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

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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 $^{13}$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 $^{13}$C-labeled hyperpolarized agents such as urea and HP001 (bis-1,1-(hydroxymethyl)-[1-(13)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 $^{13}$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 $^1$H imaging. Mapping of the transverse $^1$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 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.

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

**Methods:** A novel HP $^{13}$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 ± 0.03 s) compared with the controls (0.64 ± 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 $^{[13C,^{15N}_2]}$urea apparent T$_2$ mapping may be a useful for interrogating local renal pO$_2$ status and renal tissue alterations.

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

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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° refocusing pulses found in FSE type sequences is superior to the lower flip angle refocusing often used in balanced steady state thermal 1H imaging sequences (7). The radial ordering scheme golden ratio (111.25°) have been used for free breathing MR and recently have shown excellent abilities for 3He gas MR of the lung (12), where the transient longitudinal T1 relaxation yields similar quantitative difficulties as seen in 13C HP MR. A single shot radial FSE sequence, with slice selective excitation and refocusing pulses. The radial angle scheme used was the golden ratio of 111.25° ensuring high temporal k-space coverage allowing time resolved images to be reconstructed.

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° ensuring high temporal k-space coverage allowing time resolved images to be reconstructed.

Hyperpolarization

A total of 200 μL of [13C,15N2]urea (Sigma-Aldrich, Brøndby, DK), glycerol (Sigma-Aldrich, 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 ISMRM RD format by means of an open source MATLAB (MathWorks, Natick, MA) converter (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 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

FIG. 2. A representative image series (image 1 - image 64) of a 1H T2 weighted FSE axial slice overlaid hyperpolarized [13C,15N] 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).
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 weight 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 a significantly 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 $^{13}$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 $[^{13}\text{C},^{15}\text{N}_{2}]$urea has been shown to significantly increase $T_2$ in vivo compared with $[^{13}\text{C},^{14}\text{N}_{2}]$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 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 signals, further studies are needed for extending the view), allowing partial volume effects, smearing the ROI use of 10 mm slices thickness (mean intensity profile apparent T_2 relaxation values in vivo. Additionally, 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.

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