Cortical Perfusion and Tubular Function as Evaluated by Magnetic Resonance Imaging Correlates with Annual Loss in Renal Function in Moderate Chronic Kidney Disease

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Keywords

Chronic kidney disease · Diabetes · Fibrosis · Magnetic resonance imaging · Oxygenation · Perfusion

Abstract

Background: Chronic hypoxia is a well-recognized factor in the pathogenesis of chronic kidney disease (CKD). Loss of microcirculation is thought to lead to enhanced renal hypoxia, which in turn results in the development of fibrosis, a hallmark of progressive CKD. To evaluate the role of functional magnetic resonance imaging (MRI), we performed perfusion, oxygenation, and diffusion MRI measurements in individuals with diabetes and stage 3 CKD. **Methods:** Fifty-four subjects (41 individuals with diabetes and stage 3 CKD and 13 healthy controls) participated in this study. Data with

blood oxygenation level dependent (BOLD), arterial spin labeling perfusion and diffusion MRI were acquired using a 3T scanner. **Results:** Renal cortical perfusion was reduced in CKD compared to the controls (109.54 ± 25.38 vs. 203.17 ± 27.47 mL/min/100 g; $p < 0.001$). Cortical apparent diffusion coefficient showed no significant reduction in CKD compared to controls ($1,596.10 \pm 196.64$ vs. $1,668.72 \pm 77.29 \times 10^{-6}$ mm²/s; $p = 0.45$) but was significantly associated with perfusion. Cortical R2* values were modestly increased in CKD (20.76 ± 4.08 vs. 18.74 ± 2.37 s⁻¹; $p = 0.12$). Within the CKD group, R2*_Medulla and R2*_Kidney were moderately and negatively associated with estimated glomerular filtration rate. There was a significant association between cortical perfusion and medullary response to furosemide with annual loss of renal function, used as an estimate of CKD progression. **Conclusions:** Subjects with a moderate degree of

CKD had significantly lower renal perfusion. Diffusion and BOLD MRI showed more modest differences between the groups. Individuals with progressive CKD had lower perfusion and response to furosemide.

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Introduction

Prevalence of chronic kidney disease (CKD) in the United States is estimated to be above 10% [1]. This is primarily based on the clinical definition of CKD using estimated glomerular filtration rate (eGFR) [2]. Only a small fraction of these subjects will progress toward end-stage renal disease [3]. Hence, there is growing interest in developing ways to detect progressive disease in order to guide optimal management. Novel biomarkers [4] and risk prediction models based on multiple clinical parameters [5, 6] are being pursued.

Over the last decade, the role of chronic hypoxia in the progression of CKD has gained attention mostly based on pre-clinical data [7]. The model suggests that a primary glomerular injury initiates loss of peri-tubular capillaries leading to development of tubulo-interstitial hypoxia, which in turn leads to the development of interstitial fibrosis. The presence of fibrosis may further enhance hypoxia by limiting oxygen transport resulting in a perpetual cycle of hypoxic injury and progressive loss of renal function. While the model is widely accepted, direct evidence of enhanced renal hypoxia in humans remains limited [8], primarily due to the lack of noninvasive methods to evaluate renal oxygenation and fibrosis. Biopsy remains to be the accepted method of evaluating renal fibrosis and so there is limited data in individuals with moderate levels of CKD. Magnetic resonance imaging (MRI) offers a variety of noninvasive methods to evaluate renal perfusion, oxygenation, and fibrosis. Arterial spin labeling (ASL) perfusion MRI has been widely used in the brain [9], and recently has been applied to the kidneys [10–15]. Blood oxygenation level-dependent (BOLD) MRI is a noninvasive method to evaluate relative oxygenation status of the kidney [16]. Apparent diffusion coefficient (ADC) estimated using diffusion MRI has been shown to strongly correlate with histological measures of renal fibrosis [17, 18]. All the 3 MRI methods do not require administration of exogenous contrast agents, a great benefit to patients with reduced renal function.

