

# Hyperpolarized $^{13}\text{C}$ Urea Relaxation Mechanism Reveals Renal Changes in Diabetic Nephropathy

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**Purpose:** Our aim was to assess a novel  $^{13}\text{C}$  radial fast spin echo golden ratio single shot method for interrogating early renal changes in the diabetic kidney, using hyperpolarized (HP) [ $^{13}\text{C}$ ,  $^{15}\text{N}_2$ ]urea as a  $T_2$  relaxation based contrast bio-probe.

**Methods:** A novel HP  $^{13}\text{C}$  MR contrast experiment was conducted in a group of streptozotocin type-1 diabetic rat model and age matched controls.

**Results:** A significantly different relaxation time ( $P=0.004$ ) was found in the diabetic kidney ( $0.49 \pm 0.03$  s) compared with the controls ( $0.64 \pm 0.02$  s) and secondly, a strong correlation between the blood oxygen saturation level and the relaxation times were observed in the healthy controls.

**Conclusion:** HP [ $^{13}\text{C}$ ,  $^{15}\text{N}_2$ ]urea apparent  $T_2$  mapping may be a useful for interrogating local renal  $p\text{O}_2$  status and renal tissue alterations. **Magn Reson Med 75:515–518, 2016. © 2015 The Authors. Magnetic Resonance in Medicine published by Wiley Periodicals, Inc. on behalf of International Society for Magnetic Resonance in Medicine. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.**

**Key words:** MRI; type 1 diabetes; kidney; renal metabolism; hyperpolarization

## INTRODUCTION

The development of early diabetic nephropathy (DN) is characterized by several renal metabolic and structural changes including glomerular hyperfiltration, glomerular and renal hypertrophy and increased urinary albumin excretion (1). In contrast, overt diabetic nephropathy is characterized by a decline in renal function, decreasing creatinine clearance, glomerulosclerosis, and tubulointerstitial fibrosis. Albeit several MRI methods show great promise, lengthy acquisition times reduce the clinical impact of the methods (2). Alternatively, dissolution dynamic nuclear polarization  $^{13}\text{C}$ -labeled pyruvate was recently introduced as a possible endogenous marker to identify renal metabolic changes in diabetic rats (3–5) within seconds after the bolus injection of the bio-probe. Interestingly, the pyruvate-to-lactate conversion is seen to correlate with the progression of DN, in parallel to the structural and microvasculature changes. Additionally, several non-metabolic  $^{13}\text{C}$ -labeled hyperpolarized agents such as urea and HP001 (bis-1,1-(hydroxymethyl)-[1-( $^{13}\text{C}$ )cyclopropane-d(8)]) have unique abilities for renal angiographic and perfusion studies (6–9) and could, therefore, be used for examination of renal changes associated with DN. MR renal examination without use of gadolinium has gained increasing interest for patients with renal insufficiency and  $^{13}\text{C}$ -labeled pyruvate injections can be done without any risk of nephrogenic systemic fibrosis.

In standard proton MRI the contrast mechanism is coupled to signal relaxation. This is in contrast to hyperpolarized MR, where the signal intensity is the determinant. Hyperpolarized pulse sequences are designed to use the magnetization efficiently.  $T_2$  mapping by means of the radial fast spin echo (FSE) train has previously been shown to yield accurate  $T_2$  values (10) for thermal  $^1\text{H}$  imaging. Mapping of the transverse  $^1\text{H}$  relaxation times  $T_2$  has been shown to accurately characterize the altered composition of the renal tissue following acute kidney injury (11), while quantitative evaluation of oxygenation in venous vessels using  $T_2$ -relaxation-under-spin-tagging (TRUST) MRI has been shown to correlate with the increased oxygen consumption in the brain and has been proposed as a novel method for renal oxygen monitoring (2). We, therefore, state the hypothesis, that early renal changes are observable with hyperpolarized urea through the apparent transverse relaxation rate. The cortico-medulla urea gradient introduces different local milieus for the urea molecules and hence introduces contrast mechanisms. The present study

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Grant sponsor: The Danish Research Council; Grant sponsor: The Danish Kidney Foundation; Grant sponsor: Helen and Ejnar Bjørnøvs Foundation; Grant sponsor: the Strategic Research Council.

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Received 1 September 2015; revised 1 October 2015; accepted 15 October 2015

DOI 10.1002/mrm.26036

Published online 19 November 2015 in Wiley Online Library (wileyonlinelibrary.com).

