INTRODUCTION

Kidney diseases pose a global health burden with their steadily increasing incidence.\(^1\)\(^-\)\(^5\) Elucidating the efficacy of renoprotective strategies is essential \emph{en route} to novel treatment options and effective prophylactic regimens for acute kidney injury (AKI) and chronic kidney diseases (CKD).\(^6\) Current AKI and CKD therapies and prevention strategies are largely experience driven.\(^7\) Today’s options for targeted and stratified measures are disappointingly sparse.\(^8\) Translational approaches—although urgently warranted—are scarce. One major obstacle is the technology required to non-invasively...
disentangle the pathophysiological complexity of AKI and its progression to CKD. Closing this gap asks for approaches, which are clinically applicable, ubiquitous and non-invasive—the forte of MRI. MRI affords longitudinal studies, high anatomic detail, full kidney coverage, soft tissue contrast that helps to capture the physiological heterogeneity between and within the renal layers, and temporal resolution ranging from seconds to minutes.

Renal tissue hypoperfusion and hypoxia play a prominent role in the early pathophysiology of AKI and probably also promote its progression to CKD. Hypoxia results from an imbalance between oxygen (O$_2$) delivery and O$_2$ consumption. Insights into renal oxygenation can be derived from blood oxygenation level–dependent (BOLD) MRI, which offers a non-invasive in vivo technique. Renal BOLD-MRI methodology has progressed and a large body of literature on (pre)clinical applications is available. The growing number of reports eloquently speaking about renal BOLD-MRI and the consensus papers on technical recommendations document the value of non-invasive MRI to renal (patho)physiology. In BOLD MRI image contrast is sensitive to bulk microscopic magnetic field perturbations, which are caused by paramagnetic deoxygenated haemoglobin (deoxyHb) and impact the effective transversal MR relaxation time $T_2^*$. $T_2^*$ weighted MRI is sensitive to changes in the amount of deoxyHb per tissue volume element (voxel). $T_2^*$ decreases if the volume fraction of deoxyHb increases. Renal $T_2^*$ or its reciprocal value ($R_2^* = 1/T_2^*$) is a surrogate of renal blood oxygenation, but is frequently interpreted also as a surrogate of renal tissue oxygenation. This assumption is based upon the $T_2^*$ dependence on O$_2$ saturation of Hb (StO$_2$), the physiological relationships between StO$_2$ and blood partial pressure of O$_2$ (pO$_2$), and between blood pO$_2$ and tissue pO$_2$. Mapping of $T_2^*$ or of $R_2^*$ has been employed for the assessment of renal oxygenation in a broad spectrum of renal tissue states and interventions in human and animal studies. Renal $T_2^*$ has even been suggested to display a close correlation with renal tissue pO$_2$ levels.

Notwithstanding the encouraging progress made, questions have been raised regarding the correct interpretation of BOLD MRI data in the kidney as a surrogate of tissue oxygenation. These questions spurred the debate on “how bold is BOLD-MRI of the kidney?”. This debate was triggered by experimental observations derived from simultaneous measurements of renal $T_2^*$ and tissue pO$_2$ using an integrated approach of MRI and gold standard physiological measurements (MR-PHYSIOL). MR-PHYSIOL experiments revealed substantial discrepancies in the quantitative relationship between changes in renal $T_2^*$ and those in renal tissue pO$_2$ for the different layers of the kidney (cortex, outer medulla, inner medulla) and for various (patho)physiologically relevant interventions to the kidney. In essence, the integrated MR-PHYSIOL approach confirmed that the renal $T_2^*$ to tissue pO$_2$ relationship is not governed exclusively by renal blood oxygenation, but is also heavily influenced by a number of physiological confounders as illustrated in Figure 1.

Of particular relevance are alterations in renal blood volume fraction (BVf). As renal $T_2^*$ is a surrogate for the amount of

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**FIGURE 1** Schematic overview of the relationship between tissue partial pressure of oxygen (pO$_2$) and renal $T_2^*$, together with the confounding key factors. This includes $T_2^*$ alterations as a result of changes in the tubular compartment and/or in the intrarenal vascular compartment. Changes in the renal blood volume fraction induced by either active vasomotion or passive circular vessel distension/compression alter the amount of deoxyHb per tissue volume and may confound $T_2^*$. As the renal capsule has a rather low elasticity, changes in tubular volume fraction will often result in circular distension or compression of intrarenal vessels, which will inevitably induce changes in the renal blood volume fraction which may confound $T_2^*$. 

