Hyperpolarized Carbon (^{13}C) MRI of the Kidney: Experimental Protocol

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Abstract

Alterations in renal metabolism are associated with both physiological and pathophysiologic events. The existing noninvasive analytic tools including medical imaging have limited capability for investigating these processes, which potentially limits current understanding of kidney disease and the precision of its clinical diagnosis. Hyperpolarized ^{13}C MRI is a new medical imaging modality that can capture changes in the metabolic processing of certain rapidly metabolized substrates, as well as changes in kidney function. Here we describe experimental protocols for renal metabolic $[1-^{13}\text{C}]$ pyruvate and functional ^{13}C -urea imaging step-by-step. These methods and protocols are useful for investigating renal blood flow and function as well as the renal metabolic status of rodents in vivo under various experimental (patho)physiological conditions.

This chapter is based upon work from the COST Action PARENCHIMA, a community-driven network funded by the European Cooperation in Science and Technology (COST) program of the European Union, which aims to improve the reproducibility and standardization of renal MRI biomarkers. This experimental protocol is complemented by two separate chapters describing the basic concept and data analysis.

Key words Magnetic resonance imaging (MRI), Kidney, Mice, Rats, Hyperpolarization, ^{13}C , DNP

1 Introduction

The causal link between renal blood flow (RBF) and glomerular filtration rate (GFR), tubular reabsorption and thus oxygen/metabolic demand and the development of renal dysfunction is well-known [1]. Therefore, diagnostic modalities able to image RBF, GFR, and the metabolic effects have long been sought for.

One such method is hyperpolarized ^{13}C MR, described in the chapter by von Morze C et al. “Hyperpolarized Carbon (^{13}C) MRI of the Kidneys: Basic Concept.” In short, a transient enhancement of more than 10,000 times in spin polarization of a substrate molecule, such as $[1-^{13}\text{C}]$ pyruvate, is achieved by irradiating a supercooled mixture of ^{13}C spins and electron spins with a microwave frequency obeying the nuclear Overhauser condition—and subsequently rapidly dissolved by superheated water [2]. The

resulting mixture retains the high polarization and allows injection of metabolic and functional active molecules and subsequent imaging of the substrate and potential metabolic derivatives following cellular uptake and enzymatic conversion [3, 4]. We will describe basic experiments using hyperpolarized ^{13}C MRS and MRI for monitoring of the metabolic conversion of $[1-^{13}\text{C}]$ pyruvate and the intra-renal distribution of ^{13}C -urea in the kidney of rodents in a step-by-step experimental protocol. The rationale for choosing acquisition parameters is given in generic terms, together with specific parameter examples for 3 T, 4.7 T and 9.4 T MRI. Several imaging sequences to map renal metabolism and function are described as optional components of the experiment.

This experimental protocol chapter is complemented by two separate chapters describing the basic concept and data analysis, which are part of this book.

This chapter is part of the book Pohlmann A, Niendorf T (eds) (2020) *Preclinical MRI of the Kidney—Methods and Protocols*. Springer, New York.

2 Materials

2.1 Animals

This experimental protocol is tailored for rats (Wistar, Sprague-Dawley, or Lewis) with a body mass of 200–350 g. Advice for adaptation to mice is given as *Notes* where necessary.

2.2 Lab Equipment

1. Anesthesia has been shown to impact renal metabolism using hyperpolarized MRI [5]; An appropriate and convenient choice is the use of gas anesthesia. For in-depth description, see the chapter by Kaucsar T et al. “Preparation and Monitoring of Small Animals in Renal MRI.”
2. Gases: O_2 with or without additional N_2 , as well as a gas-mixing system to achieve required blood oxygen saturation level.
3. Hyperpolarizer equipment, typically the commercial preclinical polarizer HyperSense (Oxford Instruments Molecular Bio-tools, Oxford, United Kingdom) or newer variants of this design [6, 7] as well as the clinical intent polarizer, the so-called SPINLAB (GE Healthcare, Milwaukee, WI, USA).
4. Vein or artery access, typically tail vein access for fast bolus infusion of the hyperpolarized substrate either by manual injection or by infusion pumps [7] (see **Note 1**).
5. Phantom: A ^{13}C containing phantom is needed to confirm center frequency and power calibration. A typical phantom used: 8 M ^{13}C -urea (Sigma, St. Louis, MO, USA) in 90% H_2O and 10% Glycerol with 3 $\mu\text{l/ml}$ Gd contrast agent (Dotarem; Guerbet, Roissy, France) (see **Note 2**).

