *et al* 2003b] and the blood-cell exchange along the length of the capillaries. This is clearly seen by comparing the time courses of tracer concentrations in arterial and venous blood, $C_a(t)$ and $C_v(t)$, where the latter is clearly less dynamic (Section 3.1). Thus, the vascular tracer in the capillaries and venules is not adequately accounted for in the 1T-CM.

These limitations relate to a simplistic model that does not account for capillary concentrations gradients, which will be amended in the next section.

2.1.3 One-tissue microvascular compartment model (1T-MCM)—The 1T-MCM unifies capillary tracer concentration gradients with a compartmental modelling of the blood-cell exchange of tracer. The basic idea is to calculate the venous outlet, C_v , and to use mass conservation to predict the tracer concentration in tissue (Eqs. 1). In the derivation, we keep track of the number of uptakes from blood to cell, N , that molecules can undergo while being transported convectively forward with capillary blood perfusion. During the first uptake ($N=1$), the molecules of the input function flows through the capillary and

experiences the full surface area (S), and they either pass the capillary membrane (PS) or leave unextracted at the venous side. For each re-uptake ($N>1$), the input function consists of the backflux of molecules from $N-1$. The surface area available for re-uptake is linearly decreasing as function of N , which is equivalent to entering the capillary slightly downstream (see Figure 1), and due to the capillary flow only a finite number of uptakes, N_{max} , are allowed.

In the cells, the tracer concentration, C_I , has contributions from molecules that have been transported into the cells once or several times up to N_{max}

$$C_I = \sum_{N=1}^{N_{max}} C_I^{(N)}, \quad (6)$$

where the number in brackets refer to the number of uptakes, i.e. $C_I^{(1)}$ denotes the contribution to C_I from molecules that are transported once into a cell; $C_I^{(2)}$ denotes the contribution to C_I from molecules that are taken up two times (i.e. one re-uptake) and $C_I^{(N)}$ denotes the contribution to C_I from molecules that are taken up N times. For all N , $C_I^{(N)}$ is not used directly but is merely a hidden variable used to calculate the venous outflow C_v . For notational simplicity, we suppressed the explicit time dependence of tracer concentrations in this section.

Molecules that are not irreversibly bound in the cell must eventually leave at the venous end of the capillary with the venous tracer concentration

$$C_v = \sum_{N=0}^{N_{max}} C_v^{(N)}, \quad (7)$$

where $C_v^{(0)}$ denotes the contribution to C_v from molecules that were not extracted from capillary blood (zero uptake); $C_v^{(1)}$ denotes the contribution to C_v from molecules that were taken up once and then transported back into capillary blood and leaving at the venous side; $C_v^{(N)}$ denotes the contribution from molecules that are taken up N times. The total venous C_v is calculated (Eq.7) and inserted in Eqs.1 to analyzing C_I from a local region of interest in dynamic PET data. The next two sections will show how to calculate $C_v^{(N)}$ used in Eq.7.

Contribution to C_v from unextracted molecules: The input function available for the 1st uptake is $C_i^{(1)} = C_a$ (C_{dual} for the liver), the influx rate constant is $K_1^{(1)} = K_1$, and the rate constant for backflux from cell back to capillary blood is $K_1^{(2)} = K_2$. Thus, the 1st uptake is described by the standard 1T-CM (Eqs. 2–3) with $C_i^{(1)} = C_1$. The unextracted part of the inlet

concentration that leaves the venous end of the capillary bed after a capillary mean transit time $T_{cap} = V_{cap}/F$ is

$$C_v^{(0)}(t) = (1 - E^{(1)})C_a(t - T_{cap}) = \left(1 - \frac{K_1^{(1)}}{F}\right)C_a\left(t - \frac{V_{cap}}{F}\right), \quad (8)$$

where we explicitly show the time-dependence. This part of the venous outlet is due to molecules that passed straight through the capillary without uptake.

