Reduced cortical oxygenation predicts a progressive decline of renal function in patients with chronic kidney disease

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Renal tissue hypoxia is a final pathway in the development and progression of chronic kidney disease (CKD), but whether renal oxygenation predicts renal function decline in humans has not been proven. Therefore, we performed a prospective study and measured renal tissue oxygenation by blood oxygenation level-dependent magnetic resonance imaging (BOLD-MRI) in 112 patients with CKD, 47 with hypertension without CKD, and 24 healthy control individuals. Images were analyzed with the twelve-layer concentric objects method that divided the renal parenchyma in 12 layers of equal thickness and reports the mean R2* value of each layer (a high R2* corresponds to low oxygenation), along with the change in R2* between layers called the R2* slope. Serum creatinine values were collected to calculate the yearly change in estimated glomerular function rate (MDRD eGFR). Follow up was three years. The change in eGFR in CKD, hypertensive and control individuals was -2.0, 0.5 and -0.2 ml/min/1.73m^2/year, respectively. In multivariable regression analysis adjusted for age, sex, diabetes, RAS-blockers, eGFR, and proteinuria the yearly eGFR change correlated negatively with baseline 24 hour proteinuria and the mean R2* value of the cortical layers, and positively with the R2* slope, but not with the other covariates. Patients with CKD and high outer R2* or a flat R2* slope were three times more likely to develop an adverse renal outcome (renal replacement therapy or over a 30% increase in serum creatinine). Thus, low cortical oxygenation is an independent predictor of renal function decline. This finding should stimulate studies exploring the therapeutic impact of improving renal oxygenation on renal disease progression.

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Chronic kidney disease (CKD) is a major public health problem.[1] Diabetes, arterial hypertension (AH), and proteinuria are well-known risk factors for disease progression, but predicting the evolution of CKD remains a difficult task. For incompletely understood reasons, the glomerular filtration rate (GFR) of some CKD patients will hardly decrease over the years, whereas in others, it will rapidly progress toward end-stage renal disease. The early identification of CKD patients at risk of progressive renal function decline (progressors) would allow a more focused distribution of health care resources and improved planning of renal replacement methods. However, a multitude of circulating or urinary biomarkers has been proposed to predict outcome, so far with inconsistent results.[2]

Renal tissue hypoxia is considered as the common final pathway in the development and progression of CKD, irrespective of its cause.[3] Animal studies have provided evidence of this hypothesis, yet data in humans were limited for a long time due to the lack of a method to assess renal tissue oxygenation noninvasively in humans. This situation has changed thanks to the development of blood oxygenation level–dependent magnetic resonance imaging (BOLD-MRI).[4] In brief, BOLD-MRI uses the paramagnetic properties of deoxyhemoglobin to assess tissue oxygenation: the higher the local deoxyhemoglobin levels are, the higher the so-called apparent relaxation rate R2* (s^{-1}) is and the lower local tissue oxygen content is, assuming that blood pO_2 is in equilibrium with tissue pO_2. BOLD-MRI is performed without contrast and therefore is an ideal method for patients with CKD. Standardization of the examinations and refinements in the methods to analyze the images has resulted in a reproducible technique with low interobserver variability.[5]

Recent BOLD-MRI studies have demonstrated that the R2* values of renal parenchyma are higher in CKD patients,

suggesting lower renal tissue oxygenation.[6–8] Differences in R2* between CKD patients and healthy controls have been mainly reported in the cortex, whereas medullary oxygenation seems relatively well preserved at a lower GFR.

Acute decreases in medullary R2* levels have been described after the administration of furosemide and are greater in young persons with preserved renal function compared with older persons or patients with CKD.[9,10] The effect of furosemide has been explained by the fact that it blocks the Na^+-K^+-$2Cl^-$ transporter in the thick ascending loop of Henle, will acutely decrease oxygen-consuming active sodium transport, and increase local pO_2.[11] BOLD-MRI combined with i.v. furosemide is therefore considered by many as a functional test: the greater the change is in R2*, the more functional tubuli that are still present.[12]

To the best of our knowledge, the reported BOLD-MRI studies were all cross-sectional or short-term interventional studies, and it remains therefore unknown whether renal tissue hypoxia (high baseline R2* values) or furosemide-induced changes in R2* predict renal function decline in humans. The aim of this study was therefore to assess whether renal tissue oxygenation as measured with BOLD-MRI is associated with renal function decline in a cohort of CKD patients, hypertensive patients without CKD, and normotensive controls.

RESULTS

A baseline visit was performed in 226 participants (120 CKD patients, 62 hypertensive patients, and 44 healthy controls). Fifteen participants were excluded due to the inability to undergo BOLD-MRI (unexpected claustrophobia or other contraindication for MRI). Seven patients were excluded because of insufficient image quality. A total of 10 hypertensive patients and 11 controls did not want to return for a follow-up visit and were therefore also excluded. Baseline characteristics of the remaining 183 participants are shown in Table 1. In the CKD group, 28 patients had diabetic nephropathy, 33 had hypertensive nephropathy, and 21 had glomerulonephritis. Details of the measured radiological and arterial parameters are shown in Table 2. An example of BOLD-MRI of a healthy volunteer and a CKD patient is shown in Figure 1 (see Methods section for details). More detailed information on other causes of CKD, baseline estimated GFR (eGFR), and BOLD-MRI results according to the underlying cause of CKD are provided in Supplementary Table S1.

