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Tissue hypoxia, inflammation, and loss of glomerular filtration rate in human atherosclerotic renovascular disease

Abdelrhman Abumowad¹, Ahmed Saad^{1,3}, Christopher Ferguson¹, Alfonso Eirin¹, John R Woollard¹, Sandra M. Herrmann¹, LaTonya J. Hickson¹, Emily C. Bendel², Sanjay Misra², James Glockner², Lilach O. Lerman¹, Stephen C. Textor¹

¹Division of Nephrology and Hypertension, Mayo Clinic, 200 First Street SW, Rochester, MN, USA

²Department of Radiology, Mayo Clinic, 200 First Street SW, Rochester, MN, USA

³Department of Family Medicine, Creighton University, Omaha, NE

Abstract

The relationships between renal blood flow (RBF), tissue oxygenation, and inflammatory injury in atherosclerotic renovascular disease (ARVD) are poorly understood. We sought to correlate RBF and tissue hypoxia with glomerular filtration rate (GFR) in 48 kidneys from patients with ARVD stratified by single kidney iothalamate GFR (sGFR). Oxygenation was assessed by Blood Oxygenation Level Dependent magnetic resonance imaging (BOLD MRI), which provides an index for the levels of deoxyhemoglobin within a defined volume of tissue (R2*). sGFR correlated with RBF and with the severity of vascular stenosis as estimated by duplex velocities. Higher cortical R2* and fractional hypoxia and higher levels of renal vein neutrophil-gelatinase-associated-lipocalin (NGAL) and monocyte-chemoattractant protein-1 (MCP-1) were observed at lower GFR, with an abrupt inflection below 20 ml/min. Renal vein MCP-1 levels correlated with cortical R2* and with fractional hypoxia. Correlations between cortical R2* and RBF in the highest sGFR stratum (mean sGFR 51 ± 12 ml/min; R=−0.8) were degraded in the lowest sGFR stratum (mean sGFR 8 ± 3 ml/min; R=−0.1). Changes in fractional hypoxia after furosemide were also absent in the lowest sGFR stratum. These data demonstrate relative stability of renal oxygenation with moderate reductions in RBF and GFR, but identify a transition to overt hypoxia and inflammatory cytokine release with severely reduced GFR. Tissue oxygenation and RBF were less correlated in the setting of reduced sGFR, consistent with variable oxygen consumption or a shift to alternative mechanisms of tissue injury. Identifying transitions in tissue oxygenation may facilitate targeted therapy in ARVD.

Graphical Abstract

Corresponding author: Stephen C. Textor¹, MD, Division of Nephrology and Hypertension, Mayo Clinic, 200 First Street SW, Rochester, MN, 55905, Phone: (507)-28 4-4083, Fax: 507-266-7891, stextor@mayo.edu.

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Keywords

BOLD MR; hypertension; renal artery stenosis; renovascular disease; revascularization; hypoxia; medullary; cortical; renal blood flow; GFR; NGAL; VEGF-A

Introduction:

How changes in oxygenation develop during the evolution of clinical kidney disease has been controversial. Many diseases of the kidney include vascular compromise associated with tissue hypoxia, which some authors suggest represents a “common pathway” leading to tissue fibrosis and destruction of glomerulo-tubular structures¹²³. A recent report of human subjects with CKD indicates that measurable cortical hypoxia identified using Blood Oxygen Level Dependent (BOLD) MR predicts later progression to advanced renal failure⁴. Previous experimental studies with occlusive renovascular disease (RVD) indicate that initial hemodynamic compromise eventually transitions to inflammatory, oxidative, and pro-fibrotic mechanisms that no longer depend on blood flow alone⁵. How changes in oxygenation actually relate to changes in cortical and/or medullary blood flow in patients with RVD is poorly understood.

Relationships between cortical and medullary tissue perfusion and oxygenation within the kidney are unusually complex. Because filtration is its primary function, the normal kidney receives blood flow vastly exceeding the amount needed for metabolic demands^{6, 7}. Remarkably, measured oxygen tension within the kidney cortex is below that of the aorta and main renal artery, due in part to arteriovenous shunting^{7, 8}. Deeper regions of the medulla exhibit lower oxygen tensions due to reduced flow in post-glomerular efferent arteriolar blood vessels and oxygen consumption associated with energy-dependent active solute transport⁹. Hence, the kidney normally exhibits a gradient of developing hypoxia between the cortex and medulla. These observations have been confirmed in human subjects utilizing BOLD-MRI, which provides a non-invasive method for measuring renal parenchymal oxygenation.