2.3 MRI Hardware

The general hardware requirements for renal ^1H MRI on mice and rats are described in the chapter by Ramos Delgado P et al. “Hardware Considerations for Preclinical Magnetic Resonance of the Kidney.” The technique described in this chapter was tailored for a 3 T clinical system (GE Healthcare, Milwaukee, WI, USA) and a 9.4 Tesla MR system (Agilent, Yarnton, UK) but advice for adaptation to other field strengths is given where necessary. Additional hardware is required, for excitation and reception of ^{13}C : X-band RF amplifiers and signal preamplifiers supporting the resonance frequency of ^{13}C , ^{13}C transmit and receive RF coils, such as $^1\text{H}/^{13}\text{C}$ volume RF coils [8–10] as well as transmit/receive switches adjusted to the resonance frequency of ^{13}C .

1. A physiological monitoring system that can track the respiration, and which is connected to the MR system such that it can be used to trigger the image acquisition.

3 Methods

3.1 MRI Protocol Setup

3.1.1 Prescan

1. Single pulse and acquire sequence to identify ^{13}C phantom frequency and calibration of the ^{13}C transmitter power for a given pulse shape, width, and amplitude (*see Note 3*).

3.1.2 2D/3D Slice Selective Chemical Shift Imaging (CSI)

1. Sequence type: 2D or 3D chemical shift imaging sequence. This is a standard sequence on preclinical MRI and clinical MRI systems [9, 11, 12].
2. Repetition time (TR): choose the shortest possible with sufficient spectral resolution (which is inversely related to the duration of the data sampling window), for efficient temporal resolution.
3. Echo time (TE): choose the shortest possible for good signal-to-noise and reduction of first order phase effects (*see Note 4*).
4. Flip angle (FA): adapt to ensure a good compromise between SNR, T_1 relaxation and sufficient signal following repeated excitations. FA is often a low flip angle of $\Theta = 5\text{--}10^\circ$ leaving a factor of $\cos(\theta)^n$ longitudinal magnetization remaining, with n being the number of excitations (*see Note 5*).
5. Acquisition bandwidth (BW): set to a value in the typically range of 4000–20,000 Hz, Number of acquisition points (NP): 256–2048 [9, 11, 12]. Choose BW and NP to ensure sufficient resolution and acquisition time of approximately $2.5 \times T_2^*$.

6. K-space ordering: use conventional view ordering, which will result in the center of k-space being sampled late in the acquisition (*see* **Note 6**).
7. For an example of a specific parameter set, please *see* **Note 7**.

3.1.3 2D/3D Echo-Planar Spectroscopic Imaging (EPSI)

1. Sequence type: 2D or 3D echo-planar spectroscopic imaging (EPSI) with or without double spin echo refocusing. This approach superimposes an oscillating readout gradient onto CSI acquisition, replacing one of the phase encoding dimensions needed for CSI. This is not a standard sequence on most preclinical or clinical MRI systems [9, 13, 14].
2. Spectral bandwidth (BW): set spectral BW in the order of 500–600 Hz. The EPSI readout is limited by gradient switching and thus only much lower spectral acquisition bandwidths than CSI are achievable, with outside resonances potentially aliasing into the spectral window. Spectral bandwidth can be traded for spatial resolution but is typically in the order of 500–600 Hz (*see* **Note 8**) for a spatial resolution of ~5 mm.
3. Flip angle (FA): adapt to ensure a good compromise between SNR, T_1 relaxation, and sufficient signal following repeated excitations. FA is often a low flip of $\Theta = 5\text{--}10^\circ$ leaving a factor of $\cos(\theta)^n$ longitudinal magnetization remaining, with n being the number of excitations (*see* **Note 5**).
4. Respiration trigger: can be added to improve the spatial localization.
5. Geometry: adapt so that animal fits into FOV and both kidneys are covered. Axial or coronal directions are most often used.
6. For an example of a specific parameter set, please *see* **Note 9**.