The follow-up period (mean ± SD) for all participants was 3.0 ± 1.1 years (3.2 ± 1.2 for CKD, 2.7 ± 1.0 for AH, and 2.7 ± 0.7 for controls). On average, 5.3 ± 2.7 creatinine values were available per individual (6.6 ± 2.6 for CKD, 3.7± 1.7 for AH, and 2.9 ± 0.7 for controls).

The yearly eGFR change was, respectively, −2.0 ± 6.0, 0.5 ± 4.9, and −0.2 ± 5.3 ml/min per 1.73 m^2 per year in CKD, hypertensive patients, and controls (P_{ANOVA}= 0.027); the evolution of the eGFR over time per group is shown graphically in Supplementary Figure S1.

Table 1 | Baseline characteristics of CKD patients, hypertensive patients, and normotensive controls

Characteristics	CKD	AH	Control	P_{ANOVA}
N	112	47	24	
Age (yr)	56 ± 14	56 ± 11	47 ± 11	0.008
Sex (% female)	31.9	35	48	0.21
Diabetes (%)	25.0	16.7	0	0.004
Hypertension (%)	79.8	100	0	<0.001
Creatinine (μmol/l)	151 ± 89	75 ± 13	72 ± 15	<0.001
eGFR (ml/min per 1.73 m^2)	55 ± 29	90 ± 15	97 ± 14	<0.001
Body mass index (kg/m^2)	28 ± 5	28 ± 5	26 ± 5	0.3
Systolic BP (mm Hg)	135 ± 19	142 ± 16	121 ± 14	<0.001
Diastolic BP (mm Hg)	77 ± 12	82 ± 10	74 ± 11	0.0048
Urinary 24-hr volume (l)	2.2 ± 0.8	1.9 ± 0.7	2.1 ± 1.2	0.18
Urinary 24-hr sodium (mmol)	167 ± 88	164 ± 84	153 ± 79	0.57
Hemoglobin (g/dl)	130 ± 18	137 ± 13	135 ± 11	0.05
Hematocrit (%)	38 ± 5	40 ± 3	40 ± 3	0.04
Glycemia (mmol/l)	6.6 ± 2.3	6.2 ± 1.3	5.4 ± 1.1	0.04
Concomitant medications				
RAS blockers (%)	62.6	38.6	0	<0.001
Calcium antagonists (%)	29.8	33.9	0	<0.001
Beta-blockers (%)	28.1	37.5	0	<0.001
Diuretics (%)	22.6	19.6	0	<0.001
Statins (%)	49	32	6.3	<0.001

AH, arterial hypertension; ANOVA, analysis of variance; BP, blood pressure; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; RAS, renin-angiotensin system.

Results of uni- and multivariable regression analysis including all participants, with yearly eGFR change as a dependent (outcome) variable and age, sex, group, diabetes, renin-angiotensin system blockers, baseline eGFR, and proteinuria as independent variables, are shown in Table 3. In fully adjusted models, the yearly change in eGFR correlated negatively with baseline 24-hour proteinuria (regression coefficient β ± SE per gram of proteinuria: −1.49 ± 0.60, P = 0.012) and mean R2* value of the outer (cortical) layers (β/second^{-1}: −0.44 ± 0.16, P = 0.009), but not with the other

Table 2 | Radiologic and arterial parameters according to group

Parameters	CKD	AH	Control	P_{ANOVA}
N	112	47	24	
Magnetic resonance imaging				
Outer R2* (s^{-1})[a]	21.2 ± 3.1	20.6 ± 1.7	20.4 ± 2.5	0.04
Inner R2* (s^{-1})[b]	24.2 ± 2.6	24.4 ± 1.8	24.6 ± 2.1	0.68
R2* slope (hertz per % depth)	7.2 ± 2.8	8.7 ± 3.0	9.4 ± 3.0	<0.001
Delta inner R2* (s^{-1})[c]	2.2 ± 1.2	3.0 ± 1.4	3.2 ± 1.1	<0.001
Ultrasound				
Renal length (mm)	108.3 ± 12.6	114.9 ± 12.2	113.8 ± 8.7	0.002
Renal resistive index	0.71 ± 0.09	0.67 ± 0.07	0.64 ± 0.04	<0.001
Pulse wave analysis				
PWV car-fem (m/s)	9.5 ± 2.7	10.2 ± 2.9	7.9 ± 1.3	<0.001

AH, arterial hypertension; ANOVA, analysis of variance; car-fem; carotid-femoral; CKD, chronic kidney disease; PWV, pulse wave velocity.
[a]Outer R2*: mean R2* of the 3 most superficial, cortical layers of renal parenchyma.
[b]Inner R2*: mean R2* of the 8th to 10th layer (corresponding to medulla) of renal parenchyma.
[c]Delta inner R2* represents the change in R2* of the inner layers 15 minutes after i.v. furosemide.