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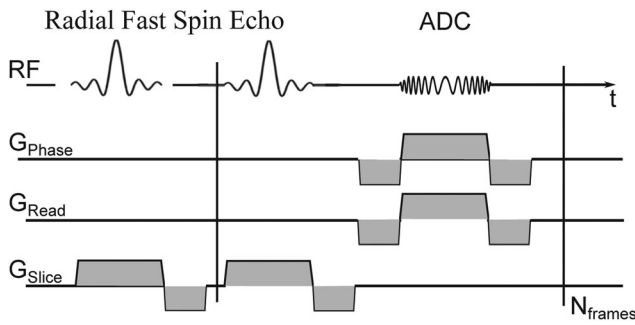


FIG. 1. A pulse sequence illustration of the radial 2D FSE sequence, with slice selective excitation and refocusing pulses. The radial angle scheme used was the golden ratio of  $111.25^\circ$  ensuring high temporal k-space coverage allowing time resolved images to be reconstructed.

addresses the potential for using  $T_2$  relaxation mechanisms of, hyperpolarized urea, a metabolic end-product, to interrogate both oxygen availability and the macroscopic renal changes seen in early diabetes.

## METHODS

A single shot radial FSE sequence (Fig. 1), with golden ratio encoding was implemented on a 9.4 Tesla (T) with VnmrJ 4.0 (Agilent, Santa Clara CA) for in vivo relaxation mapping. It has previously been shown that the  $180^\circ$  refocusing pulses found in FSE type sequences is superior to the lower flip angle refocusing often used in balanced steady state thermal  $^1\text{H}$  imaging sequences (7). The radial ordering scheme golden ratio ( $111.25^\circ$ ) have been used for free breathing MR and recently have shown excellent abilities for  $^3\text{He}$  gas MR of the lung (12), where the transient longitudinal  $T_1$  relaxation yields similar quantitative difficulties as seen in  $^{13}\text{C}$  HP MR.

Twelve 9-week-old female Wistar rats weighing  $208.6 \pm 7.5$  g, were included in this study. The rats were randomly grouped in a diabetic ( $N=6$ ) and a healthy control group ( $N=6$ ). Two rats were excluded from the study, due to erroneous or incomplete data sampling resulting in  $n=5$  in each group. The rats were kept in cages with a 12:12-h light:dark cycle, a temperature of  $21 \pm 2^\circ\text{C}$  and a humidity of  $55 \pm 5\%$  and all animals had free access to water and standard chow throughout the study. Diabetes was induced by an i.v. injection of freshly prepared streptozotocin (STZ; 55 mg/kg body weight; Sigma-Aldrich, Brøndby, DK) dissolved in 0.3 mL of 10 mmol/L cold sodium citrate buffer (pH 4.5). Blood glucose was measured in tail-capillary blood with a ContourXT blood glucose meter (Bayer Diabetes Care, Copenhagen, Denmark). Rats were considered diabetic when the blood glucose levels exceeded 15 mmol/L at 48 h after injection of STZ. Eight to 10 days after induction of diabetes, the tail vein was catheterized for hyperpolarized  $[^{13}\text{C},^{15}\text{N}]$ urea administration. The animals were anesthetized with 3% sevoflurane in 2 L/min air. Subsequently, animals were positioned in a preclinical 9.4T MRI for functional hyperpolarized imaging. The temperature was maintained at  $35^\circ\text{C}$  and respiration and peripheral capillary oxygen saturation ( $\text{SpO}_2$ ) level was monitored throughout the experiment with a MR

compatible monitoring system (SA Instruments, Inc, Stony Brook, NY).  $^1\text{H}$   $T_2$ -weighted FSE coronal and axial scans were acquired [repetition time/echo time (TR/TE) 3 s/4 ms, field of view (FOV)  $70 \times 70$ , matrix  $192 \times 192$ ] as a scout and a single shot  $^{13}\text{C}$  radial FSE sequence (FOV  $60 \times 60$  mm $^2$ , matrix  $64 \times 512$ , sw = 50 kHz, TR/TE 2.4 s/4.6 ms, 1 axial 10 mm slice) covering both kidneys was performed. After the MRI section, a 5- to 7-mL blood sample was collected from the aortic bifurcation to ethylenediaminetetraacetic acid coated tubes for determination of plasma electrolytes and osmolality. The study complied with the guidelines for use and care of laboratory animals and was approved by the Danish Inspectorate of Animal Experiments (2012-15-2934-00581). A standard solution state thermal NMR (Carr-Purcell Meiboom-Gill) CPMG experiment with hard pulses, with an inter-pulse delay  $\tau$  of 3 ms, a TR of 105 s and varying TEs of 2.5, 5, 10, 20, 40, 80 s, respectively, was run to examine the relaxation behavior of  $[^{13}\text{C},^{15}\text{N}]$ urea at 9.4T.

## Hyperpolarization

A total of 200  $\mu\text{L}$  of  $[^{13}\text{C},^{15}\text{N}]$ urea (Sigma-Aldrich, Brøndby, DK), glycerol (Sigma-Aldrich, Brøndby, DK), AH111501 (GE Healthcare, Brøndby, DK) (6.4 M concentration) mixed weight ratio (0.30:0.68:0.02) was polarized in a 5T SPINLab (GE Healthcare, Brøndby, DK) to a reproducible polarization of more than 30%.