These methods have been applied to the evaluation of individuals with different stages of CKD, either individually [12, 19–23], or in combination such as BOLD and dif-

fusion MRI [24, 25]. Recent studies have reported that the cortical oxygenation as evaluated by BOLD MRI can predict future loss of renal function [26, 27]. Another recent study evaluated BOLD and diffusion MRI in a large multicenter study in subjects with advanced CKD (mean eGFR 33.4 ± 7.2 mL/min/1.73 m²) and demonstrated reduced renal cortical oxygenation and presence of renal fibrosis consistent with the chronic hypoxia hypothesis [28].

The premise for this study stems from the hypothesis that to have an impact on clinical management, detection of progressive disease must be available at mild to moderate levels of CKD. We sought to evaluate the feasibility of the 3 parameters to detect differences in a group of individuals with diabetes and moderate (stage 3) CKD compared to healthy individuals of comparable age. We also evaluated any associations between the various MRI parameters with eGFR and urine protein concentrations used as indicators of disease severity. Furthermore, in order to evaluate the sensitivity to progressive disease, we evaluated associations of the various MRI parameters with annual loss of eGFR (eGFR_slope).

Materials and Methods

Subjects

All procedures were performed with approval from the institutional review boards (at NorthShore University HealthSystem and University of Chicago) and written subject consent prior to enrollment. Table 1 summarizes the subjects' demographic information at baseline. This preliminary study included 41 individuals with stage 3 CKD and diabetes (age: 66 ± 9 ; eGFR: 51.2 ± 12.6 mL/min/1.73 m² and median 24 h urine protein of 160 [0–520] mg). Thirteen healthy subjects (age: 59 ± 9 ; eGFR of 90.7 ± 11.9 mL/min/1.73 m²) also participated in the study. Subjects were instructed not to take non-steroidal anti-inflammatory drugs for 3 days and blood pressure medications such as angiotensin converting enzyme inhibitors or angiotensin receptor blockers (ACEi/ARBs) for 1 day prior to MRI. Both groups were instructed to fast after midnight on the day of the MRI and take half the dose of insulin if applicable. Maintenance drugs were resumed immediately following the MRI data acquisition. Blood was drawn for eGFR calculation either on the day of the scan for healthy subjects or during the screening visit prior to MRI scan for patients. The CKD Epidemiology Collaboration (CKD-EPI) equation was used for eGFR calculation [29]. Serum glucose level and blood pressure were obtained during the patients' initial visit. Twenty-four-hour urine samples were collected within a few days after MRI scan in CKD patients for determination of urine protein excretion. The body mass index (BMI) was calculated for all subjects.

MRI Image Acquisition

All experiments were performed on a 3 Tesla whole body scanner (Siemens Healthcare, Erlangen, Germany). The body coil was used as the transmitter, and the combination of spine and body

Table 1. Characteristics of study population

Variable	CKD (<i>n</i> = 41)	Control (<i>n</i> = 13)	<i>p</i> value
Age, years	66±9	59±9	0.022 [†]
Gender, male, <i>n</i> (%)	20 (49)	6 (46)	0.25 [#]
Race, <i>n</i> (%)			
White	27 (66)	7 (54)	0.061 ^{\$}
African-American	13 (32)	3 (23)	
Other	1 (2)	3 (23)	
BMI, kg/m ²	31.7±7.0	26.0±2.4 [†]	0.003 [†]
Diabetes, <i>n</i> (%)			
Type 2	34 (83)	0 (0)	
Type 1	7 (17)		
Serum glucose, mg/dL	149.0±68.7	n/a	
Systolic BP, mm Hg	135.2±18.9	n/a	
Hemoglobin, g/dL	12.6±1.3	n/a	
eGFR, mL/min/1.73 m ²	51.2±12.6	90.7±11.9 [†]	<0.01 [†]
24-h Urine protein, mg/day	160 (0–520)*	n/a	
ACEi or ARB use, <i>n</i> (%)	30 (73)	n/a	
	Enalapril (2); Accupril (1); Lisinopril (14); Lisinopril + Diovan (1); Lisinopril + Losartan (2); Diovan (3); Losartan (7)		
Loop diuretic use, %	9 (22)	n/a	

[†] By Mann Whitney U test.