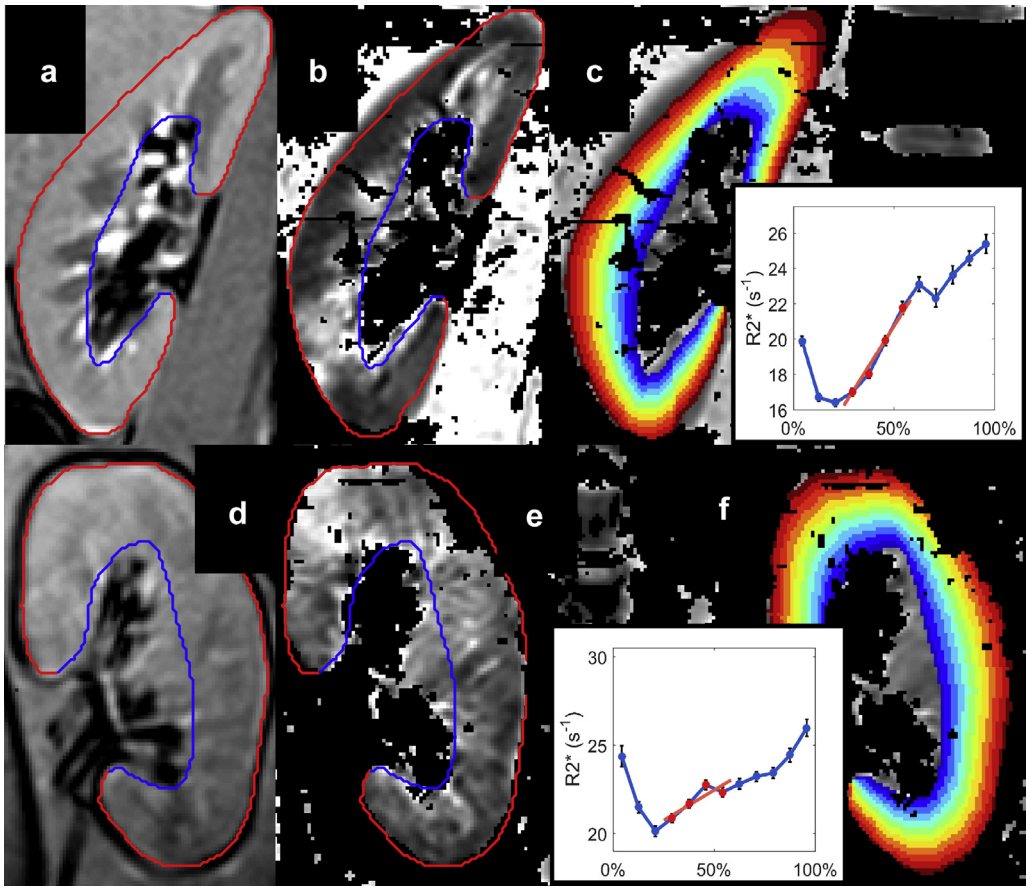

Figure 1 | Example of BOLD-MRI according to the TLCO technique. Images obtained in a healthy volunteer (**a–c**) and in a chronic kidney disease (CKD) patient (**d–f**). The outer border and inner border of the renal parenchyma are drawn manually on anatomic templates; regarding the inner borders, sections deeper than the pyramids were not included to avoid the inclusion of large vascular structures and calices (**a,d**); corresponding R2* maps are shown (**b,e**). After importation in Matlab, the selected kidney parenchyma was divided into 12 layers of equal thickness. Voxels with R2* <10 or >50 s^{-1} or voxels with badly exponentially fitted signal were suppressed (**c,f**). The graphs on the right are the corresponding R2* profiles and summarize the mean R2* of each of the 12 layers, averaged from the 4 coronal sections; the higher R2* is, the lower oxygenation is (see text for details). The percentage scale refers to the relative depth of each layer. The R2* slope (the red line) is flatter in the CKD patient (**f**) than the healthy control (**c**). BOLD-MRI, blood oxygenation level–dependent magnetic resonance imaging; TLCO, 12-layer concentric objects. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

Table 3 | Multivariable analysis including all participants showing the associations between the yearly change in eGFR (outcome variable) and clinical variables

Clinical variables	Age- and sex-adjusted β (95% CI)	*P*	Fully adjusted β (95% CI)[a]	*P*
Age (per year)	−0.007 (−0.07 to 0.056)	0.83	0.027 (−0.06 to 0.11)	0.56
Sex (female vs. male)	−0.59 (−2.34 to 1.17)	0.51	−1.23 (−3.21 to 0.76)	0.22
Diabetes (yes vs. no)	−0.80 (−2.96 to 1.35)	0.47	0.27 (−2.23 to 2.78)	0.83
Baseline eGFR (ml/min per 1.73m^2)	0.057 (0.03–0.09)	<0.001	0.013 (−0.030 to 0.057)	0.55
Proteinuria (per g/24 h)	−2.32 (−3.0 to −1.62)	<0.001	−1.49 (−2.65 to −0.33)	0.012
RAS blocker (yes vs. no)	−1.05 (−2.77 to 0.68)	0.23	0.32 (−1.63 to 2.27)	0.75
Outer R2* (per s^{-1})	−0.34 (−0.69 to −0.007)	0.045	−0.44 (−0.76 to −0.11)	0.009
Delta Inner R2* (per s^{-1})	0.67 (−0.05 to 1.38)	0.068	0.19 (−0.69 to 1.07)	0.67
Inner R2* (per s^{-1})	0.11 (−0.30 to 0.53)	0.6	−0.34 (−0.78 to 0.097)	0.126
R2* slope (per hertz per % depth)	0.48 (0.17–0.78)	0.002	0.45 (0.11–0.80)	0.010
Renal resistive index (per 0.1)	−1.37 (−2.54 to −0.19)	0.022	−0.23 (−1.63 to 1.17)	0.75
PWV (per m/s)	−1.67 (−5.98 to 2.67)	0.45	0.25 (−0.16 to 0.66)	0.23

CI, confidence interval; eGFR, estimated glomerular filtration rate; PWV, pulse wave velocity; RAS, renin-angiotensin system.
[a]Adjusted for age, sex, diabetes, baseline eGFR, proteinuria, and use of RAS blockers.

mentioned covariates. Hence, the higher cortical R2* is (corresponding to lower oxygenation), the larger the decline in yearly eGFR is. In addition, the yearly change in eGFR correlated positively with the R2* slope (β, 0.45 ± 0.17, $P = 0.010$); hence, the flatter the slope, the larger the decline in eGFR. No correlations were found between the furosemide-induced change in medullary R2* (delta inner R2*) and yearly eGFR change.

