

# **Renal blood oxygenation level-dependent (BOLD) MRI to measure renal tissue oxygenation: a statement paper and systematic review**

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## **ABSTRACT**

Tissue hypoxia plays a key role in the development and progression of many kidney diseases. Blood oxygenation-level dependent MRI (BOLD-MRI) is the most promising imaging technique to monitor renal tissue oxygenation in humans. BOLD-MRI measures renal tissue deoxyhaemoglobin levels voxel by voxel. Increases in its outcome measure  $R2^*$  (transverse relaxation rate expressed as  $\text{sec}^{-1}$ ) correspond to higher deoxyhaemoglobin concentrations and suggest lower oxygenation, whereas decreases in  $R2^*$  indicate higher oxygenation. BOLD-MRI has been validated against micropuncture techniques in animals. Its reproducibility has been demonstrated in humans, provided that physiological and technical conditions are standardized. BOLD-MRI has shown that patients suffering from chronic kidney disease (CKD) or kidneys with severe renal artery stenosis have lower tissue oxygenation than controls. Additionally, CKD patients with the lowest cortical oxygenation have the worst renal outcome. Finally, BOLD-MRI has been used to assess the influence of drugs on renal tissue oxygenation, and may offer the possibility to identify drugs with nephroprotective or nephrotoxic effects at an early stage. Unfortunately, different methods are used to prepare patients, acquire MRI data and analyze the BOLD images. International efforts such as the COST action PARENCHIMA are aiming to harmonize this process, to facilitate the introduction of this technique in clinical practice in the near future.

This article represents an extensive overview of the studies performed in this field, summarizes the strengths and weaknesses of the technique, provides recommendations about patient preparation, image acquisition and analysis, and suggests clinical applications and future developments.

## **INTRODUCTION**

Renal tissue ischemia is one of the main mechanisms leading to acute kidney injury and mounting evidence suggests that renal tissue hypoxia is a unifying factor in the development and progression of chronic kidney disease (CKD), irrespective of its cause<sup>1</sup>. Assessing renal tissue oxygenation in humans is therefore highly relevant to clinical practice. Blood oxygenation level-dependent Magnetic Resonance Imaging (BOLD-MRI) has become a widely used method to estimate renal tissue oxygenation, although its use is thus far largely restricted to the research setting.

This review paper, written by the COST action PARENCHIMA group, provides an overview of the technique and pitfalls of BOLD-MRI, as well as a summary of all renal BOLD-MRI studies performed in humans so far. These studies are summarized in tables under "Supplementary materials". This review paper also identifies gaps in knowledge and provides recommendations about patient preparation, image acquisition and analysis and possible clinical applications.

## **METHODOLOGY**

A comprehensive search of PubMed and Ovid MEDLINE was performed using the following pre-defined MeSH terms: blood, oxygenation, magnetic resonance imaging (mri) and kidney (renal). Review articles or studies performed in animals were not included, except those that validated BOLD-MRI against directly measured renal tissue oxygenation (micropuncture). Renal cancer studies were excluded. Only original articles written in English and published in peer-reviewed journals before the 31<sup>st</sup> of December 2017 were selected and divided in five categories: validation of the technique, CKD, renal artery stenosis, transplantation and dietary/drug intervention. Each study is summarized in Excel tables (Supplementary materials).