Earlier cross-sectional studies applying BOLD MR to a varied group of patients with CKD failed to identify any predictable relationship between cortical or medullary oxygenation and levels of GFR^{10, 11}. By contrast, others reported a general relationship between cortical hypoxia and reduced GFR¹². Recently, Pruijm, et.al. reported that higher levels of cortical R2* in patients with CKD predict later progression to advanced renal failure^{4, 13}. Experimental studies of renal failure associated with impaired myocardial function (cardiorenal syndrome) confirm the potential for BOLD MR to detect development of hypoxia that is associated with rising levels of hypoxia-inducible factors such as HIF-1 α ¹⁴. While such data suggest that disturbances in oxygenation can be identified in some renal disorders, few human studies have examined these findings quantitatively in relation to renal hemodynamics. Whether changes in oxygenation can identify progressive inflammatory changes in kidneys beyond a stenotic lesion in humans is unknown.

Atherosclerotic renovascular disease (ARVD) presents an opportunity to examine reductions in blood flow known to be associated with reduced GFR. Results from treatment trials for ARVD now favor depending primarily on medical therapy for the treatment of renovascular hypertension¹⁵, making it likely that more kidneys than before will be subjected to periods of chronically reduced blood flow beyond stenotic lesions. It is also recognized that restoration of blood flow at some point no longer leads to recovery of function. We undertook this study to test the hypothesis that progressively lower GFR specifically associated with reduced cortical and medullary blood flow would be identifiable with reduced tissue oxygenation. We also sought to examine whether such changes would be associated with altered response to inhibition of tubular transport (furosemide) and/or with renal vein markers of injury and inflammation. To optimize BOLD imaging, the research conditions for these studies were closely controlled for sodium and water intake, medication use, and exclusion of diabetes and other kidney diseases.

Results

Demographics, Blood pressure, and GFR:

Demographic characteristics of the entire study group (n= 48, Total) and each subgroup by single kidney GFR (G1, highest quartile of GFR), G2 (middle two quartiles of GFR) and G3 (lowest quartile of GFR) are summarized in Table 1. Average single kidney GFR values ranged from G1: 51 $\pm$ 12 (mean $\pm$ SD) [range 38–84] to G2: 25 $\pm$ 5 (range 37–14) to a low of G3: 8 $\pm$ 3 ml/min [range 2–13]. No differences were evident between the three groups in terms of age, sex, body mass index (BMI), weight, systolic blood pressure (SBP), diastolic blood pressure (DBP), hemoglobin, urine protein or serum creatinine.

Kidney volume and blood flow measurements:

Single kidney GFR correlated with the renal ultrasound peak systolic velocity Pearson correlation ($r=-5$, $P<0.05$) (Figure 1A). RBF correlated with single kidney GFR [Pearson-correlation (r) =0.87, $P<0.001$. For each 1ml/min increase in RBF Single Kidney GFR rose by 0.09 $\pm$ 0.008 ml/min as shown in Figure 1B, reflecting an average filtration fraction of 19%. Results of CT measurements of volume and blood flow are summarized in Table 2. Total kidney volume (cubic centimeters) decreased from 166 $\pm$ 24 (G1) to 53 $\pm$ 27 ml (G3), $p<$.

001 corresponding to reduced single kidney GFR. Parallel decreases in volume were observed in both the cortical and medullary volumes. Tissue perfusion (ml/min/cm^3) in cortex also fell progressively from 3.3 (G1) to 1.9 ml/min/cm^3 (G3), $p < .001$. Renal blood flow measurements were lower due both to reductions in measured volume and perfusion associated with lower single kidney GFR (G1, G2, and G3: 408 ± 126 , 201 ± 64 , and 65 ± 32 mL/min , respectively). These differences were observed in both cortical and medullary blood flow and perfusion.

BOLD MRI measurements:

Figure 2 provides an example of BOLD MRI maps of three kidneys with mild, moderate, and severe reduction in single kidney GFR. Numerical values for BOLD imaging for both axial and coronal slices of these kidneys are summarized in Table 3. Higher values for cortical $R2^*$ in both axial and coronal images were observed at the lowest levels of kidney function (G1, G2, and G3: 18 ± 1.4 , 19.8 ± 2.7 , and 22.4 ± 4 , sec^{-1} respectively, ANOVA $P = 0.003$). Similarly, larger values for the fraction of the whole kidney with $R2^*$ more than 30 sec^{-1} were observed with reduced single kidney GFR (Median (Q1, 3): G1, G2, and G3: 2.4% (1.3–7), 7.2% (1.5–9.7), and 11% (6.7–23.6), respectively, ANOVA $P = 0.005$). The fractions of these kidneys with $R2^* > 20 \text{ sec}^{-1}$ also tended to be higher with lower GFR, particularly in G3 compared to G1 (58.2% vs. 44%, $p < 0.05$). No significant differences were apparent between axial and coronal views for these parameters.

