MRI-based glomerular morphology and pathology in whole human kidneys

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1Department of Radiology, Washington University School of Medicine, St. Louis, Missouri; 2Department of Anatomy and Developmental Biology, Monash University, Melbourne, Victoria, Australia; 3School of Computing, Informatics, and Decision Systems Engineering, Arizona State University, Tempe, Arizona; 4Department of Physics, College of Natural Sciences, University of Hawaii at Manoa, Honolulu, Hawaii; 5Department of Anatomical Pathology, Monash Medical Centre, Clayton, Victoria, Australia; 6Department of Pediatrics, Division of Nephrology, University of Virginia Medical Center, Charlottesville, Virginia; 7Monash Biomedical Imaging, Monash University, Melbourne, Australia; and 8Department of Biology, College of Natural Sciences, University of Hawaii at Manoa, Honolulu, Hawaii

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Beeman SC, Cullen-McEwen LA, Puelles VG, Zhang M, Wu T, Baldelomar EJ, Dowling J, Charlton JR, Forbes MS, Ng A, Wu QZ, Armitage JA, Egan GF, Bertram JF, Bennett KM. MRI-based glomerular morphology and pathology in whole human kidneys. Am J Physiol Renal Physiol 306: F1381–F1390, 2014. First published March 19, 2014; doi:10.1152/ajprenal.00092.2014.—Nephron number ($N_{\text{glomer}}$) and size ($V_{\text{glomer}}$) are correlated with risk for chronic cardiovascular and kidney disease and may be predictive of renal allograft viability. Unfortunately, there are no techniques to assess $N_{\text{glomer}}$ and $V_{\text{glomer}}$ in intact kidneys. This work demonstrates the use of cationized ferritin (CF) as a magnetic resonance imaging (MRI) contrast agent to measure $N_{\text{glomer}}$ and $V_{\text{glomer}}$ in viable human kidneys donated to science. The kidneys were obtained from patients with varying levels of cardiovascular and renal disease. CF was intravenously injected into three viable human kidneys. A fourth control kidney was perfused with saline. After fixation, immunofluorescence and electron microscopy confirmed binding of CF to the glomerulus. The intact kidneys were imaged with three-dimensional MRI and CF-labeled glomeruli appeared as punctate spots. Custom software identified, counted, and measured the apparent volumes of CF-labeled glomeruli, with an <6% false positive rate. These measurements were comparable to stereological estimates. The MRI-based technique yielded a novel whole kidney distribution of glomerular volumes. Histopathology demonstrated that the distribution of CF-labeled glomeruli may be predictive of glomerular and vascular disease. Variations in CF distribution were quantified using image texture analyses, which is a useful marker of glomerular sclerosis. This is the first report of direct measurement of glomerular number and volume in intact human kidneys.

chronic kidney disease; magnetic resonance imaging; cationized ferritin

It is now well-known that lower nephron number is associated with higher susceptibility to kidney disease and hypertension. It is likely that a low number or a loss of nephrons leads to glomerular hypertrophy and hyperfiltration. The theory behind this is that a kidney with a low number of nephrons must filter the same amount of fluid per unit time as a kidney with a high nephron number. Therefore, to maintain an acceptable total filtration surface area and a constant glomerular filtration rate (GFR), each glomerulus in a poorly endowed kidney must increase in surface area and filter more fluid than its healthy counterpart. While this likely compensatory mechanism may maintain homeostasis in the early stages, it complicates the use of GFR measurements for early detection of kidney diseases involving changes in nephron number and glomerular volume (29). Ideally, clinical measurements of glomerular number and size could be used to detect and monitor loss of nephrons and glomerular hypertrophy in patients at risk of CKD, such as those with diabetes and hypertension.

