Kidney oxygenation under pressure

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CHAPTER

Magnetic resonance imaging derived renal oxygenation and perfusion during continuous, steady-state angiotensin-II infusion in healthy humans

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ABSTRACT

The role of kidney hypoxia is considered pivotal in the progression of chronic kidney disease. A widely used method to assess kidney oxygenation is blood oxygen level dependent (BOLD)-MRI, but its interpretation remains problematic. The BOLD-MRI signal is the result of kidney oxygen consumption (a proxy of glomerular filtration) and supply (i.e. glomerular perfusion). Therefore, we hypothesized that with pharmacological modulation of kidney blood flow, renal oxygenation, as assessed by BOLD-MRI, correlates to filtration fraction (i.e. glomerular filtration rate (GFR)/effective renal plasma flow (ERPF)) in healthy humans.

Eight healthy volunteers were subjected to continuous angiotensin-II infusion at 0.3, 0.9 and 3.0 ng/kg/min. At each dose, renal oxygenation and blood flow were assessed using BOLD and phase contrast MRI. Subsequently, gold standard GFR/ERPF measurements were performed under the same conditions. Renal plasma flow decreased dose dependently from 660±146 to 467±103 mL/min/1.73m² (F(3,21) = 33.3, p < 0.001). GFR decreased from 121±23 to 110±18 ml/min/1.73m² (F(1.8,2.4) = 6.4, p = 0.013). Cortical transverse relaxation rate (R2*; increases in R2* represent decreases in oxygenation) increased by 7.2±3.8% (F(3,21) = 7.37, p = 0.001); medullar R2* did not change. Cortical R2* related to filtration fraction (R² 0.46, p<0.001).

By direct comparison between gold standard kidney function measurements and BOLD MRI, we showed that cortical oxygenation measured by BOLD MRI relates poorly to GFR but is associated to filtration fraction. For future studies, there may be a need to include renal plasma flow measurements when employing renal BOLD-MRI.

1 This chapter contains expanded analyses using the concentric objects method.
INTRODUCTION

Based on extensive animal research, disturbances of renal oxygenation are considered pivotal in the progression of chronic kidney disease (CKD)\(^1\). Activation of the renin-angiotensin-aldosterone system (RAAS) is one of the major determinants of renal perfusion and oxygenation\(^2\). In an elegant proof of concept study, Schachinger et al. studied renal oxygenation by BOLD-MRI and demonstrated an acute decrease in cortical oxygenation during supra-physiological bolus injections of Angiotensin II (Ang-II) in healthy volunteers\(^3\). Subsequently, in two more recent studies Blankestijn and coworkers showed that blocking the RAAS system in CKD patients increases renal oxygenation. This effect was predominantly present in the renal medulla\(^4\). In a similar study in healthy subjects, ACE inhibition increased oxygenation in both cortex and medulla\(^5\). However, none of these studies were able to relate BOLD measures of kidney oxygenation among CKD patients to kidney function\(^6\). This implies that either the hypothesis on the role of hypoxia in the progression of CKD in humans is not correct, or the interpretation of the kidney BOLD signal should be revised. In this paper we focus on the latter.

The BOLD MRI signal is expressed by the transverse relaxation rate or R\(^2\)*, this value represents the ratio between oxy- and deoxygenated hemoglobin and increases in R\(^2\)* value indicate decreases in oxygenation. Therefore, it is the result of the oxygen extraction rate from the blood (i.e. oxygen demand, a proxy of glomerular filtration rate) and blood supply (perfusion)\(^7\). BOLD MRI does not differentiate between the two. Against this background, assessing oxygen demand and supply respectively is essential to understand the relation between renal oxygenation as measured by BOLD MRI and kidney function. Others have also suggested that measurement of renal blood flow (RBF) could be a prerequisite in interpreting renal BOLD MRI\(^8\), however this has not yet been shown.