## **PHYSIOLOGY OF RENAL TISSUE OXYGENATION AND PRINCIPLES OF BOLD-MRI**

Renal tissue oxygenation is the net balance between oxygen delivery and demand. Delivery depends on renal blood flow, haemoglobin levels, blood pH and oxygen saturation, whereas oxygen demand depends on tissue metabolic requirements and the oxygen-consuming active tubular transport of electrolytes<sup>2</sup>. Decreases in global renal blood flow do not necessarily lead to a reduction in oxygen delivery due to the presence of substantial arterio-venous shunting of oxygen that can vary according to metabolic needs. Inversely, an increase in renal blood flow (RBF) does not necessarily increase tissue oxygenation since it also leads, at constant filtration fraction (FF), to a higher glomerular filtration rate (GFR), higher amounts of filtered sodium and thus increased metabolic demands<sup>3</sup>. Together, these mechanisms tightly control renal tissue oxygenation. Despite the fact that each kidney receives ~10% of the cardiac output, local pO<sub>2</sub> levels are low. Micropuncture studies in animals have measured tissue pO<sub>2</sub> of around 40-50 mmHg in the cortex and as low as 10-20 mmHg in the inner medulla<sup>4</sup>. These pO<sub>2</sub> levels are at the steep part of the oxygen dissociation curve for haemoglobin, meaning that small changes in local pO<sub>2</sub> lead to relatively large changes in the percentage of saturated haemoglobin.

BOLD-MRI uses the specific paramagnetic properties of deoxyhaemoglobin to assess renal tissue oxygenation. Its outcome measure is the so-called R2\* value, which varies inversely with local oxygenation. This apparent relaxation rate R2\* (or decay rate, defined as 1/T2\* and expressed in sec<sup>-1</sup>) is measured voxel by voxel in each kidney by BOLD-MRI. The R2\* value of each voxel is influenced by microscopic inhomogeneities in the static magnetic field, largely due to the effect of deoxyhaemoglobin. As such, the decay rate R2\* in a voxel will be enhanced when the local deoxyhaemoglobin concentration is increased. Hence, assuming that blood pO<sub>2</sub> is in equilibrium with tissue pO<sub>2</sub>, low R2\* values indicate high tissue oxygenation, while high R2\* values indicate low tissue oxygenation<sup>5</sup>.

BOLD-MRI is performed without the administration of contrast agents, which makes it attractive for patients with decreased renal function.

## VALIDATION OF KIDNEY BOLD-MRI

The aim of BOLD-MRI is to measure renal tissue oxygenation. The actual 'gold standard' for tissue oxygenation is direct measurement with micro-electrodes placed in the renal cortex and medulla. For obvious reasons, this is not possible in humans, and comparison with the gold standard has only been performed in animals.

In 1996 Prasad, Edelman and Epstein published their landmark paper on the feasibility of the evaluation of intrarenal oxygenation by BOLD MRI<sup>6</sup>. They studied 7 healthy young subjects and found, as expected, a decreased  $R2^*$  (indicating increased  $pO_2$ ) during administration of furosemide; this observation was in line with prior research using microelectrodes that had shown an increase in medullary  $pO_2$  due to the furosemide-induced reduction in oxygen-consuming active electrolyte transport. In the subsequent years numerous authors confirmed the effects of furosemide both in pigs<sup>7</sup>, healthy humans and patients<sup>8</sup>.

Only a decade later (in 2005) Pedersen et al took up the challenge to quantitatively validate the technique<sup>7</sup>. They compared BOLD-MRI measurements in renal cortex and medulla to those of  $pO_2$  electrodes inserted in the contralateral kidney of 8 anesthetized pigs. They were able to establish a linear relationship between measured  $pO_2$  and  $R2^*$  over the  $pO_2$  range of 0,2-10 kPa in both the cortex and the medulla. For the cortex this resulted in a  $pO_2$  [kPa]= $R2^*$  [1/s]-21/1.2 and for the medulla  $pO_2$  =  $R2^*$ -25/1.7. A study performed by Warner et al (2011) tested the acute effects of furosemide and acetazolamide in four series of pigs (total n=21) with consecutive measurements of cortical and medullary oxygenation by BOLD-MRI and Clark electrodes 5-7 days apart<sup>9</sup>. They also found a linear relationship, namely  $pO_2$ [kPa]= $R2^*$  [1/s]-20/1.3 for the cortex and for the medulla  $pO_2$  =  $R2^*$ -20/1.5. The reported  $pO_2$  range was narrower than that of Pedersen et al., i.e. 3.1-7.6 kPa, which may explain some of the differences in calculated coefficients and Y-intercepts. Up to now the two independent studies by Pedersen et al and Warner et al serve as the most cited calibration data on renal BOLD-MRI<sup>7,9</sup>. Recently, Zhang and colleagues provided a method to relate  $R2^*$  measurements to blood and tissue  $pO_2$ .<sup>10</sup>