Contribution to C_v from extracted molecules: The input function available for the N^{th} uptake is the backflux from the previous $(N-1)^{th}$ uptake

$$FC_i^{(N)} = k_2^{(N-1)}C_1^{(N-1)} \Rightarrow C_i^{(N)} = \frac{k_2^{(N-1)}C_1^{(N-1)}}{F}. \quad (9)$$

Due to the convective flow, the surface area available for the N^{th} uptake is reduced by $1/N_{max}$ so the influx rate constant for the N^{th} uptake is

$$K_1^{(N)} = FE^{(N)} = F \left(1 - e^{-\left(\frac{N_{max} - (N-1)}{N_{max}}\right)\frac{PS}{F}}\right), \quad (10)$$

where the surface area available for uptake is reduced by $(N-1)/N_{max}$, which decreases the extraction fraction and the influx rate constant. $K_1^{(N)}$ and K_I are related through their Renkin-Crone equations

$$1 - \frac{K_1^{(N)}}{F} = e^{-\left(\frac{N_{max} - (N-1)}{N_{max}}\right)\frac{PS}{F}} = \left(1 - \frac{K_I}{F}\right)^{\left(\frac{N_{max} - (N-1)}{N_{max}}\right)}. \quad (11)$$

The surface area available for backflux is similarly reduced by $(N-1)/N_{max}$

$$k_2^{(N)} = \frac{N_{max} - (N-1)}{N_{max}} \frac{PS}{V_C} = \frac{N_{max} - (N-1)}{N_{max}} k_2. \quad (12)$$

For the N^{th} uptake, the bidirectional flux of tracer between the input function $C_i^{(N)}$ (Eq.9) and a tissue compartment $C_I^{(N)}$ is characterized by the rate constants $K_I^{(N)}$ and $k_2^{(N)}$

$$\frac{dC_1^{(N)}}{dt} = K_1^{(N)}C_i^{(N)} - k_2^{(N)}C_1^{(N)} \quad (13)$$

which is equivalent to the standard 1T-CM (Eq.1). Note that $K_1^{(N)}$ and $k_2^{(N)}$ are related directly to k_1 (Eq.11) and k_2 (Eq.12). The unextracted part of $c_i^{(N)}$ leaves the venous side of the capillary bed delayed by a capillary mean transit time $T_{cap} = V_{cap}/F$

$$C_v^{(N-1)} = (1 - E^{(N)})C_i^{(N)} = \left(1 - \frac{K_1^{(N)}}{F}\right)C_i^{(N)}. \quad (14)$$

This part of the venous outlet is due to molecules that were taken up $N-1$ times.

Operational equation for fitting 1T-MCM: With the 1T-MCM, we calculate the venous outlet of tracer C_v (Eq.7) and use of mass conservation (Eqs. 1a–1b) to calculate the total tissue concentration that includes both cells and capillaries

$$C_t(t) = F \left(\int_0^t C_a(t)dt - \int_0^t C_v(t)dt \right) = F \left(\int_0^t C_a(t)dt - \sum_{N=0}^{N_{max}} C_v^{(N)} \right), \quad (15)$$

where $C_a(t)$ is replaced by $C_{dual}(t)$ for liver studies. This operational equation is used to analyze measurements of the regional tissue tracer concentration (dynamic PET) using the input function (blood sampling). The 1T-MCM has four parameters F , V_{cap} , K_1 , and k_2 using the standard compartmental terminology, or equivalently by F , V_{cap} , PS , and V_c using the microvascular terminology (see list of symbols). Thus, the 1T-MCM uses the same input function as the standard 1T-CM, but the 1T-MCM has an additional free parameter. Note that the 1T-MCM can also be used directly to analyze measurements of the venous tracer concentration (blood sampling) using Eq.7 as operational equation and the input function (blood sampling), which is only relevant in rare cases when venous blood samples are available. We will validate both methods using experimental data.