Similar results were found when the CKD group was analyzed separately: in fully adjusted models, the yearly change in eGFR was associated with 24-hour proteinuria (β ± SE per gram of proteinuria −1.29 ± 0.58, $P = 0.029$), the outer R2* (β ± SE per s^{-1}: −0.31 ± 0.21, $P = 0.015$), and the R2* slope (β 0.66 ± 0.25, $P = 0.01$), whereas the inner (medullary) R2*, delta inner R2*, age, sex, baseline eGFR, diabetes status, and use of renin-angiotensin system blockers were not (Supplementary Table S2).

The percentage of progressors (eGFR decline >3 ml/min per 1.73 m^2 per year) in CKD patients, hypertensive patients, and controls was, respectively, 30.2%, 18.2%, and 16.7%. Progressors had higher outer R2* values (21.3 ± 2.6 vs. 20.2 ± 1.9 s^{-1}, $P = 0.033$) and flatter slopes (7.3 ± 2.8 vs. 8.2 ± 2.9 Hz per percentage of depth, $P = 0.038$) than nonprogressors. In multivariable logistic regression, progressor status was associated with proteinuria (odds ratio per gram: 2.87 (95% confidence interval [CI] 1.44–5.73, $P = 0.003$), outer R2* (OR per s^{-1}: 1.20 [1.03–1.40], $P = 0.019$), and R2* slope (OR per unit 0.91 [95% CI 0.81–0.99], $P = 0.04$), but not with baseline eGFR, age, sex, diabetes status, use of renin angiotensin system blockers, inner R2*, or furosemide-induced change in R2* (Supplementary Table S3).

A total of 5 patients in the CKD group died during follow-up, 13 had to start dialysis, and 6 had an increase in creatinine >30%. For patients with AH, these numbers were, respectively, 0, 0, and 3, whereas none of the controls died or experienced a severe renal adverse event. In 3 of 5 CKD patients, death occurred after the initiation of dialysis. Two CKD patients underwent transplantation, in both cases after the initiation of dialysis.

In 5 of 12 CKD patients with an outer R2* above the 90th percentile (high R2* group), a major renal adverse event developed over time versus 14 of 100 patients with an outer R2* below the 90th percentile, corresponding to a 3 times higher risk ($P = 0.037$), a positive predictive value of 42%, and a negative predictive value of 86%. Baseline characteristics according to R2* group are shown in Table 4. The results of the Kaplan-Meier analysis according to outer R2* percentile are shown in Figure 2. CKD patients from the high R2* group remained significantly less often free of a major renal event over time compared with the low R2* group (log-rank test, $P = 0.0031$). Similar results were found for the extremes of the R2* slope: in 9 of 13 CKD patients with an R2* slope lower than the 10th percentile, a major renal event developed compared with 14 of 88 CKD patients with an R2* slope above the 10th percentile, corresponding to a 4.3 times higher risk ($P < 0.001$), a positive predictive value of 69%, and a negative predictive value of 84%. This was confirmed with the Kaplan-Meier analysis (log-rank test, $P = 0.0009$).

Table 4 | Characteristics of CKD patients according to their R2* value of the outer layers (above or below the 90th percentile)

Characteristics	R2* <90th percentile	R2* ≥90th percentile	P[a]
N	100	12	
Age (yr)	56.5 ± 13	57 ± 18	0.9
Sex (% women)	32.5	25	0.6
Proteinuria (g/24 hr)	0.89 ± 1.52	0.81 ± 1.22	0.87
Diabetes (yes vs. no)	28.9	16.7	0.37
Baseline eGFR (ml/min per 1.73 m^2)	56.2 ± 29	49.4 ± 26	0.46
Yearly change in eGFR (ml/min per 1.73 m^2)	−0.8 ± 5.8	−3.9 ± 6.6	0.008
Baseline creatinine (per μmol/l)	153 ± 97	169 ± 97	0.6
RAS blocker (yes vs. no)	47.4	58.3	0.48
Outer R2* (sec^{-1})	20.3 ± 1.6	26.6 ± 4.6	<0.001

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; RAS, renin-angiotensin system.
R2* values above the 90th percentile correspond to the lowest cortical oxygenation.
[a]The *P* values were obtained with χ^2 tests for categorical variables and Wilcoxon's rank sum tests for numerical variables.

Receiver-operating characteristic curves for the variables that showed the strongest association with the occurrence of major renal events (proteinuria, outer R2*, and R2* slope) are shown in Figure 3a. Discrimination obtained with the variable proteinuria, expressed as the area under the curve (AUC), was 0.83 (95% CI 0.72–0.94) and slightly stronger than the AUC of the R2* slope (AUC: 0.80, 95% CI 0.65–0.96) or outer R2* (AUC: 0.71, 95% CI: 0.52–0.88). A further increase in the AUC was obtained by combining the 3 variables (AUC: 0.89, 95% CI 0.75–0.97) (see Figure 3b).