So far, three studies have specifically assessed the reproducibility of BOLD-MRI in humans. All used the classical ROI technique for image analysis (see below). Li et al showed a ~12% coefficient of variation (CV) for day-to-day measurements in eight healthy subjects at 1.5T<sup>11</sup>. Simon-Zoula et al evaluated the reproducibility in 18 healthy volunteers during three subsequent MRI-acquisition on a single day, with short interruptions between sessions<sup>12</sup>. They reported CVs of R2\* values of 3 and 4% in the medulla and cortex, respectively. Khatir et al reported CVs of 8% for cortex and medulla in 11 CKD patients examined at 1-2 weeks interval, as compared to 5.6 and 3.6% in controls<sup>13</sup>.

## **PATIENT PREPARATION**

Many factors may alter cortical and medullary R2\* values and should be taken into account. Prasad et al were the first to show that hydration status influences the BOLD-signal. They reported decreases up to 30% in medullary R2\* values after an acute oral water load of 20ml/kg in young healthy volunteers<sup>14</sup>. Most BOLD-MRI studies therefore standardize water intake, although approaches differ: some perform BOLD-MRI after an overnight or four-hour fast<sup>15</sup> whereas others prescribe a constant hourly water intake, in general of 1.5 ml/kg/h<sup>16</sup>. A theoretical disadvantage of hydration is that differences in R2\* may be 'washed out' by hydration, but this only occurs at extreme water intake (~1l intake in 15 minutes)<sup>16</sup>. The clear advantage of hydration is its feasibility in patients; the authors therefore prefer standardized hydration protocols.

Dietary salt intake also influences R2\* values. This has been demonstrated in a cross-over study that included young normotensive and untreated hypertensive male volunteers<sup>17</sup>. After five days of a low-salt diet, medullary R2\* values were significantly lower than after a five days high-salt diet. Despite the availability of these data in the literature, less than half of the published studies have controlled salt intake.

Furthermore, as expected, renal tissue oxygenation is influenced by carbogen and oxygen breathing<sup>18</sup>.

Some questions concerning patient preparation remain unanswered. For example, no study has investigated whether there is a circadian rhythm in the BOLD-signal. Besides, plasma osmolality, pH and temperature (affecting O<sub>2</sub> dissociation), iron status (affecting tissue para-magnetic properties) and carbon monoxide levels (tobacco use) could all potentially affect the BOLD-MRI signal. None of the latter factors have been studied for this purpose yet. However, subjects are advised to avoid smoking prior to the MRI examination and a delay of 1-2 weeks is usually maintained in case of fever or intravenous (IV) iron perfusion.

### **MRI ACQUISITION:**

The most common techniques used for human renal BOLD-MRI experiments are single shot echo planar imaging (EPI)<sup>6</sup> and multiple gradient echo (GRE) imaging<sup>19</sup>. Almost all measurements are expiratory breath-hold acquisitions because T<sub>2</sub><sup>\*</sup>-weighted renal MRI is prone to motion artefacts. To date, all published studies of the human kidney have been performed at either 1.5 or 3 Tesla field strengths. The most recent publications show preference for 3T due to improved signal-to-noise ratio (SNR) and better T<sub>2</sub><sup>\*</sup>-weighting secondary to T<sub>2</sub><sup>\*</sup> shortening. R<sub>2</sub><sup>\*</sup> increases with increasing field strength. For example, the R<sub>2</sub><sup>\*</sup> value of the healthy medulla at 3T is almost twice the one acquired at 1.5 T. However, artefacts caused by bulk magnetic susceptibility (BMS) effects and water-fat chemical shift are also magnified at 3T field strengths, but remain at an acceptable level when compared to 1.5 T.