## MRI Analysis

Data were converted to ISMRMRD format by means of an open source MATLAB (MathWorks, Natick, MA) converter ([www.mr.au.dk/software](http://www.mr.au.dk/software)) and reconstructed in the open-source software GADGETRON (13) with a time resolution of eight radial spokes per image. Reconstruction was performed on the graphics processing unit, where the non-Cartesian Fourier transform was inverted using an iterative solver with total variation regularization was added, to suppress noise (14). The images were then transferred to Osirix (15), where region of interest (ROI) analysis was performed; separating the renal segments into a cortex, medulla, and papilla segments. A single exponential fit was found to accurately fit the

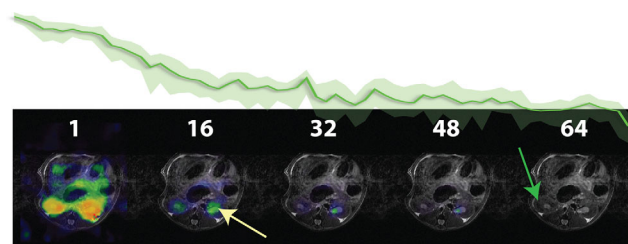


FIG. 2. A representative image series (image 1 - image 64) of a  $^1\text{H}$   $T_2$  weighted FSE axial slice overlaid hyperpolarized  $[^{13}\text{C},^{15}\text{N}]$  urea with each image constructed by eight radial spokes. The images are normalized to the first image of the series (all images are shown logarithmic scaled) and the green line (solid) is the ROI time curve (transparent illustrates SD) from the renal cortex (green arrow). An intra-renal difference in signal is observable after 16 s (yellow arrow) showing elevated signal in the medulla, while no or little signal is observable after 512 refocusing pulses (image 64).

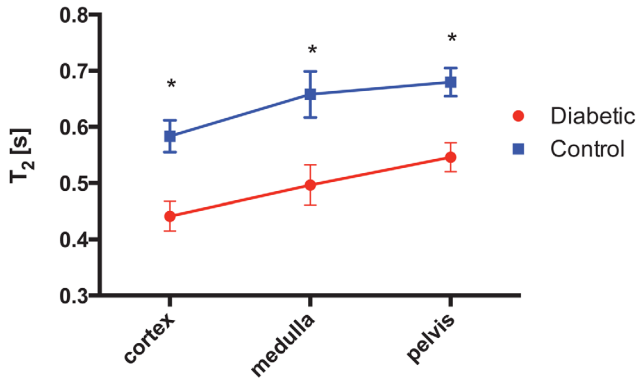


FIG. 3.  $T_2$  relaxation times as a function of kidney ROI segments, showing an overall decrease from the kidney pelvis medulla region toward the cortex in both groups, however, a significant decrease in  $T_2$  values was found in the diabetic kidney compared with the controls (mean  $\pm$  SEM).

different segments individually in MATLAB with a single exponential fit (Fig. 2).

#### Statistics

Normality was assessed with quantile plots.  $P < 0.05$  (\*) were considered statistically significant. A two-way analysis of variance (ANOVA) was used for statistical analysis of the  $T_2$  relaxation times across the kidney regions in GraphPad, Prism (GraphPad Software, Inc. La Jolla, CA). Linear regression was used to assess the correlation between  $pO_2$  and  $T_2$  values. Data on animal body and kidney weight and blood glucose were analyzed with a two-tailed student t-test with equal variance.

#### RESULTS

All rats that received STZ developed sustained hyperglycemia over 48 h. On the day of the scan, the mean body weight of the diabetic rats was  $222.4 \pm 3.6$  g and of the healthy controls  $221.3 \pm 2.7$  g ( $P = 0.81$ ). However, the weight of the diabetic kidney ( $0.960 \pm 0.025$  g) was significantly higher compared with the controls ( $0.800 \pm 0.032$ ;  $P = 0.0039$ ). The change in kidney weight was concomitant with an significant increased blood glucose level in the diabetic animals of  $24.71 \pm 0.91$  mmol/L compared with the controls  $6.97 \pm 0.39$  mmol/L ( $P < 0.0001$ ). A significant intra-renal difference in  $T_2$  relaxation times as a function of kidney region was found ( $P < 0.0001$ ) between the two groups (Fig. 3), as well as an overall significant reduction in the  $T_2$  relaxation times in the diabetic group compared with the controls ( $P = 0.011$ ). To examine the effect of oxygen availability in the investigations the correlation between the mean  $T_2$  relaxation time and the  $SpO_2$  saturation was investigated. It revealed a significant difference in elevation ( $P = 0.0045$ ) between the control group ( $R^2 = 0.98$ ;  $P = 0.016$ ) and the diabetic group ( $R^2 = 0.0003$ ;  $P = 0.98$ ), as the diabetic group shows no correlation with  $SpO_2$  (Fig. 4). Thermal phantom 1D CPMG NMR experiments confirmed the existence of a single exponential  $T_2$  decay of 6 s in solution at 9.4T.