Currently, measurements of total nephron number ($N_{\text{glomer}}$) and mean glomerular volume ($V_{\text{glomer}}$) require histological sectioning, quantitation of a fraction of a kidney and extrapolation to a total glomerular number and volume (8, 15). Studies employing these methods have provided significant insights into renal physiology and the role of $N_{\text{glomer}}$ and $V_{\text{glomer}}$ in both kidney-specific and systemic diseases (7, 9, 19, 22, 25). Unfortunately, these methods require resection and destruction of the kidney. At this time, there are no methods available for direct measurements of $N_{\text{glomer}}$, $V_{\text{glomer}}$, or protein leakage of individual glomeruli in vivo. Such methods would provide a window for early intervention and may also prove vital in assessing renal allograft viability before transplant.

One potential strategy for noninvasive measurements of glomerular morphology is the development of a magnetic resonance imaging (MRI) contrast agent that specifically targets the glomerular basement membrane (GBM). We and others recently showed that the cationized ferritin (CF) nanoparticle can be used as an intravenous MRI contrast agent to detect functional structures in fenestrated organs, including kidney glomeruli (1–6, 11, 18, 26). The application of CF in the kidney is based on electrostatic binding of CF to anionic proteoglycans of the GBM, allowing each perfused glomerulus in the kidney to be located, counted, and measured using MRI. This method has also been used to detect glomerular permeability to macromolecules in a rat model of focal and segmental

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glomerulosclerosis (5). Three-dimensional (3D) MRI, after CF injection, also enables novel measurements of glomerular size distributions and pathologies in the kidney (3, 18). Because ferritin is naturally occurring in mammalian tissue, CF may be relatively nontoxic (1).

Here, we demonstrate that CF can be used as an MRI contrast agent to visualize, count, and measure the size of glomeruli in whole excised human kidneys. We further demonstrate MRI-detectable changes in glomerular and vascular morphology with renal vascular disease and hypertension.

MATERIALS AND METHODS

Sample Preparation

Cationized horse spleen ferritin (CF; molecular weight = 475 kDa) was synthesized according to Danon et al. (16). Four human kidneys were obtained at autopsy through a donor network (The International Institute for the Advancement of Medicine, Edison, NJ) after Institutional Review Board approval and informed consent. The kidneys were deemed unsuitable for transplant by the Organ Procurement Officer. A request and informed consent for research were obtained only after the kidneys were deemed unsuitable for transplant. The kidneys were infused at autopsy with heparinized saline and stored in 10% neutral buffered formalin at 4°C.

Approximately 1 mm³ pieces of tissue were collected from the cortex of each kidney after perfusion of formalin and immediately placed in 2% glutaraldehyde/0.1 M cacodylate solution for overnight fixation. Samples were dehydrated in graded ethanol solutions ranging from 70 to 100% and then infiltrated with and embedded in epoxy resin. The resulting blocks were cut into 70-μm sections and stained with 0.2% osmium tetroxide. Osmium tetroxide precipitates were digested with 1% periodic acid for 12 min. A Philips CM12 transmission electron microscope was used to collect images at ×53,000 magnification with an accelerating voltage of 80 kV.

IF

Frozen tissue. We performed IF microscopy to confirm labeling of the GBM with CF. Because CF is a protein, it is readily detected with IF. Several ~1-mm³ tissue samples were taken from each kidney after perfusion. The biopsies were placed in 10% neutral buffered formalin for 4 h and stored in PBS overnight. The samples were cryoprotected in 15% sucrose followed by 30% sucrose and then rapidly frozen to −80°C and cut into 35-μm sections. The sections were washed in PBS, permeabilized with 0.5% Triton X-100 (Sigma, St. Louis, MO), incubated in rabbit anti-horse spleen ferritin (Sigma), immunostained with an Alexa 594 goat anti-rabbit secondary antibody and 4′,6-diamidino-2-phenylindole (Invitrogen, Carlsbad, CA), and imaged on a Zeiss 710 laser-scanning confocal microscope.