Single shot EPI is a robust approach to reduce motion artefacts and it offers the highest temporal resolution (<100 ms). Unfortunately, EPI is sensitive to BMS artefacts caused by the shape of the human body (organs) and by magnetic susceptibility variations within the body, sometimes leading to image blurring and geometrical distortions. Image distortions are amplified at higher magnetic field strengths, particularly in regions with poor magnetic field homogeneity. To address these challenges, two-dimensional multiple gradient recalled echo (2D mGRE) sequences with signal collection at multiple echoes after each excitation pulse have been introduced and are the most frequently used for renal R<sub>2</sub><sup>\*</sup> mapping. Typically 8, 12, or 16 individual T<sub>2</sub><sup>\*</sup>-weighted images of a single slice, corresponding to different echoes, are measured within a single breath-hold of about 17-20 sec. However, multislice 2D mGRE as well as 3D mGRE were also successfully implemented during a breath-hold of <23 s<sup>20</sup>. A detailed discussion of the acquisition parameters is

beyond the scope of this article, and partly based on expert opinion. The acquisition parameters (repetition time [TR], echo time [TE] interval, acquisition voxel size, flip angle, receiver bandwidth) have to be carefully chosen to maintain the minimum  $SNR > 2$  necessary to avoid errors in  $T2^*$  fitting as the result of noise at higher TE. For more details, see Supplementary data, Table S1. It should be emphasized that the  $SNR > 2$  is critical for the robust assessment of the medulla. Both 2D mGRE as well as EPI sequences suffer from magnitude and phase variations between odd and even echoes due to different phases of water and fat signal components in renal tissue (in- and out-phase effects). However, these artefacts are usually satisfactorily suppressed during mono-exponential least square fitting of  $T2^*$ .

Despite these improvements, it remains difficult to overcome all scanner-related and other non-renal factors that influence the BOLD-signal. This hampers the comparison of absolute  $R2^*$  values across sites. One way to partly overcome this limitation is to perform a dynamic test (IV furosemide for example) and assess the change in  $R2^*$  signal. Another way is the use of analysis techniques that are less dependent of the absolute  $R2^*$  values and focus more on relative changes in  $R2^*$  (TLCO and fractional tissue hypoxia technique).

## **IMAGE ANALYSIS:**

Four major methods have been used thus far to analyse the BOLD images and report the  $R2^*$  values: the region-of-interest (ROI) technique, the twelve-layer concentric objects (TLCO) or 'onion peel' technique, the fractional tissue hypoxia and the compartmental method. The basic principles, strengths and weaknesses of each technique are summarized in **Table 1**, and graphical examples are shown in **Figure 1**.

Several studies<sup>8,19,21,22</sup> have specifically assessed the inter-observer variability (image analysis of the same subject by two or more different observers) of these techniques. Piskunowicz et al reported inter-observer CVs with the ROI technique of 3.6 and 6.8% of cortical and medullary  $R2^*$  values in healthy volunteers, versus 5.7 and 12.5% in CKD. With the semi-automatic TLCO-technique, the same research group reported much lower CVs of 2.2, 2.0 and 3.1% in healthy, mild CKD and severe CKD

groups<sup>21</sup>. Cox et al reported CVs of 4.1% in healthy volunteers with the compartmental method<sup>23</sup>.

Most authors agree that the ROI technique is reproducible and easy to use in subjects with (near) normal renal function (GFR), but unreliable in advanced CKD, due to its dependence of the human eye to distinguish cortex from medulla. In CKD, the TLCO technique has the lowest inter-observer variability, whereas the fractional hypoxia technique is mostly used in patients suffering from renal artery stenosis. The compartmental method needs confirmation in CKD patients.