Inclusion of non-exchanging vessels: A tissue volume-of-interest (VOI) includes some fraction of larger vessels such as arteries, arterioles, venules and veins that do not exchange tracer with cells. In the MCM, we measure the arterial input (dual-input for the liver) and estimate the venous tracer concentration. Thus, the non-exchanging vessels may be included by expanding Eqs.1 by terms accounting for the transit of arterial tracer concentration through an arteriole volume V_a and transit of venous tracer concentrations through a venule volume V_v , e.g. Eq.1a becomes

$$C_t(t) = V_a \bar{C}_a(t) + (1 - V_a - V_v)F \left(\int_0^t C_a(t - T_a) dt - \int_0^t C_v(t) dt \right) + V_v \bar{C}_v(t) \quad (16)$$

where the bars denote the spatial average inside the non-exchanging volume. For the arterial non-exchanging vessels, the spatial average is calculated as described by Munk *et al* (2003a, 2003b)

$$\bar{C}_a(t) = \begin{cases} \frac{1}{T_a} \int_0^t C_a(t') dt', & t < T_a \\ \frac{1}{T_a} \int_{t-T_a}^t C_a(t') dt', & t > T_a \end{cases} \quad (17)$$

where $T_a = V_a/F$ is the transit time through the arterioles. The venous non-exchanging vessels are accounted for the same way using $T_v = V_v/F$ and $C_v(t)$.

Estimation of f_{HA} , time delays and N_{max} : Mass conservation (Eq.1b) was used to estimate f_{HA} and the small time-delays between the blood samples and the dynamic PET scan in a fit that used all four TACs (HA, PV, LV and liver) and had five free parameters (F , f_{HA} , and time-delays for the three blood TACs). Thus, we first estimated f_{HA} and synchronized blood samples and dynamic PET scan to a common time axis independent of the kinetic model, and then we fixed these values in subsequent fits using CM and MCM.

In the previous sections, N_{max} could be any number, but it is more realistic to relate the maximum number of uptakes to the unidirectional extraction fraction determined by F and PS (Eq.5). For ^{11}C -MG in the liver, we found that $N_{max}=6$ provided the best fits to our measured data and only around 5% of the original input function would be available for further reuptakes (Supplemental Figures 1–2). Thus, N_{max} was fixed to 6 in all fits using the MCM.

2.2 Experimental data

2.2.1 Animals and preparations—The study procedures were approved by the Danish National Ethics Committee for animal research. Five female Danish Landrace pigs (38–42 kg) were used. The pigs were fasted for 24h with free access to tap water. In each pig, catheters for blood sampling were placed in the abdominal aorta through the right femoral artery (FA), portal vein (PV) through the splenic vein, and liver vein (LV) via the right jugular vein. Liver PET data from the same animals were previously used in a study regarding dual blood supply to the liver, and the animal preparation was as described in detail elsewhere (Munk *et al* 2001).

2.2.2 Dynamic PET scans, blood samples and image analysis—The data used here included 3-min dynamic liver PET data from five pigs that were scanned on an ECAT EXACT HR-47 (CTI, Knoxville, TN) after 300 MBq ^{11}C -MG intravenous injections. In one

animal, the ^{11}C -MG injection and data acquisition was repeated. Dynamic PET data were reconstructed as a series of 10 s frames with filtered backprojection using a Hanning filter with a cut-off at 0.3 Nyquist frequency. Each image consisted of $128 \times 128 \times 47$ voxels with a size of $2.4 \times 2.4 \times 3.1 \text{ mm}^3$ and a central spatial resolution around 6.7 mm full width at half maximum. During the dynamic PET scan, manual blood samples of 1 mL each were taken from FA (corresponding to HA), PV and LV at $18 \times 5 \text{ s}$, $3 \times 10 \text{ s}$ and 30 s. It was not necessary to extract plasma samples because ^{11}C -MG does not enter the red blood cells in pigs (Zeidler *et al* 1976). Instead, plasma measurements could be derived from the blood measurements when corrected for the measured hematocrit $hct=0.26\pm0.03$. Blood tracer concentrations were measured using a well counter (Packard Instruments Co, Meriden, CT) and decay-corrected back to scan start. The well counter and PET scanner were cross-calibrated using a known ^{68}Ge solution. It has been verified that our manual blood procedure only caused minimal dispersion (Munk *et al* 2008). The arterial blood glucose concentration was in the range 5.0 – 6.7 mmol/L during the dynamic PET scans.

3-min ^{11}C -MG TACs were extracted using 200-mL VOIs defined in the liver PET images so that large intra-hepatic vessels were avoided. The liver TACs and a full set of measured blood TACs (HA, PV and LV) were analyzed using mass conservation (Section 2.1.1) and the small time-delays between blood samples and PET data were corrected. Then, the 1T-MCM was fitted to dynamic PET data (without using LV blood samples) and to LV samples (without using dynamic PET data). One-tissue models were suitable because ^{11}C -MG is not metabolized in pig livers (Bender *et al* 2001).