Cortical blood flow correlated with $R2^*$ inversely in kidneys with higher levels of GFR (Group 1: Pearson: $R = -0.8$, $p < 0.05$), although these relationships were not evident with more severely reduced GFR (Group 2 and 3, $R = -0.1$ and -0.15 , respectively, NS).

Figures 3 (A–F) illustrate the relationships between blood flow (A and B) and sGFR (C–F) with measures of tissue hypoxia (A–D) Cortex $R2^*$ (Sec^{-1}) and Fractional Hypoxia [% $R2^* > 30$] and inflammatory markers NGAL (E) and MCP-1 (F) for all kidneys studied.

Both measures of hypoxia resulted in curves which illustrate only minimal increases in tissue hypoxia associated with progressively lower single kidney GFR until an inflection region where both cortical $R2^*$ and fractional hypoxia $> 30\%$ rose abruptly near sGFR levels of 20 ml/min (Figure 3, C–F). A receiver operating characteristic (ROC) analysis examining Cortical $R2^*$ as a predictor of single kidney GFR above or below 20 ml/min identified a value of 20.6 sec^{-1} as the $R2^*$ value that maximized the sum of sensitivity (0.7) and specificity (0.5) with a significant area under the curve (AUC: 0.75, $p = .001$). Similarly, ROC analysis examining fractional hypoxia identified a value of 9.5% (% of the kidney with $R2^* > 30 \text{ sec}^{-1}$) to predict single kidney GFR above or below 20 ml/min with sensitivity (0.6) and specificity (0.5) with an AUC of (0.71, $p = 0.017$). Renal venous levels of NGAL and MCP-1 rose substantially near the same levels of sGFR (Figure 3: E–F).

Response to Furosemide:

Cortical $R2^*$ levels did not change consistently after furosemide administration in any group. The fractional hypoxia for $R2^* > 20$, which reflects data from the whole kidney section, fell from 44 ± 14 to 35 ± 6 and 50 ± 17 to 42 ± 18 in G1 and G2, $p < 0.05$, respectively, but did not change in G3 (58 ± 22 to 56 ± 25 , NS). (Figure 4A)

Similarly, the fractional hypoxia for $R2^* > 30 \text{ s}^{-1}$ fell in G1 and G2 only (Median (Q1, 3): 4.1(1.5–7.4) % to 2.7(1.9–4.2) %, and 7.2(1.5–9.7) % to 5.1(1.2–6) %, $p < 0.05$) respectively. Relative changes from baseline for each of these parameters are illustrated in Figure 4B. Measurable medullary hypoxia did not change for G3 subjects.

Renal vein markers: Renal vein levels of neutrophil gelatinase-associated lipocalin (NGAL) were elevated as compared with similarly collected values in essential hypertensive subjects $[72.5 \pm 31.5]$ ¹⁶ in all three groups: [G1: 156 ± 27 G2: 183 ± 68 and G3: 218 ± 89 ng/ml [G3 levels were elevated further compared to G1, $p < 0.05$] respectively. Levels of vascular endothelial growth factor (VEGF-A) marginally correlated with the percent of whole kidney with fractional hypoxia ($R2^*$ more than 20). Figures 5 (A) illustrate the correlation between monocyte chemoattractant protein-1 (MCP-1) and cortical $R2^*$ [$n=25$, $r=0.67$, $p < 0.1$] and (B) Fractional hypoxia ($\%R2^* > 30$) [$n=25$, $r=0.3$, $p < 0.1$]. Table 4 summarizes levels of NGAL, MCP-1, VEGF-A, and VEGF-C by individual groups of sGFR. These markers were progressively higher with the loss of sGFR [$G3 > G2 > G1$] as illustrated in Figure 3 (E and F).