## **MAIN RESULTS OF CLINICAL STUDIES:**

### Chronic Kidney Disease

According to the chronic hypoxia hypothesis, renal tissue hypoxia is an unifying pathway in the development and progression of CKD, irrespective of its cause<sup>1</sup>. Many animal studies support this hypothesis and intra-renal microelectrodes have indeed measured decreased local tissue pO<sub>2</sub> in several experimental models of CKD<sup>24,25</sup>.

Initially, numerous BOLD-MRI studies in humans failed to confirm the hypoxia hypothesis<sup>16,26</sup>. This was probably due to the lack of standardized patient preparation, suboptimal study designs, low field strength (1.5 T) and the use of the small ROI technique that is characterized by high inter-observer variability in CKD. Recent studies performed under standardized conditions and high field strength (3.0T) have reported higher cortical R2\* values in CKD compared to controls<sup>8,27</sup>. Of note, most studies have not measured renal blood flow, which complicates the interpretation of the results. In this respect, van der Bel et al have shown that R2\* values correlate strongly with the FF (GFR/effective renal plasma flow) in healthy volunteers, and they recommend performing simultaneous BOLD-MRI and phase contrast MRI to measure RBF in the renal artery<sup>28</sup>.

This field was also hampered by the lack of prospective studies, but this has recently changed with the publication of Pruijm et al. In their cohort of 112 CKD patients, 47

hypertensives and 24 healthy controls, CKD patients with high R2\* values (>90th percentile) had a faster yearly eGFR decline and needed more often renal replacement therapy than those with lower R2\* values<sup>29</sup>. This study strongly supports the hypoxia hypothesis and opens the door to more widespread implementation of renal BOLD-MRI in the clinical care for CKD patients.

### Renal artery stenosis and arterial hypertension

The decrease in RBF that characterizes renal artery stenosis (RAS) makes BOLD-MRI particularly suitable to probe tissue oxygenation in the post-stenotic kidney. Studies in swine models of acute<sup>30</sup> and chronic<sup>9</sup> reductions in RBF have confirmed that BOLD-MRI is capable of detecting changes in renal oxygenation that were independently confirmed using implanted oxygen electrodes. Studies in human subjects with over 60% obstruction of the renal artery have enabled the determination of the hemodynamic significance of the stenosis. Interestingly, cortical and medullary R2\* values were preserved in many patients with moderate obstructions of the renal artery, despite a fall in stenotic kidney RBF and GFR by 30-35%<sup>31</sup>. This finding is consistent with observed adaptability of human kidneys to moderate reductions of RBF. Repeat measurements three months later showed that their R2\* values remained highly stable<sup>32</sup>. Blunted responses to the loop diuretic furosemide in these kidneys suggested that preservation of tissue oxygenation was associated with attenuated active solute transport activity and thereby oxygen consumption.

However, kidneys distal to severe luminal obstructions showed more pronounced hypoxia<sup>33</sup> with increased tissue injury, interstitial fibrosis and inflammation<sup>34</sup>. The degree of renal hypoxia by BOLD-MRI in those stenotic kidneys correlated directly with the severity of fall in single-kidney RBF and serum injury biomarkers in the draining renal vein<sup>32,35</sup>.

The ability of BOLD-MRI to monitor therapeutic interventions has been shown in groups of patients with RAS receiving autologous mesenchymal stem cells into stenotic kidneys<sup>36</sup> or pretreated with a mitochondrial targeted peptide before undergoing renal revascularization<sup>37</sup>. In both studies, a fall in R2\* 3 months after treatment was associated with a modest increase in cortical perfusion. It should be emphasized that physiological changes in renal work may limit the ability to detect changes in R2\*. For example, in subjects with high-grade renal artery occlusion

cortical and medullary R2\* levels may appear normal due to complete lack of glomerular filtration and solute transport<sup>38</sup>. Some authors therefore propose interpretation of BOLD measurements in correlation with parenchymal renal volume and stenotic kidney GFR (measured by isotope renography) to derive a biomarker that may predict a positive renal functional response to revascularization<sup>39</sup>.