In addition, the hepatic blood volume, V_b , was estimated in each pig using a dynamic PET scan after 500 MBq [^{15}O]carbon monoxide (CO) inhalation. CO is transported by the red blood cells, which cannot cross the sinusoidal endothelium into the extravascular plasma volume, Space of Disse. During steady state, a few minutes after inhalation, the hepatic blood volume, V_b , was calculated as the liver tissue tracer concentration divided by the arterial tracer concentration, C_t/C_a . For the five pigs, the hepatic blood volume estimate was $V_b=0.25\pm0.02 \text{ mL/mL}$ as previously reported (Munk *et al* 2001). Sinusoidal blood volumes around 15% has been reported (Goresky 1963, Reichen 1993). Thus, the non-exchanging vessel volume in the VOIs is around 10%.

2.3 Parameter identifiability parameter estimation and statistical criteria

Model parameters were examined for identifiability using sensitivity functions that show the change in model prediction for a 1% change in parameter value. Identifiable model parameters are sensitive (a change in parameter value leads to a substantial effect on the model solution) and have a unique sensitivity function that is not proportional to the others. Model parameters were estimated by minimizing the residual sum of squares. Plots of residuals against time were examined for systematic errors. The statistically favorable model was found comparing the Akaike Information Criterion (AIC), which penalize models proportional to the number of model parameters (Akaike 1974). Results are given as the mean values $\pm$ standard deviation ($N=5$). Pairwise comparisons were performed using t-tests and $P < 0.05$ was considered to indicate statistically significant differences. Pearson correlation coefficients were used to assess correlation between perfusion estimates, and

Bland-Altman plots were used to evaluate the agreement between model-independent perfusion estimates and perfusion estimates using 1T-CM and 1T-MCM.

3. Results

3.1. Time activity curves and model-independent parameter estimates

Figure 2A shows examples of TACs from an artery, PV, LV and dual-input after ^{11}C -MG injection. The arterial TAC had a rapid phase with a sharp peak, whereas the PV TAC was delayed and dispersed compared to that in arterial blood during the passage through the splanchnic circulation. The calculated dual-input TAC was in between because it was the flow-weighted average of the HA and PV input curves, $C_{\text{dual}}(t) = f_{\text{HA}} C_{\text{HA}}(t) + (1-f_{\text{HA}}) C_{\text{PV}}(t)$. The dual-input function was calculated for each experiment using individual estimates of f_{HA} , and time-delays. Passage through the liver caused the LV TAC to be the least dynamic and most delayed TAC. The corresponding liver tissue TAC is shown in Figure 2B with the best fit using model-independent mass conservation (Eq.1b). For the five animals, the blood perfusion estimate was $F=1.3\pm0.3$ mL/mL/min and the hepatic arterial flow fraction was $f_{\text{HA}}=21\%\pm11\%$.

3.2 Identifiability of 1T-MCM parameters

Figure 3 shows the sensitivity functions for the 1T-MCM model parameters. For the prediction of the tissue TAC, $C_t(t)$, all parameters were identifiable having sensitivity functions that were separated and not too similar. For the prediction of the venous TAC, $C_v(t)$, however, the parameters were correlated, *i.e.* changing the value of a parameter caused a change in model prediction that could have been obtained by changing other parameters. Accordingly, the number of free parameters should be reduced to make the remaining model parameters identifiable when using MCM to fit venous TACs.

3.3 Kinetic analysis of dynamic PET data using 1T-CM and 1T-MCM

For each experiment, the dual-input function was calculated using fixed values for f_{HA} , and time-delays (see Section 3.1). First, dynamic PET data following ^{11}C -MG injection were analyzed using the dual-input function and the standard 1T-CM model including a vascular volume parameter V_0 (Eq.4). For each of the five data sets, the best fits provided zero or negative estimates for V_0 . Consequently, all data were fitted again without the V_0 term (Eq. 3), *i.e.* with only two parameters. Then, the same data were analyzed using the 1T-MCM model (Eq.15) with four parameters: F , V_{cap} , PS , and V_c . Parameter estimates and Akaike values are shown in Table 1. The 1T-MCM performed significantly better than the standard 1T-CM as shown by lower Akaike values (Figure 2C and Table 1), whereas the fits using 1T-MCM model were equally good as when using model-independent mass conservation (Eq. 1b). Parameter estimates from the animal that had two ^{11}C -MG injections were in good agreement (see Supplemental Table 1).

