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Detailing the relation between renal  $T_2^*$  and renal tissue  $pO_2$  using an integrated approach of parametric magnetic resonance imaging and invasive physiological measurements (MR-PHYSIOL)

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**Short title:** Detailing renal  $T_2^*/pO_2$  relation by MR-PHYSIOL

**Keywords:** magnetic resonance imaging, BOLD, acute kidney injury, integrative physiology, MR-PHYSIOL, renal oxygenation, renal perfusion

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## Abstract

**Objectives:** This study is designed to detail the relation between renal  $T_2^*$  and renal tissue  $pO_2$  using an integrated approach that combines parametric MRI and quantitative physiological measurements (MR-PHYSIOL).

**Materials and Methods:** Experiments were performed in 21 male Wistar rats. *In vivo* modulation of renal hemodynamics and oxygenation was achieved by brief periods of aortic occlusion, hypoxia and hyperoxia. Renal perfusion pressure (RPP), renal blood flow (RBF), local cortical and medullary tissue  $pO_2$  and blood flux were simultaneously recorded together with  $T_2^*$ ,  $T_2$  mapping and MR based kidney size measurements (MR-PHYSIOL). MRI was carried out on a 9.4 Tesla small animal MR system. Relative changes in the invasive quantitative parameters were correlated with relative changes in the parameters derived from MRI using Spearman's analysis and Pearson's analysis.

**Results:** Changes in  $T_2^*$  qualitatively reflected tissue  $pO_2$  changes induced by the interventions.  $T_2^*$  versus  $pO_2$  Spearman rank correlations were significant for all interventions, yet quantitative translation of  $T_2^*/pO_2$  correlations obtained for one intervention to another intervention proved not appropriate. The closest  $T_2^*/pO_2$  correlation was found for hypoxia & recovery. The inter-layer comparison revealed closest  $T_2^*/pO_2$  correlations for the outer medulla and showed that extrapolation of results obtained for one renal layer to other renal layers must be made with due caution. For  $T_2^*$  to RBF relation significant Spearman correlations were deduced for all renal layers and for all interventions.  $T_2^*/RBF$  correlations for cortex and outer medulla were even superior to those between  $T_2^*$  and tissue  $pO_2$ . The closest  $T_2^*/RBF$  correlation occurred during hypoxia & recovery. Close correlations were observed between  $T_2^*$  and kidney size during hypoxia & recovery and for occlusion & recovery. In both cases, kidney size correlated well with renal vascular conductance, as did renal vascular conductance with  $T_2^*$ . Our findings indicate that changes in  $T_2^*$  qualitatively mirror changes in renal tissue  $pO_2$  but are also associated with confounding factors including vascular volume fraction and tubular volume fraction.

**Conclusions:** Our results demonstrate that MR-PHYSIOL is instrumental to detail the link between renal tissue  $pO_2$  and  $T_2^*$  *in vivo*. Unravelling the link between regional renal  $T_2^*$  and tissue  $pO_2$  - including the role of the  $T_2^*$  confounding parameters vascular and tubular volume fraction and oxyHb dissociation curve - requires further research. These explorations are essential before the quantitative capabilities of parametric MRI can be translated from

experimental research to improved clinical understanding of hemodynamics/oxygenation in kidney disorders.

**Keywords:** magnetic resonance imaging, BOLD, quantitative MRI, acute kidney injury, integrative physiology, MR-PHYSIOL, renal oxygenation, renal perfusion

## Introduction

Renal tissue hypoperfusion and hypoxia are considered to be pivotal links in the pathophysiological chain of events that leads to acute kidney injury (AKI) as well as the one that promotes progression from AKI to chronic kidney diseases (CKD) [1-7]. Imbalance between renal oxygen supply and demand also appears to play a prominent role in the pathophysiology of diabetic nephropathy [8, 9]. Making ultimate statements on the role of renal hypoperfusion and hypoxia for these renal disorders is elusive since *in vivo* assessment of renal hemodynamics and oxygenation constitutes a challenge. All modalities available in today's experimental and translational research practice have inherent shortcomings and methodological constraints [2, 10-12].

Obtaining insights into renal perfusion and oxygenation under (patho)physiological conditions by means of non-invasive diagnostic imaging is conceptually appealing. Blood oxygen level dependent (BOLD) magnetic resonance imaging (MRI) and quantitative parametric mapping of the MR relaxation times  $T_2^*$  and  $T_2$  are thought to provide surrogates of renal tissue oxygenation in preclinical and clinical studies [10, 12-35]. This assumption is based upon the  $T_2^*/T_2$  dependence on  $O_2$ -saturation of hemoglobin (Hb) and motivated by the link between  $O_2$ -saturation of Hb, blood partial pressure of  $O_2$  ( $pO_2$ ) and tissue  $pO_2$ . Although  $T_2^*$  and  $T_2$  basically reflect the amount of deoxyHb while  $pO_2$  represents the concentration of oxygen, changes in renal  $T_2^*/T_2$  and tissue  $pO_2$  may be closely related. Yet their link is also known to be sensitive to the oxyHb dissociation curve, haematocrit, and to the vascular volume fraction [2, 11, 36-38]. This added complexity confounds interpretation of BOLD weighted MRI and parametric  $T_2^*$  mapping data obtained from patients with AKI and CKD [11, 39-41]. For an unambiguous physiological interpretation of renal  $T_2^*$  a calibration with quantitative physiological measurements including renal tissue  $pO_2$  is required. For this purpose an integrative multi-modality approach is essential - as underlined in a recent call for further explorations into hemodynamic influences on kidney oxygenation [4] - before the capabilities of parametric MRI can be translated from experimental research to an improved clinical understanding of hemodynamics/oxygenation in AKI and CKD.

An integrated approach that combines parametric MRI with quantitative physiological measurements is prudent to detail the link between renal  $T_2^*$  and renal tissue oxygenation. Quantitative characterization of renal hemodynamics and oxygenation comprises very well

established invasive methods that assess perfusion of the entire kidney, regional perfusion and regional oxygenation in the intact animal [2, 12, 42]. MRI offers full kidney coverage, (sub)millimeter spatial resolution, (sub)minute temporal resolution and support of longitudinal studies [10, 12-18, 39, 43]. The validity and efficacy of parametric MRI for quantitative and spatiotemporal characterization of renal tissue perfusion and oxygenation under different functional conditions has not been systematically examined yet and remains to be established. A very limited number of studies attempted to draw a link between established invasive methods and  $T_2^*$ -weighted MRI [44-46]. These studies relied on either comparing MRI and invasive physiological measurements performed in independent cohorts of animals [45, 46] or on  $T_2^*$  and  $pO_2$  recorded in the same animal but at different times [33] or in different kidneys (contralateral vs. ipsilateral) [47].

Realizing the challenges and opportunities of using an integrated multi-modality approach for detailing the relation between renal  $T_2^*/T_2$  and renal oxygenation we hypothesized that simultaneous tracking of invasive physiological parameters and MR parameters derived from the same kidney will elucidate to which extent alterations of physiological parameters are reflected by changes in renal  $T_2^*$  and  $T_2$ . To test this hypothesis an integrative hybrid approach was employed that combines established invasive measurements including renal perfusion pressure, renal blood flow, local blood flux and tissue  $pO_2$  with  $T_2^*$ ,  $T_2$  mapping and MR based kidney size measurements (MR-PHYSIOL) [12]. *In vivo* modulation of renal hemodynamics and oxygenation was achieved by standardized (patho)physiologically relevant but reversible interventions, including brief periods of aortic occlusion, hypoxia and hyperoxia. Relative changes in the invasive quantitative parameters were correlated with relative changes in the parameters derived from MRI.