In an attempt to improve the interpretation of renal BOLD we choose to translate kidney oxygenation and perfusion into terms corresponding to the physical properties of the MRI derived parameters. By doing so, we accepted a vast simplification of the very complex regulatory mechanism of renal blood flow and oxygenation a priori. Considering a stable plasma sodium concentration (e.g. during an acute intervention without fluid or electrolyte intake) GFR indirectly represents the filtered sodium load. If GFR increases, then water and sodium reabsorption shall increase to maintain volume homeostasis. While water reabsorption is metabolically neutral, sodium reabsorption is not and with increasing GFR, the tubular oxygen demand increases. Therefore, we consider glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) to represent oxygen demand and supply respectively, and then the oxygenation balance should be represented by the filtration fraction (FF)\(^9\). Because FF is the calculated GFR/ERPF ratio, it therefore should dependent on demand and supply.
Thus, implying that FF might be one of the main determinants of renal oxygenation status. Therefore, we employed a combination of renal BOLD-MRI, MRI-based renal flow measurements and radioisotope measurements of kidney function during standardized renal blood flow (RBF) modulation by continuous, steady state Ang-II infusion in healthy human subjects. We hypothesized that in healthy humans 1) Ang-II infusion causes a dose-dependent decrease in both RBF and oxygenation, 2) Oxygenation changes induced by Ang-II spatially differ between cortex and medulla and finally 3) Renal oxygenation directly correlates to FF.

METHODS

Study population
Eight healthy subjects were studied (age 20 ± 1 years, 5 males). Their medical histories revealed no significant disease, none used medication and physical examination was unremarkable. Blood pressure was 120 ± 5 / 63 ± 6 mmHg (systolic/diastolic). Plasma creatinine was 71 ± 11 μmol/L, hemoglobin level was 8.9 ± 0.6 mmol/L, hematocrit (HT) was 0.47 ± 0.03. Spot urine samples tested negative for proteinuria by dipstick. Baseline characteristics are given in Table I.

Written informed consent was obtained from all participants and the study was approved by the institutional review board of the Academic Medical Center at the University of Amsterdam.

Study design
All subjects visited our institution on two occasions. MRI was performed on visit 1 while visit 2 involved GFR and ERPF measurements. Directly before each visit, subjects collected 24-hour urine samples. To ensure a comparable sodium balance between visits, all subjects were instructed to follow their normal diet and refrain from the intake of high caloric and/or salty foods, caffeinated beverages and smoking for 12 hours before each visit. Adherence to dietary instructions was evaluated between visits by 24-hour sodium/creatinine ratio. Mean sodium/creatinine ratio was 8.9 ± 3.9 at visit 1 and 9.4 ± 5.2 at visit 2. Both the MRI and GFR/ERPF measurements were performed at baseline and during infusion of Ang-II (Angiotensin II acetate, Clinalfa, Merck Biosciences AG, Läufelfingen, Switzerland) at 0.3, 0.9 and 3.0 ng/kg/min. The half-life of Ang-II is 15-20 s, therefore after approximately 100 s a steady state exists.

Hemodynamic monitoring
During MRI, brachial artery blood pressure was measured intermittently (Accutor Plus, Datascope Corp., USA). During GFR/ERPF measurements, blood pressure was monitored by
continuous noninvasive finger artery blood pressure monitoring (Nexfin, Edwards Life Sciences, Irvine, USA). Nexfin continuous blood pressure monitoring is not MRI compatible, but has been extensively validated against intermittent brachial artery pressure measurements, making both methods comparable. From the continuous blood pressure recordings, estimations of cardiac output (CO, L/min) and systemic vascular resistance (SVR, dyn-s/cm²) were calculated using a validated pulse wave analysis algorithm (FloTrac, BMEye, Amsterdam, The Netherlands).

**Magnetic Resonance Imaging**

Magnetic Resonance Imaging was performed on a 3.0 Tesla MRI system (Ingenia, Philips Healthcare, Best, Netherlands). Survey scans, including 3D Dixon, were used to locate the position of the kidneys. Subsequently, BOLD and Phase Contrast (PC) MRI scans were acquired at baseline and at each Ang-II dose with an initial run-in period of at least 120 s after each Ang-II dose increase. Acquisition time for each set of scans was approximately 10 minutes (Fig 1), Ang-II infusion time was 12 minutes per dose.

Three-directional blood flow velocity was measured by PC MRI in a slice placed perpendicular to the right proximal renal artery, as described previously. The number of ECG-triggered cardiac phases was 30. PC MRI sequence parameters were as follows: field of view (FOV) 200×200 mm, resolution = 0.65×0.65 mm², slice thickness = 3mm, repetition time (TR)/echo time (TE) = 8.5/5.7 ms, flip angle = 10°, SENSE factor = 2, venc = 100 cm/s in all directions.

