Measurement of renal tissue oxygenation with blood oxygen level-dependent MRI and oxygen transit modeling

Jeff L. Zhang,1 Glen Morrell,2 Henry Rusinek,2 Lizette Warner,3 Pierre-Hugues Vivier,4 Alfred K. Cheung,5 Lilach O. Lerman,6 and Vivian S. Lee1

1Department of Radiology, University of Utah, Salt Lake City, Utah; 2Department of Radiology, New York University, New York, New York; 3Philips Healthcare, Cleveland, Ohio; 4Department of Radiology, Rouen University Hospital, Rouen, France; 5Division of Nephrology, University of Utah, Salt Lake City, Utah; and 6Nephrology and Hypertension, Mayo Clinic, Rochester, Minnesota

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Despite the high overall flow of blood to the kidney, the normal renal medulla works in a hypoxic state because of both its high metabolic activity (7) and also oxygen shunting due to the medullary arterial-venous counter-current mechanism (30). Medullary hypoxia plays a critical role in renal pathophysiology (12, 13). For example, with reduced blood perfusion in renovascular disease, the renal medulla becomes more vulnerable to hypoxic injury due to insufficient oxygen delivery (39). In acute renal failure (3, 4, 34), on the other hand, perfusion decreases, leading to decreased oxygen consumption. Since impaired oxygen delivery and/or consumption in the kidney may occur in acute and chronic renal diseases, a noninvasive estimate of renal PO2 could be of vital benefit in clinical practice. Oxygen content in tissue is commonly indicated by its partial pressure or tissue PO2. Medullary PO2 in healthy kidneys measured by oxygen probes is in the range of 10–20 mmHg, compared with ~50 mmHg in the cortex (6, 26). An invasive tool, oxygen microelectrodes can only be used in animal studies. To date there are no techniques for measuring kidney tissue PO2 noninvasively in human subjects.

Blood oxygen level-dependent (BOLD) MRI is an imaging technique where tissue signal reflects the concentration of deoxygenated hemoglobin molecules (28). The transverse relaxation rate, R₂*, measured using BOLD MRI has been proposed as a good surrogate for tissue oxygenation in several renal diseases (11, 16, 25, 31, 40). However, besides oxygenation, multiple factors including intrinsic spin-spin relaxation rate (R₂) and blood perfusion (F) may contribute to R₂*. These factors may vary substantially even in healthy kidneys and confound the estimation of tissue oxygenation from BOLD data. Our goal in this study is to address these potential limitations to estimate the true tissue PO2 from BOLD MRI.

Our method for estimating tissue PO2 follows two serial steps. In the first step, BOLD data are converted to hemoglobin oxygen saturation (SHb) percentage. Extrapolating from simulations that have been developed to understand brain BOLD signal behavior (5, 17, 27, 45), we developed simulations of renal BOLD signals using parameters such as vascular fraction and blood-vessel radius, specific to the kidney. These simulations provide a lookup table that relates BOLD measurements to SHb values.

SHb, combined with hematocrit and blood volume fraction, indicates the amount of oxygen in intravascular space, not that in extravascular space or tissue PO2. Hence, in the second step we use a tracer kinetic model to derive from SHb the oxygen concentration in the extravascular space, i.e., tissue PO2. Where exogenous tracers have typically been used, several models to estimate tracer kinetics from first-pass injections have been developed, such as the generalized compartmental model (41), distributed-parameter models (18, 22), and renal filtration models (23, 47). These models typically are applied to dynamic acquisitions and do not apply directly to our system. Our BOLD measurements reflect a steady state of oxygen kinetics in renal tissue, assuming constant oxygen delivery and consumption rates at the time of the MR acquisition. Therefore, we propose a distributed-parameter model for oxygen transport in renal tissue that allows estimation of tissue PO2 based on BOLD signals and other inputs.

Address for reprint requests and other correspondence: J. L. Zhang; 729 Arapene Drive, Salt Lake City, UT, 84108 (e-mail: Lei.Zhang@hsc.utah.edu).

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Once developed, we applied our models to porcine BOLD data sets. In these experiments, oxygen microprobes and Doppler flow probes were used to measure renal PO2 and renal blood flow, respectively, at baseline and after furosemide injection. The oxygen microprobe measurements served as the reference standard for the model-derived estimates of tissue PO2 using BOLD MRI. Following validation against the animal data, we then applied our models to a set of BOLD measurements performed in healthy volunteers who also received furosemide injections.

**MATERIALS AND METHODS**

In the following, we will first describe the proposed method for extracting renal tissue PO2 from BOLD MRI data, including two steps: Monte Carlo simulation of renal BOLD signal and an oxygen transit model (Fig. 1). We then describe our experiments with animals and human subjects.