Taken together, severe RAS may be characterized by either marked renal hypoxia consistent with tissue injury, and/or abolished response to furosemide, suggesting minimal residual blood flow to the kidney. BOLD-MRI may be able to assist clinical evaluation of RAS, but more data are needed to achieve clinically relevant advances.

### Transplantation

In 2005, Sadowski et al showed the feasibility of using BOLD-MRI in acute renal transplant rejection<sup>40</sup>. These authors found lower medullary R2\* values in the acute rejection group compared to normally functioning transplants and transplants with acute tubular necrosis (ATN), while cortical R2\* values were higher in ATN compared to the other groups. These observations were confirmed by several subsequent studies and the medullary-to-cortical R2\* ratio has since been proposed as a marker to distinguish between these entities<sup>41,42</sup>. The technique has been proven to be reproducible in subjects with good allograft function and its highest value appears to lie in differentiating stable well-functioning allografts from acute rejection, especially when decreased T2\* values are measured in both the medulla and cortex<sup>43</sup>. BOLD-MRI has also been combined with perfusion and diffusion-weighted MRI to better understand the natural evolution of transplanted kidneys, their degree of fibrosis as well as the episodes of rejection / acute tubular necrosis that may occur when patients are followed over time<sup>44</sup>.

### Drugs and Diet

BOLD-MRI is well suited for monitoring changes following acute dietary and/or pharmacologic manipulation. There are numerous reports evaluating different drugs or diets and the supplementary tables provide representative examples. Below is a brief discussion summarizing drug studies to date.

As mentioned, intravenous furosemide leads to an acute decrease in medullary R\* values and the response to furosemide is currently used as a functional parameter in studies with CKD<sup>16</sup> and RAS<sup>38</sup>.

Drugs that modulate the renin-angiotensin system are commonly used by renal patients and have been shown to decrease R2\* values in most but not all studies. Patients taking beta blockers showed lower R2\* values compared to those that do not<sup>45</sup>. Inversely, increases in R2\* have been observed after the infusion of angiotensin II<sup>46</sup>. In addition, one could observe indirect effects of cyclooxygenase inhibitors resulting in blunted response to water-loading in healthy young individuals<sup>14</sup>

Overall, there is good experience to date in evaluating effects of several drugs and diets with renal BOLD-MRI. Acute drug effects on renal oxygenation may provide early insights in the mode of action of these drugs and their potential nephrotoxic/ -protective effects.

## **DISCUSSION AND CONCLUSION**

Overall, BOLD-MRI is a powerful tool to evaluate tissue oxygenation within the kidneys in humans. The method has been validated against the gold standard (direct measurement of tissue pO<sub>2</sub>) in animal studies, although more studies would be welcome. The possibility to report per-kidney values is of particular interest for patients with RAS. Huge technical progress has been achieved that has resulted in reduced acquisition times (~5-15 minutes) and semi-automatic analysis protocols. However, the current lack of head-to-head comparison with the gold standard in humans remains the main limitation of the technique.

Recent proof-of concept BOLD-MRI studies in CKD patients have provided strong arguments in favor of the chronic hypoxia hypothesis<sup>8,27</sup>, and one study has illustrated its potential to predict renal function decline<sup>29</sup>. BOLD-MRI provides a wealth of

information in patients with severe vascular disease, although interpretation of the results is difficult, and combination with measures of RBF, GFR, or tissue injury will clearly increase its usefulness.

BOLD-MRI is also a powerful tool to gain insight into the renal effects of drugs, thanks to the ability to repeat the exam as often as needed without side effects or ionizing radiation burden. In this regard, BOLD-MRI is of interest to pharmaceutical companies, since it has the potential to identify possible nephrotoxic or nephroprotective properties of drugs at an early stage. The information obtained with BOLD-MRI is increased when used in combination with other MRI modalities that are able to assess RBF (phase contrast, arterial spin labelling [ASL]), oedema or tissue fibrosis (diffusion-weighted imaging [DWI], T<sub>1</sub> mapping), as discussed elsewhere in this NDT Supplement (Selby et al, 2018). However, further standardization is needed, especially when it comes to patient preparation and image analysis. The recommended way to perform BOLD-MRI, depending on the research question and the examined patient group, is summarized in Table 2. This statement is based on the available literature and expert opinion, and may therefore be subject to change in the future.