## Materials and Methods

### Animal Preparation

All investigations were approved by the Animal Welfare Department of [REDACTED] State Office of Health and Social Affairs in accordance with the [REDACTED] Animal Protection Law. The spatial constraints dictated by the MR environment required the use of relatively small rats. For this reason, experiments were performed in 21 male Wistar rats (aged 12-13 weeks, body mass (BM)  $336 \pm 27$  g; Harlan-Winkelmann, Borchon, Germany). The animals were allowed ad libitum food (standard diet) and water and were housed under standard conditions with environmental enrichment. For anesthesia urethane (20% in distilled water;  $6 \text{ mL kg}^{-1}$  BM i.p.; Sigma-Aldrich, Steinheim, Germany) was used throughout the surgical preparation and the MRI examination. This approach provides anesthesia for several hours and leaves cardiovascular reflexes largely undisturbed. Body temperature was maintained at  $37 \text{ }^\circ\text{C}$  by means of a mat through which warm water circulates.

Monitoring of absolute arterial blood pressure that corresponds with renal perfusion pressure (RPP; in mm Hg) was achieved by placing a catheter into the femoral artery with its tip towards the aorta [12]. The catheter was connected to a pressure transducer (DT-XX, Viggo-Spectramed, Swindon, UK) and amplifier (TAM-A Plugsys Transducer; Hugo Sachs Elektronik – Harvard Apparatus GmbH, Mach-Hugstetten, Germany).

Absolute measurement of renal blood flow (RBF; in  $\text{mL min}^{-1}$ ) used an ultra sound transit time flow probe (MC2PSB-MRI, Transonic Systems Inc., Ithaca, USA), equipped with a customized ceramic reflector, positioned around the left renal artery. Extra care was taken for the probe positioning [12] since pressure of the relatively large flow probe on the aorta, the renal artery and vein and/or the kidney itself bears the risk to cause ischemia or congestion of the kidney. In order to prevent these complications RBF values and the overall condition of the kidneys (e.g., surface coloring and its homogeneity) were carefully monitored during the entire preparation. After positioning of the animal in the MR scanner a low renal  $T_2^*$  (in particular medullary  $T_2^*$ ) was used as an additional criterion for early detection of any flow probe-induced renal ischemia. In two cases the flow probe was alternatively placed around the renal vein. In three animals even this probe position was not feasible so that the probe was omitted entirely. To achieve appropriate coupling of the ultrasound flow probe to the tissue,



the abdominal cavity was filled with saline via a catheter (38°C; replenished throughout experiment).

Renal vascular conductance [ $\text{ml min}^{-1} \text{mmHg}^{-1}$ ] (the inverse of resistance) was calculated by dividing RBF [ $\text{ml min}^{-1}$ ] by RPP [ $\text{mmHg}$ ].”

Measurement of absolute tissue  $\text{pO}_2$  (in mm Hg) and erythrocyte flux (arbitrary units) was enabled by combined laser-Doppler-flux/ $\text{pO}_2$  probes ( $\text{pO}_2$  E-Series Sensor; Oxford Optronics, Oxford, UK) that were inserted into the renal tissue. One probe was placed in the medullary region, at 4-mm depth. Another probe was placed in the cortical region by advancing it from the caudal extremity, centrally through the kidney to the cortical layer of the cranial extremity [12].

For induction of aortic occlusion during the MR study a remotely operated inflatable cuff was positioned around the aorta right above the renal arteries [12]. Core body temperature was monitored by means of a fiber-optic temperature probe (T1S-02-B05, Neoptix, Quebec, Canada) placed in the rectum.

The flux/ $\text{pO}_2$  probe extensions were passed through the abdominal wall using a small incision in the left inguinal region. All other cables together with the lines for the aortic cuff, for saline supply, cables of the perivascular flow probe and fiber-optic connection of the body temperature probe were passed through the caudal cutting edge of the median abdominal incision. The abdominal wall was closed by a continuous suture. For a more detailed description and discussion of the probe implantation and fixation please refer to [12].

## **MR Imaging and Parametric Mapping**

MR imaging was carried out on a 9.4 Tesla small animal MR system (Bruker Biosec 94/20; Bruker Biospin, Ettlingen, Germany) equipped with a linear polarized birdcage volume resonator used for RF transmission in conjunction with a curved four channel receive surface RF coil array (Bruker Biospin, Ettlingen, Germany) customized for rats.  $T_2$  weighted pilot scans for geometrical planning and slice positioning were acquired first. Local volume selective shimming of the magnetic field homogeneity on a voxel accommodating the kidney only was conducted using an automatic optimization algorithm based on FID length. To visualize the position of the  $\text{pO}_2$  and Laser-flux probes 3D multi gradient echo (MGE) imaging of the entire kidney was performed (repetition time = 20 ms, echo time = 2.85 ms, total acquisition time = 2 min 55 s, field of view (FOV) =  $(38.2 \times 48.5 \times 21.9) \text{ mm}^3$ , matrix

size = 126 x 120 x 72, spatial resolution = (303 x 404 x 304)  $\mu\text{m}^3$ ). The same 3D image set was used for slice positioning applied in the parametric mapping protocols.

Interleaved  $T_2^*$  and  $T_2$  mapping were performed with respiratory gated (Model 1025, SA Instruments, New York, NY, USA) imaging protocols. For  $T_2^*$  mapping a multi gradient echo (MGE) sequence (repetition time = 50 ms, number of echoes = 10, first echo time = 1.43 ms, echo time increment = 2.14 ms, averages = 4) with a total acquisition time of 1 min 20 s was used.  $T_2$  mapping employed a multi-echo spin-echo (MSME) sequence (repetition time = 550 ms, number of echoes = 7, first echo time = 10 ms, echo spacing 10 ms, averages = 1), resulting in a total acquisition time of 1 min 40s. A coronal oblique slice was placed across the kidney so that the cortical and medullary  $p\text{O}_2$  and Laser-flux probes were located within the imaging plane as illustrated in Figure 1. An in-plane spatial resolution of (226 x 445)  $\mu\text{m}^2$  (FOV = (38.2 x 50.3)  $\text{mm}^2$ , matrix size = 169 x 113 zero-filled to 169 x 215) and a slice thickness of 1.4–1.5 mm were employed.

The presence/absence of blood flow in the major renal blood vessels was confirmed with time-of-flight (TOF) MR angiography (MRA) performed at baseline and immediately after onset of aortic occlusion and onset of reperfusion. For TOF-MRA a spoiled gradient echo technique (2D FLASH, TR = 11 ms, TE = 3 ms, flip angle = 80 degree) with a spatial in-plane resolution of (200 x 268)  $\mu\text{m}^2$  and 15 slices (slice thickness = 1.0 mm) was applied.

## **Experimental Protocol and Standardized Reversible Interventions**

Throughout the experiments the rats were continuously provided with air (or other gas mixtures) at a rate of 1000  $\text{mL min}^{-1}$  provided by a respiratory mask placed around the muzzle of the spontaneously breathing rat. Following positioning of the rat in the isocenter of the MR scanner, scanner adjustments and slice positioning was conducted followed by baseline  $T_2^*$  and  $T_2$  mapping. Subsequently, short-term reversible interventions were performed: hyperoxia, aortic occlusion, and hypoxia, each followed by recovery.