**Monte Carlo simulation of renal BOLD signal.** The goal of the first step of the modeling is to determine a relationship between renal BOLD measurements (R2P, where R2P = R2* - R2 and which is the component of BOLD-measured R2* caused by deoxyhemoglobin) and deoxyhemoglobin concentration or hemoglobin saturation (SHb). As detailed further below, to simulate the formation of BOLD signals in a voxel typical of kidney tissue, we simulate the kidney as a collection of randomly oriented cylindrical blood vessels surrounded by extravascular tissue and specify parameters such as vascular fraction (\(v_v\)), hematocrit (Hct), blood oxygen saturation (SHb), and water diffusion coefficients (D) in intra- and extravascular spaces. Deoxyhemoglobin is paramagnetic, meaning that its magnetic susceptibility is greater than zero. In contrast, diamagnetic substances such as oxyhemoglobin, blood plasma, and extravascular fluid have magnetic susceptibility less than or equal to zero. In a magnetic field B0, two adjacent media with different susceptibility generate an abrupt change in the field, thus creating local field inhomogeneity (AB) near the boundary. In a voxel of human tissue, such boundaries include the surfaces of red blood cells and of blood vessels. By running Monte Carlo simulations, where orientation of blood vessels, positions of red blood cells, and diffusion trajectories of protons are randomized, we compute the predicted effect on local field inhomogeneity and derive predicted BOLD signals. We then fit these values exponentially to obtain R2P, the component of transverse relaxation caused by deoxyhemoglobin. R2P contributes to R2* according to R2* = R2 + R2P, where R2 is the intrinsic spin-spin relaxation that occurs in the absence of deoxyhemoglobin. Repeating the simulations with different SHb values, we obtain the relationship between R2P and SHb.

More specifically, simulating a red blood cell as a sphere of radius \(r_s\), the field inhomogeneity induced at point \(\vec{r}\) (the cell center as origin) can be calculated analytically as (9, 27),

\[
\Delta B_z(\vec{r}) = B_0 \frac{\Delta \chi}{3} \left( \frac{r_s}{r} \right)^3 \left[ 3 \cos^2(\theta) - 1 \right]
\]

where \(\Delta \chi\) is the susceptibility difference between red blood cell and plasma. Other parameters are defined in Fig. 2A. Since the susceptibility difference between fully deoxygenated Hb and plasma is \(\Delta \chi = 0.27\) parts per million (ppm) (37), \(\Delta B_z\) in Eq. 1 equals 0.27(1 - SHb) ppm, where SHb is blood oxygen saturation. At a point surrounded by multiple red blood cells, the field inhomogeneity computed with Eq. 1 can be summed for all the cells to obtain the overall homogeneity at this point.

In the extravascular space, the magnetic field inhomogeneity can be more conveniently computed by considering the effects of blood vessels instead of individual red blood cells. The susceptibility difference \(\Delta \chi\) between inside and outside of a blood vessel depends on hematocrit (Hct), i.e., \(\Delta \chi = 0.27(1 - \text{SHb})/\text{Hct}\) ppm. The magnetic field induced by a blood vessel, as modeled by a cylinder, at point \(\vec{r}\) can be calculated according to Chu et al. (9),

\[
\Delta B_z(\vec{r}) = B_0 \Delta \chi \left( \frac{r_s}{r} \right)^2 \cos(2\phi) \sin^2 \theta
\]

where the parameters are defined in Fig. 2B.

The MR signal measured from a voxel with a BOLD sequence decays with longer echo time due to the dephasing of water molecules that are distributed throughout both the intravascular (IV) and extravascular (EV) spaces of the voxel.

For a location in the EV space, the magnetic field magnitude can be calculated by summing up the BOLD effect from all blood vessels. To simulate EV component of BOLD signal, we construct a voxel (a cube with side length L) and fill it with cylinders of infinite length and radius \(r_v\), so that the intravascular volume reaches a predefined fraction \(v_v\) of the cube’s volume. Only the part of each cylinder that lies within the cube is counted in calculating the vascular fraction. For this study, we assume a random orientation of blood vessels relative to the cube. The location and the orientation of each cylinder are recorded. With the use of Eq. 2, the magnitude of the magnetic field at any point of EV space can be estimated.

Next, we simulate the diffusion of water molecules in the voxel within the echo time (TE) that elapses between excitation and readout. A number \(N_0\) of random locations within the voxel are selected for analysis, representing individual protons. Diffusion is simulated over a series of \(N_D\) time points. As time of diffusion increases incrementally with a step of \(TE/N_D\), these protons diffuse randomly with Gaussian distribution and diffusion coefficient \(D_{\text{EV}}\). Each proton’s trajectory during TE is recorded in an \(N_0 \times 3\) matrix. Because of the inhomogeneity of the magnetic field, protons of different diffusion trajectories accumulate different phase shifts, and the resulting incoherence of phase between protons results in magnitude decay in the collective signal \((S_{\text{EV}})\) from all protons.

