UCLA

UCLA Previously Published Works

Title

Quantitative mouse renal perfusion using arterial spin labeling.

Permalink

<https://escholarship.org/uc/item/5782257h>

Journal

NMR in biomedicine, 26(10)

ISSN

0952-3480

Authors

Rajendran, Reshmi
Lew, Si Kang
Yong, Cai Xian
et al.

Publication Date

2013-10-01

DOI

10.1002/nbm.2939

Peer reviewed

Quantitative mouse renal perfusion using arterial spin labeling[†]

Reshmi Rajendran^a, Si Kang Lew^a, Cai Xian Yong^a, Jolena Tan^a,
Danny J. J. Wang^b and Kai-Hsiang Chuang^{a,c,d,*}

Information on renal perfusion is essential for the diagnosis and prognosis of kidney function. Quantification using gadolinium chelates is limited as a result of filtration through renal glomeruli and safety concerns in patients with kidney dysfunction. Arterial spin labeling MRI is a noninvasive technique for perfusion quantification that has been applied to humans and animals. However, because of the low sensitivity and vulnerability to motion and susceptibility artifacts, its application to mice has been challenging. In this article, mouse renal perfusion was studied using flow-sensitive alternating inversion recovery at 7 T. Good perfusion image quality was obtained with spin-echo echo-planar imaging after controlling for respiratory, susceptibility and fat artifacts by triggering, high-order shimming and water excitation, respectively. High perfusion was obtained in the renal cortex relative to the medulla, and signal was absent in scans carried out *post mortem*. Cortical perfusion increased from 397 ± 36 (mean $\pm$ standard deviation) to 476 ± 73 mL/100 g/min after switching from 100% oxygen to carbogen with 95% oxygen and 5% carbon dioxide. The perfusion in the medulla was 2.5 times lower than that in the cortex and changed from 166 ± 41 mL/100 g/min under oxygen to 203 ± 40 mL/100 g/min under carbogen. T_1 decreased in both the cortex (from 1570 ± 164 to 1377 ± 72 ms, $p < 0.05$) and medulla (from 1788 ± 107 to 1573 ± 144 ms, $p < 0.05$) under carbogen relative to 100% oxygen. The results showed the potential of the use of ASL for perfusion quantification in mice and in models of renal diseases. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: kidney perfusion; MRI; arterial spin labeling; flow-sensitive alternating inversion recovery (FAIR); carbogen; translational

INTRODUCTION

The quantification of kidney perfusion can provide valuable information on the status of many renal diseases, such as renal artery stenosis, renal transplant nephropathy, chronic ischemic nephropathy and drug nephropathy (1), and can even help to ascertain the suitability of the kidney for transplant (2). Studies on transgenic mouse models provide insight into the understanding of the influence of genetics in pathogenesis, disease progression and development (3,4). However, the measurement of mouse renal perfusion has been challenging. Perfusion can vary widely across various regions within an organ. The kidney is a typical example, with the renal cortex in adult humans receiving 93% of the total blood flow to the organ and the inner zone of the medulla about 1% (5). Existing quantitative techniques are invasive and include either the injection of radioactively labeled microspheres or the inhalation of either nitrous oxide or chemically stable radioactive inert gases (6). In MRI, kidney perfusion has been assessed using dynamic contrast-enhanced MRI (7). The major drawback with the use of clinically available low-molecular-weight gadolinium chelates for the measurement of perfusion is that they are easily filtered by renal glomeruli and hence it is difficult to obtain absolute quantification. Known side-effects, such as the development of nephrogenic systemic fibrosis, also limit their application to patients with renal deficits (8).

Arterial spin labeling (ASL) MRI is a noninvasive and quantitative technique that allows the measurement of perfusion by magnetically labeling blood water spins with inversion or saturation as a freely diffusible endogenous tracer (9). With radiofrequency (RF) irradiation at the feeding artery, the proton magnetization in arterial

blood can be inverted ('labeled') and can cause tissue magnetization to be reduced relative to the control condition in which the arterial spins are not altered. The subtraction of the control and label images yields a signal difference which reflects tissue perfusion

* Correspondence to: K.-H. Chuang, Singapore Bioimaging Consortium, 11 Biopolis Way, #02-02, Singapore 138667.
E-mail: Chuang_Kai_Hsiang@sbic.a-star.edu.sg

a R. Rajendran, S. K. Lew, C. X. Yong, J. Tan, K.-H. Chuang
Magnetic Resonance Imaging Group, Singapore Bioimaging Consortium,
Agency for Science, Technology and Research, Singapore

b D. J. J. Wang
Department of Neurology, University of California, Los Angeles, CA, USA

c K.-H. Chuang
Clinical Imaging Research Centre, National University of Singapore, Singapore

d K.-H. Chuang
Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

[†] Part of the results in this article were presented at the Joint Meeting of the International Society for Magnetic Resonance in Medicine–European Society for Magnetic Resonance in Medicine and Biology, Stockholm, Sweden, 2010.

Abbreviations used: AIR, Automated Image Registration; ASL, arterial spin labeling; bpm, breaths per minute; EPI, echo-planar imaging; FAIR, flow-sensitive alternating inversion recovery; HASTE, half Fourier acquisition single-shot turbo spin echo; ROI, region of interest; RF, radiofrequency; SD, standard deviation; SE-EPI, spin-echo echo-planar imaging; SNR, signal-to-noise ratio; TI, inversion time; TrueFISP, true fast imaging with steady-state precession; UFLARE, ultrafast low-angle rapid acquisition and relaxation enhancement.

directly. As the perfusion-weighted signal is low and sensitive to gross movement, only a few studies have demonstrated ASL for the measurement of kidney perfusion, mainly in humans (5,10–19), rats (20,21) and pigs (22,23). Good reproducibility of ASL has been shown in several human studies (14,18,24), but the performance of ASL in small animals, especially in mice, has been difficult.