Formalin-fixed tissue. A second round of IF was performed in formalin-fixed tissue after MRI and stereological analysis. Two approaches were used for tissue sampling and paraffin embedding: 1) targeted sampling and 2) random sampling. Targeted sampling, based on MRI, was used for analysis of the CF2 kidney to extract 2-mm³ samples from areas of cortex with good ferritin labeling and areas of cortex with poor ferritin labeling. For CF1 and CF3, similar size blocks were randomly cut from the cortex of the formalin-fixed tissue. All formalin-fixed tissue samples were embedded in paraffin. Three serial sections (4-μm-thick each) were cut from each block. The first section was used for IF while the second section was used for periodic acid Schiff (PAS) staining. For IF, sections were rehydrated in 100% ethanol (5 min), 70% ethanol (5 min), and then PBS (5 min). Sections were then subjected to an antigen retrieval step, which involved immersion in Target Retrieval Solution (DAKO, S1699) for 20 min at a controlled temperature of 90°C in a DAKO PT Link PT10126 system. After being cooled, slides were washed in buffer (DAKO, K8007) and then in 1% filtered BSA in PBS for 1 h. Sections were then immunostained using an antibody against Wilms’ Tumor-1 (WT1) antigen (monoclonal mouse anti-human WT1-DAKO, M356101, clone 6F-H2), a well-known podocyte marker that allowed us to confirm glomerular localization and the same rabbit anti-horse spleen ferritin (Sigma), as previously described. After 1-h incubation at room temperature, sections were labeled with goat anti-mouse Alexa 488 (1:2,000; Invitrogen A-11008) and goat anti-rabbit Alexa 555 (1:1,000; Invitrogen A-11001) for another hour at room temperature under light protection. Finally, Prolong Gold with DAPI (Invitrogen P-36931; anti-fade mounting medium) was used for permanent coverslipping and left for 24 h. Confocal images were taken on a Leica SP5 laser confocal microscope (Leica Microsystems, Mannheim, Germany).

Images were obtained using a ×40 objective lens (1.25 NA), using sequential imaging for 488 nm, 555 nm and UV light.

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Stereology and Histopathology

The MRI-based measurements of $N_{\text{glomer}}$ and $V_{\text{glomer}}$ were validated (after MRI) using the physical disector/fractionator design-based stereological method described by Cullen-McEwen et al. (12–14). In brief, kidneys were weighed and a series of sampling and subsampling steps were applied to select a systematic uniform random sample of 10–15 tissue blocks from the cortex. These blocks were embedded in glycolmethacrylate (Kulzer GmbH), serially sectioned at 20 μm, and every 10th and 11th section pair was collected and stained with PAS. The section pairs were viewed with a pair of light microscopes modified for projection. Glomeruli present in one section (the reference section) but not in the paired section (look-up section) were counted according to the disector principle. At the same time glomeruli were counted with the disector principle, stereological grid points overlaying glomeruli were counted and used to estimate $V_{\text{glomer}}$.

PAS-stained glycolmethacrylate sections were assessed by a specialist renal pathologist (JD). Sections from ~10 blocks per kidney were examined. Seventy two, 61, and 66 glomeruli were assessed in kidneys CF1, CF2, and CF3, respectively.

Image Processing

$N_{\text{glomer}}$ and the individual volumes of all glomeruli were calculated from the MR images using an in-house algorithm written for MATLAB (The Mathworks, Natick, MA). First, a Hessian for each voxel of the raw MRI volume (in 3D) was used to flag candidate glomerular regions and discern glomeruli in close proximity to each other. This step populated candidate regions and, as a result, dramatically reduced the data size. Five features, including average intensity, divergence, region volumes, shape index, and the Laplacian of Gaussian, were extracted to remove false positive glomeruli. With those features, a Gaussian mixture model clustering algorithm was used to group candidate regions (black dots) into several clusters throughout the volume. Next, all clusters were overlain individually onto the original MRI volume, and clusters that did not identify populations of glomeruli were identified manually and eliminated from further analyses. The remaining black dots were counted as glomeruli and their sizes were measured based on the number of voxels comprising each dot. Clusters of black dots in the control kidney of similar locations and appearance to those of CF-labeled kidneys were counted as false positives. Donor data were investigated to establish possible reasons for any variability in nephron number and CF accumulation in the kidneys measured by MRI (see Pathology section). These data are shown in Table 1.