Discussion:

These data identify quantitative relationships between reductions in single kidney cortical and medullary blood flow, GFR, and kidney parenchymal oxygenation determined by BOLD MR for patients with atherosclerotic RVD (ARVD). Clinical selection criteria and the fact that individual GFR was correlated with RBF and measured duplex velocities reinforce the likelihood that ARVD was the primary basis for kidney dysfunction in this cohort. Our results demonstrate that elevations in cortical $R2^*$ indeed were associated with reduced tissue perfusion, but suggest that measurable hypoxia only became evident with far advanced disease (G3). Our data also indicate that severely reduced blood flow was associated with medullary tissue hypoxia, manifest as enlarged fractions of kidney tissue functioning at overtly hypoxic levels ($R2^*$ above 20 or 30 s^{-1}). Measurable hypoxia was associated with higher renal venous levels of VEGF-A, MCP-1, and NGAL. We interpret the correlation between venous levels of VEGF-A with the percent of whole kidney with $R2^*$ above 20, to reflect an independent measure of hypoxic signaling from the kidney as an angiogenic stimulus¹⁷. Based on measured blood flow, this stimulus did not itself restore the renal circulation, but may be a marker of microcirculatory “deficit”. This interpretation is consistent with studies of adjunctive angiogenic maneuvers, such as injection of vascular growth factors¹⁸ or infusion of adipose-derived mesenchymal stem cells that increase blood flow to post-stenotic kidneys that have been associated with a fall in renal venous VEGF-A¹⁹. MCP-1 is an inflammatory cytokine implicated in the pathogenesis of kidney diseases in both animal models²⁰ and humans²¹. Our results indicate that tissue hypoxia measured by BOLD MR was associated with higher MCP-1 levels in the renal vein. Taken together, these data support the concept of a progressive increase in inflammatory injury as ARVD leads to loss of GFR. Targeting MCP-1/CCL2 with specific inhibitors reduces injury in a murine RVD model²⁰. Whether targeting this pathway or other inflammatory mediators will alleviate progressive injury in human ARVD warrants further study.

The association between blood flow and oxygenation was degraded with advanced loss of GFR, as were functional changes induced by furosemide. Measured levels of hypoxia were

variable at extreme reductions in sGFR, with some G3 kidneys having near normal levels of $R2^*$. (Figure 3 C and D). Some authors have suggested that kidneys may be “hibernating” with little filtration and thereby reduced oxygen consumption²². We have observed that some kidneys with total arterial occlusion have near normal levels of tissue $R2^*$, suggesting that totally non-functioning kidneys may consume little metabolic energy²³. However, most kidneys with sGFR below 20 ml/min exhibited elevated $R2^*$ and fractional hypoxia and had consistently higher renal vein inflammatory biomarkers (Figure 3 C–F). We interpret these findings to better define the limits of perfusion that can be sustained by the kidney to retain both filtration and measurable oxygen consumption due to tubular transport. These detailed studies for individual kidneys in ARVD were possible due to the relative preservation of overall two-kidney GFR associated with the presence of a contralateral kidney. While renal revascularization restores blood flow and occasionally leads to recovery of GFR in the post-stenotic kidney²⁴, it fails to increase overall GFR in most cases^{25, 26}. Experimental studies indicate that loss of GFR related initially to reduced blood flow transitions to progressive injury associated with oxidative stress, active inflammation and interstitial fibrosis that no longer is reversed by improving blood flow^{27,28,29}. The loss of connection between measures of blood flow, hypoxia, and GFR in our patients reported here suggests that such a transition may develop for individual kidneys between G2 and G3.

Experimental studies and human research indicate that oxygen tensions within the kidney remain substantially preserved despite reductions in main renal artery blood flow approaching 30–40%^{30, 31}. Hence, multiple compensatory physiologic pathways lead to renal adjustments including afferent artery diameter, filtered load, arteriovenous shunting, and oxygen consumption related to active reabsorption and secretory processes^{7, 30}. The capacity of the kidney for acute regulatory changes has limits, however, beyond which the kidney parenchyma develops measurable hypoxia, as reflected by rising levels of deoxygenated hemoglobin³². Our results extend these observations to underscore the limits of adaptation to reduced blood flow during chronic vascular occlusion in human subjects. Previous studies identified measurable changes in cortical hypoxia with advanced disease but did not measure associated alterations in blood flow.³³

Our results also underscore the presence of considerable heterogeneity between sampling volumes within individual kidneys (Figure 2). We interpret this variability both to reflect sampling variations of deeper medullary tissue and biologic variation observed in both normal³⁴ and diseased organs³⁵. It is important for investigators to account for these variations as they apply BOLD imaging methods for assessing kidney oxygenation. While cortical regions often demonstrate little variation between slices, deeper medullary sections certainly have more heterogeneity.