The first challenge for ASL in the abdomen is the presence of artifacts caused by motion, especially respiratory and peristaltic motion. To reduce motion artifacts, the most common techniques used are breathing strategies, such as breath-holding in humans (13,18) or respiratory gating, and overnight fasting to reduce motion artifacts from the colon (20). Respiratory gating, however, can lead to variability in TR, and hence signal fluctuation when TR is not sufficiently long for spins to fully relax. Another way to reduce motion artifacts is to use background suppression (18), but this may reduce the perfusion signal and cause an underestimation of perfusion (13). The second challenge is the large susceptibility artifacts from the air–tissue interface and chemical shift artifacts from fat in the body. The field inhomogeneity and shorter T_2/T_2^* will cause distortion and/or artifacts in ultrafast imaging, such as echo-planar imaging (EPI). Fat saturation techniques may be employed to tackle fat artifacts, but large field inhomogeneity in the abdomen makes saturation at a single frequency insufficient. Third, the location and orientation of the feeding arteries limit the positioning of the labeling coil or labeling slab, such that the descending aorta is typically selected (18,21). However, when the labeling is too distal from the kidney, the signal-to-noise ratio (SNR) will be lower and the quantification will be less accurate with longer and variable arterial transit times. This is not a concern in the flow-sensitive alternating inversion recovery (FAIR) ASL method, which uses global inversion to label all the blood spins outside the imaging slab. Nonetheless, if the feeding artery lies across the selective inversion slab, which is likely to happen in multi-slice acquisition, this will lead to perfusion signal loss. Oblique coronal slices can be used, but the slice orientation needs to be restricted (13). Compared with larger animals, the smaller size of the mouse makes the above issues more stringent; in addition, the higher spatial resolution required is particularly challenging for a low-sensitivity technique, such as ASL. Although SNR can be increased with the use of a high-field system, artifacts, such as those due to susceptibility, become more serious.

In this article, quantitative renal perfusion imaging in the mouse was investigated using FAIR ASL with spin-echo EPI (SE-EPI) acquisition at 7 T. First, the image quality of SE-EPI in mouse kidney was optimized. Second, the signal change observed was ascertained to be caused by perfusion. Pronounced perfusion signals obtained from the renal cortex and medulla were subsequently quantified and compared. Last, the sensitivity to the change in perfusion induced by vessel dilatation via the inhalation of carbogen was examined.

METHODS

Experimental design

The animal study was approved by the local Institutional Animal Care and Use Committee (Biomedical Sciences Institutes, Agency for Science, Technology and Research (A*STAR), Singapore). Two experiments were carried out in male C57BL/6 mice (20–30 g, $n=7$). The first experiment was performed to verify that the

ASL signal difference observed was indeed caused by perfusion. In this experiment, two animals were euthanized in the scanner by a mixture of isoflurane and carbon dioxide, and the measurements were repeated *post mortem*. The second experiment was to test the sensitivity of renal perfusion to vasodilatation induced by the inhalation of carbogen (mixture of carbon dioxide and oxygen). Perfusion was first measured when animals were given 100% oxygen, and then the same measurement was repeated after the physiology was stabilized under 95% oxygen and 5% carbon dioxide. Measurements were carried out on all seven animals. The ratio of oxygen to carbon dioxide was based on studies assessing the amount of carbon dioxide that could be administered without causing undue stress and physiological instability to the animal (25). As perfusion is highly sensitive to the physiological status of the animal, care was taken to ensure that the respiration rate and temperature were maintained stable. The mice were anesthetized with a mixture of 3% isoflurane in air and O_2 (approximately 2 : 1) for induction, which was reduced to 1–1.5% for maintenance in MRI, via a nose cone to maintain regular breathing at a frequency of 80 ± 10 breaths per minute (bpm) monitored by an MRI-compatible sensor (SA Instruments Inc., New York, NY, USA). The temperature was maintained by an air heater at about 37 °C (SA Instruments Inc.).

MRI parameters

The experiments were carried out on a 7-T MRI scanner (ClinScan, Bruker Biospin GmbH, Ettlingen, Germany) with a bore size of 20 cm and a high-performance gradient and shim coil (gradient strength, 63 G/cm; slew rate, 6300 T/m/s) interfaced to a Siemens console (Erlangen, Germany). A birdcage mouse body coil (diameter, 38 mm) was used for RF transmit and receive. As the effective blood labeling and quantification can be complicated by the limited B_1 profile of the transmit coil (26), instead of positioning the kidneys in the isocenter, the mouse body was positioned in a manner in which the majority of the body, especially the heart and liver, was covered inside the coil.