Offline image processing for PC MRI was performed using dedicated software (GTFlow version 2.2.9, GyroTools LLC, Switzerland). After correction for background phase-offset errors and aliasing, mean RBF (mL/s) was calculated in the right proximal renal artery using manual vessel segmentation in each cardiac phase (Fig. 1D-F). Further RBF analysis assumed equal perfusion of both right and left kidney. To make direct comparison between the radioisotope ERPF and MRI derived renal blood flow measurements possible, MR renal blood flow was corrected for body surface area (BSA, according to Haycock) and hematocrit (Ht) to calculate a PC MRI-based renal plasma flow (PC-RPF). Simultaneously PC-RPF was extrapolated to both kidneys, according to the following formula: MRI Flow [mL/s] · 60 [s] · 2 [kidneys] · (1-Ht) · 1.73 [m²] / V[body weight [kg] · height [cm]] / 3600) = PC-RPF [mL/min/1.73m²].

Changes in the BOLD MRI signal were quantitatively assessed by measuring the transverse relaxation rate (R2*) within different regions of interest (ROIs). The R2* value is the rate of exponential signal decay during MRI acquisition, partly as result of the presence of local field inhomogenities. The R2* value is influenced by magnetically active particles such as deoxyhemoglobin. A decrease in oxygenation therefore leads to a relative increase in deoxyhemoglobin and an increase in R2*. We measured R2* using a multi-echo single-slice fast
Figure 1 Diagram of BOLD and PC MRI acquisition and analysis
A-C, BOLD MRI scan and segmentation (A – blue with circles in the medulla, red with triangles in the cortex), followed by R2* heat maps at baseline (B) and maximal Ang-II dose (C - 3.0 ng/kg/min) with cortical and medullary T2* relaxation curves. D-F, PC MRI of the right renal artery, plane positioning perpendicular to the renal artery in transversal and coronal planes (D), followed by profiles of renal artery blood flow velocity (vectors) and curves at baseline (E) and maximal Ang-II dose (F – 3.0 ng/kg/min). Depicted velocity contours show maximal flow indicated by the arrows in the graphs below for each condition.
field-echo MRI sequence with the following parameters: FOV = 400x400, resolution = 1.2 × 1.2 mm², slice thickness = 4mm, TR = 140 ms, flip angle = 70°, TE = 2 ms, ΔTE = 5 ms; number of echoes = 16. Image acquisition was performed during a single expiratory breath hold in a coronal slice where the kidney cross section was largest and cortical/medullary definition best visible on the survey scans (Fig. 1A).

Offline image processing for BOLD imaging was performed according to the traditional circular ROI method and a novel concentric objects (CO) method, both using routines written in Matlab (The MathWorks, Natwick, USA). For the traditional method, eight circular ROIs with an eight voxel diameter were identified at regular intervals throughout the renal cortex and medulla (Fig. 1A-I) in the baseline scan of each kidney. For each subject, the resulting mask were then applied to the three subsequent BOLD images acquired during staged Ang-II infusion. Where necessary, ROIs were adjusted vertically or horizontally to match any movement of the subject during the scan. Renal R2* values were calculated for cortex and medulla separately, via mono-exponential fitting to the multi-echo data (Fig. 1B and C). For the CO method, the cortex and medulla were delineated as a whole. After which the area was automatically divided into twelve concentric ROIs from the outer cortex to inner medulla, as described previously. Two observers independently performed the CO analysis to assess inter-observer variation.

**Glomerular Filtration Rate**

GFR and ERPF were measured from the clearance of the constantly intravenously infused tracers 125I-Iothalamate (Glofil-125, ISO-TEX diagnostics, Friendswood, USA) and 131I-Hippuran (Radioisotope Centre POLATOM, Otwock, Poland) respectively, as described previously. After loading doses, tracer clearances were calculated at baseline and during step-wise increased Ang-II doses, i.e. 0.3, 0.9 and 3.0 ng/kg/min for 40 minutes each. Plasma 125I-Iothalamate and 131I-Hippuran were assumed to have reached new steady states at the end of each 40-minute phase. Ang-II infusion time was 40 minutes per dose.