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**TABLE 1**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>pO$_2$</td>
<td>Partial pressure of oxygen</td>
</tr>
<tr>
<td>StO$_2$</td>
<td>Oxygen saturation of Hb</td>
</tr>
<tr>
<td>$T_2^*$</td>
<td>MR relaxation time</td>
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<tr>
<td>BVf</td>
<td>Blood volume fraction</td>
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of deoxyHb per tissue volume, any change in BVf confounds the interpretation of $T_2^*$ changes commonly exclusively attributed to alterations in blood oxygenation, and even more so as tissue oxygenation. This confounding role of BVf has not received careful consideration and assessment so far, although it was recently outlined in reports on alterations in renal vascular conductance, local haemoglobin concentrations and kidney size triggered by physiological interventions.\textsuperscript{30} The impact of renal BVf changes on $T_2^*$ exceeds that of most other organs as a result of the substantial and readily alterable blood volume fraction in the renal cortex.\textsuperscript{41} Taking this into account, $T_2^*$ mapping alone cannot provide an unambiguous examination of renal oxygenation. However, if an assessment of renal BVf is integrated into a comprehensive MR imaging protocol, the combination of renal $T_2^*$ and BVf mapping could provide a more quantitative surrogate of renal blood oxygenation \textit{en route} to MR oximetry tailored for the in vivo and non-invasive characterization of renal tissue oxygenation and haemodynamics.\textsuperscript{24,42}

Realizing the opportunities, challenges and caveats of assessing renal haemodynamics and tissue oxygenation in vivo and non-invasively, probing renal BVf is highly relevant if not essential for the pursuit of renal MR oximetry.\textsuperscript{42} Intravenously applied superparamagnetic iron oxide nanoparticle (USPIO) preparations can be used as MRI visible blood pool markers for probing alterations in BVf, in particular ferumoxytol, which is increasingly used off-label as an intravascular MRI contrast agent.\textsuperscript{42-47} To promote these advancements at the interface between physics, physiology and patient care, this review discusses the opportunities of ferumoxytol-enhanced assessment of renal BVf. For this purpose, the specifics of renal oxygenation and perfusion are outlined first. Secondly, alterations in renal BVf upon interventions and their impact on $T_2^*$ are surveyed. The basic physical and biophysical principles of probing BVf using ferumoxytol as a blood pool marker are then described. Next, acute effects of ferumoxytol on controlling renal haemodynamics and oxygenation are considered. Preclinical applications of ferumoxytol-enhanced MRI are presented for monitoring of renal BVf in physiological settings where significant variations in both, renal BVf and oxygenation, occur. Considerations for clinical implementation of ferumoxytol-based assessment of renal BVf changes are provided. We conclude by exploring future directions of MRI-based assessment of renal BVf.

All the animal experiments performed by the authors and shown in the figures were carried out in accordance with published guidelines.\textsuperscript{48,49}

2 | SPECIFICS OF RENAL PERFUSION AND OXYGENATION

Renal haemodynamics and oxygenation offer a number of striking differences when compared with non-renal tissue.\textsuperscript{24,38} First, total renal blood flow (RBF) is huge when compared with virtually all other organs: the kidneys receive about 20% of the cardiac output under resting conditions. Accordingly, the kidneys’ oxygen extraction (the difference between the $O_2$ content in the renal arterial and the renal venous blood) is low. Yet blood perfusion within the kidneys is quite heterogeneous: while 100% reaches the cortex, only 15% of blood that previously passes through the cortex will reach the medulla. This unequal distribution is one reason behind the very low pO$_2$ in the medulla. Secondly, the kidney differs from all other organs with regard to the relationship between metabolism and perfusion. More than 26 thousand millimoles of sodium (Na$^+$) are filtered in the human glomeruli every day: this is equivalent to more than 1.5 kg of table salt. To achieve sodium balance, the amount of salt excreted via the urine must exactly match the amount of ingested salt minus that amount of extrarenal loss. Thus, more than 99% of the filtered sodium must usually be reabsorbed from the tubules. Tubular resorption relies on active transport processes, which account for about 90% of the kidney’s energy expenditure, and, thus, its $O_2$ consumption. The more Na$^+$ is filtered in the glomeruli, the more must be reabsorbed. As glomerular filtration rate (GFR), in general, increases with increased renal blood flow, renal $O_2$ consumption also usually increases with increased renal perfusion. This is in contrast to all other organs, where metabolism determines perfusion. However, the kidney is equipped with efficient mechanisms of autoregulation, that is, the ability to maintain RBF and GFR relatively constant in the face of changes in renal artery pressure. Renal autoregulation is suggested to serve the purpose of balancing $O_2$ delivery, that is, RBF, with the metabolic needs and $O_2$ demands arising from tubular reabsorption.

In addition to the heterogeneous intrarenal blood perfusion, other factors substantially contribute to the low tissue pO$_2$ and, in particular, the physiological hypoxia in the medulla as summarized in a recent review.\textsuperscript{24} First, there is a considerable shunt diffusion of $O_2$ from arteries to veins in the cortex and from descending to ascending vasa recta in the medulla. Second, the Fahræus-Lindqvist effect reduces the haematocrit in the vasa recta supplying the medulla, which results in a reduction in the $O_2$ content of blood perfusing the medulla. Third, plasma skimming at intrarenal vessel branches induces different haematocrit and $O_2$ content of blood perfusing the daughter vessels.