3.1.4 Multi-echo Gradient-Echo Sequence for IDEAL Mapping

1. Sequence type: 2D multi-gradient echo sequence. This is a standard sequence on preclinical MRI systems; alternatively, single timepoint acquisitions such as EPI or spiral readout can be used [15–18] (*see* **Note 10**).
2. Repetition time (TR): choose the shortest possible with sufficient distinct echo times, for highest temporal resolution.
3. Flip angle (FA): adapt to ensure a good compromise between SNR, T_1 relaxation and sufficient signal following repeated excitations. FA is often a low flip of $\Theta = 5\text{--}10^\circ$ utilizing $\cos(\theta)^n$ of excitations of the nonrecoverable magnetization. n being the number of excitations.
4. Echo time (TE): set the number of echoes to $N + 1$ echoes with N being the number of peaks to be resolved. TE is determined by the ability to separate the a priori known peaks to resolve. This can be calculated using the number of signal averages

(NSA) [17]. A rule of thumb is that minimally $N + 1$ echoes are needed to accurately resolve N peaks.

5. Acquisition bandwidth (BW): use a high BW to shorten ΔTE , to ensure good separation of the peaks according to NSA.
6. Respiration trigger: on (per slice), can be added to improve the spatial localization.
7. Geometry: adapt so that animal fits into FOV and both kidneys are covered. Axial or coronal directions are most often used.
8. For an example of a specific parameter set, please *see* **Note 11**.
9. Use double spin echo (DSE) or flow gradients to suppress flowing spins [19, 20] (optional).

3.1.5 Spectral-Spatial Imaging

1. Sequence type: 2D spectral-spatial excitation with Cartesian, EPI or spiral trajectory readout [8, 21–23] or 3D sequences with or without slice selection and thus spectrally selective narrow band pulses [24] (*see* **Note 12**).
2. Repetition time (TR): adapt to the respiration. If respiration is triggered, the effective TR will be given by the respiration trigger and will be the respiration interval, that is, one excitation per breath. Choose TR to be a little shorter (about 100 ms) than the average respiration interval that is displayed on the physiological monitoring unit (*see* **Note 13**).
3. Flip angle (FA): use a low FA (in the range of $3\text{--}8^\circ$) on the substrate peak ($[1\text{-}^{13}\text{C}]\text{pyruvate}$) and high FA (in the range of $30\text{--}90^\circ$) on the metabolic derivatives, to preserve magnetization for metabolic conversions allowing a longer temporal window in dynamic studies, and allowing saturation recovery kinetics to be fitted.
4. For an example of a specific parameter set, please *see* **Note 14**.

3.1.6 Perfusion Imaging

1. Sequence type: 2D/3D balanced steady state free precession (bSSFP) sequence [25–27].
2. Repetition time (TR): use shortest possible TR for high temporal resolution. If triggering respiration, adapt to the respiration. The effective TR will be given by the respiration trigger and will be the respiration interval, that is, one frame per breath. Ideally choose $\text{NP} \times \text{TR}$ to be shorter (about 100 ms) than the average respiration interval that is displayed on the physiological monitoring unit.
3. For perfusion assessment use full saturation of the inflowing spins or (optional) a lower flip angle (in the range of $10\text{--}30^\circ$) [28, 29].

Table 1
bSSFP sequence parameters [23–25]

Sequence	TR/TE, ms	Matrix size	FOV, mm	Acquisition bandwidth, kHz	Flip angle (°)	Preparation	References
2D at 3 T	11.5/ 5.7	32 × 32	80	20	15	$\alpha/2$	[23]
2D at 9.4 T	4.8/ 2.4	32 × 32	60		15	–	[24]
3D at 9.4 T	5.4/ 2.7	48 × 48 × 48	60	20	15	–	[25]

- 4. Use preparation pulses (optional) to improve magnetization utilization; typically, $\alpha/2$ pulses before and after the bSSFP train to achieve faster pseudo-steady state and to retain the magnetization for subsequent scans [30].
- 5. For an example of a specific parameter set, please *see* Table 1.