A total of 68 CKD patients (mean ± SD age, 56.7 ± 13.7 years, 46% female) underwent a second MRI at the 1-year follow-up visit, and 34 underwent a third MRI at the 3-year follow-up visit. Twenty-two AH patients and 25 healthy controls underwent a second MRI at the 3-year follow-up visit. Results of their outer and inner R2* values, R2* slopes, and furosemide-induced changes according to the study visit are shown in Supplementary Table S4. There were no significant changes in the R2* values or their slopes between the baseline and 1- and 3-year study visits. Of note, the yearly eGFR decline in the CKD patients who underwent follow-up MRI after 1 year was −1.7 ± 7.7 ml/min per 1.73 m^2 and −0.3 ± 2.8 ml/min per 1.73 m^2 in the 34 CKD patients who underwent a follow-up MRI after 3 years. The outer R2* values of the CKD patients with rapid renal function decline (>3 ml/min per 1.73 m^2 per year, $N = 16$) increased slightly over time (from 21.0 ± 2.5 to 21.7 ± 3.2 s^{-1} after 3 years, $P = 0.16$), whereas no change occurred in the CKD patients without rapid renal function decline (from 20.6 ± 2.1 to 20.7 ± 1.7 s^{-1}, $P = 0.92$).

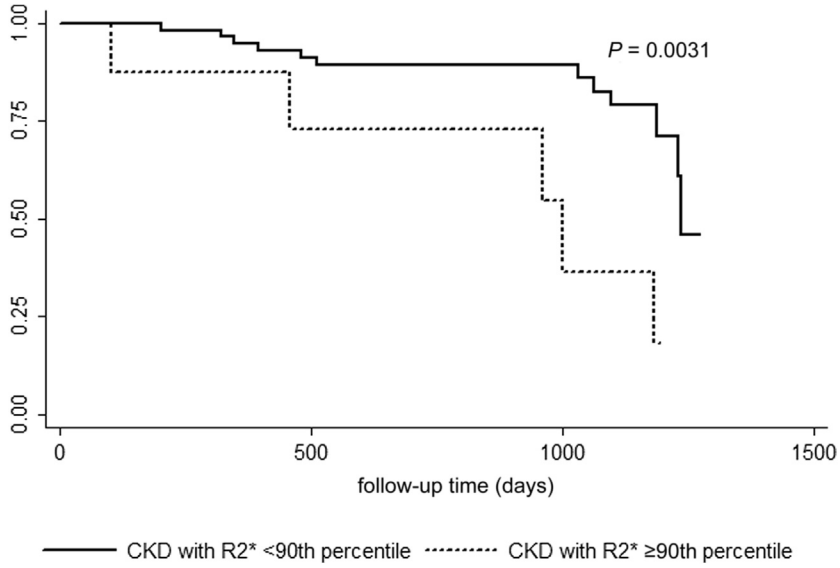

Figure 2| Kaplan-Meier curve depicting the proportion of chronic kidney disease (CKD) patients free of major renal events (initiation of renal replacement therapy or increase in serum creatinine >30%) in CKD patients according to their cortical R2* value over time (days).

DISCUSSION

To the best of our knowledge, this is the first report of an association between renal tissue hypoxia and renal function decline in humans. Using BOLD-MRI, we indeed show that the lower the oxygenation of the cortex (corresponding to high R2* values) is, the faster the yearly decline of the eGFR. In this study, renal tissue oxygenation was also associated with hard renal endpoints. CKD patients with the lowest cortical oxygenation were 3 times more likely to experience a major renal event (the need for renal replacement therapy or an increase of >30% in baseline serum creatinine) than those with preserved renal tissue oxygenation. The R2* slope, which depends less on the absolute R2* values but merely on their intracompartmental distribution, was also strongly associated

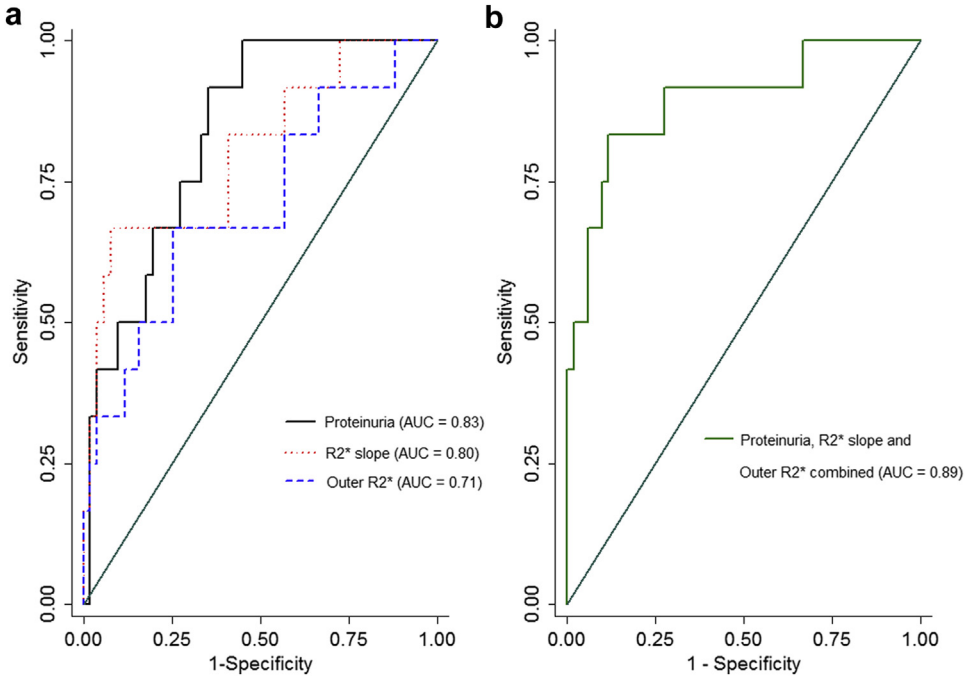

Figure 3| (a) Receiver operating characteristic (ROC) curve and area under the curve (AUC) of 24-hour proteinuria (in black), outer R2* values (in blue), and the R2* slope (in red) for their prediction of a major renal event in CKD patients. (**b**) ROC curve and AUC of the 3 variables combined.