[#] By Fischer exact probability test: 2 × 2.

^{\$} By Fischer exact probability test: 2 × 3.

* Shown as median (inter-quartile range).

eGFR, estimated glomerular filtration rate; BP, blood pressure; n/a, not available; BMI, body mass index; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker.

array coils was used as the receiver. Subjects were placed feet first in supine position. After baseline scans, each subject received 20 mg of furosemide (Hospira, Lake Forest, IL, USA) intravenously. Post-furosemide BOLD MRI scans were acquired for 20 min.

MRI Imaging Protocol

Blood Oxygenation Level Dependent

A multiple gradient-recalled-echo sequence (TE = 3.1–30.5 ms; FOV = 380–400 mm; TR = 50 ms; bandwidth = 260 Hz/pixel; flip angle = 30°; matrix = 256 × 256; #slices = 5; slice thickness = 5 mm) [30] was used to acquire 8 T2* weighted images in the oblique-coronal plane during breath-hold at end-expiration. Relaxation rate R2* served as the BOLD MRI index; higher values of which represent decreased oxygenation [31].

Perfusion MRI

A 2D retrospective navigator-gated flow-sensitive alternating inversion recovery True-FISP sequence (FOV = 380 mm, slice thickness = 8 mm, TR = 3s; echo spacing/TE = 4.0/2.2 ms, flip angle = 60°, 128 × 128 matrix, bandwidth = 501 Hz/pixel, number of measurements for patient was 100 and for control was 50, inversion delay was 2,000 ms for patient and 1,500 ms for control) was

used to acquire renal perfusion images [14]. An oblique-coronal slice was carefully prescribed based on the scout image. A 30 mm slice selective inversion band was placed over the imaging slice for labeling so as to avoid major vessels. A 10.24 ms adiabatic frequency offset correction inversion pulse ($\mu = 6$, $\beta = 1,078$) was applied for selective inversion [32]. A low resolution 2D navigator data was acquired immediately following the imaging readout using a FLASH readout (TR/TE = 2.2/1.2 ms; FA = 5°; FOV = 400 × 400 mm²; matrix = 96 × 96; BW = 1,000 Hz/pixel; 5/8 partial encoding along phase direction; parallel acceleration = 2). Additional proton density images were acquired to measure M_0 in order to scale the perfusion-weighted images appropriately to obtain absolute quantitative blood flow.

Quantitative renal blood flow map was calculated pixel by pixel using a single compartment model [33]:

$$f = \frac{\lambda}{2\alpha TI} \frac{\Delta M(TI)}{M_0} \exp\left(\frac{TI}{T_1}\right) \quad (1)$$

where f is the perfusion rate (in the unit of mL/100 g/min), λ is the blood-tissue-water partition coefficient, which was assumed to be

80 mL/100 g [34], α is the inversion efficiency, which was assumed to be 0.95, $\Delta M(TI)$ is the perfusion weighted image, and M_0 is the equilibrium magnetization of the tissue (proton density). The $T1$ value of 1.15 s for the renal cortex [35] was assumed to be the $T1$ of blood.

Diffusion MRI

Echo planar imaging technique (TE = 78 ms; FOV = 380–400 mm; TR = 3,000 ms; bandwidth = 1,628 Hz/pixel; matrix = 192×154; #slices = 5; slice thickness = 5 mm) was used to acquire diffusion weighted images. Diffusion sensitizing gradient pulses were applied along 3 different directions for calculating diffusion trace. Five different b values (200, 300, 500, 700 and 1,000 s/mm²) were acquired and 5 acquisitions were averaged for improving signal to noise ratio and to minimize motion artifacts. ADC was used as the diffusion MRI index; lower values of which may be due to increased fibrosis [17]. ADC map was generated inline.

