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Repeatability and Sensitivity of High Resolution Blood Volume Mapping in Mouse Kidney Disease

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Abstract

Purpose—To evaluate the repeatability of MRI-derived relative blood volume (RBV) measurements in mouse kidneys across subjects and days and to evaluate sensitivity of this approach to renal pathology.

Materials and Methods—A 7T MRI system and an intravascular iron-oxide contrast agent were used to acquire spin-echo based renal RBV maps in ten healthy mice on two consecutive days. Renal RBV maps were also acquired in the Alport and unilateral ureteral obstruction mouse models of renal disease.

Results—The average renal RBV measured on consecutive days was 19.97 ± 1.50 and 19.86 ± 1.62 , yielding a concordance correlation coefficient of 0.94, indicating that this approach is highly repeatable. In the disease models, the RBV values were regionally dissimilar and substantially lower than those found in control mice.

Conclusion—*In vivo* renal iron-oxide based RBV mapping in mice complements the physiological information obtained from conventional assays of kidney function and could shed new insights into the pathological mechanisms of kidney disease.

Keywords

Susceptibility contrast MRI; renal models of disease; renal function; renal blood volume; renal perfusion

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INTRODUCTION

Genetic engineering and transgenic technology have led to advances in the generation of mouse models that develop aspects of common renal pathologies. In conjunction with traditional measures of renal function, the physiological assessment of such models would benefit greatly from the development of noninvasive imaging methods capable of evaluating disease progression. Given that many renal pathologies involve vascular and/or perfusion abnormalities, imaging methods that specifically provide robust, serial measurements of kidney hemodynamic status could be of great utility. Towards this end, several Magnetic Resonance Imaging (MRI) methods, employing either endogenous or exogenous contrast mechanisms, have demonstrated great promise for the non-invasive assessment of kidney perfusion and oxygen delivery (1–6). However, the application of these methods has primarily been limited to humans, pigs, rabbits and rats. Thus, there is a compelling interest to optimize and validate MRI-based measures of hemodynamics in the context of mouse models of renal disease.

A common approach to assess renal perfusion and function is through the use of paramagnetic gadolinium based contrast agents. Such small molecular weight contrast agents typically require high temporal resolution imaging methods in order to track their dynamic passage across the vascular endothelium and through the glomeruli as they are excreted. However, given the relative small size of the mouse kidney (0.9 cm × 0.5 cm × 0.2 cm), high-resolution imaging methods are essential for regional characterization of the renal vasculature thereby limiting the potential of utilizing small molecular weight contrast agents for this purpose.

In contrast, the use of intravascular superparamagnetic iron oxide nanoparticles, which are advantageous due to their long plasma half-life and lack of filtration at the glomerulus, enables the acquisition of high-resolution steady state measures of tissue blood volume (7). Iron oxide nanoparticles induce substantial susceptibility differences between the intra- and extravascular space and decrease both T_2 and T_2^* relaxation times. In tissue, the change in the MRI signal before and after contrast agent injection is linearly proportional to the local blood volume fraction. Recently, Prasad et al demonstrated the sensitivity of iron-oxide nanoparticle based renal blood volume measurements in rats to adenosine-induced vasodilation (8).

The purpose of the present study was to assess the repeatability of renal blood volume measurements in mice and determine their sensitivity to disease induced vascular abnormalities. To our knowledge, the repeatability of renal blood volume measurements in animal models has yet to be determined. Such studies are warranted given that several factors could influence their ability to reliably assess serial changes in the renal vasculature. Given the same contrast agent dosage per kg body weight, RBV differences could occur due to individual variations in animal body composition, body temperature, carbon dioxide levels and response to anesthesia (9). Due to these potential sources of uncertainties it is important to evaluate the repeatability of RBV measurements in mice. To evaluate the sensitivity of this approach to disease, RBV measurements were acquired in mouse models of Alport's Syndrome (10,11) and unilateral ureter obstruction (UUO) (12–14).