RESULTS

To investigate the use of CF as a glomerulus-specific MRI contrast agent in humans, CF was injected into the renal artery of three viable (but untransplantable) human donor kidneys within 24 h of resection. Saline was injected into one kidney instead of CF as a control. Donor data were investigated to establish possible reasons for any variability in nephron number and CF accumulation in the kidneys measured by MRI (see Pathology section). These data are shown in Table 1. Notably, the donor of kidney CF2 suffered from severe, untreated hypertension, and the donor of kidney CF1 suffered from mild, treated hypertension.

MRI

We imaged the intact, fixed donor kidneys on a 7T MRI scanner using a 3D GRE pulse sequence. As shown in Fig. 1A, the MR images exhibited dark spots throughout the renal cortex of the CF-labeled kidneys. Each dark spot in the cortex is ~50–80% darker than the surrounding cortex. These dark spots were not present in the unlabeled control kidney (Fig.

Table 1. Clinical data, stereological estimates, and MRI-based data

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Gender</th>
<th>Race</th>
<th>Cause of Death</th>
<th>Initial Creatinine, mg/dl</th>
<th>Initial GFR, ml/min*</th>
<th>Peak Creatinine, mg/dl</th>
<th>Last Creatinine, mg/dl</th>
<th>$N_{\text{glomer}}$ ($\times10^6$)</th>
<th>$V_{\text{glomer}}$ ($\times10^{-3}$ mm³)</th>
<th>Kidney Wt, g</th>
<th>$aV_{\text{glomer}}$ ($\times10^{-3}$ mm³)</th>
<th>Median $aV_{\text{glomer}}$ ($\times10^{-3}$ mm³)</th>
<th>$N_{\text{glomer}}$ %</th>
<th>$V_{\text{glomer}}$ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF1</td>
<td>68</td>
<td>M</td>
<td>Cardiac arrest</td>
<td>1.6</td>
<td>43</td>
<td>2.9</td>
<td>2.5</td>
<td>1.13</td>
<td>5.01</td>
<td>167</td>
<td>1.27</td>
<td>4.8</td>
<td>1.5</td>
<td>12</td>
</tr>
<tr>
<td>CF2</td>
<td>45</td>
<td>F</td>
<td>AA Hypertensive stroke</td>
<td>1.1</td>
<td>65</td>
<td>2.7</td>
<td>2.7</td>
<td>0.74</td>
<td>4.68</td>
<td>110</td>
<td>0.92</td>
<td>3.2</td>
<td>2.2</td>
<td>25</td>
</tr>
<tr>
<td>CF3</td>
<td>37</td>
<td>F</td>
<td>Cardiac arrest</td>
<td>1.9</td>
<td>30</td>
<td>6.05</td>
<td>6.05</td>
<td>1.46</td>
<td>2.82</td>
<td>186</td>
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<td>Ctrl</td>
<td>55</td>
<td>F</td>
<td>Stroke</td>
<td>1.6</td>
<td>45</td>
<td>1.8</td>
<td>1.2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.057</td>
<td>1.6</td>
<td>3.2</td>
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</table>