Aortic occlusion was initiated by inflating the remotely controlled suprarenal aortic occluder and verified by absence of the flow-based blood signal in renal TOF-MRA. Occlusion lasted 3 minutes. One set of  $T_2^*/T_2$  maps was acquired before the occluder was deflated to re-establish renal blood flow. The recovery period comprised 9 minutes and three sets of  $T_2^*/T_2$  mapping.

Hypoxia was induced by decreasing the inspiration fraction of oxygen ( $\text{FiO}_2$ ) to 8% via changing the gas flow through the respiratory mask to 8%  $\text{O}_2$  / 92%  $\text{N}_2$ .  $\text{FiO}_2$  was monitored

using a Capnomac AGM-103 (Datex GE, Chalfont St. Giles, UK).  $T_2^*/T_2$  mapping were performed twice during hypoxia. The first set was acquired immediately after onset of hypoxia, the second set was started 5 minutes after onset of hypoxia. Following hypoxia of approximately 12 minutes  $FiO_2$  was restored to 21% (normoxia, room air). The recovery period of 15 minutes comprised four sets of  $T_2^*/T_2$  maps. Hyperoxia was induced using the same protocol, with the exception that  $FiO_2$  was increased to 100% by changing the gas mixture to pure oxygen.

Throughout the experiment invasive physiological parameters RPP, RBF, cortical and medullary tissue  $pO_2$  and Laser-flux were simultaneously monitored together with  $T_2^*$ ,  $T_2$  and kidney size derived from MRI.

## Data Processing and Analysis

Parametric maps of  $T_2^*$  and  $T_2$  were calculated by pixel-wise mono-exponential fitting to the signal intensities derived from a series of  $T_2^*$  and  $T_2$  weighted images acquired at different echo times (in-house developed program; MATLAB, R2010a, MathWorks, Natick, WA, USA).

Kidney movement throughout the experiment was corrected by image registration (FLIRT, FSL, [www.fmrib.ox.ac.uk/fsl](http://www.fmrib.ox.ac.uk/fsl)). For this purpose the first echo images of the multi echo MGE and MSME acquisitions were used. These images were registered onto the baseline scan. The resulting spatial transformation matrices were applied to the corresponding parametric maps.

For quantitative analysis of  $T_2^*$  and  $T_2$  regions of interest (ROIs) were defined according to the morphological features of the kidney. For this purpose, the layers (cortex (C), outer medulla (OM), inner medulla (IM)) were identified and measured in a series of freshly extracted rat kidneys [18]. The layers' dimensions were related to the individual kidney's length and width in the coronal view. From these measurements, a standardized model was derived that comprises a rectangular frame that tightly encloses the kidney and predefined sizes and positions of the ROIs relative to this frame such that the ROIs were accurately located within the respective layer (Figure 2). The positions of nine ROIs were defined: three ROIs in C (C1-C3), three ROIs in OM (O1-O3), and three ROIs in IM (I1-I3) as illustrated in Figure 2. The mean size of the ROIs was: C1, C3, O1, O3: 17 pixel, C2, O2: 46 pixel, I1, I3: 25 pixel, I2: 70 pixel. The segmentation software limited the operator interaction to the placement of a rectangular reference frame around the kidney. All ROIs were placed in safe

distance from the borders between these kidney layers to avoid any ‘contamination’ from the neighboring layers (partial segment effects) and to allow for inter-individual variations in morphology without the need to change the ROI position. The earlier described kidney segmentation model [18] was adapted for the current study to avoid overlap of the ROIs with the locations of the pO<sub>2</sub>/Laser-flux probes (Fig. 2). Mean T<sub>2</sub>\* and T<sub>2</sub> values were calculated over the three ROIs placed in each kidney layer. The size of the reference rectangle, which tightly encloses the kidney, was used as an estimate for kidney size.

The invasively measured physiological data were averaged over the time period of the corresponding MR scans with the exception of the Laser-flux signals. Here 5s data intervals starting 1 s after the end of the MR acquisitions were used. This approach helped to exclude MR induced artifacts in the Laser-flux signals [12].

## **Statistical Analysis**

To test our hypothesis the relation between invasively measured physiological parameters and parameters derived from MRI was assessed. For this purpose relative changes in the physiological parameters and in kidney size were tested for correlation with relative changes in T<sub>2</sub>\* and T<sub>2</sub> for all renal layers and for the three interventions. Spearman’s analysis (non-parametric correlation on ranks) was used, by which the strength of relationships is assessed that follow a monotonous function. If such a significant correlation was observed, additionally Pearson’s analysis (parametric correlation) was applied, by which the strength and parameters of linear relationships are assessed. A p-value p<0.05 was considered to be statistically relevant.

## Results

### Animal Preparation

Notwithstanding the experimental challenges dictated by the space constraints of the small bore MR scanner, surgical preparation was successfully performed in 15 out of 21 animals. The most frequent complication during surgery was unintended obstruction of renal blood flow caused by the vascular flow probe. To identify and exclude invalid data due to surgical, technical or physiological reasons all *in vivo* data were thoroughly examined. MR imaging scouts were used to check the positioning of the perivascular flow probe and the cortical and medullary Laser-flux/pO<sub>2</sub> probes. All renal T<sub>2</sub>\* maps were carefully checked for susceptibility artifacts induced by the surgical preparation or by the probes, which yielded data sets free of severe susceptibility artifacts for each renal layer for 15 animals. The invasively acquired physiological parameters were benchmarked against data and experience derived from our previous studies which included physiological measurements in large cohorts of animals [48-51].

### Renal T<sub>2</sub>\* and T<sub>2</sub>

Figure 3 shows exemplary T<sub>2</sub>\* and T<sub>2</sub> maps obtained during baseline, aortic occlusion, hypoxia, and hyperoxia together with  $\Delta T_2^*$  and  $\Delta T_2$  difference maps. The latter were determined by subtracting T<sub>2</sub>\* and T<sub>2</sub> maps acquired at the last time point of the intervention phase from baseline. **At baseline** the inner medulla including the papilla revealed T<sub>2</sub>\*/T<sub>2</sub> values that were markedly higher versus T<sub>2</sub>\*/T<sub>2</sub> observed for the cortex and outer medulla. Parametric maps showed a cortical intra-layer T<sub>2</sub>\*/T<sub>2</sub> pattern that indicates that the spatial resolution affords visualization of spatial variability in intra-layer pO<sub>2</sub>, which is related to the distance to the vascular bundles [52, 53].

**Hyperoxia** induced rather uniform increase in renal T<sub>2</sub>\* and T<sub>2</sub> across the kidney as outlined by exemplary maps in Figure 3. Averaged over all animals maximum T<sub>2</sub>\* changes were 19±3% (C), 22±2% (OM), 7±4% (IM) and maximum T<sub>2</sub> changes were 7±1% (C), 9±1% (OM), 8±2% (IM). **Aortic occlusion** caused a T<sub>2</sub>\*/T<sub>2</sub> decrease for all renal layers. Averaged over all animals maximum T<sub>2</sub>\* changes were -11±2% (C), -42±2% (OM), -17±5% (IM) and maximum T<sub>2</sub> changes were -17±3% (C), -22±3% (OM), -6±6% (IM). **Hypoxia** induced

rather uniform reduction in renal  $T_2^*$  and  $T_2$  with maximum  $\Delta T_2^*$   $-47\pm 3\%$  (C),  $-59\pm 2\%$  (OM), and  $-37\pm 5\%$  (IM)), and maximum  $\Delta T_2$   $-28\pm 3\%$  (C),  $-36\pm 4\%$  (OM), and  $-20\pm 6\%$  (IM)).