To simulate the signal from the intravascular space of a voxel (or IV component of BOLD signal), we start with a randomly oriented
Table 1. Typical parameter values for normal kidneys

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Renal Medulla</th>
<th>Renal Cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>$v_o$</td>
<td>0.25</td>
<td>0.40</td>
</tr>
<tr>
<td>Hct</td>
<td>0.20</td>
<td>0.40</td>
</tr>
<tr>
<td>$r_o$, μm</td>
<td>10 (30%), 4 (70%)</td>
<td>10</td>
</tr>
<tr>
<td>$D_{EV}$, $D_{IV}$, $D_{DIV}$</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>$D_{EX}$, $D_{IX}$</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>SHb</td>
<td>0.40 [0.30, 0.80]</td>
<td>0.90 [0.85, 0.95]</td>
</tr>
</tbody>
</table>

Values in brackets are ranges within which the values were randomly selected for the regression procedure. $v_o$, volume fraction; Hct, hematocrit; $r_o$, radius fraction; $D_{EV}$, extravascular diffusion coefficient; $D_{DIV}$, intravascular diffusion coefficient; SHb, blood oxygen saturation.

cylinder of radius $r_o$ representing the blood vessel. Spheres of radius $r_o$ representing red blood cells are added in the cylinder until the volume taking up by the spheres reaches a predefined hematocrit (Hct) value. The location of each sphere within the cylinder is randomly chosen and is fixed during the process (blood flow can be ignored during TE, on the order of milliseconds). At time zero, $N_o$ protons are randomly distributed throughout the axial plane orthogonal to the cylinder axis at the middle point of the cylinder, representing protons with the same phase at any arbitrary location along a vessel. As the simulation time increases incrementally, protons are allowed to diffuse from this plane in both directions along the long axis of the cylinder, with diffusion coefficient $D_{EV}$. Similar to the EV simulation, the collective signal magnitude $S_{IV}$ is computed.

The BOLD signal from an entire voxel is the sum of the signals from intravascular and extravascular compartments,

$$S = (1 - v_o) \cdot S_{EV} + v_o \cdot \varepsilon \cdot S_{SV} \cdot S_{IV},$$

where $\varepsilon$ is the ratio of intrinsic signals, including the effect of relaxation weighting (27), and $v_o$ is blood volume fraction in a voxel. The weighting of $S_{IV}$ in the second term reflects the contribution of all other vessels to the signal decay of IV protons that we did not take into account in the above section.

Exponential fitting of the $S$ vs. TE curve results in a decay time constant that equals $1/R_{2P}$. In our simulation, we repeated the entire process $N_{ave}$ times, and all $R_{2P}$ values were averaged. We used the following parameters values for simulation: $L$ 160 μm, $r$ 3 μm, $N_r$ 1,000, $N_o$ 1,000, $r_o$ 1.4, TE from 0 to 60 ms with even interval of 2 ms, and $N_{ave}$ 10. Other parameters related to renal tissue, such as SHb in renal cortex and medulla, are given in Table 1. All components for the simulation were programmed with Matlab (Mathworks, Natick, MA). Each run ($N_{ave} = 10$) took $\sim$150 min on a personal computer (Intel Core i7@2.8GHz, RAM 4.0 GB).

We performed the Monte Carlo simulation of R2P with a series of SHb values (Table 1) and created a look-up table relating SHb to $R_{2P}$. All other parameters besides SHb related to renal tissue were fixed at their typical values for cortex or medulla (Table 1).

Since we found that for the parameter values we used in this study, the relationship between SHb and $R_{2P}$ is approximately linear, we performed a linear regression for the discrete points [$R_{2P}$, SHb] to obtain a linear equation that provides a SHb estimate for any given $R_{2P}$ value.

While we refer to the $R_{2P}$ as the relaxation rate derived from the BOLD MR measurements, in reality BOLD MR measurements ($R_{2P}$) include both the effects of $R_2^*$ as well as the effects of $R_1$. $R_{2P}$ values or maps are obtained by subtracting $R_2$ from BOLD $R_{2P}^*$, $R_2$ is measured with single-shot turbo spin-echo (TSE) sequence repeated for multiple different echo times.

Oxygen transit in renal tissue: link between intra- and extravascular oxygen. Oxygen is transported in blood in two ways: most bound with hemoglobin in red blood cells in form of oxyhemoglobin and a little dissolved in plasma (~0.003 ml O2 per 100 ml blood for each mmHg PO2). The equilibrium between the oxygen saturation of hemoglobin (SHb) and the oxygen partial pressure of plasma ($P_1$), can be expressed by Hill’s equation (24),

$$SHb = \frac{P_1^4}{P_1^4 + P_{50}^4}$$

where $h$ ($\sim 2.55$) is Hill’s coefficient and $P_{50}$ ($\sim 26$ mmHg) is oxygen partial pressure at SHb of 0.50. With Eq. 4, we can estimate SHb for a known $P_1$. Alternatively, we can estimate $P_1$ for a known SHb.

The overall concentration of oxygen in blood, $C_1$, can be calculated as

$$C_1 = (1 - Hct) \cdot \alpha_1 \cdot P_1 + Hct \cdot C_{hgb} \cdot SHb \cdot 4$$

where Hct is hematocrit, $\alpha_1$ is oxygen solubility in plasma (unit: mmol·ml⁻¹·mmHg⁻¹), and $C_{hgb}$ is hemoglobin concentration in red blood cell (unit: mmol/ml RBC). The factor 4 in Eq. 5 reflects the capacity of each hemoglobin molecule to carry four oxygen molecules. With Eqs. 4 and 5, we can estimate $C_1$ from SHb.

