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Quantitative mouse renal perfusion using arterial spin labeling†

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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 ± 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. T1 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.
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 slice, 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. 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 O2 (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 B1 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.mmrrcc.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, 2023, 2026).
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):

\[
\Delta M(TI) = 2M_02f/\lambda \left[ \exp\left(-\frac{TI}{T_{1\text{app}}} \right) - \exp\left(-\frac{TI}{T_{1a}} \right) \right]
\]

where \(1/T_{1\text{app}} = 1/T_1 + f/\lambda\), \(f\) is the perfusion (mL/100 g/min) and \(\lambda\) is the blood–tissue partition coefficient. In the above equation, \(M_0\) (the equilibrium magnetization), \(T_1\) (the tissue longitudinal relaxation time) and \(\lambda\) (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. \(\lambda\) 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 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.loni.ucla.edu/air5/) (33) to minimize the potential movement. The registered control and labeled images were subtracted to obtain the \(\Delta M\) 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 $\Delta M$ 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% $\Delta M$ 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 $\Delta M$ images at TI = 1700 ms, was $8.88 \pm 2.16$. 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 $0.93 \pm 0.03$ in the abdomen. The quantified perfusion values in the kidney cortices and medullas are listed in Table 1. Under 100% oxygen, the perfusion was $397 \pm 36$ mL/100 g/min (mean $\pm$ SD; $n = 7$) in the renal cortex and 2.5 times lower ($166 \pm 41$ 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, 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 $1570 \pm 164$ to $1377 \pm 72$ ms, $p < 0.05$, two-tailed Student’s $t$-test) and medulla (from $1788 \pm 107$ to $1573 \pm 144$ 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.
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.

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 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 multislice 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).

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

<table>
<thead>
<tr>
<th></th>
<th>Cortex</th>
<th>Medulla</th>
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<th>Cortex</th>
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<td></td>
<td>Perfusion (mL/100 g/min)</td>
<td>$T_1$ (ms)</td>
<td>Perfusion (mL/100 g/min)</td>
<td>$T_1$ (ms)</td>
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<td></td>
<td>100% oxygen</td>
<td>Carbogen</td>
<td>100% oxygen</td>
<td>Carbogen</td>
<td>100% oxygen</td>
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<tr>
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<td>1515</td>
<td>1427</td>
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<td>1626</td>
<td>1289</td>
<td>156</td>
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</table>

Figure 4. Quantified perfusion maps under 100% oxygen (a) and carbogen (b) and the corresponding anatomical image (c).
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 $\sim 1.5$ 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 $\lambda$, 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 $\Delta M$ 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 reanalyzed 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 $397 \pm 36$ 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.

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