### **Time Course of Physiological and MR Parameters during Interventions**

Figure 4 outlines the time course of physiological and MR parameters monitored during **hyperoxia & recovery**. After onset of hyperoxia tissue  $pO_2$  started to increase and reached 240% over baseline in the cortex and 60% over baseline in the medulla. This  $pO_2$  increase was accompanied by small rises in RPP (7%) and RBF (8%). Laser-fluxes, renal conductance and kidney size remained largely unchanged.  $T_2^*$  increased versus baseline with maxima of 19% in the cortex, 22% in the outer medulla, and 7% in the inner medulla.  $T_2$  maps revealed smaller increases (C: 7%, OM: 9%, IM: 8%). After switching to normoxia tissue  $pO_2$  slowly returned towards baseline.  $T_2^*$  and  $T_2$  returned to baseline more rapidly. RBF and RPP even fell somewhat below baseline.

Figure 5 illustrates the time course obtained for physiological and MR parameters during **aortic occlusion & recovery**. With onset of occlusion RBF immediately ceased and kidney size decreased by 3.9%. Tissue  $pO_2$  rapidly decreased: within 90 seconds cortical  $pO_2$  had dropped by 90% and medullary  $pO_2$  by 97%.  $T_2^*$  became also reduced, but much less than  $pO_2$ .  $T_2^*$  displayed a different time course among the layers. Outer medullary  $T_2^*$  declined instantly by 42% while cortical and inner medullary  $T_2^*$  decreased only modestly by 11% and 17%. After onset of reperfusion cortical and inner medullary  $T_2^*$  initially continued to decrease and reached a level of 71% of baseline in the cortex and 69% of baseline in the inner medulla before starting to re-increase. Excursions of  $T_2$  were even smaller than those of  $T_2^*$ . In response to the occluder's deflation RPP returned to baseline within 5 minutes while RBF's return to baseline was more slowly. Consequently renal conductance started significantly below baseline (not measurable during the occlusion) and slowly approximated baseline. Kidney size followed this trend and regained baseline within 8 minutes after reperfusion. Cortical and medullary  $pO_2$  followed the gradual recovery of RBF and reached baseline level only 8 minutes into reperfusion. While cortical and tissue  $pO_2$  started to improve immediately after onset of reperfusion,  $T_2^*$  initially remained unchanged (OM) or was even further reduced (C, IM) before restoration to baseline values began.

Figure 6 depicts the time course obtained for physiological and MR parameters in response to the **hypoxia & recovery** maneuver. With the onset of hypoxia RPP declined by 46% and RBF decreased even more (by 70%). Renal conductance dropped by 53% and kidney size by 5%.

Tissue  $pO_2$  gradually decreased: within 7 minutes of hypoxia medullary  $pO_2$  had decreased by 96% and cortical  $pO_2$  by 66%.  $T_2^*$  and  $T_2$  changes were found to be in sync with the  $pO_2$  alterations. The drop in  $T_2^*$  and  $T_2$  observed during hypoxia was markedly larger than during occlusion. During hypoxia  $T_2^*$  fell by 47% in C, 59% in OM, and 37% in IM. After switching to normoxia RPP, RBF, conductance and renal tissue  $pO_2$  started to recover immediately and returned to baseline. The tissue hypoxia to normoxia transition was faster versus aortic occlusion-recovery.  $T_2^*$  and  $pO_2$  time courses slightly deviated during recovery from hypoxia as cortical and medullary  $pO_2$  displayed some oscillations.

### **Correlations of Physiological and MR Parameters**

Table 1 provides a synopsis of the correlations between physiological parameters and MR parameters in response to hyperoxia & recovery, occlusion & recovery, and hypoxia & recovery. Significant Spearman rank correlations between  $T_2^*$  and tissue  $pO_2$  were observed for all interventions and all renal layers as illustrated in Figures 7, 8, and 9. The weakest  $T_2^*/pO_2$  correlation was found during hyperoxia & recovery, a closer one in response to occlusion & recovery, and the closest  $T_2^*/pO_2$  correlation during hypoxia & recovery (Table 1). The inter-layer comparison revealed weakest  $T_2^*/pO_2$  correlations for the cortex (Figure 7) and closest correlations for the outer medulla (Figure 8).

Pearson's analysis revealed significant linear correlations of  $T_2^*$  with outer medullary tissue  $pO_2$  for all interventions. Cortical  $T_2^*$  and  $pO_2$  showed a significant linear correlation during occlusion & recovery only. This is plausible since the average  $O_2$  saturation of Hb in most cortical vessels will be above 75% (i.e. in the non-linear range of the sigmoid oxyHb dissociation curve) under most conditions but not during the occlusion period, whereas saturation in medullary vessels will be lower (i.e., in the range of the curve that approaches linearity).

Significant Spearman correlations of  $T_2^*$  to RBF were deduced for all renal layers and for all interventions (Figures 7-9). The closest correlation was observed for the outer medulla followed by the cortex and the inner medulla. The  $T_2^*/RBF$  correlations for cortex and outer medulla were even superior to those between  $T_2^*$  and tissue  $pO_2$ . The closest  $T_2^*/RBF$  correlation occurred during hypoxia & recovery.

$T_2^*/RPP$  correlations were similar to  $T_2^*/RBF$  correlations as outlined in Table 1. Surprisingly close correlations were observed between  $T_2^*$  and kidney size during hypoxia & recovery and

for occlusion & recovery. In both cases, kidney size correlated well with renal vascular conductance (Table 2), as did renal vascular conductance with  $T_2^*$  (Table 1). Significant correlations of RBF to tissue  $pO_2$  were observed for all kidney layers during hypoxia & recovery and for occlusion & recovery (Table 2). These correlations were superior to the  $T_2^*/pO_2$  correlations observed for occlusion & recovery and hypoxia & recovery.



## Discussion

Our results demonstrate that MR-PHYSIO is instrumental to detail the link between renal tissue  $pO_2$  and  $T_2^*$  *in vivo*. This is of essence to address the weakness of MRI, its qualitative nature, by benchmarking the surrogate MRI biomarkers against invasive methods while putting MRI's ability to non-invasively capture the physiological heterogeneity between and within the renal layers to good use. The technical challenges and practical obstacles of the MR environment were successfully offset. Remotely controlled standardized interventions were implemented in the MR scanner in order to systematically examine the validity and efficacy of parametric MRI as a surrogate marker for renal oxygenation. These efforts are of high relevance for research into the pathogenesis of renal diseases that are induced or promoted by renal tissue hypoperfusion and hypoxia.