ERPF was calculated according to plasma clearance of 131I-Hippuran: \( I_u \cdot V/P_u \), where \( I_u \) is the 131I-Hippuran concentration in the infusion solution in counts/min/ml, \( V \) the volume infused in ml/min and \( P_u \) the plasma concentration of 131I-Hippuran in counts/min/ml. GFR was calculated by the renal clearance of 125I-Iothalamate: \( U_r \cdot V/P_r \), where \( U_r \) is the 125I-Iothalamate concentration in the urine in counts/min/ml and \( P_r \) the plasma concentration of 125I-Iothalamate in counts/min/ml. GFR was corrected for inaccurate urine collections according to: \( (I_u \cdot V/P_u)/(I_u \cdot V/P_u) \) as described previously. ERPF and GFR values were corrected for BSA and are presented as ml/min/1.73m². FF is calculated as the GFR/ERPF ratio. Renal vascular resistance (RVR; ml/min/mmHg) was calculated as RBF/MAP.
Statistical analysis
The primary outcome of this study was cortical and medullary R2* during staged Ang-II infusion. The effect size was estimated at 0.5, based on data reported by Visser et al. and Gloviczki et al. A priori, the study was powered at 0.8 to detect a significant R2* effect during Ang-II infusion with an alpha of 0.05 by ANOVA for repeated measures. Levine’s Test was used to assess normal distribution of the data. Normally distributed variables are presented as mean ± standard error, and non-normally distributed as median (range) or as proportion where appropriate.

One-way ANOVA for repeated measures was used to assess the dose dependent effect of Ang-II on systemic (blood pressure, heart rate, CO and SVR) and renal (GFR, ERPF, FF and RVR) hemodynamic parameters, renal artery blood flow, cortical and medullar R2*. In case the sphericity assumption was violated, degrees of freedom were corrected according to Greenhouse-Geisser. Pairwise comparison between baseline and each Ang-II dose was performed using post hoc analyses with Bonferroni correction.

Taking into account the clustered nature of the repeated measures, we used linear mixed model analysis to test the association between cortical or medullar R2* and radioisotope measurements of GFR, ERPF or FF. The model included the Ang-II dose as co-variate and the measurement number as factor, in order to take the within subject relation of the measurements into account. All variables were included as fixed variables into the model which was corrected for random intercept variation. All statistical analyses were performed using SPSS Statistics 22 (IBM, Chicago, USA). A p-value < 0.05 was considered significant.

RESULTS

Systemic and renal hemodynamic changes induced by Ang-II
Systemic and renal hemodynamic changes during Ang-II infusion are shown in Figure 2. On both study visits, there was a significant effect of Ang-II infusion on the mean arterial blood pressure (MAP) increased dose dependently during Ang-II infusion. During visit 1, MAP increased from 82 ± 2 at baseline to 90 ± 2 mmHg at maximal Ang-II dosage, F(3, 21) = 27.9, p < 0.001. At visit 2, MAP increased similarly from 82 ± 2 to 98 ± 2 mmHg at maximal Ang-II dosage (F(3, 21) = 24.0. p < 0.001, Fig. 2A). There was a dose-dependent increase in SVR, from 1018 ± 34 dyn·s/cm² by 22.7% ± 6.5%, F(1.8, 12.7) = 5.1, p = 0.05) and renal vascular resistance (RVR), from 0.07 ± 0.005 mL/min/mmHg by 72.5% ± 10.7% (F(3, 21) = 28.9, p < 0.001, Fig. 2D and H). CO showed no significant change (baseline 6.5 ± 0.24 L/min; during 3.0 ng/kg/min Ang-II 6.4 ± 0.2 L/min, F(3, 21) = 0.7, p = 0.56, Fig 2C).
MRI derived PC-RPF decreased dose dependently from 660 ± 48 to 467 ± 36 mL/min/1.73m² (F(3, 21) = 33.3, p < 0.001, Fig. 3C). Radioisotopic ERPF decreased dose dependently from 354 ± 17 ml/min/1.73m² at baseline to 275 ± 12 ml/min/1.73m², at maximal Ang-II dosage (F(1.62, 11.36) = 38.3, p < 0.001, Fig. 2F). FF increased from 0.37 ± 0.02 at baseline to 0.44 ± 0.03 (F(3,21) = 53.4, p < 0.001, Fig. 2G). GFR change was less overt (121 ± 7.6 to 110 ± 6.6 ml/min/1.73m², F(1.8, 2.4) = 6.4, p = 0.013, Fig 2E).

Renal oxygenation changes induced by Ang-II
At baseline, cortical R2* (CR2*) value was 17.4 ± 1.1 and medullary R2* 29.3 ± 1.5 sec⁻¹ (Figs. 3A and 3B). CR2* increased to 20.0 ± 0.8 sec⁻¹ (i.e. 7.2 ± 5.4%) at maximal Ang-II dose (F(3, 21) = 7.37, p = 0.001, Fig. 3A). Medullary R2* did not change during Ang-II infusion (F(3, 21) = 1.38, p = 0.29, Fig. 3B). There was no association between CR2* and GFR (F(20.3, 142) = 0.014, p=0.58) or between CR2* and ERPF (F(25.9, 182) = -5.1·10⁻⁴, p = 0.97). Also, there were no associations between medullary R2* and GFR or ERPF.