with renal function decline and hard renal endpoints. As such, a flatter slope corresponded to a greater yearly decline in renal function and vice versa.

In contrast, we did not find any association between the furosemide-induced change in medullary R2* values as assessed at baseline and the eGFR decline.

A possible role of renal ischemia as a major determinant of renal disease progression was first proposed by Fine *et al.*[13] in 1998. The so-called chronic hypoxia hypothesis states that loss of glomeruli leads to loss of peritubular capillaries, which induces interstitial hypoxia. The latter triggers local inflammation and fibrosis, leading to further ischemia and renal function decline, thus producing a vicious circle. This hypothesis has been supported by many animal studies,[14,15] yet direct evidence in humans was still lacking. The results of this prospective study using BOLD-MRI to assess renal tissue oxygenation support the role of chronic tissue hypoxia in CKD development and progression in humans.

Associations between baseline renal R2* values, eGFR decline, and adverse renal outcome were confined to the outer, more cortical layers of the renal parenchyma, and no association was found between eGFR decline or renal outcome and baseline R2* values of inner medullary layers. This is in line with previous BOLD-MRI observations from our and other groups showing that cortical, but not medullary, oxygenation is decreased in CKD.[7,8] The cortical hypoxia can be explained by the combination of a reduction in cortical blood flow and intact nephrons, oxygen-dependent glomerular hyperfiltration in residual nephrons, and reduced metabolic efficiency of proximal tubular cells, as encountered in CKD.[16] Proximal tubular cells are the predominant cell type in the cortex. They are also the most energy-consuming tubular cells containing a great number of mitochondria with intense transport activity. Therefore, they are very susceptible to changes in oxygenation,[3] whereas reduction in renal blood flow mainly occurs at the cortical level in different forms of CKD.[17,18] Although hypothetical, this might explain why cortical oxygenation has a greater impact on eGFR decline than medullary oxygenation.

Furosemide blocks active sodium transport in Henle's loops and leads to an acute decrease in oxygen consumption in the medulla. Some authors have suggested that the BOLD-MRI response to furosemide could be used as a test to assess residual renal function (the response to furosemide being a marker of tubular functional reserve) in animals and humans with ischemic renal disease due, for example, to renal artery stenosis.[13,19] In this study, we also assessed the clinical interest of measuring renal oxygenation after the administration of furosemide as a prognostic marker of renal function decline. Thus, BOLD-MRI was repeated 15 minutes after the administration of furosemide in all participants, and the changes in R2* were analyzed in each of the 12 layers. As expected, furosemide-induced changes in R2* (called Delta R2*) were greater in deep medullary layers and greater in healthy controls, as reported previously.[10] Changes in R2*-induced by furosemide were smaller in CKD patients, most likely due to the fact that they have less functional tubuli per volume of renal mass but also because the tubular secretion of furosemide, which is necessary for its activity, is reduced in CKD. However, we did not find any association between the furosemide-induced change in R2* and eGFR decline or progressor status, limiting the future application of the furosemide test as a predictor of renal function decline in nonstenotic kidneys. Due to the specific structure of the kidneys with the columns of Bertin, the inner layers, as assessed with the 12-layer concentric objects (TLCO) technique, most likely also contained cortical tissue. This may have diluted the effect of furosemide and partly explain the lack of predictive power of the furosemide test.

In contrast, the R2* slope was strongly associated with eGFR decline and an adverse renal outcome. This is relevant for clinical practice because it is known that the R2* values *per se* are influenced by MRI-related characteristics,[5,20] and this limits comparisons of measurements among different centers. The R2* slope possibly overcomes this shortcoming because it merely depends on the intracompartmental distribution and less on the absolute R2* values. Additional multicenter studies are needed to validate this statement and to assess the usefulness of measuring the R2* slope in a clinical context.

A subgroup of participants underwent a second or third MRI, enabling us to assess whether long-term changes in renal tissue oxygenation do occur. We found that the R2* values did not change significantly over time, although there was a trend toward higher outer R2* values over time among CKD patients with a rapid decline in eGFR. The stability of R2* values over time in hypertensive patients and controls underlines the reproducibility of BOLD-MRI if performed under standardized conditions. In addition, this finding also illustrates that those with reduced renal tissue oxygenation at baseline remain hypoxic over long periods of time, which will, in theory, expose them to the vicious circle, as stated by the chronic hypoxia hypothesis.

This study must be seen in the perspective of its strengths and limitations. Among its strength are the monocentric prospective design, the inclusion of a control group, the use of a standardized hydration protocol, and a well-validated, reproducible method of BOLD-MRI analysis. A limitation is the lack of a histologic "gold standard" to validate BOLD-MRI, which is inherent to most studies in this field. Besides, the TLCO technique cannot differentiate with certainty cortex from medulla. Yet, even renal biopsy has limitations in its ability to predict the decline in renal function over time.