3.4 Kinetic analysis of venous blood samples using 1T-MCM

The microvascular models can be used to predict a measured venous time-activity curve (TAC) using Eq.7 (Figure 2D). For all pigs, the liver vein blood TACs were fitted using the 1T-MCM where the number of free parameters were reduced by fixing values for the blood

perfusion, F , and the capillary blood volume V_{cap} to the parameters estimates obtained from the MCM fits to C_t (Table 1). Parameter estimates of PS and V_c are shown in Table 1. Parameter estimates from the animal that had two ^{11}C -MG injections were in good agreement (Supplemental Table 1).

3.5 Effect of including non-exchanging vessels using 1T-MCM

Dynamic PET data were analyzed with 1T-MCM including V_a and V_v for non-exchanging vessels that were always set to fixed values in order not to have too many free parameters. As discussed in section 2.2.2, it is reasonable to expect that our liver VOIs include around 10% non-exchanging vessels. Thus, the dynamic PET data were fitted with V_a and V_v fixed to 5% in Eq.16. Furthermore, the dynamic PET data was fitted with non-exchanging vessel volumes fixed in steps of 5%, $V_a=[0\%, 5\%, 10\%, 15\%]$ and $V_v=[0\%, 5\%, 10\%, 15\%]$, and parameter estimates were reported from the best of the 16 fits (lowest AIC). The best combination of V_a and V_v could vary between animals. The results from fits including non-exchanging vessels are shown in Table 2. Perfusion estimates using 1T-MCM with and without non-exchanging vessels were compared with model-independent perfusion estimates (Figure 4A). Parameter estimates from the animal that had an additional ^{11}C -MG injection were in good agreement (Supplemental Table 1).

3.6 Comparison of flow estimates

All 1T-MCM-derived perfusion estimates and the 1T-CM-derived K_I estimates were significantly correlated to the model-independent perfusion estimates. However, all MCM-derived perfusion estimates were less biased and less variable than K_I estimates from 1T-CM. For the perfusion estimates plotted in Figure 4A, the differences (in mL/mL/min) were -0.18 ± 0.04 ($V_a = V_v = 0\%$), -0.07 ± 0.05 ($V_a = V_v = 5\%$), -0.01 ± 0.05 (best V_a, V_v) using 1T-MCM for ($V_a = V_v = 0\%$), and 0.30 ± 0.13 using 1T-CM. Figure 4B shows Bland-Altman plots of the best 1T-MCM results and 1T-CM results.

Discussion

The MCM amends known deficiencies of the standard CM, and the MCM includes capillary tracer concentration gradients, backflux into the capillary, multiple re-uptakes, and a physiologically meaningful vascular volume parameter. MCM predicts the venous TAC, $C_v(t)$, calculated from the well-known compartmental equations, and the tissue TAC, $C_t(t)$, calculated using mass conservation. In contrast to existing distributed models, MCM is easy to implement and does not require numerical solution.

The 1T-CM produced statistically poor fits (Table 1), and the model configuration without vascular volume (Eq.3) described data better than a model with a vascular volume (Eq.4), which conflicts with the facts that the liver has a large blood volume and that a significant amount of ^{11}C -MG leaves through liver veins (Figure 2). Thus, the 1T-CM predicted a wrong distribution of the measured tracer concentration in liver tissue, i.e. no vascular tracer, which led to overestimated parameter estimates for the influx rate constant K_I (Figure 4B) and the extravascular distribution volume K_1/k_2 . We explain this behavior by the simplistic treatment of the vascular volume where the input function is directly used as the vascular

concentration profile. In addition, when CM underestimates capillary plasma concentration by presenting tracer only once, it has to overestimate K_1 to make up the uptake.