MRI Analysis Methods

A custom image processing toolbox written in Python (Python Software Foundation, Wilmington, DE, USA) [36, 37] was used to define regions of interest manually in entire cortex, medulla or whole renal parenchyma. Renal cortex is the outer layer of kidney and typically segmented by a ring shaped region of interest (ROI) encompassing the entire cortex tissue, similar to a previous report [38]. Multiple small ROIs were used to define medulla. Whole kidney ROI was defined on entire renal parenchyma excluding any voxels from areas near strong susceptibility changes or other artifacts. ROIs were placed on anatomic images then copied to R2* map to read R2* values. The ROIs for each region were averaged for all slices and both kidneys as the representative measure for each subject for each time point. Two readers (J.M.T., W.L.) performed the ROI analysis for BOLD MRI data. In a sub-group of studies where both readers analyzed the same datasets independently and in a blinded fashion, there was a high degree of agreement between the readers (ICC = 0.93 for cortex, 0.94 for kidney and 0.79 for medulla, by 2-way mixed effects model where reader effects were assumed to be random and measurement effects were assumed to be fixed).

Cortical ROIs were placed on ASL and ADC map directly to read perfusion and ADC values respectively.

eGFR Slope Calculation

Serum creatinine levels were used to calculate eGFR using CKD-EPI formula. Clinical serum creatinine values were obtained from electronic medical records for a period of up to 2 years prior to enrollment date and continued past enrollment. The time range of eGFR readings was 2–7 years (4.4 ± 1.3 years including before and following the MRI). The number of eGFR measurements available for each individual varied between 8 and 101 (23.3 ± 16.6) for the calculation of eGFR_slope. A least squares regression method was used to calculate the eGFR_slope over time, with a negative slope representing an annual reduction in eGFR.

Statistical Analysis

Continuous variables were summarized as mean $\pm$ SD. The comparison of various MRI derived parameters between the CKD and control groups was evaluated by nonparametric Mann-Whitney U Test.

Spearman correlation coefficients were calculated among the MRI parameters, eGFR, urine protein level (Upr), and eGFR_slope. Because the distribution of Upr values was skewed, Log (Upr) was

used for the analysis. For those parameters that showed significant correlation with eGFR, Upr, and eGFR_slope, multiple variable linear regression analysis was used to examine for any effects of potential confounders. Associations between MRI measurements were also evaluated. For the parameters that showed significant associations with yearly loss of renal function, we performed receiver operating characteristic (ROC) analysis to evaluate their sensitivity.

All of the statistical analyses were carried out using SPSS Statistics 22 (IBM Corporation, Armonk, NY, USA), and p value <0.05 was regarded as statistically significant.

Results

Even though the number of subjects in the control group was smaller, the demographic information was comparable. The number of male and female subjects in each group was comparable. Both groups had more Caucasians than African American and other races. Of the 41 CKD subjects with diabetes, 34 had type 2 diabetes and the rest type 1. The average systolic blood pressure was 135 mm Hg in CKD patients and 30 were taking either ACEi or ARB while only 9 out of 41 were treated with loop diuretics.

Figure 1 shows MR images in a representative individual from the CKD and control groups. Table 2 summarizes the measurements in CKD and healthy control groups. Among the MRI parameters, only blood flow and medulla to cortex ratio in R2* (R2*_MCRatio) showed a significant difference between CKD and control groups. Even though MRI data was acquired in all the 41 patients, technical and/or quality issues limited the analysis of some of the data in a few subjects. This is reflected in a lower n shown in Table 2. Although the initial recruitment was based on eGFR estimated using Modification of Diet in Renal Disease formula (routinely used in the clinic), the estimates reported in the study are based on CKD-EPI formulation. This resulted in some values to be above 60 mL/min/1.73 m² in the CKD group.

Table 3 summarizes Spearman correlation coefficients between MRI measurements and eGFR, Upr, and eGFR_slope in the CKD cohort. Further, associations between BOLD MRI parameters with ASL and diffusion MRI measurements were also evaluated. Only medullary and whole kidney R2* demonstrated moderate negative association with eGFR. A novel finding is that cortical blood flow and medullary response to furosemide showed significant positive associations with eGFR_slope, while urine protein was negatively associated with eGFR_slope. The association of $\Delta R2^*_\text{Medulla}$ and $\Delta R2^*_\text{Kidney}$ with eGFR_slope remained significant even after adjusting for eGFR, age, BMI, sex, systolic blood pressure, blood glucose, and the use of loop diuretics and ACEi/ARB (Table 4a). In those