3 | ALTERATIONS IN RENAL BVF UPON INTERVENTIONS AND THEIR IMPACT ON $T_2^*$

Changes in renal BVf, that is, in vessel volume per tissue volume, induced by either active vasomotion or passive circular
vessel distension/compression alter the amount of deoxyHb per tissue volume and thus confound the renal $T_2^*$ to tissue $pO_2$ relationship (see Figure 1). If considered per tissue weight, total renal blood flow is much higher than that of most other tissues. It is to be expected that renal vasomotion, at least of cortical vessels, results in renal BVf changes that exceed those documented for other tissues.\textsuperscript{10,21,32,35} Substantial changes in the renal BVf were observed for various experimental settings.\textsuperscript{7,11,20} Moreover, the tubules are a unique feature of the kidney. Their volume fraction is quite large and can rapidly change as a result of changes in glomerular filtration, alterations in tubular outflow towards the pelvis, changes in tubular fluid resorption and modulation of the transmural pressure gradient. As the renal capsule has a rather low elasticity, changes in tubular volume fraction should often result in circular distension or compression of intrarenal vessels, which will inevitably change the renal BVf.\textsuperscript{10}

Figure 1 provides a schematic overview of the confounders of the renal $T_2^*$ to tissue $pO_2$ relationship as derived from various studies, in particular studies that assessed the impact of alterations in renal haemodynamics, renal blood and tissue oxygenation, haematocrit, and tubular and vascular volume fractions on $T_2^*$ under various (patho)physiological conditions.\textsuperscript{24} The confounders include (i) changes in $O_2$ diffusion between blood and tissue, (ii) shifts of the oxyHb dissociation curve, (iii) alterations in Hb concentration per blood volume (haematocrit), (iv) changes in the tubular compartment and (v) changes in the intrarenal vascular compartment (blood volume fraction, BVf). The impact of changes in BVf was demonstrated in pre-clinical studies that revealed suprarenal aortic occlusion (or that of the renal artery) induces only a minor decrease in renal $T_2^*$, in contrast to a massive $T_2^*$ decrease upon occlusion of the renal vein.\textsuperscript{24} Simultaneous occlusion of the renal vein and artery yielded an intermediate $T_2^*$ decrease.\textsuperscript{24} In each of these three cases, tissue $pO_2$, blood $pO_2$ and StO$_2$ approach zero. Yet the BVf is decreased in case of arterial occlusion, augmented for venous occlusion and remains approximately unchanged during common arterial-venous occlusion. Contrary to vasoconstriction, vasodilatation induces $T_2^*$ shortening governed by a BVf increase, although renal tissue $pO_2$, blood $pO_2$, and StO$_2$ primarily increase owing to improved $O_2$ delivery. Vasoconstriction causes a $T_2^*$ prolongation despite a decline in $O_2$ delivery as a result of a reduced BVf. Distension of tubules results in increased $T_2^*$ in the face of primarily unchanged StO$_2$, blood and tissue $pO_2$ caused by reduced BVf.\textsuperscript{24} Anaemia or an increase in plasma skimming evoke an increase in $T_2^*$ despite the drop in $O_2$ delivery and tissue $pO_2$. Finally, alterations in the inspiratory $O_2$ fraction (FiO$_2$) change renal $T_2^*$. Hypoxia decreases $T_2^*$ in parallel with StO$_2$, blood and tissue $pO_2$. Changes in BVf may either enhance or diminish this effect on $T_2^*$, depending on whether the FiO$_2$ falls moderately or severely below 21%, leading either to intrarenal vasodilatation or vasoconstriction respectively. The effect of hyperoxia on $T_2^*$ is much less pronounced.

## 4 | BASIC PRINCIPLES OF RENAL BVF ASSESSMENT USING FERUMOXYTOL IN AN EQUILIBRIUM CONCENTRATION REGIMEN

Ferumoxytolt-enhanced MRI is susceptible to bulk microscopic magnetic field perturbations around blood vessels, which are induced by the USPIO nanoparticles. These microscopic magnetic field inhomogeneities affect the (effective) transversal MR relaxation times $T_2^*$ and $T_2$ as a function of the USPIO concentration. $T_2^*$ is governed by $1/T_2^* = 1/T_2 + 1/T_2'$, with $T_2$ being the tissue-dependent transverse relaxation time observed in spin-echo MR measurements and $T_2'$ embodying susceptibility-related contributions.\textsuperscript{51} While the magnetic field perturbations caused by Fe atoms (in deoxyHb or USPIO nanoparticles) directly reduce $T_2^*$, diffusion effects in the proximity of blood vessels also lead to a decrease in $T_2$. When the USPIO concentration increases, a $T_2^*/T_2$ decrease occurs and hence a signal attenuation in $T_2^*$-weighted and $T_2$-weighted MR images. Parametric mapping provides quantitative data of the relaxation times $T_2^*$ and $T_2$. Renal $T_2^*$ maps are commonly obtained with multi-echo gradient echo techniques. With this approach a train of gradient refocused echoes is acquired after the initial excitation, whereby each echo is independently $T_2^*$ weighted. In vivo $T_2^*$ mapping of the kidney permits high anatomical detail employing an in-plane spatial resolution as good as 50-100 µm in mice and about 250 µm in rats with dedicated small animal MR systems equipped with state-of-the-art radiofrequency antenna technology\textsuperscript{52} (Figure 2). Parametric mapping of the reciprocal value of renal $T_2^*$ ($R_2^* = 1/T_2^*$) is used for renal BVf mapping in an equilibrium concentration regime. For this purpose, renal BVf can be calculated by comparing pre-ferumoxytolt data ($R_2^*$-maps) with post-ferumoxytolt data ($R_2^*$-USPIO maps):\textsuperscript{53}