ASL was performed using the FAIR technique (27,28) interleaving a nonselective inversion with a slice-selective inversion on the imaging slice. A hyperbolic secant adiabatic pulse, generated using MATPULSE software (<http://www.mmrrc.upenn.edu>), with a duration of 15.36 ms was used for inversion. Volume-selective high-order shimming was conducted using the Siemens three-dimensional shim to optimize the field homogeneity. Single-shot SE-EPI was used for data acquisition to achieve high SNR and to minimize motion artifacts. Water excitation was used to minimize the fat artifact in EPI. The motion artifact caused by respiration was controlled by respiratory triggering at the beginning of the inversion pulse. Triggering at the inversion pulse was chosen over triggering at image acquisition or double triggering to ensure consistency in inversion times (TIs). To ensure complete relaxation of the spins between acquisitions, a long TR of 10 s was used. TE was kept to a minimum of 18 ms. An oblique coronal slice with a thickness of 1.5 mm, covering both kidneys, was selected. A matrix size of 64×64 , pixel bandwidth of 3906 Hz, echo train length of about 16 ms and an in-plane resolution of 0.41×0.41 mm² were used in SE-EPI. A selective inversion slice thickness of 4 mm was used to maintain an inversion to imaging slice thickness ratio of more than 2.5 : 1 to avoid artifacts from spins that are not fully inverted at the margins of the readout slice (14). To better quantify the flow, FAIR with multiple TIs (200, 400, 500, 600, 800, 900, 1100, 1400, 1700, 2000,

2300, 3000 and 4000 ms) was performed. TIs were chosen to obtain denser sampling in the fast changing part of the perfusion signal kinetics. For each TI, data were averaged for 10 pairs of control and labeled images. This leads to a total scan time of about 46 min. As the multi-TI perfusion data were collected over a long period, to reduce errors in T_1 measurement caused by movement, a T_1 map was also acquired using an inversion recovery SE-EPI of the same imaging parameters but with fewer TIs (10, 100, 500, 1000, 2000, 5000 and 8000 ms) and four averages in 6 min. Both the FAIR and T_1 mapping were respiratory gated at the beginning of the inversion pulse. The anatomical reference image was obtained at the same slice location, but with a slice thickness of 1 mm, using a two-dimensional true fast imaging with steady-state precession (TrueFISP) sequence with TR = 3 ms, TE = 1.07 ms, flip angle of 70° and 16 averages.

Flow quantification

The perfusion-weighted images were calculated by pairwise subtraction of the control and labeled images. The perfusion-weighted signals at different TIs [$\Delta M(TI)$] were fitted to the following kinetic function by a nonlinear least-squares routine in Matlab (Mathworks, Natick, MA, USA) (29):

$$\Delta M(TI) = 2M_0\alpha f/\lambda \left[\frac{\exp(-TI/T_{1app}) - \exp(-TI/T_{1a})}{(1/T_{1a} - 1/T_{1app})} \right] \quad [1]$$

where $1/T_{1app} = 1/T_1 + f/\lambda$, f is the perfusion (mL/100 g/min) and λ is the blood–tissue partition coefficient. In the above equation, M_0 (the equilibrium magnetization), T_1 (the tissue longitudinal relaxation time) and α (the inversion efficiency) were determined from the three-parameter fit of the inversion recovery T_1 mapping data. The mean inversion efficiency of each animal was calculated from a homogenous tissue area, and this value was used in flow quantification of the animal. λ values from 0.52 to 0.94 (30) have been reported, but 0.9 mL/g was chosen based on studies in which the blood water content was also adjusted for the density of blood (31). T_{1a} is the longitudinal relaxation time of arterial blood, and the value of 2210 ms was used (32). The transit time was ignored as the delivery of spins is almost instantaneous with the 1.25-mm gap between the imaging and inversion slices used in this study. This was verified in preliminary experiments and with a kinetic model incorporating the transit time, in which the transit time was found to be negligible (data not shown).

Data analysis

The ASL data of each TI were split into control and labeled images and averaged. The control and labeled images of all the TIs were registered using Automated Image Registration (AIR 5; <http://bishopw.ionu.edu/air5/>) (33) to minimize the potential movement. The registered control and labeled images were subtracted to obtain the ΔM images for quantification in Matlab using Equation [1].

Regions of interest (ROIs) in the cortex and medulla of the kidneys were manually defined using the freehand tool in AMIDE (34) based on the high-resolution anatomical images, the pre-subtraction ASL images and the T_1 map (Fig. 1). The medulla has slightly longer T_1 and T_2 than the cortex, and hence can be differentiated (35). In addition, the renal pelvis, which showed high intensity in the TrueFISP and T_1 map because of its long T_1 and T_2 , was used to define the boundary of the medulla. Care was also taken to avoid areas near susceptibility artifacts and high-intensity spots, which may correspond to blood vessels, so as to avoid an overestimation of perfusion. Blood vessels were found to have about 150% higher signal intensity on the perfusion-weighted image compared with the renal cortex under normal conditions. The mean and standard deviation (SD) of perfusion from the seven mice were calculated, and statistical significance was tested using a two-tailed paired Student's *t*-test.

RESULTS

Evaluation of image quality in kidney

The use of single-shot SE-EPI and respiratory triggering minimized the motion artifacts within and across scans. The specially controlled respiration rate, which was maintained at 80 ± 10 bpm, avoided variations in breathing and, subsequently, perfusion that may change with the depth of anesthesia. Distortion as a result of the susceptibility artifacts caused by the various air cavities was examined by drawing outlines of the kidneys in the anatomical image and then superimposing these on the SE-EPI image. Good correspondence between the SE-EPI and anatomical images, with little distortion between them, was observed (Fig. 1a, b). To reduce chemical shift artifacts from the fat, both traditional fat saturation and water excitation were compared. Much less residual fat artifact was observed in ASL pairwise subtracted images using water excitation, and hence this method was used to reduce the fat artifact in both the perfusion measurements and T_1 maps.

Figure 1. Anatomical true fast imaging with steady-state precession (TrueFISP) (a), the corresponding spin-echo echo-planar imaging (SE-EPI) (b) and the T_1 map of mouse kidneys (c). The outlines of the kidneys in (a) and (b) indicate minimal distortion of SE-EPI. The regions of interest for perfusion quantification in the cortex and medulla are shown in (c).