BOLD MR imaging examines levels of deoxyhemoglobin (expressed as $R2^*$ (sec⁻¹) within a defined volume (voxel) of tissue. Typically, images are obtained for a defined sample (“slice”) of kidney tissue aligned either with the axial or coronal axis in cross-section, often near the mid-section defined by hilar blood vessels (Figure 6). Cortical and medullary regions differ with respect to both blood flow and levels of oxygen consumption. How best to define and express $R2^*$ for different regions has been controversial and has led to multiple analytical schemes, as we and others have reviewed^{11, 35, 36}. Most often, BOLD images are

acquired for multiple slices and the results averaged to express a composite $R2^*$ value for cortex and medulla. Although average $R2^*$ levels are reproducible between observers, less attention has been directed at the variability of oxygenation between different slices or sampling regions. We believe that differences between slices likely represent both variable proportions of deep medullary segments included in a given sample and heterogeneities related to biologic variation and disease.

Atherosclerotic vascular disease, in particular, is subject to patchy, localized areas of severe disease, sometimes with local infarctions²³. Initial reports of BOLD imaging set a precedent for using localized operator-selected ROI's within visually apparent cortex or medullary tissue. While these methods define differences between abundantly perfused and oxygenated cortex and the less perfused medullary segments, they can fail to depict the extent of reduced oxygen tension in different regions³⁷. Semi-automated analytic maneuvers have been introduced to identify distributions of voxel oxygenation, computer-generated "concentric objects", or fractional hypoxia that can limit subjective bias and facilitate quantitative analysis. The sensitivity of BOLD measurements to study conditions has been a practical limit to using such measurements in clinical medicine. Recent reviews emphasize the importance of avoiding confounding effects related to differences in sodium and water intake, medication use, and other kidney diseases, including diabetes, which itself modifies kidney oxygenation^{37,38}. Our studies required a known, standardized sodium and water intake and adjustment of medications to control the likelihood of these factors modifying BOLD MR measurements. We believe the attention to these elements likely improved the identified relationships between measured cortical and fractional tissue hypoxia and independent measures of blood flow, volume, and single-kidney GFR.

This study had limitations. We excluded individuals with diabetes and far advanced two-kidney loss of GFR (creatinine above 2.5mg/dL). The asymmetric nature of RVD allowed the study of individual kidneys with severe GFR reductions with the benefit of function from the contralateral kidney for excretion of contrast.

Our results provide important insights into the evolution of ischemic kidney injury associated with ARVD. They underscore the adaptability of renal hemodynamics to gradually developing reductions in cortical perfusion and blood flow that develop with atherosclerotic disease. Decrements of cortical oxygenation and functional responses to inhibition of solute transport were most evident with severe loss of blood flow and GFR with a transition zone identifiable near 20 ml/min in our series. Results from multiple clinical trials in the last decade have fostered dependence on optimized medical therapy as the primary management strategy for patients with atherosclerotic RVD¹⁵. This necessarily translates into many more patients living with chronically underperfused kidneys, some of which will progress to advanced loss of GFR. Further studies are needed to define specific features that identify kidneys at risk for ischemic damage and/or no longer likely to respond to clinical measures to recover function, including revascularization. Our results suggest that both measures of tissue hypoxia and inflammatory injury might assist in this effort. Clinicians need additional resources to evaluate functional reserve and viability of such kidneys as one seeks to identify both the optimal role and potential for adjunctive therapy, including cell-based therapy^{39,19} and mitochondrial protection⁴⁰, in addition to

revascularization maneuvers. Our data suggest that quantitative assessment of both cortical and medullary fractional hypoxia may help identify reversible versus irreversible microvascular injury in this disorder.

Methods:

Study population

In this cross sectional study, Fifty-five kidneys from 28 subjects were analyzed from patients participating in a study of atherosclerotic renovascular disease registered with [ClinicalTrials.gov](https://clinicaltrials.gov) (). Subjects with ARVD greater than 60% stenosis as identified by clinical duplex ultrasound and/or quantitative C.T imaging were enrolled between July 2013 to Sep 2017. Patients were admitted to the Clinical Research and Trials Unit (CRTU) of St. Mary's Hospital for four days. Seven kidneys were excluded due to solitary kidney, multi-cystic kidneys, or insufficient image quality leaving 48 kidneys for analysis. These 48 kidneys were classified into 3 groups based on single kidney GFR measured by iothalamate clearance and partitioned by the percentage of RBF, as previously described²⁶. Inclusion criteria included age between 40 and 80 years, hypertension (systolic BP>155 mmHg), and/or requirement for two or more antihypertensive medications. Pre-study serum creatinine levels were below 2.5 mg/dL. There were no restrictions on antihypertensive agents, although loop diuretics were changed to diluting site agents (e.g. hydrochlorothiazide, indapamide, metolazone) prior to the study. Angiotensin converting enzyme (ACE inhibitor) or angiotensin receptor blocker (ARB) therapy was maintained during these studies. Subjects with the following criteria were excluded: diabetes requiring insulin or oral hypoglycemic medications, known allergy to furosemide, pregnancy, or a cardiovascular event within three months including myocardial infarction, stroke, or congestive heart failure. Cardiac ejection fraction less than 30% was an exclusion as well.