$$ BVf = \frac{3}{4\pi} \cdot \frac{\Delta R_{2,\text{USPIO}}}{\Delta \chi_{\text{USPIO}} \cdot B_0 \cdot \gamma} = \frac{3}{4\pi} \cdot \frac{\Delta R_{2,\text{USPIO}}}{\Delta \chi_{\text{USPIO}} \cdot B_0 \cdot \gamma} $$

where $\gamma$ is the gyromagnetic ratio, which is 2.675 · 10$^8$ rad/ (s T), $B_0 = 9.4$ T and $\Delta \chi_{\text{USPIO}}$ is the susceptibility difference between blood with and without added USPIO: $\Delta \chi_{\text{USPIO}} = 0.024$ ppm (cgs units) × [USPIO dose in mg Fe/ kg body weight].\textsuperscript{54} The equilibrium concentration regime facilitates the monitoring of renal BVf over several hours, supported by the long half-life time of ferumoxytolt.\textsuperscript{55} Assuming that the magnetic field inhomogeneity is negligible and constant throughout the experiment so that $R_2^*$ is governed by microscopic rather than by macroscopic susceptibility gradients, renal StO$_2$ can be estimated using a multi-parametric
MR approach including renal $R_2^*$, $R_2$ and BVf and a solution of the model equation $s(t) = f(t, BVf, SO_2, ...)$ for $StO_2 = f(R_2^*, R_2, BVf, ...)$.\textsuperscript{56-58}

\begin{equation}
StO_2 \approx 1 - \left( \frac{3}{4\pi} \cdot \frac{(R_2^* - R_2)}{\gamma \cdot \Delta \chi_0 \cdot BVf \cdot Hct \cdot B_0} \right)
\end{equation}

with $\Delta \chi_0$ representing the susceptibility difference between deoxygenated and oxygenated red blood cells ($\Delta \chi_0 = 0.264$ ppm at a magnetic field strength of $B_0 = 9.4$ tesla).\textsuperscript{59} $StO_2$ is given in arbitrary units as magnetic field inhomogeneities $B_0$ are not corrected. A haematocrit of Hct = 0.40 can be used for the renal cortex and for the outer medulla (85%-95% of systemic Hct, which was assumed to be 0.45).\textsuperscript{60}

Further to the equilibrium concentration regimen, renal blood volume can also be derived from dynamic susceptibility weighted imaging, where signal intensity changes in the kidney are monitored during the first passage of a ferumoxytol bolus as demonstrated in human studies.\textsuperscript{61} This approach requires the measurement of the arterial concentration-signal-intensity-time-curve of ferumoxytol, designated as the arterial input function (AIF) and its deconvolution from the renal tissue signal intensity time curves. This technique is better suited for clinical applications than preclinical studies in small animals, where the small size of the relevant vessels makes the AIF measurement rather challenging.

5 | FERUMOXYTOL DOES NOT AFFECT REGULATION OF RENAL HAEMODYNAMICS AND OXYGENATION

Ferumoxytol is an approved intravascular iron supplementation therapy in the USA targeting patients with iron deficiency anaemia related to CKD. The preparation consists of USPIO nanoparticles encapsulated by a polyglucose sorbitol carboxymethylether coating. Ferumoxytol is superparamagnetic, which makes it a viable candidate as an off-label MRI contrast agent for a broad spectrum of preclinical and diagnostic imaging applications.\textsuperscript{44,45,62} Ferumoxytol does not readily extravasate and is not filtered in the glomeruli, so it exhibits a long intravascular half-life of > 14 h in humans and about 2 h in small rodents. Ferumoxytol can be administered by intravenous injection.

A recent report studied acute intrinsic effects of ferumoxytol on the control of renal haemodynamics and oxygenation in rats.\textsuperscript{63} This study was of high relevance as basic methodological principles require that a method intended to measure a certain variable per se does not alter this variable. For this purpose, in vivo measurements of arterial blood pressure, total renal blood flow (RBF, transsonic probe), local perfusion (laser-Doppler-flux) and tissue PO$_2$ (fluorescence quenching optodes) in the renal cortex and medulla of healthy rats were employed upon application of ferumoxytol.\textsuperscript{20,63,64} Modulation of renal haemodynamics