Evaluation of perfusion signal

High ASL ΔM signal was observed in the kidneys and was particularly high in the renal cortex relative to the medulla (Fig. 2a). High perfusion signal was also observed in the large vessels, such as the renal artery, which exhibited 200–500% ΔM signal increase in the subtracted perfusion image under both oxygen and carbogen breathing. The perfusion SNR of the renal cortex, which was measured in the ΔM images at $TI=1700$ ms, was 8.88 ± 2.16 . In order to ascertain that the ASL signal difference observed was indeed a result of perfusion, measurements were repeated in two animals *post mortem* using an overdose of isoflurane and carbon dioxide. It was observed that the ASL signal was absent *post mortem*, confirming the detection of perfusion *in vivo* (Fig. 2b). The artifact-free image *post mortem* also indicates that no residual artifact is present.

Quantification of perfusion

Figure 3 shows examples of $\Delta M/M_0$ signals at the various TIs , and the fitted results from the renal cortex and medulla of a mouse. The good correspondence between the experimental data and the model in both regions showed low signal variation caused by motion and reasonable quality, even in the low-signal region of the medulla. The inversion efficiency estimated from the three-parameter fit to the T_1 mapping data was 0.93 ± 0.03 in the abdomen. The quantified perfusion values in the kidney cortices and medullas are listed in Table 1. Under 100% oxygen, the perfusion was 397 ± 36 mL/100 g/min (mean $\pm$ SD; $n=7$) in the renal cortex and 2.5 times lower (166 ± 41 mL/100 g/min) in the medulla.

Perfusion change under carbogen

The sensitivity to changes in renal perfusion was evaluated by manipulating blood flow via carbogen (95% oxygen and 5% carbon dioxide). As expected, with vasodilatation, increased perfusion was observed in both the cortex and medulla (Table 1). A significant increase in perfusion by about 20% was seen in the cortex and 25% in the medulla under carbogen (Fig. 4). However,

Figure 3. The normalized arterial spin labeling (ASL) difference signal ($\Delta M/M_0$) versus the inversion time (TI) shows a good fit with the kinetic model in renal cortex (blue) and medulla (red).

the variation in perfusion also increased because of the higher variability in animal physiology under carbogen, as observed by differences in respiration rate. T_1 decreased significantly in both the cortex (from 1570 ± 164 to 1377 ± 72 ms, $p < 0.05$, two-tailed Student's t -test) and medulla (from 1788 ± 107 to 1573 ± 144 ms, $p < 0.05$) under carbogen relative to 100% oxygen.

DISCUSSION

The feasibility of quantitative perfusion in the mouse kidney using FAIR ASL was evaluated. We showed that, with water excitation and respiratory triggering, single-shot SE-EPI of good quality can be obtained in mouse abdomen at 7 T. To verify whether the ASL signal was caused by perfusion or artifacts in EPI, two animals were scanned *post mortem*, instead of a phantom, to ascertain that the susceptibility and chemical shift artifacts in the

Figure 2. Arterial spin labeling (ASL) difference signal at an inversion time of 1700 ms in a live mouse (a) compared with *post mortem* (b). Note that the intensity scales are six times different.

Table 1. Renal perfusion in the cortex and medulla and the corresponding tissue T_1 and inversion efficiency (α) under 100% oxygen and carbogen

	Cortex				Medulla				α
	Perfusion		T_1 (ms)		Perfusion		T_1 (ms)		
	(mL/100 g/min)				(mL/100 g/min)				
	100% oxygen	Carbogen	100% oxygen	Carbogen	100% oxygen	Carbogen	100% oxygen	Carbogen	
Mouse 1	385	443	1515	1427	144	140	1798	1617	0.93
Mouse 2	338	460	1626	1289	156	168	1800	1430	0.9
Mouse 3	396	400	1520	1417	154	204	1724	1854	0.93
Mouse 4	423	508	1446	1346	199	244	1690	1512	0.94
Mouse 5	375	397	1553	1496	241	256	1722	1546	0.95
Mouse 6	452	526	1422	1345	142	205	1772	1613	0.96
Mouse 7	409	600	1909	1319	123	203	2011	1442	0.89
Mean	397	476	1570	1377	166	203	1788	1573	0.93
Standard deviation	36	73	164	72	41	40	107	144	0.03

Figure 4. Quantified perfusion maps under 100% oxygen (a) and carbogen (b) and the corresponding anatomical image (c).

body were suppressed. Pronounced perfusion signal was observed in both the renal cortex and medulla. The perfusion change detected under carbogen further shows good sensitivity to physiological variations.

Optimal spin labeling using FAIR relies on homogeneous inversion across the body during labeling and minimal inversion outside the imaging slice during control. However, even with nonselective adiabatic inversion, the actual inversion is determined by the RF B_1 profile of the volume transmit coil. The key to achieving high perfusion signal using FAIR ASL is to optimize the coil coverage. In our previous study in rat brain, an optimal coil coverage of the body to include heart and liver increased the perfusion signal by almost 40% relative to the positioning of the imaging region at the isocenter (36). Similarly, in this study, to increase the ASL signal in the kidney, the mouse was placed so that the heart was covered in the 38-mm transmit/receive coil. Although the B_0 field inhomogeneity at the kidney might be larger when it is not positioned at the isocenter, the image distortion was minimal (Fig. 1). The current experimental set-up was thus a trade-off between spin labeling of the FAIR sequence and the B_0 field homogeneity. This concern can be eliminated if a larger volume coil is used for transmission and a surface coil for receiving.