MATERIALS AND METHODS

Animals

Male C57BL/6N mice, aged 6 weeks, were purchased from Harlan Laboratories (Indianapolis, IN). Animals were fed a standard diet and allowed free access to water. All animal procedures were conducted in accordance with institutional guidelines and approved

by the Institution of Animal Care and Use Committee. For the Unilateral Ureteral Obstruction (UUO) mode, mice were anesthetized with 2% isoflurane and the left kidney was exposed through the site of the left flank incision. The ureter was obstructed completely near the renal pelvis using a 4-0 silk tie suture at two points. Sham-operated mice underwent the same surgical procedure except for the ureter ligation. The mice were imaged at 0 (3 hours), 1, 3, and 6 days after surgery. The Alport mice with 129X1/SvJ background (10,11) were imaged at seven weeks of age.

In Vivo imaging

MRI studies were performed on a Varian 7T horizontal bore imaging system. Anesthesia was induced and maintained with a 1.5%/98.5% isoflurane/oxygen mixture, and a constant body temperature of 37°C was maintained throughout the experiment using heated air flow. A specific-shaped holder was designed for mouse and a bite bar was applied to reduce motion. Animals were placed in a prone position and imaged using a birdcage 25-mm inner diameter radiofrequency coil. A multi-slice gradient echo imaging sequence (repetition time (TR) = 25 ms; echo time (TE) = 2.5 ms; 128 × 128 matrix; 25.6 × 25.6 mm² FOV; 2-mm slice thickness) was used to acquire 3-plane multi-slice images for proper positioning and slice selection. A coherent gradient echo sequence was used for T₁-weighted anatomic imaging (TR = 45 ms; TE = 2.25 ms; flip angle = 35°). Post-contrast enhanced images were collected using multi-gradient echo multi-slice sequence (TR = 350 ms; TE_{min} = 2.8 ms; echo spacing (ESP) = 3 ms; 8 echoes). Respiration gating was applied with 500 ms delay and 100 ms duration to reduce motion artifacts.

The imaging protocol for the assessment of RBV consisted of a T₂-weighted multi-slice fast spin echo (FSE) sequence (TR = 2000ms; effective TE = 48 ms; RARE-factor 8, 256 × 256 matrix; 25.6 × 25.6 mm² FOV; 0.5-mm slice thickness, scan duration ~ 10 min). The use of T₂-weighted images for the RBV acquisition minimizes sensitivity to both large blood vessels and static magnetic field inhomogeneities.⁽¹⁵⁾ To improve the signal to noise for RBV mapping we also evaluated a reduced resolution of 0.2×0.2×0.5 mm³. Images were collected before and after the administration of the superparamagnetic iron-oxide contrast agent, Molday-ION (Biophysics Assay Laboratory, MA, USA), which has a colloidal size of 30 nm. To determine the optimum contrast agent dose we systematically varied the injected dose of Molday-ION between 2 and 12 mg/kg. To assess the repeatability of the RBV measurements, MRI data was acquired in the control animals on two consecutive days. To verify that Molday-ION remains within the blood vessels over the duration of the RBV mapping sequence a fast gradient echo sequence (TR = 15 ms; echo time (TE) = 4 ms; 64 × 64 matrix; 25.6 × 25.6 mm² FOV; 1 mm slice thickness) with at high temporal resolution (~1s per volume) was used to assess the dynamics of Molday-ION transit through the kidney.