As blood transits through renal capillaries, oxygen in the blood diffuses into the extravascular interstitial space, causing a decrease in $C_1$. The diffusion of oxygen out of blood is driven by the lower oxygen partial pressure in the interstitial space (termed $P_2$) than $P_1$. $P_2$ is commonly known as tissue PO2 and a key parameter of tissue function. For animal studies, $P_2$ has been measured by inserting oxygen microprobes directly into tissues of interest.

In the previous section, we described a Monte Carlo approach for estimating the oxygen saturation of hemoglobin (SHb) from BOLD MRI data (Fig. 1). To determine tissue PO2 ($P_2$) from SHb, a separate model is needed to take into account the transit of oxygen across the capillary wall and into the interstitial space. A schematic diagram for such a model is shown in Fig. 3. During a BOLD MRI scan, the kidney can be assumed to have constant oxygen input from arterial inflow and steady venous outflow. The vascular space is modeled as a cylinder of length $L$, parameterized by variable $x$ that ranges from $x = 0$ at the arterial entry to $x = L$ at the venous exit. The cylinder is surrounded by an outer layer that represents renal tissue. $C_1(x)$ is the concentration of $O_2$ within the vascular space (Eqs. 4 and 5). Its change along $x$ can be characterized by,

$$\rho \cdot F \cdot \frac{dC_1(x)}{dx} = -\frac{K}{L} \left[ \alpha_1 \cdot P_1(x) - \alpha_2 \cdot P_2 \right]$$

where $\alpha_1$ and $\alpha_2$ are oxygen solubility coefficients in plasma and in interstitial fluid, $F$ is blood perfusion rate (ml·min⁻¹·g tissue⁻¹), and $\rho$ is tissue density (g/ml). Parameter $K$ denotes the transfer rate of oxygen from vascular to interstitial space and reflects both the physiological area product of vessel membranes and oxygen diffusion in the interstitial fluid. The relationships among $P_1$, SHb, hematocrit (Hct), and $C_1$ are characterized in Eqs. 4 and 5. Parameters $\alpha_1$ and $\alpha_2$ are oxygen solubility in plasma and in interstitial fluid, respectively.
meability-surface area product of vessel membranes and oxygen diffusion in the interstitial fluid. Within the vascular space, the oxygen content C(T) decreases along the axial direction. Since within the extravascular space oxygen is most likely mixed due to the convoluted blood vessels, we assume a constant transfer constant K and same tissue PO2 (P2) for an entire voxel for simplification.

To solve Eq. 6 for P2, we discretize the vascular space from x = 0 to x = L, into N discrete steps. With the use of an optimization technique, P2 is adjusted and P1(x) is calculated repeatedly, until the average P1 (interchangeable with SHb by Eq. 4) matches the value estimated from BOLD. We use literature values for other tissue parameters: \( \alpha_1 = 1.3 \times 10^{-5} \) mmol·ml\(^{-1}\)·mmHg\(^{-1} \), \( \alpha_2 = 1.25 \times 10^{-6} \) mmol·ml\(^{-1}\)·mmHg\(^{-1} \), \( C_{\text{dib}} = 2.13 \times 10^{-2} \) mmol/ml RBC, Hill’s coefficient \( h = 2.55 \), half-saturation PO2 \( P_{0,2} = 26 \) mmHg, arterial PO2, \( P_1 = 100 \) mmHg. Some parameters are likely to differ across subjects in a physiologically important way, including perfusion P (measurable with either contrast-enhanced MRI or arterial spin labeling MRI), oxygen transfer constant K and capillary hematocrit Hct. Below we describe an approach for estimating K and capillary Hct using animal data.

Animal experiments with BOLD MRI and oxygen probe measurements. Porcine experiments were approved by the Animal Care and Use Committee at Mayo Clinic. A total of 20 domestic pigs (after overnight fast) were examined, while the animals were under general anesthesia with mechanical ventilation. An ear vein catheter was inserted in the pig for saline infusion (5 ml/min), and a bladder catheter was placed to collect urine output. The pig was placed in an MRI scanner (Signa TwinSpeed EXCITE 1.5T system; GE Healthcare, Waukesha, WI). For BOLD imaging, a gradient multiecho sequence was used to acquire 16 echoes with parameters: TR/TE/flip angle/BW/FOV/matrix/thickness/NEX = 85 ms/2.3–37.2 ms/40/32 cm/63.95 kHz/256 × 256/5–7 mm/1. At each TE we acquired five to six oblique slices. To eliminate motion artifact, respiration as controlled by mechanical ventilation was suspended for short periods of time for BOLD scan. BOLD measurements were performed at baseline and repeated within 15 min after a bolus of either furosemide (0.05 mg/kg, 12 pigs) or the same volume of saline (8 “control” pigs). The same volume of saline (8 “control” pigs).