Our findings indicate that changes in  $T_2^*$  qualitatively reflect changes in renal tissue  $pO_2$  induced by hyperoxia, aortic occlusion, and hypoxia. This is in alignment with the link between  $T_2^*$ ,  $O_2$ -saturation of Hb, blood  $pO_2$ , and tissue  $pO_2$ . A closer examination of the quantitative relation between relative changes in  $T_2^*$  and in tissue  $pO_2$  revealed discrepancies that point at factors other than the known shifts of the deoxyHb dissociation curve and changes in haematocrit, which may also confound the renal  $T_2^*/$  tissue  $pO_2$  relationship. Major differences in the  $T_2^*/pO_2$  correlations together with the stark differences in the linear regression of  $T_2^*/pO_2$  indicate that simple translation of quantitative results obtained for one intervention of renal hemodynamics and oxygenation to another intervention is falling short from being appropriate. Also, taking the perfusion and oxygenation heterogeneity within any given kidney layer into account, extrapolation of results obtained for specific renal regions to other renal areas must be made with due caution.

**Hyperoxia & recovery** yielded weak correlations for tissue  $pO_2$  changes versus  $T_2^*$  changes for all kidney layers. This observation is plausible since almost all of the available Hb in arterial blood is already  $O_2$  saturated under normoxic conditions. Increasing the inspiratory oxygen fraction to 100% barely lifts the  $O_2$  saturation of Hb in arterial blood. It does substantially increase arterial blood  $pO_2$  though, which enhances the driving force for  $O_2$  diffusion from vessels to tissue so that renal tissue  $pO_2$  increases dramatically. The difference in the  $pO_2$  increase between cortex and medulla is likely due to arterio-venous diffusive  $O_2$  shunting, which reduces the oxygen content of arterial blood that perfuses the medulla [3, 38, 52]. Benchmarked against the massive  $pO_2$  increase  $T_2^*$  changes were much smaller. Hyperoxia-induced increase of arterial blood oxygen content are barely detectable by  $T_2^*$ -

mapping since the deoxyHb concentration is virtually unaffected. However, hyperoxia increases blood  $pO_2$  in intrarenal veins due to increasing arterio-venous oxygen shunting in case of a higher arterio-venous  $pO_2$  difference [3, 38]. As renal venous Hb is far from being completely saturated with  $O_2$  under normoxic conditions hyperoxia induced increase in venous blood  $pO_2$  translates into the small increase in  $T_2^*$  observed in our study.

**Aortic occlusion & recovery** showed closer tissue  $pO_2/T_2^*$  correlations for all renal layers versus those observed in response to hyperoxia & recovery. Occlusion of the suprarenal aorta results in abrupt cessation of blood flow into the kidney while renal  $O_2$  consumption remains unaltered at this early stage. This leads to a rapid and massive decline in renal tissue  $pO_2$ , which reduces blood  $pO_2$  and  $O_2$  saturation of Hb in the intrarenal (micro-)vasculature. This intrarenal Hb deoxygenation is aggravated by a progressive rightward shift of the oxyHb dissociation curve during the occlusion due to intrarenal accumulation of  $CO_2$ . The increase in deoxyHb is reflected by the  $T_2^*$  decrease observed during occlusion for all layers. The  $T_2^*$  decrease was mild and slow as compared to the decline in  $pO_2$  though, and displayed different characteristics for the kidney layers. These findings can be attributed to blood volume fraction changes which occurred during occlusion. While blood flow into kidney is abruptly stopped by the occlusion, outflow of blood via the renal vein will continue until pressures in intrarenal vessels and in the vena cava are equalized. This results in a reduction of intrarenal blood volume [54] and manifests itself in the immediate drop in kidney size shown here. Since the total volume of the other renal fluid compartments including the tubular, interstitial, and cellular fraction is probably largely unchanged, the blood volume fraction becomes markedly reduced. As  $T_2^*$  is linked to the volume fraction of deoxyHb, the reduction in blood volume fraction compensates some of the increased deoxyHb blood concentration induced changes in  $T_2^*$ . Consequently, the  $T_2^*$  decrease turns out to be smaller than the actual change in blood (and tissue) oxygenation. It is plausible that this effect is much more pronounced in the cortex than in the medulla, due to the much larger blood volume in the cortex [4, 38].  $T_2^*$  in the cortex follows the declining blood (tissue)  $pO_2$  less closely than in the outer medulla. The inner medulla's metabolism is largely anaerobic [55], hence the remaining oxygen in the stationary blood is sufficient for a longer time span so that  $pO_2$  and  $T_2^*$  decline only slowly.

By deflating the aortic occluder the kidney is reperfused. Complete restoration of RBF took about 8 minutes so that oxygen delivery recovers only gradually. At the same time, glomerular filtration, which was arrested during occlusion, is gradually restored. The ensuing restoration of tubular reabsorption necessitates higher  $O_2$  consumption. Limited oxygen delivery and increased demand result in the observed slow recovery of tissue  $pO_2$ . The

substantial O<sub>2</sub> extraction from the freshly inflowing blood is embodied by a low T<sub>2</sub>\*. Although tissue pO<sub>2</sub> started to improve immediately after onset of reperfusion, cortical T<sub>2</sub>\* continued to decline even further before it started to recover. Again, this discrepancy most probably relies on a change in the blood volume fraction. With reperfusion renal blood volume increases while the total volume of the other fluid compartments is largely unchanged. Increased blood volume fraction at the onset of reperfusion leads T<sub>2</sub>\* to an overestimation of tissue hypoxia. The increase in blood volume is somewhat attenuated by renal vasoconstriction triggered by renal autoregulatory mechanisms [56], as evident from renal vascular conductance being significantly below baseline right after the onset of reperfusion. The attenuated recovery of intrarenal blood volume is also mirrored by the somewhat delayed restoration in kidney size.

**Hypoxia & recovery** revealed the strongest renal tissue pO<sub>2</sub>/T<sub>2</sub>\* correlation for all interventions applied. Switching the inspiratory oxygen fraction to 8% resulted in arterial hypoxemia so that tissue pO<sub>2</sub> gradually decreased and reached a steady state. The major reason behind the pronounced decrease in medullary pO<sub>2</sub> is that the medulla is predominantly perfused by blood that had already traversed the cortex, where blood oxygen content is lowered by oxygen extraction and arterio-venous oxygen shunt diffusion [3, 4, 38, 52]. As expected, the pO<sub>2</sub> decrease during hypoxia was slower and with regard to the cortex also less pronounced than during aortic occlusion. In contrast, the T<sub>2</sub>\* response to hypoxia was significantly more pronounced versus aortic occlusion. It is evident that aortic occlusion must lower intrarenal blood oxygenation much more and faster than hypoxia. In addition, intrarenal pCO<sub>2</sub> increases during occlusion due to CO<sub>2</sub> accumulation, but decreases during hypoxia due to hyperventilation triggered by systemic hypoxemia [57]. Decreased pCO<sub>2</sub> shifts the deoxyHb dissociation curve to the left so that at given pO<sub>2</sub> the O<sub>2</sub> saturation of Hb is larger. Increased pCO<sub>2</sub> induces opposite effects. For all these reasons, T<sub>2</sub>\* decrease should be more pronounced during aortic occlusion versus hypoxia. Our results indicate that the T<sub>2</sub>\* response to FiO<sub>2</sub> 8% overestimates the actual degree of blood (and tissue) hypoxia. The decrease in renal conductance during hypoxia suggests that renal vasoconstriction lowered intrarenal blood volume. Yet the vasoconstriction-related drop in renal blood volume during hypoxia is most likely smaller versus the outflow-induced drop during aortic occlusion. Intriguingly, the decrease in kidney size was somewhat larger during hypoxia than during occlusion. This observation points at an additional volume loss of renal tissue compartments other than blood. Contrary to aortic occlusion, where glomerular filtration ceases and with it the pressure gradient that drives tubular fluid toward the renal pelvis, filtration and tubular fluid flow will

decrease but not cease during hypoxia. The outflow lowers tubular volume. Moreover, reabsorbed fluid cannot be drained by peritubular capillaries during occlusion because of the arrested blood flow such that the sum of the tubular plus interstitial volumes remains constant. As peritubular capillary blood flow is not arrested during hypoxia, reabsorbed fluid is continued to be drained from the interstitium. Since the decrease in tubular volume is not counterbalanced by an increase in the interstitial volume, the sum of both volumes decreases. This translates into a larger decrease in the blood volume fraction during occlusion versus hypoxia which might explain the discrepancies in the  $T_2^*$  and  $pO_2$  response to hypoxia and aortic occlusion.