Data Analysis

All data were analyzed using Matlab 2009a (The Mathworks Inc., Natick, MA, United States). Pre- and post-contrast images were registered using a rigid registration algorithm. The RBV maps were computed as:

$$RBV = -\frac{1}{TE} \log \frac{S_{post}}{S_{pre}} \quad [1]$$

where S_{post} and S_{pre} are the post- and pre-contrast signals, respectively. The Contrast-to-noise ratio was defined as:

$$CNR = -\frac{1}{TE} \log(S_{post}/S_{pre})/\sigma_{post} \quad [2]$$

where σ_{post} is the standard deviation. The high-resolution anatomic images were used to manually segment the kidneys. The following regions of interest were identified and segmented on the high-resolution MR images: the cortex, the outer stripe of the outer medulla, the inner strip of outer medulla, inner medulla and papilla (Figure 1). The average RBV values and standard deviation across voxels in both kidneys and in different ROIs were calculated based on the above ROI definition. The mean RBV values and standard deviation across subjects and days were obtained afterwards. Statistical differences between measurements and groups were evaluated using t-test. The repeatability between RBV measurements on consecutive days was also assessed using the concordance correlation coefficient.

RESULTS

Representative *in vivo* MR images of normal mouse kidneys are shown in Figure 1. The pre-contrast T_1 (Fig. 1A) and T_2 (Fig. 1B) weighted images enable differentiation between the major anatomical regions of the kidney including the cortex, outer medulla, inner medulla and papilla. The high-resolution pre-contrast T_2 -weighted images ($0.1 \times 0.1 \times 0.5 \text{ mm}^3$) shown in Figure 1B clearly delineate fine structures such as the medullary rays. The post-contrast T_2 -weighted image (Fig. 1C) exhibits the expected signal drop throughout the kidney. The RBV map in Fig. 1D demonstrates that regional variations in renal microvascular density can be resolved in mice.

Figure 2 illustrates an example average time course of Molday-ION kinetics through a whole kidney region of interest. As expected for an intravascular contrast agent that is not filtered at the glomerulus, the gradient echo signal dropped rapidly after injection and reached a steady-state level in less than one minute. Furthermore, no substantial regional variations in signal shape (i.e. delayed signal variations in medulla or papilla that would indicate filtration) were observed. The signal remained at steady-state for more than 30 minutes (data not shown), which is substantially longer than the scan duration of the approach used herein.

Optimization of Contrast Agent Dose

The change in $1/T_2$ (ΔR_2) was computed at different Molday-ION doses (2, 4, 6, 8, 10, and 12 mg/kg). As shown in Figure 3A the average ΔR_2 of the kidneys increased substantially as the Molday-ION dose increased from 0 to 6 mg/kg. Smaller changes were observed as the dose increased from 6 to 12 mg/kg. The bars show the standard deviation across voxels in a single mouse kidney. The standard deviation across voxels increases with Molday-ION dose. To determine the optimal dose we computed the Contrast to Noise Ratio (CNR) between the papilla plus inner medulla, outer medulla, and cortex at different Molday-ION doses (Figure 3B). The CNR of the cortex and outer medulla increases rapidly between 0 and 4 mg/kg and starts to plateau at higher doses of the contrast agent. The CNR of papilla and inner medulla rises to a peak around 4 mg/kg and begins to drop at higher doses. Taken together this data indicates that the optimum Molday-ION dosage at 7T is in the range of 4–8 mg/kg. Henceforth, all RBV maps were acquired using a dose of 6 mg/kg.

Repeatability of RBV Measurements

The RBV was first evaluated across all the voxels (10 coronal slices) in both kidneys of the same mouse. No significant difference was observed in RBV between the left and right kidneys. The region of interest (16) results for a representative normal mouse is shown in

Figure 4A. The outer medulla outer stripe and the combination of papilla and inner medulla exhibited the lowest and highest RBV values, respectively. As shown in Figure 4B, the average RBV in cortex, outer stripe of outer medulla, inner stripe of outer medulla, and inner medulla and papilla across ten mice are 14.7 ± 1.7 , 11.2 ± 1.1 , 26.1 ± 1.2 , and 44.3 ± 1.9 respectively.

A comparison of the average RBV values across the ten subjects and two days is shown in Table 1. In general, the difference in RBV across days in the same subject is small. Eight out of ten subjects have RBV differences across days smaller than 0.4, which reflects a change of less than 0.03%. Subject 3 has an unusually large difference in RBV across days, which may be due to an experimental loading error of contrast agent. The average RBV measured on consecutive days is 19.97 ± 1.50 and 19.86 ± 1.62 , yielding a concordance correlation coefficient of 0.94, indicating that this approach is highly repeatable.