Taken together, this study describes for the first time associations between renal tissue oxygenation and eGFR decline and confirms that renal tissue oxygenation is an important determinant in CKD progression, thus confirming the chronic hypoxia hypothesis. This study also opens the road to further studies exploring functional MRI as a tool to investigate new therapeutic strategies that may retard renal disease progression. However, several shortcomings still need to be addressed. First, BOLD-MRI is not widely available and

remains expensive. Second, the procedure has not been universally standardized. Third, as illustrated by the receiver-operating characteristic curve analysis, the ability of BOLD-MRI to distinguish progressors from nonprogressors is weaker than proteinuria, which is an easy and inexpensive approach to assess the renal risk in daily practice. Nevertheless, the combination of the classic biomarker proteinuria with BOLD-MRI parameters increased the AUC. One may, therefore, expect that the ongoing development of multiparametric renal MRI protocols that combine different MRI techniques such as phase-contrast MRI, arterial spin labeling, BOLD-MRI, and diffusion-weighted imaging will further improve our capacity to predict renal outcome while performing only 1 MRI session.[7,21] This will eventually lead to a better and earlier identification of CKD patients at the highest risk of end-stage renal disease.

METHODS

This monocentric research project was approved by the local institutional review committee (Ethical Committee of the Canton de Vaud, Switzerland) and conducted according to the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Study population

Patients with CKD stages 1 through 4 or with AH without CKD followed at the outpatient clinic of the Nephrology and Hypertension Service at the University Hospital of Lausanne (CHUV) were eligible for this study. CKD was defined as an eGFR ≤60 ml/min per 1.73 m^2 or the presence of albuminuria, irrespective of its cause.[22,23] AH was defined as a mean office blood pressure ≥140/90 mm Hg measured on more than 1 occasion or an office blood pressure <140/90 mm Hg while taking 1 or more antihypertensive drugs. All patients willing to participate had to be at least 18 years old and able to understand the study protocol. A healthy control group was also included. Controls were recruited by local advertisement and were normotensive, untreated healthy individuals without a history of kidney disease or other concomitant morbidity and without structural renal abnormalities on a screening ultrasound scan. Exclusion criteria for all participants were age <18 years, a contraindication to MRI such as claustrophobia or the presence of a pacemaker or other implanted metallic device, or the presence of life-threatening comorbidities with a short life expectancy. CKD patients could not participate if they were on renal replacement therapy, had a single kidney, had autosomal dominant polycystic kidney disease, or acute kidney injury during the previous 6 months, according to the KDIGO (Kidney Disease: Improving Global Outcomes) definition.[24]

Study protocol

CKD and AH patients had a study visit at baseline, 1 year, and 3 years after study inclusion. Healthy controls only had a baseline visit and a 3-year study visit. At each follow-up visit, patient records were reviewed for intercurrent medical events (cardiovascular complications, hospitalization), and, if necessary, the treating physician was contacted for further details. Major events (e.g., initiation of renal replacement therapy, death) were immediately reported to the principal investigator.

All participants were instructed to perform a 24-hour urine collection and not to drink alcohol the day before the study visit. All participants were allowed to eat a light breakfast on the day of the study visit. Patients continued their regular medication intake.

An identical oral hydration protocol was followed by each participant at home on the day of the study visit. The study visit was performed between 11 AM and 2 PM and included a questionnaire, a complete physical examination, blood and urine sampling, a renal ultrasound scan, and a BOLD-MRI examination. Blood pressure was measured 5 times after 10 minutes of rest with an automated Omron 705IT oscillometric device, according to the recommendations of the European Society of Hypertension.[25]

Serum and urine creatinine values were measured in the central laboratory of CHUV using the IDMS-traceable Jaffe kinetic compensated method (Roche Diagnostics, Rotkreuz, Switzerland, maximum intra- and interbatch CV: 2.9%–0.7%), and the MDRD (Modification of Diet in Renal Disease) formula was used to calculate the eGFR. All renal ultrasound scans were performed by the same experienced operator in gray-scale and Doppler mode using the Aplio XG device (Toshiba Systems, AG/Sa, Volketswil, Switzerland). The renal resistive indexes were measured on 3 segmental arteries (superior, middle, and inferior) in each kidney. The values were then averaged to obtain the mean value for each participant.

BOLD-MRI measurements were carried out on a 3-T whole-body MR system (MAGNETOM Trio, Siemens Medical Systems, Erlangen, Germany), as previously described.[8] In brief, 4 coronal slices obtained in expiration were selected from morphologic images for functional evaluation with BOLD-MRI. Twelve T2*-weighted images were recorded within a single breath hold of 12.4 s with a modified Multi Echo Data Image Combination sequence (MEDIC) with the following parameters: repetition time (TR), 65 ms; echo time (TE), 6-52.2 ms (equidistant echo time spacing 4.2 ms); flip angle, 30°; field of view, 400 × 400 mm^2; voxel size, 0.8 × 0.8 × 5 mm^3; slice thickness, 5 mm; slice distance, 5.5 mm; bandwidth, 331 Hz/pixels; and matrix, 256 × 256 (interpolated to 512 × 512). Images were analyzed with Matlab 7.11 (The MathWorks Inc., Natick, MA). R2* maps were calculated voxel by voxel using the linear least square fit of the logarithm of the signal.