Fig. 1. Representative parametric maps in a healthy volunteer (**a**) and an individual with CKD (**b**). The color bars for each of the parameters apply for both control and CKD. Also included are anatomic MR images (short TE images from BOLD MRI acquisition) as a reference. ADC, apparent diffusion coefficient; ASL, arterial spin labeling; CKD, chronic kidney disease.

individuals with fast progression (eGFR decline of >3 mL/min/year), medullary response to furosemide and cortical blood flow were significantly lower compared to the rest of the group (1.90 ± 2.53 vs. 5.39 ± 3.65 s $^{-1}$; $p = 0.007$) and (88.99 ± 30.31 vs. 117.11 ± 34.76 mL/min/100 g; $p = 0.045$) respectively. However, eGFR and urine protein levels were not different among these individuals. We did not adjust for multiple testing in Table 3. The reported p values are of nominal significance and serve only as guides for possible associations that are hypothesis-generating.

ADC was positively associated with blood flow (Table 4b). Even though $R2^*$ _Medulla and $R2^*$ _Kidney were marginally correlated with eGFR, they were not linearly related

to eGFR (Table 4c). Figure 2 shows a visual representation of the changes observed in cortical perfusion and medullary response to furosemide as a function of the annual change in eGFR. ROC analysis showed an area under the curve (AUC) of 0.88, 0.77, and 0.32 for medullary response to furosemide, cortical perfusion, and proteinuria respectively.

Discussion and Conclusions

Among the 3 functional MRI parameters that were investigated in this study, ASL perfusion MRI was the most sensitive for detecting differences between kidneys of sub-

Table 2. Comparison of means between CKD and control groups

		<i>n</i>	Mean ± SD	<i>p</i> value*
R2* _Cortex s ⁻¹	Control	13	18.7±2.4	0.12
	CKD	38	20.8±4.1	
R2* _Kidney s ⁻¹	Control	13	22.1±2.3	0.20
	CKD	38	23.8±3.7	
R2* _Medulla s ⁻¹	Control	13	29.0±3.9	0.50
	CKD	38	28.1±5.2	
R2* _MC Ratio	Control	13	1.6±0.3	0.042
	CKD	38	1.4±0.3	
ΔR2* _Cortex s ⁻¹	Control	13	0.42±0.86	0.11
	CKD	38	1.18±2.08	
ΔR2* _Kidney s ⁻¹	Control	13	2.04±1.13	0.34
	CKD	38	1.85±1.26	
ΔR2* _Medulla s ⁻¹	Control	13	6.28±3.26	0.15
	CKD	38	4.65±3.71	
Perfusion _Cortex, mL/min/100 g	Control	12	203.2±27.5	6.5E-07
	CKD	35	109.5±25.4	
ADC _Cortex, ×10 ⁻⁶ mm ² /s	Control	13	1,668.7±77.3	0.45
	CKD	37	1,596.1±196.6	

* By Mann-Whitney Test; *p* < 0.05 are shown in bold.

CKD, chronic kidney disease; ADC, apparent diffusion coefficient.

jects with CKD and healthy control groups. The relative reduction in mean Perfusion_Cortex in CKD (109.54 vs. 203.17 mL/min/100 g) was well matched with relative reduction in mean eGFR (51.22 vs. 90.7 mL/min/1.73 m²). This is in good agreement with a recent report that evaluated ASL perfusion MRI in diabetic nephropathy [21]. It is also consistent with the report by Khatir et al. [39] on renal artery blood flow using phase contrast MRI. The novel finding in this study is the significant association of renal perfusion with eGFR_slope, a marker of progressive CKD (Tables 3, 4a; Fig. 2). ROC analysis indicated an AUC of 0.77, which is considered moderate sensitivity. In individuals with fast progression, renal perfusion was significantly lower. These observations are consistent with the chronic hypoxia hypothesis where one of the early changes in progressive CKD is loss of peritubular capillaries [7, 8].