Significant rank correlations of RBF to  $T_2^*$  were observed for all renal layers and for all interventions. These correlations were closer than those of tissue  $pO_2$  to  $T_2^*$  for the cortex and outer medulla. Tissue  $pO_2$  primarily reflects the balance between  $O_2$  supply and  $O_2$  demand. Due to  $O_2$  shunt diffusion, blood  $pO_2$  in larger arterial and venous vessels exceeds that in capillaries and tissue  $pO_2$ . The amount of  $O_2$  that is shunted depends on  $O_2$  consumption but also on RBF and arterial  $O_2$  content [3, 4, 38, 52]. In the cortex, the effect of changes in RBF on shunting appears to be enhanced: the correlations of RBF to cortical tissue  $pO_2$  were much weaker than those of RBF and medullary tissue  $pO_2$ . When comparing RBF/ $T_2^*$  versus  $pO_2/T_2^*$  it should be taken into account that the  $pO_2$  probes cover a rather small tissue volume ( $r \approx 120 \mu m$ ) subjacent to the probes' tip [2, 12]. The individual position of a given probe in relation to larger vessels versus capillaries will therefore determine the individual absolute  $pO_2$  values, and may also impinge on the relative changes during interventions. Unlike  $pO_2$  measurements RBF measurements are unaffected by intrarenal spatial variabilities.

To summarize, this work presents valuable insights into the mechanisms behind alterations in renal  $T_2^*$  by detailing the link between renal  $T_2^*$  and renal tissue  $pO_2$ . Yet, a singular report eloquently refers to simultaneous measurements of renal  $R_2^*$  ( $R_2^* = 1/T_2^*$ ) and tissue  $pO_2$  [47]. The authors modulated  $FiO_2$  (range: 5-70%) in pigs and reported  $R_2^*$  changes to be linearly related with  $pO_2$  changes. This conclusion appears somewhat premature, since  $T_2^*$  was measured in the contralateral kidney with the  $pO_2$  probe being placed in the ipsilateral kidney. It should be also noted that correlation analysis was based upon group means rather than individual data pairs as used here.

The overall qualitative agreement of  $T_2^*$  and  $pO_2$  changes observed in the present study encourages further research into calibration of renal  $T_2^*$  alterations using MR-PHYSIOL. Notwithstanding this success, our findings generated novel questions about the renal

$T_2^*$ /tissue  $pO_2$  relation. It stands to reason that  $T_2^*$  is directly related to the amount of deoxyHb per tissue volume and hence linked with tissue  $pO_2$  via blood  $pO_2$  and the oxyHb dissociation curve. However, the  $T_2^*$  to tissue  $pO_2$  correlation differences between the interventions and our renal vascular conductance and kidney size data indicate that changes in the blood volume fraction considerably influence renal  $T_2^*$ . Renal vascular conductance changes point at changes in intrarenal blood volume that occur by passive circular distension induced by changes in the transmural pressure gradient, or by active vasomotion. Changes in kidney size may stem from volume changes in any of the renal fluid compartments. There are at least two compartments besides the vascular one that can experience rapid volume changes, which in turn modulate the blood volume fraction: the interstitial and the tubular compartment, with the latter being a particularity of the kidney. The tubular volume fraction is quite large and can rapidly change due (i) changes in filtration, (ii) alterations in tubular outflow towards the pelvis, (iii) modulation of the transmural pressure gradient, and (iv) changes in resorption. A recent report recognized that changes in blood volume fraction induced by changes in tubular volume may impact renal  $T_2^*$  [17] by showing that the renal  $T_2^*$  response indicated an increased oxygenation immediately after x-ray contrast agent administration rather than decreased oxygenation [17]. The dependence of  $R_2=1/T_2$  on alterations in tubular volume has also been observed upon administration of vasoactive substances [58].

Unravelling the link between regional renal  $T_2^*$  and tissue  $pO_2$  - including the role of the  $T_2^*$  confounding parameters vascular and tubular volume fraction and oxyHb dissociation curve - requires further research. Blood volume fraction, tubular volume fraction and oxyHb dissociation curve  $T_2^*$  contributions must be differentiated from renal BOLD  $T_2^*$  changes in order to provide quantitative means for interpretation of renal hemodynamics/oxygenation. These efforts should make use of the advanced capabilities of MR-PHYSIOL by including parallel imaging techniques to improve the temporal resolution [59-62], by investing into dual contrast techniques for simultaneous  $T_2^*/T_2$  weighted MRI [63], by driving  $T_2^*$  mapping techniques free of image distortion [64] but also MR based assessment of renal blood volume [65] and by probing tubular volume fraction using diffusion weighted or intra-voxel incoherent motion techniques [43, 66-69], while blood sampling may be employed to examine the role of shifts in the oxyHb dissociation curve. These explorations are essential before the quantitative capabilities of parametric MRI can be translated from experimental research to improved clinical understanding of hemodynamics/oxygenation in kidney disorders.