Taking into account the fact that FAIR is unable to label the blood flowing within the selective inversion slice, care was taken to orient the imaging slice to avoid the crossing of the major feeding arteries, including the aorta, and especially the heart,

in the selected slice. However, this strategy is difficult to carry out on a coronal slice, especially with the small dimensions of a mouse. If part of the feeding artery is affected by the selective inversion slice, less blood will be labeled, leading to an underestimation of perfusion. This issue will be more serious for multi-slice acquisition, which will have greater inversion slice thickness. One way to overcome this is to use axial (where the attenuation is much less) or sagittal (where most of the artery can be avoided) slices. The other option is to use another method for spin labeling, such as pseudo-continuous ASL (37,38).

Respiratory motion was minimized by triggering at the beginning of the inversion pulse, and the residual movement was further reduced by motion correction. The other alternatives are to trigger at the image acquisition or at both inversion and acquisition. Triggering at the image acquisition ensures minimal movement among the scans; however, the actual TI can be increased by one respiratory cycle. Considering the respiration rate of 80 bpm in our study, this could result in up to a 750-ms deviation from the desired TI. As a well-defined TI is crucial for perfusion quantification, this triggering strategy could result in considerable errors in quantification. Moreover, triggering at either inversion or acquisition alone may lead to a mismatch between the imaging and inversion slices, which can, in turn, cause variation in the control signal. To evaluate the influence of the residual movement under triggering at inversion alone, we compared control images obtained at TIs differing by about one-half of a respiratory cycle, e.g. 500 and 900 ms. The maximum movement

between these two phases of the respiratory cycle was about 0.34 mm in-plane. As the displacement was less than the in-plane resolution and much less than the slice thickness, its influence on the ASL signal will be negligible. Considering the larger impact of variable TI, it was decided to carry out triggering at the inversion.

The kinetic model for perfusion quantification was based on infinitely long TR and negligible arterial transit time. The TR of 10 s was determined based on four times the tissue T_1 of ~1.5 s with the longest TI (4 s) used for data acquisition. With the maximal volume transmitter coverage and hyperbolic secant adiabatic inversion pulse, one might assume the inversion efficiency to be unity. However, from our calculations, it was observed that the measured inversion efficiency varied between 0.9 and 0.96 in the abdomen across animals, which is slightly lower than the value reported in mouse brain (39). Therefore, it is preferable to estimate the inversion efficiency to avoid the underestimation of perfusion. In addition, the tissue T_1 estimated using the nonselective inversion may be overestimated depending on the volume fraction of the blood vessel, because of the generally longer T_1 of blood than tissue (32,40). However, T_1 may be underestimated as a result of the noninverted blood outside the coverage of the coil. Moreover, it should be noted that the kidney T_1 decreased under hypercapnia (Table 1). Decreased renal cortical T_1 under hyperoxia and hypercapnia relative to normoxia has been reported in healthy humans (41). Therefore, appropriate estimation of T_1 or T_{1app} is important in the quantification. The blood–tissue partition coefficient λ , which is different from that in the brain because of the high vascularization of the kidney, was assumed to be a higher constant value of 0.9 mL/g, used in many animal studies (21,22), rather than a lower value used in human studies. Using an inversion to imaging slice thickness ratio of about 2.5 : 1 helps to avoid artifacts from spins that are not fully inverted at the margins of the read-out slice (14). However, this introduces an arterial transit time during which unlabeled inflowing blood moves into the imaging slice (27,40). In our study, the transit time in the cortex was found to be negligible from preliminary experiments, and hence taken to be zero in our kinetic model. There may be some transit delay in the medulla; however, it was not well resolved with the minimum TI of 200 ms used in this study. Shorter TIs are needed to measure the transit time in the medulla. Another issue that may lead to quantification error is the signal from the artery. In particular, large vessels, which are present around the medulla, may cause overestimation of perfusion. This was minimized by avoiding regions with arteries when drawing ROIs based on the percentage changes in the ΔM signal. One may further suppress the arterial signal using bipolar gradients, but this leads to decreased perfusion signal from the capillaries as well.

Although the use of single-shot SE-EPI can minimize the scan time and avoid certain motion artifacts, it has limitations, such as susceptibility artifacts, geometric distortion (caused by the low bandwidth in the phase-encoding direction), rapid signal dropout (in particular, because of the short intrinsic T_2/T_2^* of the abdomen) and inferior quality at higher resolutions (the long echo train leads to signal decay), which are further deteriorated by the magnetic field inhomogeneity at high field (13). Another drawback with EPI is the relatively long TE, which may cause decreased signal intensity in tissues with relatively short T_2 . In this study, these artifacts were controlled for using a high pixel bandwidth, a small matrix size and a minimum TE of 18 ms. As the average T_2 values measured in our preliminary study were around

39 ms in the cortex and 49 ms in the medulla, image quality degradation caused by a short T_2 is not an issue. For higher resolution imaging, multi-shot EPI may be used, but shot-to-shot variation could affect the ASL data quality. Therefore, single-shot or other ultrafast imaging is still preferable. Previous kidney perfusion studies on rats, swine and humans used acquisition methods such as gradient echo (10,20,21), TrueFISP (5,15,42,43), half Fourier acquisition single-shot turbo spin echo (HASTE) (11) and ultrafast low-angle rapid acquisition and relaxation enhancement (UFLARE) (14). The only other animal study on renal perfusion that used single-shot EPI was a study on *ex vivo* porcine samples (22). These acquisition methods come with their own limitations. A short T_2 at high field strengths renders HASTE impractical. UFLARE has been reported to lead to a blurring of the image, which makes it difficult to delineate the cortical segment walls, ultimately leading to an overestimation of medulla perfusion (14). TrueFISP has been demonstrated in many clinical studies at 3 T and has been shown to be superior to SE-EPI under inhomogeneous field (44). Although artifacts, such as dark bands caused by field inhomogeneity, may be more severe at higher fields, the TrueFISP image acquired for anatomy in this study showed good quality, and hence may have potential for ASL at 7 T. However, caution should be exercised, as the long echo train length at larger matrix size becomes closer to the respiratory cycle, and hence becomes sensitive to motion.