Figure 2 Systemic and renal hemodynamics during staged Ang-II infusion
Lines represent the absolute mean or mean percentage difference to baseline; error bars indicate SEM. In panels A and B dashed lines represent measurements at visit 1 (Accutor Plus), solid lines at visit 2 (Nexfin). * indicates significant difference from baseline p<0.05.

Figure 3 Cortical (A), medullary (B) BOLD and flow MRI data (C)
Lines represent mean percentage difference to baseline ± SEM. * indicates significant difference from baseline p<0.05.
To account for the influence of tissue perfusion on kidney function, we explored the association between CR2* and radioisotope measured FF. This resulted in a positive association depicted in Figure 4A (F (7.69, 53.8) = 18.15, p = 0.049). In order to make it possible to associate BOLD MRI to GFR, we corrected CR2* for renal plasma flow by substituting the MRI derived parameters: CR2* and PC-RPF for the FF and ERPF in the following formula for the filtration fraction (FF = GFR / ERPF). With these assumptions we calculated the CR2*-PC-RPF product, subsequently this product did associate positively to GFR (F(15.9, 111) = 69.3, p = 0.022, Fig. 4B).

To evaluate the dependence of the CR2*-PC-RPF product’s relation to GFR on the Ang-II dose, additional regression analyses at each Ang-II dose is included in Figure 4B. The figure shows regression lines for each Ang-II dose with similar β coefficients; 98, 119, 113, 121 for baseline, 0.3, 0.9 and 3.0 ng/kg/min Ang-II respectively.

**Figure 4** BOLD MRI and kidney function correlations
A Correlation between cortical R2* and filtration fraction, with fitted mixed model regression line (black) (F (7.69, 53.8) = 18.15, p = 0.049). B Correlation between the product of PC-RPF and cortical R2* versus GFR, with fitted regression lines for mixed model analysis (black line, F(15.9, 111) = 69.3, p = 0.02) and for each separate Ang-II dose (dotted lines). Shapes of datapoints correspond to Ang-II doses.

**Concentric objects analysis method**
The BOLD MRI data was according to the CO method as well. Two observers first assessed the both kidney in all baseline scans (Fig. 5). The average observer bias was -0.045 sec⁻¹ with 95% limits of agreement of -1.28 and 1.19 sec⁻¹. In figure 6, the response to Ang-II is visualized for each concentric layer by both observers (Fig. 6A and B respectively).
Figure 5 Analysis using the twelve layer concentric objects method of the baseline BOLD MRI scan of the left and right kidneys each individual subject S1 to S8 by two observers. Observer 1 in blue, observer 2 in red. Right kidneys circles, left kidneys squares.
**DISCUSSION**

**Major findings**
In the present study, we corroborate that continuous steady-state Ang-II infusion causes a dose-dependent decrease in renal blood flow in healthy humans. However, the flow reduction is only accompanied by a minor decrease of oxygenation in the cortex, and not in the medulla. As a composite of oxygen supply and demand, the filtration fraction is an important determinant of cortical oxygenation. These data imply that kidney BOLD MRI on its own is most associated with FF and renal blood flow measurement may be prerequisite to interpret renal BOLD MRI in terms of glomerular filter function.

**Angiotensin II and renal hypoxia**
The systemic effects of the Ang-II infusion that we found are, although not all statistically significant, similar to those reported previously in young healthy volunteers. As to the magnitude of the renal blood flow decrease (which was highly statistically significant): this was adequate to test our hypotheses regarding changes in renal oxygenation. BOLD MRI-derived R2* values were also comparable to previously reported values in the kidney cortex and medulla, both in magnitude and range. Ang-II induced an increase in cortical R2*, reaching a plateau phase that did not change between 0.9 ng/kg/min and 3.0 ng/kg/min Ang-II. Interestingly, this maximal cortical R2* change is similar to those found by Schach-
inger et al. during bolus infusion with 8 ng/kg Ang-II and by Gloviczki et al. in patients with severe renal artery stenosis.