Inpatient Study Protocol: On the first day, subjects were admitted to the Clinical Research and Trials Unit CRTU to establish a specified sodium intake (150mEq daily). On the second day, GFR was measured using iothalamate clearance with three consecutive, timed periods. On the following day, BOLD MRI was performed³⁷. On the fourth day, renal vein blood samples were obtained from each kidney for measurement of neutrophil gelatinase-associated lipocalin (NGAL), monocyte chemoattractant protein-1 (MCP-1), vascular endothelial growth factor(VEGF-A) and VEGF-C as previously described¹⁹. NGAL was tested by ELISA according to the manufacturer's protocol (BioPorto Diagnostics, catalog no. KIT 036), VEGF-A and VEGF-C and MCP-1 were tested Luminex (Millipore, Billerica, MA, USA). Cytokine values measured in the renal venous samples were expressed as amount per individual kidney volume, which varied between 28–265 cubic centimeters (cm³), the venous catheter was then advanced to the inferior vena cava for bolus contrast injection before obtaining helical multi-detector CT imaging for the measurement of cortical and medullary perfusion and blood flow¹⁹.

BOLD imaging technique: BOLD MR scans were performed on a 3.0-T MRI (GE Twin Speed Signa EXCITE; GE Medical Systems, Waukesha, WI) with a twelve-channel torso-phased array coil. A fast 2-dimensional gradient echo BOLD sequence with twelve echo

times was used. Imaging was performed in both axial and coronal planes during patient instructed breath-hold intervals (20 seconds). Axial and coronal images were acquired at TR/TE/Flip Angle/FOV/Slice thickness/Slice amount/Matrix= 90ms, 3.5–60ms, 20°, 32–40cm, 4mm, 2–4, 352×224 to 192. BOLD acquisition was performed both before and fifteen minutes following the administration of intravenously injected furosemide (20mg) followed by a 20mL saline flush¹⁹.

BOLD data were quantified using a Matlab Graphical User Interface (The MathWorks, Natick, Mass) program developed in our laboratory by drawing ROIs on T2* images and then transferring that ROI to R2* BOLD maps. For each slice of the kidney where BOLD images were acquired, one ROI was drawn to include the total-kidney (cortex and medulla) and another to be limited to the cortex alone. Cortical oxygenation was assessed by the average cortical R2* value and whole kidney levels of hypoxia by measuring the fractional tissue hypoxia (percentage of the whole slice >20 and 30 sec⁻¹), which sample primarily the medulla as previously described³⁷.

MDCT imaging technique: For MDCT measurement of renal blood flow, the femoral vein was cannulated with a 6F sheath followed by the placement of a 5-F pigtail Cobra catheter (Cook, Bloomington, Ind.) in the right atrium used for injection of iodinated contrast. Functional and Volume studies were performed using a 64-slice dual-source (SOMATOM Definition, Siemens Medical Solutions, Forchheim, Germany) shortly after an injected bolus of iopamidol 370 (0.5mL/kg not exceeding 40mL). Images of four adjacent 7.2-mm slices were obtained at each of the successive 45 time-points over approximately 2.5 minutes. After fifteen minutes, a second bolus of contrast was injected. A helical study was then performed in the axial plane with a slice thickness of 5-mm, which covered the entirety of both kidneys and was used to determine cortical and medullary volumes^{19, 41, 42}.

MDCT images were reconstructed and quantified using the Analyze software package (Biomedical Imaging Resource; Mayo Clinic Rochester, Minn). In brief, regions of interest (ROI) were traced on the helical scans in the cortex and medulla on every slice of both kidneys to measure the volume. Additionally, ROIs were drawn in the cortex, medulla, and aorta during the contrast transit images for hemodynamic measures. ROIs drawn on the functional MDCT images were used to plot time-attenuation curves which were then quantified using in an in-house Matlab (The MathWorks, Natick, Mass). (Graphical user interface) where a gamma-variate curve fitting model was used to determine perfusion and renal blood flow^{19, 41}.