Stereological estimates and magnetic resonance imaging (MRI)-based measurements for total nephron number ($N_{\text{glomer}}$) and mean glomerular volume ($V_{\text{glomer}}$) follow a similar trend, although differences between the 2 techniques are apparent. It is important to note that, due to the difference in the number of glomeruli sampled using each method and the heterogeneous nature of human kidneys, we do not expect these 2 measurements to be in perfect agreement. *MDRD Study Equation was used to calculate glomerular filtration rate (GFR).
Intravenously injected cationized feritin (CF) specifically labels glomeruli in perfused human donor kidneys, making them visible with 7T magnetic resonance imaging (MRI). A 2D slice of a 3D MR image of a CF-labeled human kidney contains punctate, hypointense spots throughout the cortex (A). Images of an unlabeled control kidney contained no hypointense spots but did show minimal image darkening caused by residual blood (D). Immunofluorescence confirmed the accumulation of CF (red) in glomeruli (B, top arrow) and leakage of CF into tubules of CF-perfused kidneys (B, bottom arrow). Unlabeled glomeruli of the control kidney were clear of CF (E). Cell nuclei are shown in blue (DAPI). Transmission electron microscopy (TEM) confirmed the accumulation of CF in the glomerular basement membrane (GBM) and endothelial glycocalyx (arrows, C). The glomerular capillary walls of the unlabeled control kidney were clear of CF (F). White scale bars = 50 μm. Black scale bars = 0.2 μm.

To assess the possibility of detecting glomeruli in typical clinical MRI systems, CF-labeled kidneys were also imaged at lower resolution on a clinical 3T MRI scanner (Fig. 2). While individual glomeruli were not visible at the lower resolution of 3T MRI, the average image magnitude in the cortex of CF-labeled kidneys was ~20% lower than in the medulla. Minimal difference between image magnitude in the cortex and medulla (<2%) was seen in the unlabeled control kidney. Thus, CF labeling can be detected with typical clinical MRI systems by measuring the ratio of cortical to medullary image intensity.

Leakage of CF through the GBM into the proximal tubule was visible in 7T MRI and IF images of kidneys CF1 and CF2 (Fig. 3A, Fig. 4G, Fig. 5, A and D, and magnified MRI panels of Fig. 6). The leakage of CF past the glomerular capillary wall appeared as diffuse darkening of the MR image, similar to leakage previously observed in a rat model of focal and segmental glomerulosclerosis (5). CF was visible by IF in the tubules (Fig. 1B, lower arrow) and Bowman’s capsules (Fig. 4N) of kidneys CF1 and CF2, consistent with the MRI-visible CF leakage in kidneys CF1 and CF2. Based on MRI (Fig. 3A)
Histopathology

Histopathology of kidney CF1 revealed four sclerotic glomeruli of the 72 examined. There was widespread patchy fibrosis and tubular dilation and atrophy with mild diffuse lymphohistiocytic leukocytic infiltration within the interstitium. The arteries were sclerotic with variable hyalinosis. Overall, there was minor nephrosclerosis and acute tubular injury.

In CF2, five totally sclerosed glomeruli of the 61 were examined and one with perihilar segmental sclerosis. In the regions of CF-labeled tissue (Fig. 3B), the interstitium had widespread mild, patchy fibrosis, tubules were mildly dilated and atrophic, and a moderate lymphohistiocytic infiltrate was present that included eosinophils. The arterioles were tortuous and showed marked hyalinosis with intimal sclerosis. Within the unlabeled regions of CF2, the degree and number of severely sclerotic glomeruli were striking (Fig. 3C). Vascular involvement was evident, with both the arteries and arterioles severely thickened. In some unlabeled areas, it was impossible to distinguish sclerotic glomeruli from obstructed arterioles. The tubules in the unlabeled areas were unaffected overall, but did occasionally contain cast material. We conclude that the observed lack of CF-labeled glomeruli in some regions of kidney CF2 was correlated with focal sclerosis and vascular damage at those locations.

Kidney CF3 had one sclerotic glomerulus of the 66 glomeruli examined, with no mesangial proliferation or segmental sclerosis within the glomeruli. The interstitium showed slight fibrosis and tubular atrophy with minimal lymphohistiocytic interstitial inflammatory infiltrate. The arteries were either normal or had mild sclerosis of the intima. The tubules were mildly dilated with scattered uromodulin casts. There were only very mild changes of acute tubular injury and very mild background nephrosclerosis.