During postprocessing, a monoexponential function was fitted to the multiple gradient echoes (mGRE) to generate a map of \( R_{2*} \). On the \( R_{2*} \) map, regions of interest (ROI) were placed in cortex and medulla in both kidneys, and ROIs of a same compartment (cortex or medulla) were averaged for both kidneys. Five to seven days after the MRI exam, the same animals were prepared for PO2 measurement before and after injection of furosemide or saline. Through a catheter position in the left carotid artery, arterial PO2 was sampled and mean arterial pressure was measured. The left and right ureters were exposed and cannulated through small flank incisions. The right kidney was also exposed and surrounded by cotton wool soaked in saline and mineral oil and was placed in a lexan holder. An ultrasound flow probe (T206 Flowmeter; Transonic, Ithaca, NY) was placed around the renal artery to measure renal blood flow. To estimate cortical and medullary perfusion, renal blood flow measured with ultrasound was split for cortex and medulla and then divided by their respective volumes based on previous CT scans of the same type of animals (10). Ventilation rate and tidal volume were adjusted to maintain arterial PO2 between 90 and 110 mmHg, PCO2 between 35 and 50 mmHg, and pH between 7.3 and 7.5, respectively. After calibration, tissue PO2 was measured every second for the duration of the experiment from the kidneys with Clark electrodes (Unisense, Aarhus, Denmark) inserted into the right kidney to a depth 0.5–0.8 cm for cortex and 1.0–1.2 cm for outer medulla. The probe placements were subsequently verified with histologic staining of cortex and medulla.

With the above animal data, we calibrated the proposed oxygen transit model by determining the two parameters that are difficult to measure in vivo, oxygen transfer constant K and capillary hematocrit Hct. Specifically, we used the data from eight “control” animals and a backward fitting approach to determine the values for K and Hct that minimized the differences between the model-computed tissue oxygenation (P3) and the microprobe-measured value. The optimized K and Hct values were used to compute the P2 values from BOLD images in the 12 pigs both before and after furosemide. The differences (average ± SD) between the model-computed MRI-based P2 and the microprobe-measured P2 values from the 12 pigs were used to calculate the prediction error of the model.

Healthy human subjects with furosemide. This study was Health Insurance Portability and Accountability Act compliant and Institutional Review Board approved (New York University). Informed consent was obtained from nine healthy human subjects (age: 25.7 ± 3.0 yr, 5 males) with no history of kidney disease. The subjects fasted overnight and were asked to void before the experiment. MRI was performed on a 3-T MRI scanner (Tim Trio; Siemens Medical Solutions, Erlangen, Germany) with a multielement phased array coil. BOLD measurements were made using a two-dimensional (2D) mGRE sequence with a water-selective excitation pulse (TR/flip angle/BW/FOV/slice thickness, 70 ms/300/300 Hz/pixel/420 × 336 mm/320 × 272/7 mm) to acquire 12 coronal images with TE from 4.3 to 42.7 ms. The acquisition was performed within a 15-s breath hold. For R2 measurement, a 2D multiple spin-echo (mSE) sequence was performed to acquire images with TE from 22 to 153 ms. Other parameters were TR/flip angle/BW/parallel acquisition/turbo factor 700 ms/180°/521 Hz/pixel/GRAPPA 2/4. The imaging slice and spatial resolution were matched to the BOLD sequence. The acquisition was performed within a 22-s breath hold. Before furosemide injection, mGRE and mSE were performed twice at time points separated by ~10 min. Twenty milligrams of furosemide were injected intravenously over 10 s and flushed with 10 ml of saline. mGRE and mSE imaging were started 5 min after furosemide and repeated seven times over ~50 min. The subject was allowed to get out of the scanner for voiding, after which MR acquisition resumed immediately.

In postprocessing, an exponential function was fitted to each set of mGRE data pixel-by-pixel to obtain a map of R2*. And mSE data were similarly fitted to give a map of R2. Each R2* map was manually registered to the R2 map that was acquired at a same time point. The registered R2* and R2 maps were then subtracted to obtain an R2P map. In the R2 map acquired at baseline, where corticomedullary contrast was relatively large, ROIs were drawn manually in renal cortex and medulla by a radiologist (Vivier P.-H., 10 years experience in kidney imaging), with care taken to avoid susceptibility artifact in R2*. The same ROIs were then copied to R2P maps from other time points and were manually adjusted if necessary. Finally, all ROIs were copied to R2P maps of the same time points. R2P values from ROIs of the same tissue type (cortex or medulla) and the same time point were averaged.

Using the R2P–SHb relationship derived from the Monte Carlo simulation for Bo of 3.0 T, we converted the R2P values measured from the human subjects to SHb estimates, which were further input into the oxygen transit model for computing tissue PO2 (P2) for renal cortex and medulla. To implement the oxygen transit model, we used the K and Hct values previously determined from pig data. Without measuring renal perfusion for the healthy subjects, we assumed values from literature (1, 36), 300 ml·min\(^{-1}\)·100 g\(^{-1}\) for cortex and 50 ml·min\(^{-1}\)·100 g\(^{-1}\) for medulla.