In this study, the ASL data with 13 TIs were obtained in 46 min. The time efficiency (SNR per unit time) of our scans can be further optimized by reducing TR and the number of TIs and increasing the number of averages. A shorter TR can allow more data averaging to be made, and hence the SNR per unit time can be increased (29). Together with a global saturation, the labeled signal may not depend on TR; however, respiratory triggering will still lead to a variable recovery time and hence variation in the labeled signal. The number of TIs used could also be reduced and optimized to shorten the scan time. Many of the animal experiments on kidney perfusion and almost all human experiments used a single TI. Larger variation and much higher flow in the renal cortex were observed when the data were re-analyzed with a single TI of 1700 ms. However, this comparison was performed by taking one of the data points from multiple TIs, and hence required less averaging. With a comparable scan time, perfusion estimation should be similar, regardless of whether single or multiple TIs are used (39), although measurements with multiple TIs are still preferable for the estimation of the transit time. Comparison with matching scan times will be needed to determine the optimal number of TIs required. The other limitation is the slice coverage, which could be improved by multi-slice acquisition, but this will again lead to a loss of sensitivity and an increase in the transit time, which will complicate the quantification further.

The renal cortical perfusion was quantified to be about 397 ± 36 mL/100 g/min under 100% O_2 . This is comparable with, but lower than, the values from the literature for rats (average values of 392–728 mL/100 g/min) (6,21). One difference might be the gas used. Although hyperoxia may lead to slight vasoconstriction, it has been shown that renal perfusion under 100% O_2 is the same as normoxia in rats (47) and rabbits (48). The perfusion in the medulla was 2.5 times lower than that in the renal cortex, and the increase with vasodilatation was also pronounced. Medullary perfusion has not been well documented, except in humans, where a ratio of about 1 : 4 to the cortex was reported. The inner zone of the medulla has a much smaller

perfusion (1% of the total was reported), and this is attributed to the high resistance to flow within the extremely long vasa recta and the increase in viscosity associated with the passage of blood through the hypertonic milieu of the papillary region. It has also been well established that only a small portion of the microcirculation in the kidney goes to the medulla (49). This relatively low blood flow and blood volume lead to reduced labeled spins in medullary tissue, making the signal change very small. The flow could also be underestimated because of the faster clearance of labeled spins from the capillary bed. A human study estimated that approximately 15% of the labeled spins may be lost because of glomerular filtration, outflow or both (20). Another reason for the low perfusion signal could be the signal decay with a longer arterial transit time (22). More averaging and the use of longer TIs optimized for perfusion detection in the medulla, combined with a model that takes into account the microcirculation of the kidney, may improve the quantification of medullary flow (22). Nevertheless, there are still differences between the perfusion measured in this study and that reported in rodents. A major factor could be the anesthesia used. Though isoflurane is known to increase cerebral blood flow, it has been shown to reduce both renal cortical and medullary perfusion in a dose-dependent manner (45,46). It has also been shown that the ratio between cortical and medullary perfusion changes with the anesthesia used. For example, the cortical to medullary perfusion ratio is 4.4 under propofol and becomes 2.7 under isoflurane (46).

The wide accessibility of MRI and the growing availability of ASL sequences on commercial scanners make ASL a good choice for the measurement of perfusion in order to assess renal function and to monitor changes following therapy or renal transplant. The development and validation of this quantitative method from mice to humans will facilitate translational studies and treatment development. Some of the areas in which this would be potentially useful are renal hypertension caused by renal arterial stenosis or the detection of allograft rejection. With the noninvasive ASL technique, studies can be carried out repeatedly in the same subject over time. In addition, it can be easily compared with other MR techniques for the comprehensive assessment of tissue perfusion, morphologic features, metabolism and function, thus providing a more complete understanding of pathophysiological mechanisms.

Acknowledgement

This work was supported in part by the JCO grant CCOGA02_010_2008 from the Agency for Science, Technology and Research, Singapore.