Quantitative Morphology

We developed and applied custom software to measure glomerular number and individual glomerular volume from the MR images. The custom 3D image processing software identified (Fig. 5, B, E, H, K) and measured the size of labeled glomeruli (Fig. 5, C, F, I, L) in the MR images of CF-labeled kidneys.

The number of glomeruli identified in the MR images by the software yielded the total apparent number of glomeruli per kidney ($N_{\text{glom}}$). These data were compared with stereological estimates of $N_{\text{glom}}$ (Table 1). Both MRI- and stereology-based measurements were consistent with the range of $N_{\text{glom}}$ reported in the literature (24). The algorithm counted $0.057 \times 10^6$ false glomeruli in the one unlabeled control kidney, yielding an estimated false positive rate of the algorithm of $\sim 6\%$. 

Fig. 3. 7T MR image of the CF2 kidney revealed large regions of cortex lacking CF-labeled glomeruli (A). Histopathology performed on the cortical regions that lacked CF-related signal darkening revealed severe sclerosis of glomeruli and arterioles (C), which likely prevented perfusion of glomeruli in these regions. The regions of the CF2 cortex that did have CF-labeled glomeruli showed mild sclerosis. These glomeruli and arterioles appeared substantially healthier than those located in regions that lacked CF-related contrast (B). Scale bars = 400 μm.
Using the same software, we estimated the median apparent glomerular volumes ($aV_{\text{glom}}$) using the MR images and compared them with stereological estimates (Table 1). These median volumes are consistent with those reported in the literature (24). The MRI-based measurements were used to generate the glomerular size distribution for each CF-labeled kidney, which cannot be obtained with other techniques (Fig. 5, C, F, I, L). We observed a large number of glomeruli in these distributions with volumes of $2.4 \times 10^{-3}$ mm$^3$ or less. This was unexpected, because prior stereological estimates of glomerular volumes in human kidneys suggest that only $\sim 10\%$ of the total number of glomeruli in a kidney should have volumes this small (data not shown). In MRI, these glomeruli represented $\sim 30\%$ of the total number of glomeruli.

**Image Texture Analysis**

To detect morphological differences between MR images of CF-labeled donor kidneys, we performed image texture analysis (Fig. 6). This analysis consisted of spatial power spectra associated with line profiles randomly drawn in the cortex in the MR images. The line signal profiles in the CF1 kidney,
which showed only mild nephrosclerosis, were composed of a mix of high- and low-frequency oscillations with CF-related spatial spectral peaks at $k = 0.8$ mm$^{-1}$ (4.5% of total signal power) and $k = 1.2$ mm$^{-1}$ (5% of total signal power). The line signal profiles in kidney $CF_2$, histopathologically assessed as the least healthy kidney of the group, were composed of low-frequency CF-related oscillations corresponding to a CF-related spatial spectral peak at $k = 0.8$ mm$^{-1}$, which accounted for 5% of the total signal power along the line profiles. The line signal profiles in $CF_3$, defined by histopathology as the healthiest kidney of the group, demonstrated high-spatial frequency oscillations between $k = 1.2$ and 1.5 mm$^{-1}$ and account for 10% of the total signal power along the line profiles.

DISCUSSION

This work demonstrates that individual glomeruli in human kidneys can be detected using intravenous injection of CF, followed by MRI. With this approach, the apparent number ($aN_{\text{glom}}$) and volume ($aV_{\text{glom}}$) of glomeruli can be measured in the whole kidney. We refer to these as “apparent” measurements of glomerular number and volume because they are based on detection of glomeruli by an exogenous agent and on a computer algorithm to measure the sites of agent accumulation. Nevertheless, this technique expands the number of glomeruli that can be practically sampled by many orders of magnitude; from hundreds of glomeruli using stereology to all functioning glomeruli in the kidney (of the order of $10^5$-$10^6$ glomeruli). The MRI technique has potential for direct translation to clinical practice to aid in the evaluation of transplant allografts, the diagnosis of kidney disease, and the quantitation of nephron endowment in children born early or with low birth weight.