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Coronal views of rat kidneys: (A) photograph of a freshly excised rat kidney, (B) $T_1$-weighted anatomical MR image acquired ex vivo using an in-plane spatial resolution of 50 μm (cortex COR; outer medulla OM; inner medulla IM, the 1 cm scale bar illustrates the size of the rat kidney), (C) $T_2^*$-sensitized MR image acquired ex vivo using an in-plane spatial resolution spatial resolution 50 μm, (D) $T_2^*$-weighted in vivo MR image acquired with an in-plane spatial resolution of 300 μm prior to ferumoxytol application, (E) $T_2^*$-weighted in vivo MR image acquired with an in-plane spatial resolution of 300 μm after ferumoxytol application, (F) in vivo $T_2^*$ map acquired at baseline with an in-plane spatial resolution of 300 μm and (G) in vivo $T_2^*$ map acquired post–ferumoxytol administration with an in-plane spatial resolution of 300 μm. The $T_2^*$ shortening caused by the superparamagnetic effect of ferumoxytol can be readily recognized.}
\end{figure}
and oxygenation was accomplished by dedicated test interventions. The dose study included three (cumulative) doses of ferumoxytol: two low doses adapted from previous reports on monitoring the blood volume fraction of the brain and kidneys in rats, and a high-dose regimen that mimics the human equivalent dose (based upon body surface area) for the therapeutic application of ferumoxytol. The main finding of these investigations in rats was that intravenously injected ferumoxytol has no sustained impact on renal haemodynamics and oxygenation under baseline physiological conditions as well as on control of haemodynamics and oxygenation as studied by hypoxic and hyperoxic stimuli and a brief suprarenal aortic occlusion (Figure 3). The only significant finding reported was a moderate reduction in the hyperoxia-evoked increase in arterial pressure at a cumulative dose of 41 mg Fe/kg. This dose mimics the human equivalent dose for iron supplementation and is well beyond the dose commonly used for MR-based assessment of the renal BVf. The results demonstrated no immediate risk of impaired control of renal oxygenation or perfusion upon application of ferumoxytol. This observation rendered ferumoxytol as an intravascular contrast medium suitable for the quantification and monitoring of changes in renal blood volume fraction.

6 | MONITORING RENAL BVF DURING EXPERIMENTAL PHYSIOLOGICAL INTERVENTIONS

The applicability of ferumoxytol-enhanced MRI for probing renal BVf during physiological interventions that are concomitant with variations in BVf was recently demonstrated in healthy rats in conjunction with correcting renal T₂* for BVf alterations. For this purpose a dose finding study was performed first in rats to ensure that BOLD effects were detectable after ferumoxytol administration considering that the signal-to-noise ratio may be further diminished owing to T₂* shortening attributed to the de-oxygenation of blood. Balancing the sensitivity of T₂* to ferumoxytol injection and the T₂* sensitivity to the (patho) physiological intervention (occlusion of the renal vein) a
ferumoxytol dose of 2-4 mg Fe/kg BW was found to be best suited for $R_2^*$, BVf and StO$_2$ mapping. $\Delta R_2$ and $\Delta R_2^*$ analyses revealed that BVf is higher in the outer medulla versus the renal cortex (Figure 4).42

Changes in the intrarenal BVf and StO$_2$ were studied in rats in response to renal venous occlusion. Examples of renal cortical and outer medullary $T_2^*$ maps are depicted in Figure 5. Renal BVf and StO$_2$ maps obtained at baseline, during renal venous occlusion and during the recovery phase are illustrated in Figure 5. BVf and StO$_2$ derived from MRI in rats were benchmarked against BVf and StO$_2$ references provided by near infrared spectroscopy (NIRS) conducted in rats. The MRI data were in qualitative accordance with the NIRS data: cortical StO$_2$ dropped substantially during the short-term venous occlusion for both approaches. The almost twofold increase in cortical BVf deduced from MRI during venous occlusion was in qualitative agreement with the rise in haemoglobin concentration per tissue volume derived from NIRS.

The main findings of the assessment of renal BVf during experimental physiological interventions in rats at 9.4 Tesla were fourfold42: (i) a 4 mg Fe/kg dose of ferumoxytol suits BVf assessment at 9.4 T during baseline, venous occlusion and recovery, (ii) the proposed approach permits reproducibility for BVf and StO$_2$ examination as validated by test/re-test experiments,42 (iii) relative changes in cortical BVf and cortical StO$_2$ derived from MRI were in qualitative accordance with relative changes in BVf and StO$_2$ deduced from NIRS and (iv) without the monitoring of BVf MRI overestimates the StO$_2$ decrease during renal venous occlusion.42 To conclude, this experimental work permitted a non-invasive detection of renal BVf increase in rats upon venous occlusion and a removal of its effects on blood oxygenation-sensitized renal MR.

Probing renal BVf with ferumoxytol-enhanced MRI can be integrated with gold standard physiological measurements such as MR-PHYSIOL.37-39 The insertion of invasive probes (combined pO$_2$- and laser-flux probes) into the kidney in the preclinical hybrid MR-PHYSIOL setting might induce a minor change in the intrarenal (tubular and blood) volume fractions. The volume of a kidney in the rats we usually study ranges between 1200 μl and 1500 μl. The combined volumes of two probes inserted (usually one into the cortex, one into the medulla) ranges between 30 μl and 50 μl. The amount of tissue (including the tubular and blood volume) “displaced” by two probes is about 2%-4%. This “volume shift” can be neglected for renal BVf assessment.