RESULTS

Relationships between R2P and SHb derived from Monte Carlo simulation. Using typical values for tissue parameters of healthy kidneys, we ran the Monte Carlo simulation and obtained a linear relationship between R2P and SHb. The simulation was performed for two different Bo levels, 1.5 and 3.0 T, separately. The magnitude of Bo has an impact on the
relationship because the same magnetic susceptibility difference (determined by concentration of deoxyhemoglobin in our case) produces different levels of magnetic field inhomogeneity for different B0 levels and thus different signal decay rates. Figure 4 shows the results for Monte Carlo simulation at both 1.5 and 3.0 T and the regression lines. The linear regression equations for B0 of 1.5 and of 3.0 T are listed in Table 2. For comparison, we also show the equations that were originally derived with analytic method (45). We adapted these equations for renal cortex and medulla by using parameter values in Table 1. To compare the equations, we computed R2P values at relatively low SHb values (0.8 for renal cortex, 0.3 for medulla), which represent hypoxic state in some renal diseases. The differences between R2P values of analytic method and of numerical method were not more than 3.2 s−1, except for renal medulla at 3.0 T, where the analytic formula R2P estimate was 12.1 s−1 or 61% higher than our numerical formula.

Model calibration and validation with animal experiment. From the animal experiments with both BOLD data and probe-measured Po2, we estimated two parameters in the oxygen transit model, capillary Hct and transfer constant K. Table 3 shows the Hct and K values estimated from the eight control pigs. The transfer constant K was lower in the cortex than that in the medulla. Renal K values (4.5 ml·s−1·ml−1 for cortex, 18.9 ml·s−1·ml−1 for medulla) were small compared with K for the heart, which has been estimated as ~50 ml·s−1·ml−1(6). These results are supported by the findings by previous studies that a countercurrent arrangement of blood vessels in the kidney, especially the medulla, contributes to the low net oxygen transfer rate to the cells (50). The coefficient of variation of Hct across the individual animals was 26−28%, and the coefficient of variation for K was 18−19%, most likely due to individual differences and measurement error in R2*

and Po2.

Applying the model with calibrated K and Hct values to the pig data with furosemide resulted in Po2 estimates comparable to probe measured values (Table 4). Mean absolute Po2 errors for baseline data before furosemide injection averaged 2.3 mmHg for cortex and −0.1 mmHg for medulla, indicating accurate prediction by the model. Although the model used K and Hct values optimized with baseline data only, it worked reasonably well for the postfurosemide data, with Po2 prediction error of 2.6 ± 4.0 mmHg for medulla and 6.9 ± 3.9 mmHg for cortex. For baseline and furosemide data combined, the prediction error for renal cortex Po2 averaged 4.6 ± 5.1 mmHg and for renal medulla 1.3 ± 4.4 mmHg.

Human subjects with furosemide injection. In our experiment with nine healthy human subjects (Table 5), baseline R2P in renal medulla averaged 19.5 ± 3.3 s−1. The injection of furosemide reduced medullary R2P to 12.7 ± 2.0 s−1 at 5 min after furosemide injection. Medullary SHb, converted from R2P by our Monte Carlo model, increased from 31 ± 11% at baseline to 53 ± 6% at 5 min after furosemide administration, while the converted tissue Po2 values increased from 16.0 ± 4.9 to 26.2 ± 3.1 mmHg. At 1 h after injection, the medullary parameters recovered towards their baseline level (Table 5). The parameters for renal cortex did not respond to the injection of furosemide: R2P 6–7 s−1, SHb 92–93%, and P2 57–59 mmHg.
DISCUSSION

Due to its invasive nature, probe measurements of tissue PO2 are not applicable for human kidneys in vivo. BOLD MRI offers a noninvasive technique to monitor intravascular deoxyhemoglobin concentration in each voxel. Because of the many factors other than tissue PO2 that contribute to BOLD signal, BOLD data require more complex analytic interpretations to derive renal tissue PO2. We proposed a two-part model: a Monte Carlo simulation model that simulates the formation of BOLD signal and enables the estimation of blood oxygen saturation SHb, and an oxygen transit model that estimates extravascular tissue PO2 based on intravascular SHb. Animal experiments show high agreement between probe-measured and MRI-estimated tissue PO2 using our method. For young healthy human subjects, tissue PO2 in renal medulla, as estimated by our method, increased from ~16 to ~26 mmHg after injection of furosemide, while that in renal cortex remained at ~58 mmHg.

Previous studies have shown that furosemide, water loading, and many renal diseases can reduce or even eliminate the normal corticomedullary differences in R2* (25, 32, 44). Our experiment with healthy volunteers found similar result: following furosemide, medulla R2* dropped to a level only 4 s−1 (~20% of medulla R2*) higher than cortex R2*. If R2* is considered to be a surrogate for tissue oxygenation, then the reduction found in this and other previous studies would seem to imply that furosemide eliminates the difference in tissue PO2 between renal cortex and medulla. However, after applying our models relating BOLD signals to tissue oxygen level, we found that after furosemide challenge tissue PO2 in renal medulla was still <50% of that in renal cortex (~26 vs. ~58 mmHg; Table 5). This discrepancy between R2* and the more physiologically meaningful parameters can be explained by noting that with less vascularity and lower hematocrit in renal medulla (i.e., less hemoglobin), a similar level of R2P or R2* actually corresponds to much lower tissue PO2 in medulla than in cortex. Recent studies have shown that parameter “corticomedullary R2* ratio” (CMR) is promising in diagnosing multiple renal diseases (14, 40, 43, 46). Based on our current study, it should be noted that CMR does not equal to corticomedullary ratio of tissue PO2 or oxygen consumption rate.