MRI-based measurements of $aN_{\text{glom}}$ and $aV_{\text{glom}}$ for $CF_1$ and $CF_3$ agreed well with the estimates obtained using stereology. Due to the difference in the number of glomeruli sampled using each method and the heterogenous nature of human kidneys, we did not expect these two measurements to be in perfect agreement. Furthermore, we counted 57,000 glomerulus-like dark spots in the unlabeled control kidney, most of which were likely due to small regions of residual blood. This ~6% false positive rate of this counting technique is of the order of previous reports in rat kidneys (3).

For $CF_2$, the kidney with marked glomerular, interstitial, and vascular pathology, the MRI and stereological estimates of glomerular number and volume were more disparate. Surprisingly, the MRI estimate of glomerular number was higher than the stereological estimate for this kidney, despite the fact that ~20% of the cortex lacked CF-labeled glomeruli. This patient...
had significant and uncontrolled hypertension resulting in vascular and glomerular pathology. Histopathology revealed that glomeruli and arterioles were severely sclerosed in the regions of the CF2 cortex that lacked CF-labeled glomeruli. The glomeruli in these regions were likely underperfused or abnormal. We observed glomeruli with poor ferritin labeling, sclerotic glomeruli with no open capillaries, and possibly atubular glomeruli (27) or obstruction of collecting ducts; all of them with little, if any, ferritin signal and therefore representing a population of nonfunctioning glomeruli. To the best of our knowledge, this is the first time large regions of nonfunctioning glomeruli have been detected in an intact kidney. We also found evidence of vascular remodeling and of CF accumulation at sites of vascular remodeling in this kidney. The over counting of glomeruli in the CF2 kidney can likely be attributed to CF accumulation to vasculature undergoing remodeling, which would appear glomerulus-like to the image processing algorithm. Future work will focus on separating CF-related MRI signal changes in glomeruli from those that occur due to vascular changes and blood- and CF-artifact (discussed below). These structures will likely be distinguishable based on their morphology.

The 3D MRI-based technique also enabled calculation of the glomerular volume distribution in CF-labeled kidneys. This is the first report of the glomerular size distribution in human kidneys. The glomerular size distributions show a large number of small “glomeruli.” From previous stereological estimates of individual glomerular volumes (IGV) in human kidneys, we predict that ~10% of the glomeruli should have volumes of $2.4 \times 10^{-3}$ mm$^3$ or less (20), yet from MRI we find ~30% of apparent glomeruli have volumes in this range. It is thus likely that ~1/3rd of the small apparent glomeruli that make up ~10% of all glomeruli in the kidney. We speculate that a remaining 1/6th of the small apparent glomeruli are due to the same systematic artifacts observed in the unlabeled control kidney. There are then two possible sources of the remaining 1/2 of the small of apparent glomeruli: spurious labeling of CF in nonglomerular structures or partial CF labeling in some glomeruli, or both. Future work will focus on...
improving image analysis and MRI acquisition to reduce the number of falsely identified glomeruli. It may be possible to exclude these erroneous glomeruli by setting a strict lower image processing threshold on the IGV measurements. Our data illustrate that caution is required in applying this threshold to ensure that IGV is not simply adjusted to give a “correct” result. Nonetheless, the intrarenal distribution of glomerular volumes may emerge as a powerful new parameter for assessing glomerular hypertrophy and shrinkage in health and disease.