3.2 In Vivo ¹³C MR

3.2.1 Preparations

- 1. Anesthetize the animal and transfer it to the scanner.
- 2. Place ¹³C enriched phantom in the FOV (*see* **Note 15**).
- 3. Set up the temperature monitoring (rectal probe) and respiratory monitoring (balloon on chest) unit.
- 4. Perform anatomical imaging as described in the chapter by Pohlmann A et al. “Essential Practical Steps for MRI of the Kidney in Experimental Research.”
- 5. Perform localized shimming on the kidney imaging as described in the chapter by Pohlmann A et al. “Essential Practical Steps for MRI of the Kidney in Experimental Research” (*see* **Note 16**).
- 6. Perform ¹³C frequency calibration and ¹³C power calibration (*see* **Note 17**).

3.2.2 Hyperpolarized ¹³C Metabolic and Functional Imaging

- 1. Load the ¹³C sequence of choice, adapt the slice orientation to provide a coronal or axial view with respect to the kidney (in scanner coordinates this is double-oblique).
- 2. Triggering (¹³C): in the monitoring unit set the trigger delay so that the trigger starts at the beginning of the expiratory plateau (no chest motion) and the duration such that it covers the entire expiratory phase, that is, until just before inhalation starts.
- 3. Run the ¹³C imaging scan (*see* **Note 18**). Examples of images are shown in Figs. 1, 2, 3, and 4.

Fig. 1 Series of axial metabolic maps (color) from a 2D CSI overlaid on ^1H anatomical scans (grayscale), showing the individual metabolites after integration of the individual peaks

Fig. 2 Examples of axial IDEAL spiral sequence in the kidney at 3 T

3.3 Emerging Sequences

Several novel alternative strategies have been proposed for various imaging applications and just to mention a few there are control of the inflowing spins with bolus tracking [31, 32], spatiotemporal encoding [33], improved resolution by spatially shaped excitations [32, 34]. Variations of the balanced steady state sequences [35, 36] hold potential for further improvement by optimally designing the imaging strategy toward the long T_2 's seen in most ^{13}C molecules. Relaxation contrast is another potential renal contrast mechanism, showing substantial promise to improve the diagnostic potential of hyperpolarized MR [37–40].

Fig. 3 Examples of coronal spectral spatial (spsp) excitation sequence in the kidney at 3 T

Fig. 4 2D BSSFP dynamic coronal imaging of hyperpolarized [^{13}C , ^{15}N]urea at 3 T. Using a 180 flip angle, 0.92 mm in plane resolution, no slice select. The imaging durations were 1 s separated by 4 s delays, with catalyzation/decatalyzation pulses before and after each image

4 Notes

1. In order to allow optimal usage of the substrate, keep dead-volume as small as possible for injection line; injection speed is typically 0.1 ml/s and up to 2 ml for a rat. If the sequence is single timepoint imaging method or flow artifacts are corrupting the images with a minimum of 20 s time delay from start of injection it is an appropriate choice for intrarenal metabolism and function of the rodent kidneys.