Single kidney GFR: Iothalamate clearance (iothalamate meglumine, Conray, Mallinckrodt) was determined over three 30-minute timed collection periods after oral hydration (20ml/kg) ^{19, 43}. Single kidney GFR was calculated by partitioning the iothalamate clearance for both kidneys by the percent of MDCT-derived renal blood flow for each kidney.

Statistical Analysis: Statistical analysis was performed using JMP software version 13.0.0 (SAS Institute Inc., Cary, NC). Distribution of the data was assessed by Shapiro-Wilk test. Normally distributed data were expressed as mean values and SD while skewed data

were expressed as median values (interquartile range). Wilcoxon rank-sum test and t-tests were used as appropriate. Qualitative variables were expressed as number (percentage), Chi-squared test was used for categorical variables as appropriate. For comparisons among groups parametric (analysis of variance (ANOVA)) and non-parametric (Kruskal-Wallis) tests were used, with $p < 0.05$ considered statistically significant. Pearson and Spearman correlation methods were used to determine the correlations as appropriate. A receiver operating characteristic was used for sensitivity analysis. Fitting of the curves was done mathematically using JMP Software Graph Builder interface (SAS Institute Inc., Cary, NC).

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Figure 1.

(a) Levels of single-kidney glomerular filtration rate (sGFR) were lower in relation to the severity of vascular stenosis as measured by duplex peak systolic velocities. Pearson correlation: $r = -0.5$, $P < 0.05$. (b) Relationship between sGFR (ml/min) and renal blood flow (ml/min). Pearson correlation: $r = 0.87$, $P < 0.001$. Regression coefficient (β): 0.09 ± 0.008 ml/min. We interpret these data to support atherosclerotic renovascular disease as the primary basis for reduced GFR in this cohort. US, ultrasound.

Figure 2.

Representative blood oxygenation level-dependent magnetic resonance imaging parametric maps of 3 kidneys with mild (G1), moderate (G2), and severe (G3) reduction in single-kidney glomerular filtration rate, respectively. (a–c) Representative angiograms from patients in this group. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

Figure 3.

(a,b) These plots illustrate the higher levels of cortical hypoxia (cortical R2*) and percentage of the kidney with R2* >30 with lower levels of renal blood flow in the single kidneys. (c,d) Similarly, levels of cortical and whole-kidney tissue hypoxia rose only slightly with reduced single-kidney glomerular filtration rate (sGFR) until a transition limit where hypoxia rose abruptly. This was evident beginning near an sGFR of 20 ml/min (red dashed circle). Fitting these points into a curve was done mathematically using JMP software (SAS Institute, Cary,

NC). (e,f) Higher levels of monocyte-chemoattractant protein-1 (MCP-1) and neutrophil-gelatinase-associated-lipocalin (NGAL) with reduced sGFR. Higher levels were most apparent at severe GFR reductions near the levels associated with overt hypoxia.

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Figure 4.

(a,b) The change in fractional of kidney with $R2^* > 20/\text{sec}$ and $R2^* > 30/\text{sec}$ after administration of i.v. furosemide. Kidneys in both G1 and G2 groups had substantial decrements in fractional hypoxia in medullary zones (cortical levels did not change in any group), whereas the change after furosemide was minimal in kidneys with very low glomerular filtration rate (G3).

Figure 5.
(a,b) Monocyte-chemoattractant protein-1 (MCP-1; n = 25) correlated with the cortical R2* and fractional hypoxia (% R2* >30); Pearson correlation: $r = 0.65$, $P < 0.001$ and Spearman correlation: $r = 0.35$, $P = 0.1$, respectively.

Figure 6.

(a) Schematic diagram illustrating the axial slice location for acquisition of blood oxygenation level–dependent magnetic resonance imaging and blood flow (multidetector computed tomography). Three or 4 kidney planar slices were acquired in the midsection of the kidney defined by the hilum. (b) A T2* image of 1 plane with demarcation of 2 regions of interest (ROI). One ROI was drawn to include the whole kidney slice used to estimate the degree of fractional hypoxia. Another ROI was drawn in the cortex to measure the cortical R2* over the circumference of this slice. (c) Parametric map for R2* levels within this slice from which cortical R2* and fractional hypoxia (both for fraction >20 sec⁻¹ and fraction >30 sec⁻¹) were obtained. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

Table 1.