## References

1. Singh P, Ricksten SE, Bragadottir G, et al. Renal oxygenation and haemodynamics in acute kidney injury and chronic kidney disease. *Clin Exp Pharmacol Physiol*. 2013;40:138-47.
2. Evans RG, Gardiner BS, Smith DW, et al. Methods for studying the physiology of kidney oxygenation. *Clin Exp Pharmacol Physiol*. 2008;35:1405-12.
3. Evans RG, Goddard D, Eppel GA, et al. Factors that render the kidney susceptible to tissue hypoxia in hypoxemia. *Am J Physiol Regul Integr Comp Physiol*. 2011;300:R931-40.
4. Evans RG, Ince C, Joles JA, et al. Haemodynamic influences on kidney oxygenation: clinical implications of integrative physiology. *Clin Exp Pharmacol Physiol*. 2013;40:106-22.
5. Chawla LS, Kimmel PL. Acute kidney injury and chronic kidney disease: an integrated clinical syndrome. *Kidney Int*. 2012;82:516-24.
6. Seeliger E, Sendeski M, Rihal CS, et al. Contrast-induced kidney injury: mechanisms, risk factors, and prevention. *Eur Heart J*. 2012;33:2007-15.
7. Legrand M, Mik EG, Johannes T, et al. Renal hypoxia and dysoxia after reperfusion of the ischemic kidney. *Mol Med*. 2008;14:502-16.
8. Friederich-Persson M, Thorn E, Hansell P, et al. Kidney hypoxia, attributable to increased oxygen consumption, induces nephropathy independently of hyperglycemia and oxidative stress. *Hypertension*. 2013;62:914-9.
9. Hansell P, Welch WJ, Blantz RC, et al. Determinants of kidney oxygen consumption and their relationship to tissue oxygen tension in diabetes and hypertension. *Clin Exp Pharmacol Physiol*. 2013;40:123-37.
10. Prasad PV. Functional MRI of the kidney: tools for translational studies of pathophysiology of renal disease. *Am J Physiol Renal Physiol*. 2006;290:F958-74.
11. Evans RG, Leong CL, Anderson WP, et al. Don't be so BOLD: potential limitations in the use of BOLD MRI for studies of renal oxygenation. *Kidney Int*. 2007;71:1327-8; author reply 8.
12. Pohlmann A, Cantow K, Hentschel J, et al. Linking non-invasive parametric MRI with invasive physiological measurements (MR-PHYSIOL): towards a hybrid and integrated approach for investigation of acute kidney injury in rats. *Acta Physiol (Oxf)*. 2013;207:673-89.
13. Prasad PV, Epstein FH. Changes in renal medullary pO<sub>2</sub> during water diuresis as evaluated by blood oxygenation level-dependent magnetic resonance imaging: effects of aging and cyclooxygenase inhibition. *Kidney Int*. 1999;55:294-8.
14. Prasad PV, Priatna A, Spokes K, et al. Changes in intrarenal oxygenation as evaluated by BOLD MRI in a rat kidney model for radiocontrast nephropathy. *J Magn Reson Imaging*. 2001;13:744-7.
15. Li LP, Halter S, Prasad PV. Blood oxygen level-dependent MR imaging of the kidneys. *Magn Reson Imaging Clin N Am*. 2008;16:613-25, viii.
16. Haque M, Franklin T, Prasad P. Renal oxygenation changes during water loading as evaluated by BOLD MRI: effect of NOS inhibition. *J Magn Reson Imaging*. 2011;33:898-901.
17. Arakelyan K, Cantow K, Hentschel J, et al. Early effects of an x-ray contrast medium on renal T(2)\*/T(2) MRI as compared to short-term hyperoxia, hypoxia and aortic occlusion in rats. *Acta Physiol (Oxf)*. 2013;208:202-13.
18. Pohlmann A, Hentschel J, Fechner M, et al. High temporal resolution parametric MRI monitoring of the initial ischemia/reperfusion phase in experimental acute kidney injury. *PLoS One*. 2013;8:e57411.
19. Peng XG, Bai YY, Fang F, et al. Renal Lipids and Oxygenation in Diabetic Mice: Noninvasive Quantification with MR Imaging. *Radiology*. 2013;269:748-57.

20. Morrell GR, Zhang JL, Lee VS. Science to Practice: Renal Hypoxia and Fat Deposition in Diabetic Neuropathy-New Insights with Functional Renal MR Imaging. *Radiology*. 2013;269:625-6.
21. Menzies RI, Zammit-Mangion A, Hollis LM, et al. An anatomically unbiased approach for analysis of renal BOLD magnetic resonance images. *Am J Physiol Renal Physiol*. 2013;305:F845-52.
22. Pruijm M, Hofmann L, Charollais-Thoenig J, et al. Effect of dark chocolate on renal tissue oxygenation as measured by BOLD-MRI in healthy volunteers. *Clin Nephrol*. 2013;80:211-7.
23. Pruijm M, Hofmann L, Vogt B, et al. Renal tissue oxygenation in essential hypertension and chronic kidney disease. *Int J Hypertens*. 2013;2013:696598.
24. Pruijm M, Hofmann L, Zanchi A, et al. Blockade of the renin-angiotensin system and renal tissue oxygenation as measured with BOLD-MRI in patients with type 2 diabetes. *Diabetes Res Clin Pract*. 2013;99:136-44.
25. Liss P, Cox EF, Eckerbom P, et al. Imaging of intrarenal haemodynamics and oxygen metabolism. *Clin Exp Pharmacol Physiol*. 2013;40:158-67.
26. Gloviczki ML, Lerman LO, Textor SC. Blood oxygen level-dependent (BOLD) MRI in renovascular hypertension. *Curr Hypertens Rep*. 2011;13:370-7.
27. Rognant N, Lemoine S, Laville M, et al. [Evaluation of renal oxygen content by BOLD MRI]. *Nephrol Ther*. 2012;8:212-5.
28. Park SY, Kim CK, Park BK, et al. Evaluation of transplanted kidneys using blood oxygenation level-dependent MRI at 3 T: a preliminary study. *AJR Am J Roentgenol*. 2012;198:1108-14.
29. Donati OF, Nanz D, Serra AL, et al. Quantitative BOLD response of the renal medulla to hyperoxic challenge at 1.5 T and 3.0 T. *NMR Biomed*. 2012;25:1133-8.
30. Ebrahimi B, Gloviczki M, Woollard JR, et al. Compartmental analysis of renal BOLD MRI data: introduction and validation. *Invest Radiol*. 2012;47:175-82.
31. Chrysochou C, Mendichovszky IA, Buckley DL, et al. BOLD imaging: a potential predictive biomarker of renal functional outcome following revascularization in atheromatous renovascular disease. *Nephrol Dial Transplant*. 2012;27:1013-9.
32. Rognant N, Guebre-Egziabher F, Bacchetta J, et al. Evolution of renal oxygen content measured by BOLD MRI downstream a chronic renal artery stenosis. *Nephrol Dial Transplant*. 2011;26:1205-10.
33. Warner L, Glockner JF, Woollard J, et al. Determinations of renal cortical and medullary oxygenation using blood oxygen level-dependent magnetic resonance imaging and selective diuretics. *Invest Radiol*. 2011;46:41-7.
34. Gloviczki ML, Glockner J, Gomez SI, et al. Comparison of 1.5 and 3 T BOLD MR to study oxygenation of kidney cortex and medulla in human renovascular disease. *Invest Radiol*. 2009;44:566-71.
35. Rossi C, Sharma P, Pazahr S, et al. Blood oxygen level-dependent magnetic resonance imaging of the kidneys: influence of spatial resolution on the apparent R2\* transverse relaxation rate of renal tissue. *Invest Radiol*. 2013;48:671-7.
36. Ogawa S. Finding the BOLD effect in brain images. *Neuroimage*. 2012;62:608-9.
37. Griffeth VE, Buxton RB. A theoretical framework for estimating cerebral oxygen metabolism changes using the calibrated-BOLD method: modeling the effects of blood volume distribution, hematocrit, oxygen extraction fraction, and tissue signal properties on the BOLD signal. *Neuroimage*. 2011;58:198-212.
38. Evans RG, Gardiner BS, Smith DW, et al. Intrarenal oxygenation: unique challenges and the biophysical basis of homeostasis. *Am J Physiol Renal Physiol*. 2008;295:F1259-70.
39. Michaely HJ, Metzger L, Haneder S, et al. Renal BOLD-MRI does not reflect renal function in chronic kidney disease. *Kidney Int*. 2012;81:684-9.