The 1T-MCM produced significantly better fits than the 1T-CM (Table 1). Furthermore, 1T-MCM provided parameter estimates that all were physiologically meaningful. The 1T-MCM blood perfusion estimates were similar to model-independent estimates using mass conservation (Table 1 and Figure 4). The liver capillaries (sinusoids) are lined with fenestrated endothelial cells that contain the large red blood cells but allow plasma and most molecules, such as plasma-borne ^{11}C -MG, to rapidly diffuse into an extended plasma volume, Space of Disse (Goresky 1963). The extended plasma volume is around half of the sinusoidal blood volume depending on the size of the tracer molecule (Goresky 1963, Blouin *et al* 1977). Thus, the vascular volume estimate around 0.4 mL/mL was reasonable when analyzing ^{11}C -MG tissue data using blood samples and accounting for hematocrit and the extended plasma volume.

The 1T-MCM prediction of the venous TAC was used to fit liver vein blood samples (Table 1). Our estimated $PS=4.1\pm 1.7$ mL/mL/min and $V_c=0.30\pm 0.04$ mL/mL were comparable to estimates made in perfused rat livers by Sweet *et al* (1996) who reported $PS=4.6\pm 0.33$ mL/g/min and $V_c=0.27\pm 0.03$ mL/g using 3- O -[^3H]MG when analyzing liver venous outflow concentration curves. The 1T-MCM allows inclusion of non-exchanging vessels, and reasonable estimates of their volumes, such as $V_a=V_v=5\%$, led to improved perfusion estimates (Figure 4). In comparison, the standard 1T-CM cannot fit the venous TAC and cannot include non-exchanging venules because it does not account for the tracer concentration gradient during the passage through the capillary.

The goal of a comprehensive kinetic analysis is to obtain reliable parameter estimates, but noisy measurements and correlated parameters may prevent unambiguous parameter estimation. In addition, model parameters cannot be reliably estimated when they do not cause substantial changes to the model prediction. We verified the identifiability of the 1T-MCM model parameters by examining their sensitivity functions (Figure 3) and found that the 1T-MCM parameters were identifiable when used to analyze dynamic PET data, whereas constraints were needed when used to analyze liver vein blood samples without using PET. It should be noted that 1T-MCM include extra parameters than the 1T-CM. Thus, it might be an advantage to constrain or fix parameters to avoid local minima and unphysiological fits when using the 1T-MCM to analyze noisy data, e.g. when calculating voxel-based parametric maps. In this paper, however, we analyzed data with relatively low noise (Figure 2) and obtained stable unconstrained fits.

The MCM model has limitations even though it amends shortcomings of the standard CM. MCM deals with capillary concentration gradients and multiple reuptakes by returning backflux from cells to the capillary further downstream for each re-uptake so that subsequent reuptakes have a linearly decreasing PS . This approach is probably not a perfect model of the blood-cell exchange, but it did lead to a model that is easy to implement and that includes more physiology than the standard CM. Another limitation is the choice of N_{max} that determines the number of reuptakes allowed and affects how the backflux re-enters the capillary. We did not choose N_{max} based on organ-specific structural

considerations, but we empirically found that $N_{max}=6$ produced the best fits to data (Supplemental Figure 1). In practice, the choice of N_{max} value is not critical because the MCM parameter estimates were slowly varying as function of N_{max} (Supplemental Figure 3).

We compared the 1T-MCM and 1T-CM for the analysis of 3-min dynamic PET data of pig livers following injection of ^{11}C -MG, which are data that should be affected by capillary concentration gradients because the liver has a large vascular volume and MG is readily transported across the hepatocyte membrane. Thus, analyses of these data should be able to reveal shortcomings in a model's description of the fast blood-cell exchange. We found that the 1T-MCM outperformed the 1T-CM. The results demonstrate the benefit of using models that include more physiology than the standard CM, and we believe that models including concentration gradients, such as MCM, should be preferred when analyzing the blood-cell exchange of any tracer in the liver. The advantage of using MCM would probably be less pronounced for dynamic PET data with less pronounced capillary concentration gradients. However, we foresee that other dynamic PET applications will benefit from being analyzed using more realistic models because of continued improvements in PET technology leading to PET scanners with better sensitivity and higher temporal resolution.