ADC showed that the least difference between the control and CKD groups and was not associated with eGFR. This may be partly due to moderate level of CKD in this study. Advanced CKD was shown to have significantly lower values compare to healthy controls [28]. We have used *b* values >200 s/mm² to estimate ADC, which

would be more specific to the structural changes associated with fibrosis and less influenced by blood flow [17]. The ADC values in control group are consistent with the slow component estimated using bi-exponential fit in a recent report [40]. A previous study also showed low ADC values to be associated with low eGFR [40]. A novel finding of the current study is the strong positive association with cortical perfusion (Tables 3, 4b). This is consistent with the pathophysiology suggested in chronic hypoxia hypothesis, that is, fibrosis is associated with low perfusion.

Only R2*_MC ratio showed significant differences between the CKD and control groups. The modest increase in cortical R2* is consistent with prior studies [26, 27]. It is not yet clear if such modest change is due to the inherently lower sensitivity of BOLD MRI in evaluating cortical oxygenation [31] or whether the kidney compensates for the lower perfusion and maintains the oxygenation status. Unlike previous studies [26, 27] reporting a significant association of cortical R2* with eGFR and eGFR_slope, we did not observe similar associations. We suspect this may be partly related to the moderate CKD in our study, limited range of eGFR values and the smaller number of subjects. It should be noted that the differences between fast progressors and others in the earlier report [26] was small (21.3 ± 2.6 vs. 20.2 ± 1.9 s⁻¹, *p* = 0.033), even though reaching statistical significance. Whole kidney and medullary R2* were moderately and negatively associated with eGFR (Table 3). The novel finding was the significant positive associations of medullary and whole kidney response to furosemide with eGFR_slope (Tables 3, 4a; Fig. 2). These associations remained significant, even after adjusting for age, sex, BMI, blood glucose, systolic blood pressure, and use of loop diuretics and ACEi/ARB (Table 4a). ROC analysis indicated an AUC of 0.88, which is considered good sensitivity. In those individuals with faster progression (>3 mL/min/year), the medullary response to furosemide was lower even though the baseline R2*_Medulla was not different compared to the rest of the group and the controls. This is different from individuals with advanced CKD where R2*_Medulla was lower than in controls [28]. A previous study indicated the responses to furosemide to be lower in those individuals being treated with loop diuretics [41]. Only 2 of the 9 individuals with fast progression were taking loop diuretics at the time of our study. So the reduced response to furosemide observed in our study cannot be explained by prior use of loop diuretics. *What does the response to furosemide tell us?* The concept of kidney stress testing is being considered in nephrology with protein intake or amino acid infusion as a glomerular stress and furosemide as a tu-

Table 3. Spearman correlations between MRI measurements, eGFR, eGFR_slope, and urine protein levels in CKD

		eGFR	Perfusion_cortex	ADC_cortex	eGFR slope	Log (Upr)
eGFR	ρ	1.000	-0.120	-0.015	0.206	0.704**
	p		0.492	0.930	0.202	4.12E-05
	n	41	35	37	40	27
R2*_cortex	ρ	-0.269	-0.110	-0.007	0.069	0.210
	p	0.102	0.550	0.967	0.683	0.324
	n	38	32	35	37	24
R2*_kidney	ρ	-0.346*	0.083	0.196	0.180	0.157
	p	0.034	0.651	0.260	0.285	0.465
	n	38	32	35	37	24
R2*_medulla	ρ	-0.322*	0.136	0.247	0.255	0.073
	p	0.048	0.459	0.152	0.127	0.734
	n	38	32	35	37	24
R2*_MC ratio	ρ	-0.060	0.076	0.271	0.178	-0.133
	p	0.721	0.681	0.116	0.291	0.537
	n	38	32	35	37	24
Δ R2*_cortex	ρ	0.051	0.149	-0.145	0.257	0.065
	p	0.761	0.416	0.407	0.125	0.763
	n	38	32	35	37	24
Δ R2*_kidney	ρ	0.256	0.091	-0.116	00.427**	-0.333
	p	0.121	0.622	0.507	0.008	0.112
	n	38	32	35	37	24
Δ R2*_medulla	ρ	0.168	0.082	-0.025	0.401*	-0.270
	p	0.313	0.654	0.888	0.014	0.202
	n	38	32	35	37	24
Perfusion_cortex	ρ	-0.120	1.000	0.458**	0.441**	-0.427*
	p	0.492		0.008	0.009	0.042
	n	35	35	32	34	23
ADC_cortex	ρ	-0.015	0.458**	1.000	0.116	-0.475*
	p	0.930	0.008		0.499	0.019
	n	37	32	37	36	24
eGFR_slope	ρ	0.206	0.441**	0.116	1.000	-0.393*
	p	0.202	0.009	0.499		0.042
	n	40	34	36	40	27