**FIGURE 4** Illustration of blood volume measurement procedure using ferumoxytol as an exogenous blood pool marker. Shown are parametric maps of renal $R_2$ and $R_2^*$ prior (left) to and after (middle) ferumoxytol administration.42 Quantitative $R_2$ and $R_2^*$ maps permit comparisons over time and between animals, as they are not biased by external factors such as the RF coil sensitivity ($B_1^-$) or the location of the subject with respect to the RF coil. The difference between $R_2/R_2^*$ maps acquired before and after USPIO is closely related to the local BVf. The area with apparently very high BVf stretches from the papilla via the inner medulla to the central outer medulla. This phenomenon represents the influence of large iron-rich vessels located close to the image slice, rather than the actual medullary tissue properties. The locations of apparently high BVf in papilla and inner medulla co-localize with the renal artery and vein, and the interlobar arteries and veins.
FIGURE 5  Maps of renal cortical and outer medullary $T_2^*$, together with estimated maps of blood volume fraction (BVf) and oxygen saturation of Hb ($SO_2$) at baseline, during renal venous occlusion and the recovery phase. The maps at baseline and after 10 minutes of recovery are almost indistinguishable, confirming that the effects of venous occlusion were reversible. Within-subject repeatability is demonstrated for renal BVf and SO2 by comparing the maps derived from two different experimental phases, namely, following 4 mg Fe/kg USPIO and following 6 mg Fe/kg USPIO. Test-retest reliability (repeatability) was high—the differences between both iterations are nearly negligible, even though more USPIO had been injected in between. In addition, SO2 maps were calculated assuming that renal BVf remains constant and identical to the baseline condition (bottom row): the missing compensation for BVf changes during venous occlusion results in SO2 values considerably lower than when BVf was monitored.
Notwithstanding these encouraging first results, benchmarking BVf and StO₂ results deduced from MRI against a quantitative reference is essential for further rigorous validation of the MRI approach. Currently, a perfect quantitative equivalent for validation of renal MR-derived BVf and StO₂ is not readily available. Invasive tissue pO₂ probes are very well established but cover very small renal regions. Another constraint is that invasive tissue pO₂ probes measure tissue pO₂ rather than blood oxygenation. NIRS affords estimation of StO₂ and tissue concentration of haemoglobin—an established surrogate for BVf—but the necessary mathematical modelling is rather complex and it is currently constrained to reflection mode probing of the renal cortex because of penetration depth limitations. However, benchmarking the occlusion-induced changes in cortical BVf and StO₂ derived from MRI with NIRS obtained for the renal cortex show good agreement. This observation provides encouragement for further advancement and integration of the MR approach into a comprehensive renal MR oximetry protocol.

7 TOWARDS CLINICAL IMPLEMENTATION OF FERUMOXYTOL-ENHANCED MRI ASSESSMENT OF RENAL BVF

The R₂ and R₂* mapping protocols employed for renal BVf assessment in small rodents at ultrahigh magnetic field strengths are readily available on clinical MR scanners, which facilitates a swift translation into clinical use of ferumoxytol-based renal BVf examination. This development is fuelled by an ever-growing number of studies that report on the off-label use of ferumoxytol for a wide portfolio of preclinical and diagnostic imaging applications. In a clinical setting, renal T₂* and T₂ mapping is commonly employed at time points that are longitudinally propagated by at least hours if not days or months. With this clinical context, a temporal resolution of 2-3 min for combined T₂* and T₂ mapping and the 1-2 min time shift between the measurements of both parameters are negligible. MRI protocols affording breath-hold acquisitions facilitated by accelerated imaging using parallel acquisition techniques or compressed sensing approaches would make respiratory triggering obsolete, shorten examination times and enhance image quality by reducing the propensity to respiratory motion-induced image artefacts.

*En route* to clinical implementation ferumoxytol was demonstrated to be useful for differentiation between acute and chronic inflammatory kidney diseases based on different patterns of parenchymal ferumoxytol depositions in a rat model using a clinical whole-body 3.0 T MR scanner. The sensitivity of ferumoxytol-enhanced R₂* mapping to dynamic BVf changes in the rat kidney was demonstrated using a 3.0 Tesla human MR scanner. Steady-state MR angiography using incremental doses of up to 4 mg/kg body weight of ferumoxytol as intravenous contrast agent was performed in patients with advanced kidney disease due for transplant listing, which implicitly supports the feasibility of renal BVf mapping in patients. T₂* mapping has been also used to demonstrate that allografts undergoing acute rejection in paediatric kidney transplant patients show T₂* prolongation in ferumoxytol-enhanced MRI versus non-rejecting allografts. This observation was attributed to reduced perfusion and increased oedema in rejecting allografts. Normal R₂* values obtained for post-ferumoxytol administration (4 mg/kg body weight) at 1.5 T and 3.0 T were reported for the human kidney. Pharmacokinetics of ferumoxytol in the kidney were examined in healthy subjects using R₂* relaxometry at 1.5 T and 3.0 T. Renal R₂* increased at post-injection and peaked on day 1 (R₂*baseline = 13 s⁻¹ vs R₂*ferumoxytol = 31 s⁻¹). Clearance of ferumoxytol from the kidney occurred on day 2 for a ferumoxytol dose of 2mg/kg body weight. For a ferumoxytol dose of 4mg/kg body weight clearance from the kidney was reported on day 4 post-injection. Renal T₂* decrease caused by ferumoxytol accumulation was demonstrated for healthy subjects and for type 1 diabetes patients. A recent multicentre safety study enrolled 3215 patients who received 4240 ferumoxytol injections (dose range: 1–11 mg/kg body weight) for MRI. The authors reported a positive safety profile for ferumoxytol use in MRI and concluded that diagnostic ferumoxytol was well tolerated and associated with no serious adverse events. Ferumoxytol-enhanced dynamic MRI was employed in healthy subjects to assess the blood volume in the renal cortex (mean rBVrenal cortex = 41±8 ml/100 g). When implementing ferumoxytol-based renal BVf assessment in clinical renal imaging protocols, caution should be taken to prevent interference of ferumoxytol with other MRI techniques and MR contrasts. For example, ferumoxytol shortens the longitudinal relaxation time T₁, which affects T₁ contrast.