Sensitivity of RBV Measurements to Vascular Abnormalities

To evaluate the sensitivity of RBV maps to disease induced vascular abnormalities, MRI was performed in two models of kidney disease: Alport's Syndrome and Unilateral Ureter Obstruction (UUO) (Figure 5). As shown in Figure 6A, the RBV in the experimental kidneys of Alport mice was substantially lower than that found in control mice (p value 0.0122). Even with the small sample size used in this preliminary study, a regional analysis in the Alport mice indicates that the RBV values in the cortex and the inner stripe of the outer medulla were markedly lower than that found in control mice (Figure 6B). No differences were found in the outer stripe of the outer medulla or in the inner medulla and papilla.

In the UUO model six days after the surgery, the UUO kidney was enlarged showing a marked expansion of the renal pelvis and thinning of the cortex and medulla. The contra-lateral (CL) kidney retained its normal size (right kidney in Figure 5B) with preserved corticomedullary differentiation. Due to the expansion of the ureter and significant thinning of the medulla, RBV values could only be assessed in the cortex and outer strip of the outer medulla. The average RBV value (10.1) in these regions of the UUO kidney was significantly lower than that of sham mice (a decrease of 49.4%, with p value at 0.0064). There were no significant changes in the RBV values found in the contra-lateral kidney (20.1) as compared to sham mouse (19.95).

DISCUSSION

Steady-state, iron-oxide based relative blood volume measurements are commonly used to assess vascular status in brain tissue, tumors and more recently in structures like muscle and kidney. The studies described herein are a first step towards the validation of such measures as biomarkers of renal vascular abnormalities in mice. First, consistent with previous studies in non-murine animal models, when acquired at sufficient spatial resolution the iron-oxide contrast agent based RBV measures in mice exhibit high sensitivity to variations in regional microvascular density. Second, when a consistent contrast agent dose is administered the RBV values are highly repeatable across mice and days. Third, mouse renal RBV maps are able to differentiate between healthy and diseased kidneys. Accordingly, these studies support the use of RBV maps as a valuable, non-invasive tool for the serial characterization of regional hemodynamic status in mouse models of renal disease.

The regional assessment of RBV in mice requires high-resolution anatomic imaging with contrast optimized for structural delineation. Using a CNR analysis similar to that used for RBV, the pulse sequence parameters for T_1 and T_2 -weighted imaging were optimized and ultimately enabled the segmentation of renal cortex, outer medulla, inner medulla and

papilla. The optimized scans were found to be markedly sensitive to morphological changes in these structures in the Alport and UUO mice. The combination of the RBV maps and high-resolution anatomic images facilitated the investigation of renal structure and function.

Unlike traditional angiographic measures of the renal vasculature within the kidney, which reflect larger caliber vessels, the RBV values acquired in this study primarily reflect the microvasculature owing to the use of a T₂ weighted pulse sequence (15). Previous studies have shown that such measures are maximally sensitive to capillary sized vessels and exhibit decreasing sensitivity to larger vessels. Furthermore, such contrast enhanced susceptibility based RBV measurements can also be influenced by local hematocrit and the vessel distribution within a voxel. In general, we found that the blood volume fraction in the cortex was higher than that in outer stripe of the outer medulla but lower than that found in the inner stripe of the outer medulla and in the inner medulla and papilla. While this regional variation seems to counter (e.g. higher blood volume in the inner medulla as compared to the cortex) measurements acquired with methods sensitive to the total vascular volume (e.g. gradient echo based RBV or contrast enhanced ultrasound), it does agree with that recently reported in rats (8) and likely reflects the unique sensitivity of the spin echo approach used herein.