The 12-layer concentric objects method (TLCO) was used to analyze the images, as previously described.[8] In each coronal slice, the circumference of the renal parenchyma was drawn manually on anatomic templates. Concerning the inner border, care was taken not to include vascular structures or calyces. Hereafter, an automatic algorithm divided the selected renal parenchyma in 12 layers of equal thickness; the R2* values of each layer were combined per kidney with the voxels present in the same layer of the other 3 coronal slices. By averaging the R2* values per layer of all 4 coronal slices, the mean R2* of each layer was obtained for the kidney. (See Figure 1a–c for an example of a healthy control and Figure 1d–f for a patient with CKD.) The mean R2* value of the outer 3 layers was used as a proxy of renal cortical oxygenation (called Outer R2*), whereas the mean R2* value of the inner 8th to 10th layers was used as a proxy of medullary oxygenation (called Inner R2*). The mean R2* values of all 12 layers at increasing depth were plotted as a curve, called the R2* radial profile. The R2* radial profiles were shifted down to the *x*-axis, such that the value of the third point was 0 for every curve. This shift permitted a comparison of the shapes of the curves instead of their absolutes values. The slope of the linear part of this curve (R2* slope) was expressed as the change in hertz per percentage of change of depth and its regression coefficient β was calculated with the linear least-square regression technique.

The procedure was repeated for all 4 coronal series 15 minutes after the administration of 0.3 mg/kg of i.v. furosemide.

The interobserver agreement (Lin's concordance correlation coefficient) of the TLCO technique for each layer was between 0.85 and 0.99 for the layers and between 0.78 and 0.80 for the R2* slope, depending on the CKD stage.[8]

Statistics

Statistical analysis was performed with STATA 14.0 (StataCorp, College Station, TX). Quantitative values were expressed as mean ± SD or median values (range). Qualitative variables were expressed as number (percentage). Normality was assessed with skewness and kurtosis tests. Analysis of variance, Student *t* test, Wilcoxon rank sum test, or χ^2 test was used as appropriate to compare clinical characteristics and R2* values of the groups. Because no significant differences were observed between the R2* values of the right and left kidneys, the mean of both kidneys was used in all analyses shown.

The rate of change of eGFR slope was based on all available creatinine-based eGFR values from baseline until the last follow-up visit. Creatinine values of CKD and AH patients obtained during routine ambulatory control visits were also included. For participants who died, underwent transplantation, or initiated dialysis during follow-up, the creatinine value of the last study visit or ambulatory visit when the patient was in stable condition was used to assess the eGFR slope. The steepness of the eGFR slope was calculated with linear regression analysis. Based on a predefined power calculation, a minimum of 150 participants had to be included to have 80% power to detect (a combination of) variants explaining 2.4% (~1.5 ml/min per 1.73 m^2) of GFR variance. Multivariate linear regression analysis was performed to explore the associations of clinical and radiologic variables (renal resistive indexes, outer and inner R2*, and furosemide-induced changes in inner R2* and R2* slope) with the eGFR slope.

We further explored the association of eGFR with clinical and radiologic variables by stratifying individuals according to a yearly eGFR decline of less than or greater than 3 ml/min per 1.73 m^2 (nonprogressors vs. progressors), according to the definition used by the US Food and Drug Administration to indicate a clinically important decline in kidney function.[26] Multivariable logistic regression, adjusted for age, sex, baseline eGFR, diabetes, use of renin-angiotensin system blockers, and 24-hour proteinuria, was used to determine the independent association of the radiologic variables with progressor status.

Kaplan-Meier analysis was used to assess the proportion of CKD patients free of major renal events (defined as an increase in serum creatinine >30% or the need for renal replacement therapy[27]), according to their outer R2* level. Because it was recently demonstrated that a minority of CKD patients accounts for the higher R2* values observed in CKD patients,[7] those with outer R2* values above the 90th percentile were compared with CKD patients whose R2* values were below the 90th percentile threshold in this Kaplan-Meier analysis. For participants in whom a major renal event did not develop, data until December 31, 2015 were included and censored hereafter. The log-rank test was used to compare the proportion free of renal event in the 2 groups. For all analyses, $P < 0.05$ was considered significant.

Receiver-operating characteristic curves were built to assess the discriminative accuracy of different variables to predict major renal events. The comparison (c-statistic) was performed according to the nonparametric approach proposed by DeLong *et al.*[28]

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Figure S1. The evolution of the estimated glomerular filtration rate (eGFR) over time in each group. In this figure, the 95% confidence intervals are presented as colored areas that overlapped for arterial hypertension (AH) and control groups. CKD, chronic kidney disease.
Table S1. Detailed information on the underlying causes of chronic kidney disease (CKD) and their respective baseline estimated glomerular filtration rate (eGFR), yearly eGFR decline, and blood oxygenation level–dependent magnetic resonance imaging (BOLD-MRI) results.
Table S2. Multivariable analysis including participants suffering from CKD showing the associations between the yearly change in eGFR (outcome variable) and clinical variables.
Table S3. Multivariable logistic regression analysis showing the associations between the risk of renal progression (estimated glomerular filtration rate [eGFR] decline ≥3 ml/min per 1.73 m^2) and clinical variables, expressed as odds ratio (OR) and corresponding 95% confidence interval (CI).
Table S4. Changes in renal R2* and slope values over time, per patient category (the mean R2* value of the right and left kidneys is shown on each occasion).
Supplementary material is linked to the online version of the paper at www.kidney-international.org

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