In conclusion, BOLD-MRI has come a long way and is almost ready for prime time; international collaborations such as the on-going COST action PARENCHIMA offer unique opportunities to accelerate its entrance in to clinical practice, most likely in combination with other functional MRI modalities within a single MRI session.

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**Figure legends:**

**Figure 1:** Examples of different ways to analyze the images obtained with BOLD-MRI. A) small ROI (region of interest, ROI) technique. B) Twelve layer concentric objects (TLCO) technique. C) R2\* profile: the curve links the mean R2\* value of each of twelve layers shown under B, and the red line depicts its steepness. D) Fractional tissue hypoxia. E) Compartmental method.

**Table 1:** Overview of the actually used techniques to analyze BOLD-images.

Technique	Principle	Strengths	Weakness
ROI	Manual placement of circles (called regions of interest, ROIs, 20-40 voxels each) in cortex and medulla	<ul style="list-style-type: none"> <li>- Provides separate information on cortical and medullary R2*</li> <li>- Most frequently used method, easy to perform</li> </ul>	<ul style="list-style-type: none"> <li>- Difficult to differentiate cortex from medulla in advanced CKD</li> <li>- Low reproducibility in CKD</li> <li>- Medullary ROI selection may be skewed by hypoxic regions in the cortex</li> </ul>
Fractional hypoxia	Reports the percentage of R2* values of manually selected renal parenchyma above a certain threshold (usually $30 \text{ s}^{-1}$ or $>2.5$ standard deviations above the average R2*)	<ul style="list-style-type: none"> <li>- This parameter is easily understood by clinicians</li> </ul>	<ul style="list-style-type: none"> <li>- The threshold for hypoxia is artificial</li> <li>- No formal assessment of medullary hypoxia</li> </ul>
Compartmental method	The heterogeneity of renal oxygenation and its gamma distribution of R2* values is used to differentiate medulla from the cortex	<ul style="list-style-type: none"> <li>- Smaller inter-observer variability than classical ROI technique</li> <li>- Allows analysis of separate cortical and medullary response to drug challenge</li> </ul>	<ul style="list-style-type: none"> <li>- Vulnerable to abdominal susceptibility artefact</li> <li>- Inaccurate estimation in advanced CKD</li> </ul>
TLCO (Twelve layer concentric objects) or onion peel	Division of renal parenchyma from each slice in twelve layers of equal thickness, reporting mean R2* of each layer	<ul style="list-style-type: none"> <li>- Semi-automatic procedure with lowest inter-observer variability</li> <li>- Large surface of renal parenchyma analyzed</li> <li>- The steepness of the slope of the R2* curve connecting mean values of each layer depends less of absolute R2* values</li> </ul>	<ul style="list-style-type: none"> <li>- No formal anatomic distinction between cortex and medulla</li> </ul>

**Table 2:** Key points of BOLD-MRI.

Patient preparation	<ul style="list-style-type: none"><li>- Screen for contra-indications for MRI</li><li>- Standardize and record water- and salt-intake</li><li>- Standardize and record medication intake</li><li>- Record blood pressure and blood oxygen saturation</li><li>- Measure blood hematocrit</li></ul>
MRI acquisition	<ul style="list-style-type: none"><li>- Favor field strength of 3T</li><li>- Either coronal or axial plane</li><li>- Expiratory breath hold</li><li>- Standardize and record the time of the MRI acquisition</li></ul>
MRI analysis	<ul style="list-style-type: none"><li>- Whatever method used, perform internal validation</li><li>- Automate the process as much as possible</li></ul>
Limitations	Susceptible to environmental and internal factors (scanning parameters, movement and other artefacts).