$p < 0.05$ are shown in bold.

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease; MRI, magnetic resonance imaging.

bular stress agent [42]. Furosemide stress test (FST) is thought to test the overall integrity of the tubular function because the delivery of furosemide to the thick ascending limbs requires adequate blood flow to the proximal tubule and function of the proximal tubule to transport furosemide into tubular lumen. FST has been utilized in acute kidney injury, measuring urine output over 2–4 h as the

response to its administration [43, 44]. Evaluating responses with BOLD MRI can demonstrate faster responses, typically within 15 min [45]. The recent study that reported increased cortical R2* to be associated with loss of renal function [26], found no association of response to furosemide with eGFR_slope. Even though the exact reason is not yet clear, we suspect this may be related to the use of con-

Table 4.**a.** Linear regression of MRI parameters and urine protein with eGFR_slope in CKD

Dependent variable	Predictor	β	SE	<i>p</i> value	Adjusted*		
					β	SE	<i>p</i> value
eGFR_slope	Perfusion_cortex	0.040	0.016	0.021	0.036	0.018	0.062
	$\Delta R2^*$ _medulla	0.377	0.136	0.009	0.442	0.140	0.004
	$\Delta R2^*$ _kidney	0.902	0.415	0.037	0.957	0.443	0.040
	Log(U protein)	-2.908	0.978	0.006	-3.599	1.695	0.049

*Adjusted for eGFR, age, BMI, sex, systolic blood pressure, blood glucose, loop diuretic and ACEi/ARB use.

p < 0.05 are shown in Bold.

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; ACEi, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blockers; BMI, body mass index.

b. Linear regression of ADC_cortex and log(Upr) with perfusion_cortex in CKD

Dependent variable	Predictor	β	SE	<i>p</i> value	Adjusted*		
					β	SE	<i>p</i> value
ADC_cortex	Perfusion_Cortex	2.809	0.898	0.004	1.666	1.133	0.156
Log(Upr)		-0.006	0.004	0.138	-0.003	0.003	0.283

*Adjusted for eGFR, age, BMI, sex, systolic blood pressure, blood glucose, loop diuretic and ACEi/ARB use.

p < 0.05 are shown in Bold.

ADC, apparent diffusion coefficient; ACEi, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blockers; eGFR, estimated glomerular filtration rate; BMI, body mass index; CKD, chronic kidney disease; Upr, urine protein.

c. Linear regression $R2^*$ _medulla, $R2^*$ _kidney, and log(Upr) with eGFR in CKD

Dependent variable	Predictor	β	SE	<i>p</i> value	Adjusted*		
					β	SE	<i>p</i> value
eGFR	$R2^*$ _Medulla	-0.632	0.403	0.126	-0.120	0.436	0.785
	$R2^*$ _Kidney	-1.001	0.565	0.085	-0.621	0.657	0.352
	Log(Upr)	-14.014	2.950	7.81E-05	-12.063	4.045	0.008

* Adjusted for age, BMI, sex, systolic blood pressure, blood glucose, loop diuretic and ACEi/ARB use.

p < 0.05 are shown in Bold.

eGFR, estimated glomerular filtration rate; Upr, urine protein; CKD, chronic kidney disease; ACEi, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blocker; BMI, body mass index.

centric rings as ROIs, which combine cortical and medullary tissue, resulting in reduced sensitivity to detect changes in the medulla. Consistent with the prior report [26], we did find proteinuria to be negatively associated with eGFR_slope (Tables 3, 4a). However, the ROC analysis showed an AUC of 0.32, indicating poor sensitivity.