REFERENCES

- Huang AJ, Lee VS, Rusinek H. MR imaging of renal function. *Radiol. Clin. North Am.* 2003; 41(5): 1001–1017.
- Tesi RJ, Elkhammas EA, Davies EA, Henry ML, Ferguson RM. Pulsatile kidney perfusion for evaluation of high-risk kidney donors safely expands the donor pool. *Clin. Transplant.* 1994; 8(2 Pt 1): 134–138.
- D'Agati V, Schmidt AM. RAGE and the pathogenesis of chronic kidney disease. *Nat. Rev. Nephrol.* 2010; 6(6): 352–360.
- Brosius FC 3rd, Alpers CE, Bottinger EP, Breyer MD, Coffman TM, Gurley SB, Harris RC, Kakoki M, Kretzler M, Leiter EH, Levi M, McIndoe RA, Sharma K, Smithies O, Susztak K, Takahashi N, Takahashi T. Animal models of diabetic complications C. Mouse models of diabetic nephropathy. *J. Am. Soc. Nephrol.* 2009; 20(12): 2503–2512.
- Martirosian P, Boss A, Schraml C, Schwenzer NF, Graf H, Claussen CD, Schick F. Magnetic resonance perfusion imaging without contrast media. *Eur. J. Nucl. Med. Mol. Imaging.* 2010; 37(Suppl 1): S52–S64.
- Yarger WE, Boyd MA, Schrader NW. Evaluation of methods of measuring glomerular and nutrient blood flow in rat kidneys. *Am. J. Physiol.* 1978; 235(5): H592–H600.
- Grenier N, Basseau F, Ries M, Tyndal B, Jones R, Moonen C. Functional MRI of the kidney. *Abdom. Imaging.* 2003; 28(2): 164–175.
- Grobner T. Gadolinium—a specific trigger for the development of nephrogenic fibrosing dermopathy and nephrogenic systemic fibrosis? *Nephrol. Dial. Transplant.* 2006; 21(4): 1104–1108.
- Williams DS, Detre JA, Leigh JS, Koretsky AP. Magnetic resonance imaging of perfusion using spin inversion of arterial water. *Proc. Natl. Acad. Sci. USA.* 1992; 89(1): 212–216.
- Abd Ellah M, Kremser C, Pallwein L, Aigner F, Schocke M, Peschel R, Pedross F, Pinggera GM, Wolf C, Alsharkawy MA, Jaschke W, Frauscher F. Changes of renal blood flow after ESWL: assessment by ASL MR imaging, contrast enhanced MR imaging, and renal resistive index. *Eur. J. Radiol.* 2009; 76(1): 124–128.
- Berr SS, Haghspeil KD, Mai VM, Keilholz-George S, Knight-Scott J, Christopher JM, Spinosa DJ, Angle JF, Matsumoto AH. Perfusion of the kidney using extraslice spin tagging (EST) magnetic resonance imaging. *J. Magn. Reson. Imaging.* 1999; 10(5): 886–891.
- Fenchel M, Martirosian P, Langanke J, Giersch J, Miller S, Stauder NI, Kramer U, Claussen CD, Schick F. Perfusion MR imaging with FAIR true FISP spin labeling in patients with and without renal artery stenosis: initial experience. *Radiology.* 2006; 238(3): 1013–1021.
- Gardener AG, Francis ST. Multislice perfusion of the kidneys using parallel imaging: image acquisition and analysis strategies. *Magn. Reson. Med.* 2010; 63(6): 1627–1636.
- Karger N, Biederer J, Lusse S, Grimm J, Steffens J, Heller M, Gluer C. Quantitation of renal perfusion using arterial spin labeling with FAIR-UFLARE. *Magn. Reson. Imaging.* 2000; 18(6): 641–647.
- Lanzman RS, Wittsack HJ, Martirosian P, Zgoura P, Bilk P, Kropil P, Schick F, Voiculescu A, Blondin D. Quantification of renal allograft perfusion using arterial spin labeling MRI: initial results. *Eur. Radiol.* 2009; 20(6): 1485–1491.
- Ritt M, Janka R, Schneider MP, Martirosian P, Horneberger J, Bautz W, Uder M, Schmieder RE. Measurement of kidney perfusion by magnetic resonance imaging: comparison of MRI with arterial spin labeling to para-aminohippuric acid plasma clearance in male subjects with metabolic syndrome. *Nephrol. Dial. Transplant.* 2010; 25(4): 1126–1133.
- Roberts DA, Detre JA, Bolinger L, Insko EK, Lenkinski RE, Pentecost MJ, Leigh JS, Jr. Renal perfusion in humans: MR imaging with spin tagging of arterial water. *Radiology.* 1995; 196(1): 281–286.
- Robson PM, Madhuranthakam AJ, Dai W, Pedrosa I, Rofsky NM, Alsop DC. Strategies for reducing respiratory motion artifacts in renal perfusion imaging with arterial spin labeling. *Magn. Reson. Med.* 2009; 61(6): 1374–1387.
- Wu WC, Su MY, Chang CC, Tseng WY, Liu KL. Renal perfusion 3-T MR imaging: a comparative study of arterial spin labeling and dynamic contrast-enhanced techniques. *Radiology.* 2011; 261(3): 845–853.
- Wang JJ, Hendrich KS, Jackson EK, Ildstad ST, Williams DS, Ho C. Perfusion quantitation in transplanted rat kidney by MRI with arterial spin labeling. *Kidney Int.* 1998; 53(6): 1783–1791.
- Williams DS, Zhang W, Koretsky AP, Adler S. Perfusion imaging of the rat kidney with MR. *Radiology.* 1994; 190(3): 813–818.
- Warmuth C, Nagel S, Hegemann O, Włodarczyk W, Ludemann L. Accuracy of blood flow values determined by arterial spin labeling: a validation study in isolated porcine kidneys. *J. Magn. Reson. Imaging.* 2007; 26(2): 353–358.
- Artz NS, Wentland AL, Sadowski EA, Djamali A, Grist TM, Seo S, Fain SB. Comparing kidney perfusion using noncontrast arterial spin labeling MRI and microsphere methods in an interventional swine model. *Invest. Radiol.* 2011; 46(2): 124–131.
- Cutajar M, Thomas DL, Banks T, Clark CA, Golay X, Gordon I. Repeatability of renal arterial spin labelling MRI in healthy subjects. *MAGMA.* 2012; 25(2): 145–153.
- Rijkema M, Kaanders JH, Joosten FB, van der Kogel AJ, Heerschap A. Effects of breathing a hyperoxic hypercapnic gas mixture on blood oxygenation and vascularity of head-and-neck tumors as measured by magnetic resonance imaging. *Int. J. Radiat. Oncol. Biol. Phys.* 2002; 53(5): 1185–1191.