Several issues could confound the repeatability of high-resolution blood volume mapping in mice including the small size of the kidney, susceptibility artifacts from the bowels and respiratory motion. In this study, the use of a fast spin echo pulse sequence with navigator and respiratory gating was found to yield RBV maps with minimal artifacts. As indicated above, across ten subjects the average RBV on consecutive days was 19.97 ± 1.50 and 19.86 ± 1.62 . This observation indicates that RBV measurements are a robust tool for renal functional studies over time, and the renal abnormalities can be characterized if the average RBV changed by more than 1.82 (with 99.99% confidence based on t-test). It is also noteworthy that there was no apparent accumulation of the iron-oxide contrast agent within the kidney in any of the mice between the two days. Macrophage-based accumulation can confound the use of such contrast agents for the serial assessment of tumor hemodynamics.

Substantial changes in RBV values were found in the whole kidney and regionally in two mouse models (Alport and UUO) of kidney disease. In general, the renal RBV values were lower in both models as compared to controls. In the Alport mice, the regional variation in RBV values decreased markedly with a loss of contrast between the cortex and medulla. These findings are consistent with the progression of glomerular fibrosis and decreased renal function known to occur in the Alport mice. In UUO model (day 6), no evident RBV increase was observed in contra-lateral kidney, while a dramatic RBV decrease was observed in the UUO kidney. These preliminary findings indicate that RBV can potentially be used to characterize the degree of disease induced vascular abnormalities *in vivo*. Validation against gold-standard metrics of microvascular density is warranted for future studies.

In conclusion, our experimental results demonstrate that renal RBV measurements in mice are robust and sensitive to variations in the vasculature that may occur during disease progression. The optimized RBV method used herein is ideally suited for evaluating mouse renal vasculature because it employs high-resolution, steady-state acquisition sequences, and avoids the use of CA kinetic models. The imaging efforts could complement the physiological information obtained from conventional assays of kidney function and facilitate the understanding of pathological mechanisms of kidney disease.

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Figure 1. Differentiation of mouse kidney based on *in vivo* high resolution T1W (A), pre-contrast T2W (B), post-contrast T2W images (C), T2*W images, and the computed RBV map (E). C-cortex, OM-outer medulla (IS-inner stripe, OS-outer stripe), IM-inner medulla, P-papilla.

Figure 2. Gradient echo signal intensity time course for a whole kidney region of interest after Molday-ION injection (6mg/kg). Consistent with the first pass-kinetics of an intravascular contrast agent, the signal dropped rapidly after injection and reached a steady-state level in less than one minute.

Figure 3.
Average and standard deviation of ΔR_2 across voxels of both kidneys (A), and Contrast to Noise Ratio (CNR) as a function of Molday-ION dose (B).

Figure 4. Region of interest analysis comparing the average RBV in specific regions of one subject (error bars show the STD across voxels) (A), and (B) a comparison of the average RBV across subjects (error bars show the STD across subjects).

A. Alport model

B. UUO model

Figure 5. Representative T₁- and T₂-weighted images and RBV maps of (A) Alport mouse at week 7, and (B) UUO mouse 6 days after surgery.

Figure 6.

(A) A comparison of average whole-kidney RBV in Alport (n=2), UUO mice (n=6) and sham mice. (B) Regional RBV measurements in Alport and control mice (error bars show the STD across subject).

Table 1

Reproducibility of mean RBV across subjects and days

Subject	1	2	3	4	5	6	7	8	9	10	Mean $\pm$ Std.
Day 1	21.69	20.94	20.75	19.56	19.56	17.56	18.19	21.13	21.75	19.31	19.97 $\pm$ 1.50
Day 2	21.63	20.69	16.56	19.13	19.44	17.75	18.69	21.44	22.00	18.00	19.86 $\pm$ 1.62
Mean	21.66	20.81	18.66	19.34	19.50	17.66	18.44	21.28	21.88	18.66	19.91 $\pm$ 1.54
Std.	0.04	0.18	2.96	0.31	0.09	0.13	0.35	0.22	0.18	0.93	0.27 $\pm$ 0.26