Conclusion

We present a general microvascular compartment model that includes capillary tracer concentration gradients and backflux from cells into the perfused capillaries, allowing multiple re-uptakes of tracer. It incorporates only parameters with clear physiological meaning, it is easy to implement and it does not require numerical solution. The 1T-MCM was validated for ^{11}C -methylglucose in pig liver, and it clearly outperformed the standard compartment model. 1T-MCM fitted dynamic PET data better and extracted physiologically reasonable information including perfusion estimates that were in close agreement with model-independent perfusion estimates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the staff at the Department of Nuclear Medicine & PET Center, Aarhus University Hospital for their dedicated work during the experimental studies that formed the basis of this work. This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases (R01-DK074419).

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Figure 1.

Schematic drawing of the 1T-MCM. N is the number of uptakes. The first unidirectional uptake corresponds to $N=1$. For the first uptake, the input function flows through the entire capillary and the molecules either pass the capillary membrane (PS) or leave unextracted at the venous side. The capillary concentration gradient is described by the Renkin-Crone relation. For $N=2$, the input function consists of backflux of molecules from $N=1$. These molecules experience a capillary surface area S that is reduced, which is equivalent to entering the capillary slightly downstream. Subsequent uptakes $N=3 \dots N_{max}$ enter the

capillary further downstream proportional to N . The total venous outlet is the sum of venous outlets for all N .

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Figure 2.

Measured and fitted TACs following ^{11}C -MG injection in Pig no. 1. **A)** Blood TACs measured by manual sampling. The HA TAC was sampled in the abdominal aorta. The dual-input TAC was the flow-weighted mean of the HA TAC and PV TAC. **B)** Liver TAC measured by dynamic PET. The solid line shows the best fit using mass conservation and the blood TACs. This fit estimated the hepatic blood perfusion $F=1.08$ mL/mL/min, hepatic arterial fraction $f_{HA}=0.31$ and the small delays on the blood TACs ($td_{HA}=7$ sec, $td_{PV}=9$ sec, $td_{LV}=12$ sec) relative to the liver PET TAC. The f_{HA} and delays were used to estimate the dual-input shown in Figure 2A. **C)** The PET-measured liver TAC is shown with open circles. The solid line shows the best fit using 1T-MCM and the dual-input TAC, and the dashed line shows the best fit using the standard 1T-CM and the dual-input TAC. The Akaike value was lower (better) for the 1T-MCM ($AIC=51$) than for the 1T-CM ($AIC=74$). Parameter estimates are shown in Supplemental Table 1. **D)** Liver vein TAC is shown with open circles.

The solid line shows the best fit using 1T-MCM with constrained F and the dual-input TAC. Parameter estimates are shown in Supplemental Table 1. The CM cannot predict the time course of concentrations in the venous outflow as it does not return tracer backflux from cells to capillary blood.

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Figure 3.

Sensitivity of 1T-MCM predictions to 1% changes in each of the four model parameters as function of time. Positive sensitivity means that an increase in the parameter will increase the model prediction. A model parameter is identifiable if it has a substantial effect on the model prediction and a unique sensitivity function that is not proportional to the others. **A)** Sensitivity functions for prediction of tissue TAC measured by dynamic PET. **B)** Sensitivity functions for prediction of the venous TAC measured by blood sampling.

Figure 4.

A) Perfusion estimates using 1T-MCM and 1T-CM as a function of model-independent perfusion estimates (Eq.1b). 1T-MCM perfusion estimates without non-exchanging vessels are shown with open circles. 1T-MCM perfusion estimates using $V_a=V_v=5\%$ are shown with grey circles. 1T-MCM perfusion estimates from best fits with non-exchanging vessel are shown with black circles. For comparison, 1T-CM K_1 estimates are shown with triangles. **B)** Bland-Altman plots showing the difference between the best perfusion estimates using 1T-MCM and the model-independent F estimates against the mean of the two estimates. The black lines show mean values ± 2 standard deviations. The dashed lines show the same result for K_1 from 1T-CM.

Table 1

Parameter estimates and statistical criteria for best fits to liver tissue TACs and liver vein TACs following ^{11}C -methylglucose injections in 5 pigs.