Application of ferumoxytol might impact mapping of regional perfusion in renal tissue using arterial spin labelling (ASL) techniques. The ASL technique employs magnetization-labelled water in arterial blood of the aorta or the renal arteries as a freely diffusible endogenous tracer. In a preparation module, spin labelling is applied to tag the longitudinal magnetization of arterial blood water before it enters the imaging plane in the kidney. An image covering the kidney is acquired following arterial blood labelling and a delay time between labelling and the advent of the tagged arterial blood water in the imaging plane. A control image is acquired of the same slice in the kidney using the same delay time but no labelling of the arterial blood water. Provided that the magnetization of the inflowing blood is the only difference between the control and the label image, a difference map yields a perfusion-weighted image of the kidney with the signal intensity being proportional to renal tissue perfusion.
T₁ shortening owing to the presence of ferumoxytol requires adaption of the delay or traveling time used for arterial blood water in the arterial spin labelling preparation module. ASL approaches were exploited for examination of regional renal blood flow and were shown to be suitable for the evaluation of renal injury in rats and for the assessment of patients suffering from diabetes and moderate (stage 3) CKD compared with healthy controls. ASL MRI also provides an avenue towards MR-based assessment of arterial blood volume using multiple-delay time sampling as demonstrated for the human brain. As ASL is a difference image technique and a low signal-to-noise ratio (SNR) technique, it might suffer from ferumoxytol-induced T₂* shortening and SNR degradation. To decouple the interference of ferumoxytol with other MR techniques and MR contrasts, it is thus recommended that ferumoxytol-enhanced BVf assessments be performed at the end of clinical renal MRI protocols.

8 | FUTURE DIRECTIONS OF MAPPING RENAL BVF WITH MRI

Due caution for quantitative interpretation of renal BOLD-MRI is required as renal T₂* is a surrogate that does not quantitatively reflect renal tissue oxygenation in several (patho) physiological conditions and because T₂* may not mirror blood oxygenation quantitatively in some scenarios. This requires that renal blood oxygenation level–related T₂* changes are strictly differentiated from T₂* alterations caused by changes in tubular and vasculature volume fraction, the latter being T₂* confounders. To meet this goal, it is essential to incorporate an MR-based assessment of renal BVf into renal MRI protocols to detail vasodilation, vasoconstriction and other alterations in the blood volume fraction.

It is well recognized that dynamic contrast-enhanced (DCE) methods in conjunction with tracer kinetic principles provide a viable alternative for the quantification of renal perfusion and blood volume. For this purpose, bolus injection of exogenous contrast agents like gadolinium (Gd) chelates or iron oxide nanoparticles is administered. Tracking and analysing the dynamic susceptibility contrast (DSC) changes during first-pass contrast agent bolus passage through the kidney requires fast imaging techniques with a temporal resolution of ≤ 1 s. It also requires the measurement of the arterial concentration-time-curve, designated as the arterial input function and its deconvolution from the time course of signal intensity in renal tissue. Renal DSC was successfully implemented in human studies and animal studies.

For the latter, methodological (imaging speed) and physiological (heart rate) constraints govern the achievable spatial resolution and render this approach particularly challenging in small rodents. Compartmental models used for the analysis of DCE signal intensity time course upon injection of a gadolinium-based contrast bolus commonly include an extravascular component, leading to a complex mathematical kinetic description. Intravascularly confined contrast agents benefit DCE MRI as their kinetic models exclude the extravascular space. The experimental paramagnetic gadolinium-based contrast agent P792 is a rapid-clearance intravascularly confined agent characterized by negligible interstitial diffusion but unrestricted glomerular filtration.

This intravascular contrast agent has recently been evaluated for measurements of renal function in rats using a low dosage.

Fluorine (¹⁹F) MRI offers an intriguing alternative to proton (¹H) methods such as T₂* and DCE imaging/mapping for quantifying changes in blood pO₂ and blood volume in the kidney. ¹⁹F is another MR active nucleus with similar sensitivity to ¹H. Although the absence of organic ¹⁹F in the body of humans and rodents is an advantage and any ¹⁹F compounds introduced in vivo can be detected with high selectivity and absolute specificity, its application is also challenged by typically low SNRs, especially when ¹⁹F compounds are administered systemically and distributed at low concentrations in vivo. Nonetheless, the application of fluorinated emulsions, typically prepared from perfluorocarbons (PFCs), has been explored to study vascular pathology in mice and rats and recently, their utility to assess BVf and pO₂ in the renal microvasculature was demonstrated following AKI in mice.