Table 4. Tissue PO2 prediction for 12 pigs with the proposed model

<table>
<thead>
<tr>
<th></th>
<th>Baseline PO2, mmHg</th>
<th>Furosemide PO2, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Measured</td>
<td>Predicted</td>
</tr>
<tr>
<td>Cortex</td>
<td>45.9 ± 3.6</td>
<td>48.2 ± 3.4</td>
</tr>
<tr>
<td>Medulla</td>
<td>25.0 ± 3.5</td>
<td>24.9 ± 2.1</td>
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</tbody>
</table>

From our Monte Carlo simulations, MRI-measured R2P and SHb showed an approximately linear relationship for both renal medulla and for cortex when other factors were held constant (Fig. 4). The linearity between SHb and R2P partially supports the widely used R2P or R2* as a surrogate for renal oxygenation in literature. However, it should be noted that the simulations were done with all parameters (except for SHb) fixed at their typical values. During physiologic challenge and in some renal diseases, multiple physiologic parameters could deviate substantially from their normal range and introduce complicated confounding contribution to R2*. For instance, in chronic kidney disease, the number of peritubular capillaries is decreased, which decreases the vascular fraction of the medulla and alters the relationship between R2* and PO2. To quantify the impact of the parameters to our Monte Carlo simulation, we performed sensitivity analysis with regard to each parameter (see Appendix). The analysis shows that among all the parameters tested, both vascular fraction Vv and hematocrit Hct correlate with R2P in a positive linear way (Fig. 5). For patients with renal diseases that may change one or more of the parameters substantially, some a priori knowledge about the likely changes can help determine whether our model can be applied or whether modifications need to be made and Monte Carlo simulations rerun.

Linear relationship between R2P and SHb was also found in brain functional MRI, either with analytic derivation (45) or numerical simulation (29). By using vascular parameter values typical of the kidneys, we adapted these equations and compared them with our linear equations in Table 2. The differences between our equations and the equations of literature could be due to the fact that the literature equations were developed primarily for brain where fractional blood volume is only 2–4% and SHb is much higher than 0.3. In future studies we will investigate these differences further.

The oxygen transit model was built based on our experience of tracer kinetic modeling for analyzing dynamic contrast-enhanced imaging data. A century ago, August Krogh proposed a model for oxygen supply to skeletal muscle (20, 21, 35). In Krogh’s model, a cylinder is used to approximate a bundle of muscle fibers surrounding a central blood vessel that supplies oxygen; in kidney tissue, our model is comprised of convoluted blood vessels with heterogeneously distributed concentrations of intravascular oxygen along the axial direction and supplies oxygen only to nearby surrounding tissue. This distributed-parameter model, partly inspired by the Krogh model, has been widely validated in tumor imaging literature (19, 22, 38) and is used to analyze the transit of intravenously injected tracer through tumor tissue. Because of the convoluted arrangement of the blood vessels and the steady-state conditions during the BOLD MRI scan, it is appropriate to assume well mixing of oxygen, i.e., a single tissue PO2, within the tissue.

Table 5. Tissue oxygen parameters of renal medulla in young healthy human subjects, as measured by BOLD MRI (at 3.0 T) and the proposed models

<table>
<thead>
<tr>
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<th>R2P, s−1</th>
<th>SHb</th>
<th>Tissue PO2, P2, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>19.5 ± 3.3</td>
<td>31 ± 11%</td>
<td>16.0 ± 4.9</td>
</tr>
<tr>
<td>5 min after furosemide</td>
<td>12.7 ± 2.0</td>
<td>53 ± 6%</td>
<td>26.2 ± 3.1</td>
</tr>
<tr>
<td>60 min after furosemide</td>
<td>14.3 ± 3.3</td>
<td>48 ± 10%</td>
<td>23.8 ± 5.0</td>
</tr>
</tbody>
</table>

Values are means ± SE. BOLD, blood oxygen level dependent.
For the ultimate goal of accurately estimating renal tissue P\textsubscript{O2} from BOLD data, additional imaging data including R\textsubscript{2}, perfusion and vascular fraction are necessary. Fortunately, these parameters can be measured in vivo in the same session and with the same spatial resolution as the BOLD acquisition. Some other parameters such as capillary Hct might be relatively constant across different human subjects (33, 51), and for these parameters assumed values may be adequate. The oxygen transfer constant \( K \) can be affected by multiple factors, including blood vessel permeability (or the fraction of permeable vessels in a voxel or ROI), restricted diffusion within the interstitial space, and shunting effect (e.g., due to the counter-current arrangement of blood vessels). We expect that as renal function decreases, oxygen may diffuse more slowly within the interstitial space due to tissue fibrosis (42). In the future, the relationship between \( K \) and renal function deserves further research.