These observations suggest that the effect of renal blood flow on renal oxygenation is restricted. Possibly this is due to a simultaneous and proportional effect of renal blood flow on the metabolic workload. This is further illustrated by separate linear regression analysis at baseline and each Ang-II dose of the relation between GFR and the flow corrected CR2*, showing similar β coefficients for each condition. This could indicate that both the afferent and efferent renal vasculature is equally affected with increasing Ang-II dose.

As to the spatial differentiation of the effects of impaired RBF, our data also indicate that RBF restriction does not affect medullary oxygenation but manifests as a decreased cortical oxygenation similar to observations in critical renal artery stenosis. Therefore, these observations are not in line with the proposed role of Ang-II in the development of hypoxic damage in the renal medulla in CKD. However, deterioration of kidney function is a slow and chronic process. In this respect, our results represent the reaction to acute kidney blood flow reduction and management of a kidney specific hemodynamic stressor in healthy humans as opposed to chronically impaired sodium homeostasis and subsequent renal dysfunction in CKD patients.

**Interpretation of renal BOLD MRI**

Ever since its introduction, the interpretation of renal BOLD MRI has been challenging. BOLD imaging remains an indirect measure of tissue oxygenation, resulting from the complex interplay of blood flow, hemoglobin oxygen saturation and other biological factors such as vessel geometry, hydration status, hematocrit and pH. It cannot differentiate between oxygen delivery, oxygen consumption, and efficiency of arteriovenous oxygen diffusion. Inherently, baseline BOLD signal intensity cannot be translated directly to an absolute quantitative measure of oxygenation.

To improve the interpretation of renal BOLD MRI, we compared R2* measurements to radioisotope kidney function measurements (GFR, ERPF and FF). We were unable to show an association between renal R2* values and GFR. However, we found a clear relation between cortical oxygenation measured by BOLD MRI and the FF. Therefore, for correct renal BOLD MRI interpretation simultaneous assessment of renal blood flow measurements may be obligatory. MRI based flow measurements for such a purpose are widely used and easily implemented.

In this study we adhered to the most frequently used single slice BOLD MRI analysis method with observer selected ROIs in the renal cortex and medulla, in order to allow the comparison of our results to those reported previously. These ROI based methods give limited
insight into the renal physiology and are subject to observer variations\textsuperscript{26}. Applying the novel CO method, we substantiate that this method provides much more detailed BOLD analysis by visualizing the oxygenation gradient between cortex and medulla\textsuperscript{21}. Moreover, the inter-observer variation is minimized using this technique, making this method a good candidate for a more universally applicable and comparable method for renal BOLD assessment. This method could also be applied to achieve a layer-wise assessment of other MRI modalities, such as diffusion weighted imaging and arterial spin labeling.

**Study limitations**

This study has several potential limitations that merit discussion. First, Phase Contrast MRI measurements of renal blood flow were performed in the right renal artery only and assumed equal perfusion of both kidneys. However this can be justified since, kidney perfusion should amount to approximately 20\% of CO in healthy individuals\textsuperscript{19} and we measured a baseline RBF of 19\% ± 4.4\% of CO. Secondly, we studied healthy volunteers and this limits the generalizability of our findings to CKD patients. Further studies in kidney disease require validation of the combined BOLD and PC MRI measurements. Also, we did not assess alterations in sodium hemostasis in these individuals, which might have influenced renal oxygenation.

Lastly, we acknowledge the discrepancy between the radioisotope and MRI-measured renal plasma flow. The \textsuperscript{131}I-Hippuran derived ERPF values we found are comparable to those reported by others\textsuperscript{15}. However, these values underestimate the MRI derived PC-RPF. This discrepancy can be attributed to two possible causes: one, the renal extraction of Hippuran is not 100\%, but about 85-90\%\textsuperscript{26}. Two, the remaining 25\% underestimation could be attributed to extra–nephronic perfusion as Hippuran measures effective RPF only (i.e. the amount of plasma that passes through the glomeruli). However, in proportional changes, both methods show satisfactory concurrence.

**PERSPECTIVES**

In conclusion, Ang-II causes a dose-dependent decline in renal blood flow. The observed oxygenation change differs between cortex and medulla. During an approximately 30\% perfusion reduction of the kidneys, medullary oxygenation is maintained. By direct comparison between radioisotope GFR, ERPF and FF measurements and BOLD MRI of the kidneys we showed that –out of these three renal perfusion parameters– renal BOLD MRI associates most with kidney filtration fraction and not with GFR. As the FF is the product of both the GFR and renal plasma flow, the interpretation of renal BOLD MRI might be improved with simultaneous renal blood flow measurements in future studies.
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