We quantified the spatial distribution of CF accumulation in the kidney by image texture analysis. The CF-related image darkening in the CF3 kidney—the kidney with the least reported nephrosclerosis—was punctuate and was associated with spatial power spectral peaks at k = 1.2 and 1.5 mm⁻¹. With profound arteriosclerosis and nephrosclerosis, as in kidney CF2, the CF-related signal darkening appeared diffuse and was associated with a spatial power spectral peak at k = 0.8 mm⁻¹. Previous work in rats showed that this diffuse labeling, quantified using the spatial power spectrum, can result from leakage of protein past the glomerular capillary wall into Bowman’s space and the proximal tubule (5). This analysis is supported by the histopathological and spectral analyses of kidney CF1, which exhibited only mild nephrosclerosis and spectral peaks at both k = 0.8 and 1.2 mm⁻¹, suggesting populations of healthy (k = 1.2 mm⁻¹) and sclerotic (k = 0.8 mm⁻¹) glomeruli. Image texture analysis may prove useful to quantify morphological changes with disease progression.

CF creates contrast in T2*-weighted MRI by dephasing the spins of water protons surrounding the site of CF accumulation. The volume over which this dephasing is seen in T2*-weighted MRI images depends on the amount of accumulated CF and the image acquisition parameters. It is thus important to consider imaging parameters and to minimize CF dosage when measuring avglomer with T2*-weighted MRI. While this work used a large CF dose of 300 mg/kg kidney wt, intravenous doses of just 0.6–1 mg/kg body wt of CF should be sufficient to visualize rat glomeruli with T2*-weighted MRI and have minimal effects on the kidney, liver, and immune function biomarkers (1–3). Substantial work must be done in the future to optimize dosing of donor kidney with CF. Recent advances in MRI contrast agent design have also opened the door to labeling glomeruli with a highly sensitive T1-shortening (bright) MRI “paraCF” contrast agents (11). ParaCF allows improved in vivo detection of glomeruli with T1-weighted MRI and elimination of the dephasing artifacts found in T2*-weighted MRI that might affect volume measurements, and a further 100-fold reduction in required dose. Such an agent would greatly improve glomerular detection in vivo against the dark blood background and allow for doses that may have a minimal effect on renal function.

The ability to clinically measure glomerular morphology and local protein leakage has the potential to directly improve patient care and clinical outcomes. Measurements of glomerular morphology could be used to assess the viability of kidneys from both living and deceased donors, ensuring that a donor kidney has sufficient filtration surface area. The relationship between glomerular morphology and kidney viability, measured by in vivo GFR and survival rates, will be the subject of future work in preclinical models of kidney transplants. Noninvasive glomerular morphological measurements would also allow younger recipients to receive kidneys possessing a nephron number sufficient to match their future life span. Individuals at risk for CKD could receive an individualized risk assessment using this technique, enabling early detection and regular monitoring of kidney disease. Still, the use of such a technique in the clinic requires substantial work to address scan time and contrast agent toxicity. Many of these studies are already underway. Development of highly sensitive, T1-shortening (bright) contrast agents (11) and advancements in radio frequency hardware for high-resolution in vivo MRI of the kidney (26) have made it possible to visualize glomeruli in vivo in a matter of minutes, although specialized contrast agents and semi-invasive hardware are currently required to do so. Initial studies of the toxicity and biodistribution of CF (1) suggest MRI-detectable doses of CF are minimally toxic. Production of recombinant human ferritin may further reduce toxicity (30). It will also be increasingly important to determine the minimum dose of CF needed to detect glomeruli in human studies with MRI. Based on our previous in vivo studies, and accounting for allometric differences between rats and humans, we approximate the in vivo detectable limit of intravenous CF in humans to be 0.56 mg/kg.

In conclusion, glomerular number and volume in viable human kidneys can be measured with MRI. To the best of our knowledge, this is the first technique to measure the volume of every glomerulus in the human kidney and to identify large regions of arteriolar and glomerular sclerosis. This technique is nondestructive and therefore has the potential for translation to the clinic. This study is thus a first step toward characterizing human kidney glomeruli in vivo.

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DISCLOSURES

K.M. Bennett owns Nanodiagnostics, LLC.

AUTHOR CONTRIBUTIONS

Innovative Methodology


REFERENCES

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