Owing to the virtual absence of ¹⁹F in the human/animal body, ¹⁹F spin density–weighted MRI following intravenous injection of fluorinated emulsions may provide a quantitative measure of renal BVf. Compared with the USPIO-based approach, BVf assessment using ¹⁹F requires a reference with known concentration to be scanned together with the subject, and the much lower local concentration of ¹⁹F vs ¹H requires measures to counteract the SNR challenge, such as increasing signal averaging and/or lowering spatial resolution. To this end, in the context of renal MR oximetry, conceptually it is more appealing to exploit directly the blood oxygenation sensitivity of PFC (as outlined in the following), rather than using it for probing BVf to correct for effects of BVf variations from T₂*-based blood oxygenation measurements.

PFC emulsions have a high oxygen–dissolving capacity making them ideal O₂ carriers as well as indicators of pO₂ changes. The longitudinal relaxation rate (1/T₁) of ¹⁹F in PFCs was shown to depend linearly on O₂ partial pressure. This led to the first non-invasive in vivo tissue O₂ assessments in tumour tissue and liver in mice and later in the myocardial tissue of rats. The latter study provided the first proof-of-concept study for rapid, non-invasive measurements of O₂ tension changes in response to ischaemia and reperfusion using ¹⁹F MRI. More recently, a decreased renal BVf and blood pO₂ was detected in the cortico-medullary junction 24 h following unilateral renal ischaemia reperfusion using ¹⁹F MRI, and in parallel an increased T₂*
was observed by $^1$H BOLD MRI. In the injured kidney, the $T_2^*$, $^{19}$F signal and pO$_2$ within the renal cortex were comparable with the contralateral non-injured kidney, but in the inner medulla, vascular leakage and extravascular retention of PFC NPs resulted in reduced $T_2^*$, increased $^{19}$F and unchanged pO$_2$. These results suggest a recovery in perfusion and oxygenation within the cortex but not within the inner medulla. This study shows the potential of applying PFC emulsions for studying renal disease. The relatively large size of the nanoparticles within these emulsions (> 100 nm) might provide a relatively good safety profile with respect to renal toxicity, as they are not expected to be cleared through glomerular filtration. Therefore, unlike other imaging agents that undergo renal clearance (eg iodinated x-ray contrast agents, gadolinium-based MR contrast agents), PFC emulsions are not immediately considered as nephrotoxic, thereby holding promise for in vivo tissue pO$_2$ assessment of renal tissue in animal models of AKI.

One of the most exciting areas of innovation in biomedical imaging concerns the visualization on multiple scales in space and time: imaging biological objects in size ranging from the atomic to the anatomic scale, and from nanoseconds to decades in large population imaging studies. Advances in biomedical imaging have spurred developments and applications in the fields of optical, high-frequency ultra-sound and photoacoustic imaging. The progress in photoacoustic imaging provides a trajectory into the characterization of (patho)physiological conditions of the kidney, which could allow early detection of kidney injury owing to its capacity to probe renal oxygen saturation of haemoglobin. This development underlines the potential and value of hybrid and complementary modalities in basic research and clinical science, such as optoacoustic imaging and MRI, photoacoustic imaging and intravital 2-photon microscopy or near infrared spectroscopy (NIRS) and MRI, and their calibration with quantitative gold standards.

9 | CONCLUSION

We should definitely not skip BOLD for renal imaging. What we need to do is to re-evaluate our understanding of BOLD imaging as validated bio- or imaging marker for renal tissue oxygenation. For this, we (only) need to decipher and quantify the changes in the vascular and tubulus volume fraction that are confounding BOLD imaging. This calibration will empower BOLD imaging and its application in the research setting and in the clinical arena. It can be expected that there are several (patho)physiological conditions in which the knowledge of BVf will not be as essential (eg in early stage diabetes) as for other more complex scenarios (eg in kidney transplantation). These considerations are necessary to make valid statements about renal blood oxygenation, especially in cases where BVf is not significantly different from the normal healthy physiological condition. It still remains to be determined whether errors introduced by ignoring BVf in specific settings remain within acceptable error margins or not.

To conclude, further weight should be put behind the solution of the remaining issues to advance the (pre)clinical value and the capabilities of parametric MRI for probing dynamic changes in the renal blood volume with the goal to decipher the confounding impact of the vascular compartment on $T_2^*$ en route to improving our understanding of haemodynamics/oxygenation in kidney disorders. A swift transfer of MR oximetry, including $T_2^*$ mapping and renal BVf quantification, from the research scenario into the clinic should be targeted in interdisciplinary and interinstitutional collaboration networks among forward-thinking basic researchers, application scientists and clinicians to establish a comprehensive MR protocol for the in vivo and non-invasive assessment of renal haemodynamics and oxygenation. As this approach may become increasingly used in (pre)clinical research, it should help to enhance the potential of MRI for the assessment of renal diseases.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ORCID

Thoralf Niendorf https://orcid.org/0000-0001-7584-6527
Sonia Waiczies https://orcid.org/0000-0002-9916-9572
Andreas Pohlmann https://orcid.org/0000-0002-8572-2568

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