Demographics, Blood pressure, and GFR of kidneys with mild (G1), moderate (G2) and severe reduction in single kidney GFR (G3):

	Total	(G1)	(G2)	(G3)	p-value
N (%)	48 (100%)	12 (25%)	24 (50%)	12 (25%)	
Single kidney GFR, ml/min	27±17	51 ± 12	25±6	8±3	
Age (years)	71.7±5.6	68.6±8.1	73.7±26.6	71±7	NS
Sex (Male), % [¥]	58%	58%	58%	58%	NS
BMI	31.4±5.9	34.1±6	29.9±5.7	32±5.8	NS
Weight, KG	87±17	93.6±17	82.8±18.3	88.6±15.8	NS
SBP, mm Hg	145±13	142±11.8	147.6±14.6	143±11	NS
DBP, mm Hg	70±12	71±10	71±13	68.3±12	NS
Hemoglobin, g/dl	13.8±1	13.8±1.2	13.3±1.3	13.7±1.2	NS
S. Creatinine, mg/dl [¶]	1.4(1.2–1.6)	1.3(0.9–2.4)	1.5(1.1–1.6)	1.5(1.4–1.8)	NS
Total urine proteins, mg/24h [¶]	101[50,187]	123[62,164]	95[48,212]	99[73,162]	NS
Iothalamate clearance (Both kidneys)	55±16	67±16.2	50.3±10	52.2±19	0.006

Mean± SD, Median (Q1-Q3), P values were determined by analysis of variance test

[¶]Kruskal-Wallis test

[¥]chi-squared test

Table 2:

Single kidney volume, blood flow, and U.S Doppler velocity measurements:

	Total	(G1)	(G2)	(G3)	P-value
Cortical volume, cm³	67±32	102±20	70±19	33±17	P<0.05
Medullary volume, cm³	42±19	60±10	43±14	20±11	P<0.05
Total Kidney Volume cm³	111±49	166±24	113±30	53±27	P<0.05
Cortical perfusion, ml/min/ cm³	2.4±0.9	3.3±0.9	2.4±0.7	1.9± 1	P<0.05
Medullary perfusion, ml/min/ cm	0.8±0.3	0.95±0.3	0.8±0.3	0.6±0.2	P<0.05
Cortical flow, mL/min	183±129	351±118	156±56	54±28	P<0.05
Medullary flow, mL/min	35±23	57±19	36±20	11±6	P<0.05
renal blood flow, mL/min	218±146	408±126	201±64	65±32	P<0.05
U.S Doppler velocity cm/s	277±110	204±123	261±97	338±95	P<0.05

All numbers are of single kidney measurements, Mean± SD, P values were determined by analysis of variance

Table 3

BOLD MRI measurements:

	Cortex R2* (Sec ⁻¹)		FH % of whole kidney with R2* >20		FH % of whole kidney with R2* >30	
	Axial	coronal	Axial	coronal	Axial	Coronal
G1	18±1.4	18.7±1.3	44.1±14.3	46.1±11.1	4.1(1.5–7.4)	4.6(2.2–8.3)
G2	19.8±2.7	20.3±2.7	50.2±17.2	53.4±11.1	7.2(1.5–9.7)	6.3(4–10.7)
G3	22.4±4 *	21.6±3.6 *	58.2±22 *	56.6±14.1 *	11(6.7–23.6) *	11.2(7.3–15.5) *
P-value	0.003¥	0.07¥	0.2¥	0.2¥	0.005¶	0.03¶

Kidneys with mild (G1), moderate (G2) and severe reduction in single kidney GFR (G3), All numbers are of single kidney measurements. Data are presented as Mean± SD for normally distributed values or Median (Q1-Q3) for skewed data. P values were determined by ¶ Kruskal-Wallis test and ¥ analysis of variance test as appropriate.

* Significant compared to G1 P<0.05 determined using Student's t-test

Table 4.

Renal vein inflammatory markers and cytokines values per individual kidney volume.

	G1	G2	G3	P-value
MCP-1 (pg/ml)/cm³	1.9[1.5–3.4]	3.9[3.7–5.9]	9.6[5–12.9]	0.004
VEGF-A (pg/ml)/cm³	0.8[0.2–1.6]	1.0[0.3–3.0]	2.8 [1.3–6.1]	0.008
VEGF-C (pg/ml)/cm³	2.7[2.0–3.9]	7.2[4.2–9.1]	7.8[7.2–9.7]	0.04
NGAL (ng/ml)/cm³	0.9[0.8–1.0]	1.4[1.0–1.8]	3.6[3.2–4.3]	0.0001

All numbers are of single kidney measurements, Data are presented as Median (Q1-Q3), P values were determined by Kruskal-Wallis test.