Our study has multiple limitations. First, for the human subjects, we did not measure tissue P\textsubscript{O2} with invasive oxygen probes to validate the accuracy of our MRI-estimated results. The models were calibrated with pig data and showed high accuracy for MR-derived P\textsubscript{O2} compared with probe-measured P\textsubscript{O2}. Second, our assumptions made in the Monte Carlo simulation to obtain the R\textsubscript{2P}-SHb relationship, including the literature-based values for cortical and medullary vascular fractions and values for diffusion coefficients, could cause some error in the SHb estimates. We performed sensitivity analysis for all parameters, as shown in the APPENDIX. Future work will incorporate in vivo measurement of vascular fraction and diffusion coefficients by MRI techniques (48, 49). Third, our approach did not correct for macroscopic B\textsubscript{0} inhomogeneity (8, 15), which could severely distort BOLD signals. This artifact could be minimized by better B\textsubscript{0} shimming technique (2) and by higher spatial resolution in the BOLD images. Fourth, we did not include patients with renal diseases or dysfunction in this study. In some renal diseases, some physiologic parameters of the tissue P\textsubscript{O2} model might deviate from their normal value, which would complicate the estimation of tissue P\textsubscript{O2}. Our error analysis shows that inaccuracies in tissue perfusion and vascular fraction cause the largest errors in estimated tissue P\textsubscript{O2}.

In conclusion, we have presented and tested a new analytic approach for deriving tissue P\textsubscript{O2} from renal BOLD measurements. We have shown that our P\textsubscript{O2} measurements result in different physiologic interpretations of renal oxygenation compared with conventional R\textsubscript{2*} BOLD results. After being calibrated with a group of pig data, the technique produced accurate P\textsubscript{O2} values for furosemide-stimulated pig kidneys based on renal microprobe measurements. When applied to human kidney, our BOLD method produced reasonable estimates of renal cortical and medullary P\textsubscript{O2} in healthy volunteers before and after furosemide. The proposed technique opens the door to noninvasive renal tissue P\textsubscript{O2} measurements using BOLD MRI.

**APPENDIX: MODEL SENSITIVITY ANALYSIS**

Our renal tissue P\textsubscript{O2} estimation with two serial steps, Monte Carlo simulation and then the oxygen transit model, involves multiple parameters. To evaluate how each model is impacted by its parameters (either measured or assumed), we performed sensitivity analyses as described below.

**Monte Carlo simulation model.** For the Monte Carlo simulation model, we repeated the simulation while varying each of the following parameters: vascular fraction \( v_c \), hematocrit Hct, radius of blood vessel \( r_a \), diffusion coefficients \( D_{IV} \) and \( D_{EV} \), and blood oxygen saturation SHb, and we recorded the simulated BOLD signal R\textsubscript{2P}. In this study we performed a sensitivity analysis for renal medulla only (Fig. 5). The results show that an increase in \( v_c \) caused a proportional increase in the resulted R\textsubscript{2P}, with an increased slope of \( \sim 1 \text{ s}^{-1}/\text{per } 1\% \text{ vascular fraction} \) (Fig. 5A). A similar pattern and slope were found for hematocrit (Fig. 5B). With the overall vascular fraction fixed, an increase of the radius of blood vessels, up to \( \sim 10 \mu \text{m} \), led to an increase of R\textsubscript{2P}, while any further increase did not change R\textsubscript{2P} (Fig. 5C).

**Oxygen transit model.** For the oxygen transit model, parameters include tissue perfusion F, capillary hematocrit Hct, and oxygen transfer constant K. Note that Hct shows up in both models but with
different effects: in the Monte Carlo simulation, higher Hct corresponds to more deoxyhemoglobin, while in the oxygen transit model, higher Hct corresponds to more hemoglobin-bound oxygen (Eq. 5). We computed tissue oxygenation P2 with each above parameter varying within its range and the other parameters fixed at their typical values. We also ran the model backward to test with a fixed value for tissue P02 how BOLD R2* would change as each model parameter is adjusted. Typical values used for the analysis were: FCx = 0.042 ml·s⁻¹·ml tissue⁻¹, Kcx = 4.5 ml·s⁻¹·ml tissue⁻¹, Hct cx = 0.32, P1,Cx = 62 mmHg (~ShB 90%), P1,Med = 0.040 ml·s⁻¹·ml tissue⁻¹, Kmed = 18.9 ml·s⁻¹·ml tissue⁻¹, Hct med = 0.19, and P1,Med = 33 mmHg (~ShB 65%). Each independent variable was varied within ±50% of the typical value. Results (Fig. 6) show that an increase in any parameter (F, Hct, or K) causes an increase in tissue P02. These findings are as expected since each parameter relates to oxygen delivery rate. In the renal medulla, the impact of K on P2 was weaker than the impact of F or Hct, probably because in the renal medulla F or Hct is the dominant factor limiting the delivery of oxygen to tissue. The opposite holds for the renal cortex where blood perfusion is adequate but vessel permeability is restricted. In Fig. 6B, we observe that an increase in F or Hct results in a decrease in R2*, due to the decreased amount of deoxyhemoglobin. However, a higher K increased the amount of deoxyhemoglobin in the voxel and thus R2*.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: J.L.Z., G.M., H.R., P.-H.V., A.K.C., L.O.L., and V.S.L. conception and design of research; J.L.Z., G.M., H.R., A.K.C., L.O.L., and V.S.L. inter- vened on tissue PO2 fixed. Along x-axis, independent variables (perfusion F, hematocrit Hct, or oxygen transfer rate K) varied between 0.5 and 1.5 times of their respective typical value. Note that the results for F and Hct are the same.

**REFERENCES**