Parameter	MC (C_t)	1T-CM (C_t)	1T-MCM (C_t)	1T-MCM (C_v)
F (mL/mL/min)	1.3 ± 0.3	-	1.2 ± 0.3	Fixed 1.2
V_{cap} (mL/mL)	-	-	0.39 ± 0.11	Fixed 0.39
PS (mL/mL/min)	-	-	2.6 ± 1.0	4.1 ± 1.7
V_c (mL/mL)	-	-	0.24 ± 0.04	0.30 ± 0.04
K_f (mL/mL/min)	-	1.6 ± 0.4	$1.0 \pm 0.3^*$	$1.1 \pm 0.3^*$
k_2 (1/min)	-	1.8 ± 0.3	$10 \pm 3^\#$	$13 \pm 4^\#$
AIC	58 ± 11	82 ± 8	50 ± 4	24 ± 9

MC = mass conservation (model-independent). (C_t) = fit to tissue TACs (dynamic PET data).

(C_v) = fit to venous TACs (blood samples) with fixed F and V_{cap} .

AIC from C_v fits should not be compared to C_t fits.

Results are mean $\pm$ standard deviation ($N=5$);

^{*)} Calculated as FE ;

^{#)} Calculated as PS/V_c

Table 2

Parameter estimates and statistical criteria for best fits to liver TACs following ^{11}C -methylglucose injections in 5 pigs using MCM including non-exchanging vessels.

Parameter	$V_a=V_v=0\%$	$V_a=V_v=5\%$	Best V_a and V_v
F (mL/mL/min)	1.2 ± 0.3	1.3 ± 0.3	1.3 ± 0.3
V_{cap} (mL/mL)	0.39 ± 0.11	0.34 ± 0.09	0.39 ± 0.11
PS (mL/mL/min)	2.6 ± 1.0	2.8 ± 1.1	1.9 ± 1.1
V_c (mL/mL)	0.24 ± 0.04	0.26 ± 0.04	0.26 ± 0.04
K_f (mL/mL/min)	$1.0 \pm 0.3^*$	$1.1 \pm 0.3^*$	$1.0 \pm 0.4^*$
k_2 (1/min)	$10 \pm 3^\#$	$7 \pm 3^\#$	$7 \pm 3^\#$
AIC	50 ± 4	52 ± 5	46 ± 4

Results are mean $\pm$ standard deviation ($N=5$);

^{*)} Calculated as FE ;

^{*)} Calculated as PS/V_c

Best values for V_a and V_v were $V_a=0.14\pm0.02$ and $V_v=0.08\pm0.06$

List of symbols

Symbol, unit and definition		
$C_a(t)$	[kBq / mL blood]	Tracer concentration in arterial blood (measured)
$C_t(t)$	[kBq / mL tissue]	Tracer concentration in tissue VOI (measured or calculated)
$C_{pv}(t)$	[kBq / mL blood]	Tracer concentration in portal venous blood (measured)
$C_v(t)$	[kBq / mL blood]	Tracer concentration in venous blood (measured or calculated)
$C_I(t)$	[kBq / mL tissue]	Tracer concentration in tissue compartment (calculated)
E	[unitless]	Extraction fraction
F	[mL blood / mL tissue / min]	Tissue perfusion (not a conventional flow in [mL blood per min])
K_I	[mL blood / mL tissue / min]	Influx rate constant
k_2	[1 / min]	Backflux rate constant
P	[cm / min]	Permeability of the tracer across the capillary surface
PS	[mL blood / mL tissue / min]	Permeability-Surface product
S	[cm ² / mL tissue]	Capillary surface area per mL tissue
T_{cap}	[min]	Capillary transit time $T_{cap} = V_{cap}/F$
V_a	[mL / mL tissue]	Non-exchanging arteriole volume
V_b	[mL blood / mL tissue]	Blood volume including non-exchanging vessels
V_c	[mL / mL tissue]	Cellular volume
V_{cap}	[mL blood / mL tissue]	Capillary blood volume
V_0	[mL blood / mL tissue]	Vascular volume parameter used in the standard compartment model
V_v	[mL / mL tissue]	Non-exchanging venule volume