2. Ensure short enough T_1 of the ^{13}C phantom to allow rapid frequency and power calibration. Caution, due to B_0 variation the reference ^{13}C peak is not always representative of the in vivo position.
3. Frequency calibration and power calibration can be done in a similar manner as any ^1H experiment with a centering on the phantom peak and running a power calibration using a standard nutation experiment. Calibrating with a 180 pulse is desirable as the signal changes more rapidly as a function of applied power than with a 90 pulse. Alternatively, a Bloch–Siegert shift method can be used [41].
4. As the RF pulse length contributes to the TE and the RF flip often is very low, it is often possible to shorten the TE by reducing the RF pulse length.
5. Signal decay over the phase encoding steps, either due to T_1 decay or consumption of magnetization due to RF pulses (i.e., T_2). Constant flip angle excitation constitutes a k-space filter that can introduce significant image blurring. Alternatively, an increasing flip angle can be employed to compensate for such effects [42].
6. A variety of CSI sampling patterns can be adopted to optimize temporal constraints, SNR, and partial volume effects [9].
7. Example for a 300 g rat at 9.4 T: TR = 70 ms; TE = 0.68 ms, flip angle = 10° ; pulse length 500 μs ; receiver bandwidth = 8 kHz; number of points = 512 complex points (i.e., 1024), averages = 1; slice orientation = axial oblique; FOV = (60×60) mm; matrix size = 16×16 zero-filled to 32×32 ; 1 slice with 1–2 cm thickness; TA = 17 s (without triggering).
8. The low bandwidth will typically require prior knowledge of the peaks of interest and their folding patterns to fully recover the spectral information [9].
9. Example 3D EPSI for a 300 g rat at 3 T: An EPSI sequence with double spin echo refocusing (13). Images are acquired with $(8 \times 8 \times 8)$ mm³ voxel size and a matrix size of $18 \times 10 \times 8$, coronal view, with a TR of 215 ms, TE of 140 ms, and a spectral width 543 Hz.
10. The multispectral signals are resolved by fitting to a signal model with unknown components at known frequency offsets. B_0 offset is typically an important component of the model and is fitted along with the spectral components or estimated separately (see <https://github.com/LarsonLab/hyperpolarized-mri-toolbox>).
11. Example: 2D IDEAL-spiral for a 300 g rat at 3 T (18): TR = 100 ms; TE = 0.9 ms; flip angle = 10° ; 11 IDEAL

echoes with ΔTE values 0.9 ms, determined by the NSA formalism to resolve $[1-^{13}\text{C}]$ pyruvate, $[1-^{13}\text{C}]$ lactate, $[1-^{13}\text{C}]$ alanine, $[1-^{13}\text{C}]$ pyruvate hydrate, $[^{13}\text{C}]$ bicarbonate. Slice orientation = axial oblique; FOV = (80×80) mm²; matrix size = 32×32 ; 5 mm $\times$ 5 mm real spatial resolution; 1 slice with 1–2 cm thickness covering both kidneys. Dynamic acquisition over 60–80 s.

12. Offset frequencies for individual metabolites must be specified a priori. It is important to note that the frequency might be different than the in vivo version of a given phantom metabolite and thus it is important to verify the correct frequency.
13. Monitor the respiration continuously throughout the entire experiment and if necessary adapt the TR accordingly for the ^{13}C examination.
14. Example: *2D spectral-spatial for a 300 g rat at 3 T (20)*: TR = 1 s; TE = 10 ms; flip angle = 90° on $[1-^{13}\text{C}]$ lactate, flip angle = 15° on $[1-^{13}\text{C}]$ pyruvate; flip angle = 90° on $[^{13}\text{C}]$ bicarbonate and flip angle = 90° on $[1-^{13}\text{C}]$ alanine; slice orientation = axial oblique; FOV = (80×80) mm; matrix size = 32×32 ; 5 mm $\times$ 5 mm real spatial resolution; 1 slice with 1–2 cm thickness covering both kidneys. Dynamic acquisition over 60–80 s.
15. Be careful with the frequency shift between in vivo and ex vivo phantom conditions.
16. B_0 shimming is particularly important, since macroscopic magnetic field inhomogeneities shorten T_2^* , and potentially shift the peak positions relative to the main magnetic field. Shimming should be performed on a voxel enclosing only the kidney using either the default iterative shimming method or manual shimming.
17. It is often possible to retain a similar B_1 amplitude for similar sized animals. ^{13}C Tx gain and Tx/Rx frequency is largely similar in rats and mice and thus the differences should be small between experiments (typically below 1 dB and 50 Hz).
18. A good starting point is to use the same relative resolution as for rats. For this, reduce the FOV to the mouse body width and keep the matrix size the same.

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