#### DISCUSSION

The main finding was a significant difference in the  $T_2$  relaxation time between the diabetic and healthy control. A significant correlation between  $SpO_2$  and hyperpolarized <sup>13</sup>C urea  $T_2$  relaxation was found in the healthy group. It, therefore, seems plausible to attribute the difference in  $T_2$  relaxation between the diabetic and healthy control group to a difference in local  $pO_2$  (hypoxia, increased deoxyhemoglobin), at least in part. Most likely,  $T_2$  is also affected by changes in other tissue properties such as protein content. The decrease in  $T_2$  might originate from either structural changes (hypotrophy and fibrosis) or increased oxygen consumption or both; however, further studies are needed to elucidate the origin of this relaxation mechanism. The difference between the response in the healthy control and the diabetic groups may originate from the increased oxygen consumption observed in the diabetic kidney leading to hypoxia, thus directly linking diabetes with the development of chronic kidney disease (16–18).

Of interest, it has been shown that an increased concentration of urea in the blood is directly coupled with insulin resistances and oxidative stress (19,20) creating an over stimulated situation in the diabetic kidney compared with the healthy control. The study indicates a potential for interrogating  $SpO_2$  directly with hyperpolarized bio-probes having benefit of being transient signals originating from the bio-probe itself and thus a positive contrast mechanism directly coupled to the bio-probes local milieu. The single shot nature of this current method provide beneficial speed, while the background less hyperpolarization ensures robustness in the quantification process, compared with TRUST or BOLD. Dual-isotopically enriched [<sup>13</sup>C,<sup>15</sup>N<sub>2</sub>]urea has been shown to significantly increase  $T_2$  in vivo compared with [<sup>13</sup>C,<sup>14</sup>N<sub>2</sub>]urea, thus improving the obtainable signal-to-noise ratio (21). The  $T_2$  relaxation times found here do not correspond to previous findings at 3T that showed bi-exponential and longer  $T_2$  relaxation times (21), however, does agree with findings of similar sized molecules at 9.4T finding  $T_2$  relaxation times in the range 0.1–0.6 s (22,23). This may originate from higher magnetic field and the continuous crushing of inflowing spins by

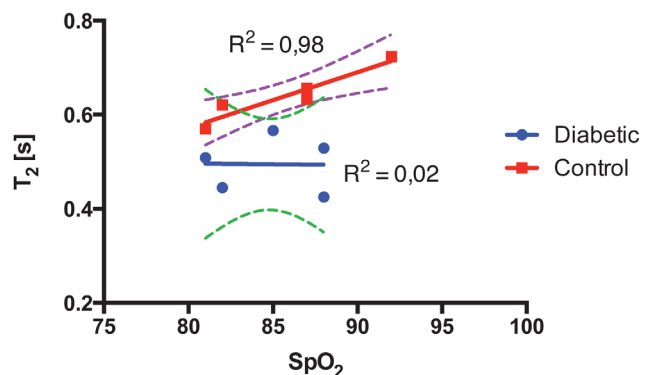


FIG. 4. A significant correlation between the healthy renal controls  $T_2$  values and  $SpO_2$  was observed, while no correlation was found in the diabetic kidneys. The cyan and green stippled lines illustrate the 95% confidence intervals for the interactions.

gradients and imperfect refocusing in this sequence, due to the use of slice selective excitation and refocusing in contrast to spatial non selective pulses in Reed et al (21). Hence, a lower overall  $T_2$  will be found with a single component.

A limitation of the current experimental setup was the use of 10 mm slices thickness (mean intensity profile view), allowing partial volume effects, smearing the ROI signals, further studies are needed for extending the method to three-dimensional. A potential alternative to urea is hyperpolarized water, which would increase the  $T_2$  relaxation and hence improve the sensitivity of the proposed method (24). The method shows the ability to get high resolution and high temporal resolution imaging of hyperpolarized compounds, allowing determination of apparent  $T_2$  relaxation values in vivo. Additionally, the radial acquisition ensures motion robustness for high temporal changes such as respiration. Our results highlight the potential for using HP relaxation contrast MR for probing cellular and macroscopic changes associated with renal disease, with a significant difference between the diabetic and control kidneys.

#### ACKNOWLEDGMENTS

Henrik Vestergaard Nielsen is acknowledged for his expert laboratory assistance.

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