Limitations

The number of subjects in our study was relatively small. The progression data included time before and after enrollment of subjects. This is primarily due to our low recruitment rate, and reliance on data from electronic medical records. Whether these measures can predict fu-

ture loss of function needs continued prospective follow-up. However, an earlier report [26] had shown that the MRI measurements were reproducible over 36 months follow-up suggesting that any one of the 3 longitudinal measurements would have shown the observed associations with annual loss of renal function. The range of eGFR is small and mostly reflective of moderate level of CKD. However, this was by design. The use of 20 mg of furosemide in all subjects may not be optimal. Dose should probably be scaled with body weight, underlying eGFR, and adjusted for chronic use of loop diuretics [43]. The maximum echo time used (30 ms) in our acquisitions may be sub-optimal for sampling the cortex with $T2^* \sim 50$ ms.

Fig. 2. Shows the association between response to furosemide in renal medulla (**a**) and cortical perfusion (**b**) with annual loss of renal function. eGFR, estimated glomerular filtration rate.

The take home messages include that cortical oxygenation as evaluated by BOLD MRI is lower in CKD, but did not reach significance. It remains an open question whether kidneys in individuals with progressive CKD have “hypoxia”. Whether this may be due to the use of $R2^*$ as the BOLD MRI parameter as suggested recently [46] is not yet clear and needs further investigations. Data from this study indicate that renal perfusion showed the largest differences between individuals with CKD and controls and was one of the parameters associated with loss of renal function. More interestingly, for the first time, FST performed using BOLD MRI showed a significant association with loss of renal function, even though it did not correlate with eGFR.

In conclusion, consistent with prior reports, we observed a significantly lower cortical blood flow and modestly higher cortical $R2^*$ values in individuals with mild to moderate CKD compared to healthy controls. Even though cortical ADC was not significantly different in CKD, it correlated with cortical perfusion. The most interesting and novel observations were the significant associations of perfusion and response to furosemide with annual loss in renal function, a parameter related to progressive CKD. There was a significantly lower response to furosemide in the medulla of those individuals with faster progression (i.e., >3 mL/min/year) compared to the rest. These observations, along with other recent reports that have shown sensitivity of functional MRI measurements to predict progressive CKD, should motivate further prospective studies in a larger number of subjects to verify these observations. The functional MRI methods also can be used for

FST and may provide better sensitivity to progressive CKD compared to baseline oxygenation, especially in subjects with moderate level of CKD. Further, the use of multi-parametric approach can provide a more comprehensive evaluation of renal functional status and in the quest to validate chronic hypoxia hypothesis in humans.

Acknowledgments

The authors thank Dr. Ivana Lazich, Ms. Shoshana Fettman, Ms. Dana Factor, Ms. Sharon Trevino, and Ms. Claire Feczko for their technical assistance during the study. We also thank Dr. Chi Wang for his expert advice on statistical analysis.

Ethics Statement

All subjects have given their written informed consent. The study protocol has been approved by the institutional committees on human research.

Disclosure Statement

The authors have no conflicts of interest to declare.

Funding Sources

Work supported in part by grants from the National Institute of Diabetes Digestive and Kidney Diseases, R21-DK079080 (P.V.P.) and R01-DK093793 (P.V.P.) and National Heart Lung and Blood Institute F31HL123360 (J.M.T.).

Authors Contribution

P.V.P.: conception and design, data analysis and interpretation, drafting and revising manuscript, final approval of manuscript, assurance for accuracy and integrity of all the work involved, and securing funding. L.-P.L.: data acquisition, analysis and interpretation of data, revision and final approval of manuscript. J.M.T.: im-

age analysis and interpretation of data, and final approval of manuscript. B.H.: data acquisition, revision and final approval of manuscript. W.L.: image analysis and interpretation of data and final approval of manuscript. O.K.: data acquisition and interpretation, revision and final approval of manuscript. S.M.S.: conception and design, data acquisition and interpretation, revision and final approval of manuscript, and securing funding.

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