26. Lipton ML, Branch CA, Hrabe J, Lewis DP, Helpen JA. RF excitation profiles with FAIR: impact of truncation of the arterial input function on quantitative perfusion. *J. Magn. Reson. Imaging*, 2001; 13(2): 207–214.
27. Kim SG. Quantification of relative cerebral blood flow change by flow-sensitive alternating inversion recovery (FAIR) technique: application to functional mapping. *Magn. Reson. Med.* 1995; 34(3): 293–301.
28. Kwong KK, Chesler DA, Weisskoff RM, Donahue KM, Davis TL, Ostergaard L, Campbell TA, Rosen BR. MR perfusion studies with T1-weighted echo planar imaging. *Magn. Reson. Med.* 1995; 34(6): 878–887.
29. Pell GS, Thomas DL, Lythgoe MF, Calamante F, Howseman AM, Gadian DG, Ordidge RJ. Implementation of quantitative FAIR perfusion imaging with a short repetition time in time-course studies. *Magn. Reson. Med.* 1999; 41(4): 829–840.
30. Kertti R, Koivumäki N, Liukko KE, Oikarinen VJ, Tolvanen T, Iida H, Turtti R, Metsärinne K, Iozzo P, Nuutila P. Parametric renal blood flow imaging using [¹⁵O]H₂O and PET. *Eur. J. Nucl. Med. Mol. Imaging*, 2009; 36(4): 683–691.
31. Herscovitch P, Raichle ME. What is the correct value for the brain-blood partition coefficient for water? *J. Cereb. Blood Flow Metab.* 1985; 5(1): 65–69.
32. Dobre MC, Ugurbil K, Marjanska M. Determination of blood longitudinal relaxation time (T1) at high magnetic field strengths. *Magn. Reson. Imaging*, 2007; 25(5): 733–735.
33. Woods RP, Grafton ST, Holmes CJ, Cherry SR, Mazziotta JC. Automated image registration: I. General methods and intrasubject, intramodality validation. *J. Comput. Assist. Tomogr.* 1998; 22(1): 139–152.
34. Loening AM, Gambhir SS. AMIDE: a free software tool for multimodality medical image analysis. *Mol. Imaging*, 2003; 2(3): 131–137.
35. de Bazelaire CM, Duhamel GD, Rofsky NM, Alsop DC. MR imaging relaxation times of abdominal and pelvic tissues measured in vivo at 3.0 T: preliminary results. *Radiology*, 2004; 230(3): 652–659.
36. Nasrallah FA, Lee EL, Chuang KH. Optimization of flow-sensitive alternating inversion recovery (FAIR) for perfusion functional MRI of rodent brain. *NMR Biomed.* 2012; 25(11): 1209–1216.
37. Dai W, Garcia D, de Bazelaire C, Alsop DC. Continuous flow-driven inversion for arterial spin labeling using pulsed radio frequency and gradient fields. *Magn. Reson. Med.* 2008; 60(6): 1488–1497.
38. Duhamel G, Callot V, Tachrount M, Alsop DC, Cozzone PJ. Pseudo-continuous arterial spin labeling at very high field (11.75T) for mouse brain perfusion imaging. *Magn. Res. Med.* 2012; 67(5).
39. Kober F, Duhamel G, Cozzone PJ. Experimental comparison of four FAIR arterial spin labeling techniques for quantification of mouse cerebral blood flow at 4.7 T. *NMR Biomed.* 2008; 21(8): 781–792.
40. Calamante F, Williams SR, van Bruggen N, Kwong KK, Turner R. A model for quantification of perfusion in pulsed labelling techniques. *NMR Biomed.* 1996; 9(2): 79–83.
41. O'Connor JP, Jackson A, Buonaccorsi GA, Buckley DL, Roberts C, Watson Y, Cheung S, McGrath DM, Naish JH, Rose CJ, Dark PM, Jayson GC, Parker GJ. Organ-specific effects of oxygen and carbogen gas inhalation on tissue longitudinal relaxation times. *Magn. Reson. Med.* 2007; 58(3): 490–496.
42. Martirosian P, Klose U, Mader I, Schick F. FAIR true-FISP perfusion imaging of the kidneys. *Magn. Reson. Med.* 2004; 51(2): 353–361.
43. Boss A, Martirosian P, Graf H, Claussen CD, Schlemmer HP, Schick F. High resolution MR perfusion imaging of the kidneys at 3 Tesla without administration of contrast media. *Rofo*, 2005; 177(12): 1625–1630.
44. Boss A, Martirosian P, Klose U, Nagele T, Claussen CD, Schick F. FAIR-TrueFISP imaging of cerebral perfusion in areas of high magnetic susceptibility differences at 1.5 and 3 Tesla. *J. Magn. Reson. Imaging*, 2007; 25(5): 924–931.
45. Gelman S, Fowler KC, Smith LR. Regional blood flow during isoflurane and halothane anesthesia. *Anesth. Analg.* 1984; 63(6): 557–565.
46. Wentland AL, Artz NS, Fain SB, Grist TM, Djamali A, Sadowski EA. MR measures of renal perfusion, oxygen bioavailability and total renal blood flow in a porcine model: noninvasive regional assessment of renal function. *Nephrol. Dial. Transplant.* 2012; 27(1): 128–135.
47. Flemming B, Seeliger E, Wronski T, Steer K, Arenz N, Persson PB. Oxygen and renal hemodynamics in the conscious rat. *J. Am. Soc. Nephrol.* 2000; 11(1): 18–24.
48. Cheng HL. Effect of hyperoxia and hypercapnia on tissue oxygenation and perfusion response in the normal liver and kidney. *PLoS One* 2012; 7(7): e40485.
49. Ulfendal HR WM (ed). *The kidney: physiology and pathophysiology*, 2nd edn. Raven, 1992.