40. Fine LG, Dharmakumar R. Limitations of BOLD-MRI for assessment of hypoxia in chronically diseased human kidneys. *Kidney Int.* 2012;82:934-5; author reply 5.
41. Inoue T, Kozawa E, Okada H, et al. Is there no future for renal BOLD-MRI? *Kidney Int.* 2012;82:934; author reply 5.
42. Dean DA, Jia CX, Cabreriza SE, et al. Validation study of a new transit time ultrasonic flow probe for continuous great vessel measurements. *ASAIO journal (American Society for Artificial Internal Organs : 1992).* 1996;42:M671-6.
43. Hueper K, Rong S, Gutberlet M, et al. T2 relaxation time and apparent diffusion coefficient for noninvasive assessment of renal pathology after acute kidney injury in mice: comparison with histopathology. *Invest Radiol.* 2013;48:834-42.
44. Juillard L, Lerman LO, Kruger DG, et al. Blood oxygen level-dependent measurement of acute intra-renal ischemia. *Kidney Int.* 2004;65:944-50.
45. dos Santos EA, Li L-P, Ji L, et al. Early changes with diabetes in renal medullary hemodynamics as evaluated by fiberoptic probes and BOLD magnetic resonance imaging. *Investigative radiology.* 2007;42:157-62.
46. Li L-P, Ji L, Santos EA, et al. Effect of nitric oxide synthase inhibition on intrarenal oxygenation as evaluated by blood oxygenation level-dependent magnetic resonance imaging. *Investigative radiology.* 2009;44:67-73.
47. Pedersen M, Dissing T, Morkenborg J, et al. Validation of quantitative BOLD MRI measurements in kidney: Application to unilateral ureteral obstruction. *Kidney Int.* 2005;67:2305-12.
48. Flemming B, Seeliger E, Wronski T, et al. Oxygen and renal hemodynamics in the conscious rat. *J Am Soc Nephrol.* 2000;11:18-24.
49. Hoff U, Lukitsch I, Chaykovska L, et al. Inhibition of 20-HETE synthesis and action protects the kidney from ischemia/reperfusion injury. *Kidney Int.* 2011;79:57-65.
50. Seeliger E, Flemming B, Wronski T, et al. Viscosity of contrast media perturbs renal hemodynamics. *J Am Soc Nephrol.* 2007;18:2912-20.
51. Seeliger E, Cantow K, Arakelyan K, et al. Low-Dose Nitrite Alleviates Early Effects of an X-ray Contrast Medium on Renal Hemodynamics and Oxygenation in Rats. *Invest Radiol.* 2013. [epub ahead]
52. Schurek HJ. [Kidney medullary hypoxia: a key to understanding acute renal failure?]. *Klin Wochenschr.* 1988;66:828-35.
53. Lubbers DW, Baumgartl H. Heterogeneities and profiles of oxygen pressure in brain and kidney as examples of the pO<sub>2</sub> distribution in the living tissue. *Kidney Int.* 1997;51:372-80.
54. Grosenick D, Steinkellner O, Wabnitz H, et al. Near-infrared spectroscopy of renal tissue in vivo. *Proc of SPIE.* 2013.
55. Edwards A, Silldorff EP, Pallone TL. The renal medullary microcirculation. *Front Biosci.* 2000;5:E36-52.
56. Seeliger E, Wronski T, Ladwig M, et al. The renin-angiotensin system and the third mechanism of renal blood flow autoregulation. *Am J Physiol Renal Physiol.* 2009;296:F1334-45.
57. Marshall JM, Metcalfe JD. Influences on the cardiovascular response to graded levels of systemic hypoxia of the accompanying hypocapnia in the rat. *J Physiol.* 1989;410:381-94.
58. Storey P, Ji L, Li LP, et al. Sensitivity of USPIO-enhanced R2 imaging to dynamic blood volume changes in the rat kidney. *J Magn Reson Imaging.* 2011;33:1091-9.
59. Niendorf T, Sodickson DK. Highly accelerated cardiovascular MR imaging using many channel technology: concepts and clinical applications. *Eur Radiol.* 2008;18:87-102.
60. Niendorf T, Sodickson DK. Parallel imaging in cardiovascular MRI: methods and applications. *NMR Biomed.* 2006;19:325-41.



61. Niendorf T, Saranathan M, Lingamneni A, et al. Short breath-hold, volumetric coronary MR angiography employing steady-state free precession in conjunction with parallel imaging. *Magn Reson Med.* 2005;53:885-94.
62. Niendorf T, Sodickson D. [Acceleration of cardiovascular MRI using parallel imaging: basic principles, practical considerations, clinical applications and future directions]. *Rofo.* 2006;178:15-30.
63. Fuchs K, Hezel F, Klix S, et al. Simultaneous dual contrast weighting using double echo rapid acquisition with relaxation enhancement (RARE) imaging. *Magn Reson Med.* 2013.[epub ahead]
64. Heinrichs U, Utting JF, Frauenrath T, et al. Myocardial T2\* mapping free of distortion using susceptibility-weighted fast spin-echo imaging: a feasibility study at 1.5 T and 3.0 T. *Magn Reson Med.* 2009;62:822-8.
65. Wang F, Jiang RT, Tantawy MN, et al. Repeatability and sensitivity of high resolution blood volume mapping in mouse kidney disease. *J Magn Reson Imaging.* 2013.
66. Eckerbom P, Hansell P, Bjerner T, et al. Intravoxel incoherent motion MR imaging of the kidney: pilot study. *Adv Exp Med Biol.* 2013;765:55-8.
67. Zhang JL, Sigmund EE, Rusinek H, et al. Optimization of b-value sampling for diffusion-weighted imaging of the kidney. *Magn Reson Med.* 2012;67:89-97.
68. Ichikawa S, Motosugi U, Ichikawa T, et al. Intravoxel incoherent motion imaging of the kidney: alterations in diffusion and perfusion in patients with renal dysfunction. *Magn Reson Imaging.* 2013;31:414-7.
69. Niendorf T, Dijkhuizen RM, Norris DG, et al. Biexponential diffusion attenuation in various states of brain tissue: implications for diffusion-weighted imaging. *Magn Reson Med.* 1996;36:847-57.

## Figure Captions

### Figure 1:

Coronal  $T_2^*$  weighted image of a rat kidney (**left**) used for control of the perivascular flow probe and the cortical and medullary Laser-flux/ $pO_2$  probes position during the *in vivo* experiments. For this purpose the imaging slice was positioned such that both probes were located within the imaging plane. The  $T_2^*$ -map obtained for the same coronal view of the kidney map (**middle**) also shows the position of the perivascular flow probe and the cortical and medullary Laser-flux/ $pO_2$  probes. Schematic view of the positions used for the perivascular flow probe and the cortical and medullary Laser-flux/ $pO_2$  probes (**right**).

### Figure 2:

Kidney segmentation model overlaid onto a photograph of a freshly excised rat kidney in coronal view (**A**) and superimposed to a  $T_2^*$ -map of a rat kidney (**B**). During analysis the rectangular reference frame is manually positioned around the kidney, followed by an automated drawing of the diagonals (yellow). After their intersections with the kidney borders are defined manually, the ROIs (I1-I2, O1-O3, C1-C3, I: inner medulla, O: outer medulla, C: cortex) are automatically placed at pre-defined relative positions with regard to these references. The numbers shown on the horizontal and vertical axis as well as on the diagonals signify percentages of the reference frame dimensions and of the diagonals.

### Figure 3:

**A,B**) Examples of renal  $T_2^*$  maps (**A**) and  $T_2$  maps (**B**) derived from baseline and during aortic occlusion, hypoxia and hyperoxia. **C,D**) Corresponding  $\Delta T_2^*$  (**C**) and  $\Delta T_2$  (**D**) maps which represent the pixel-by-pixel  $T_2^*$  and  $T_2$  difference between the last time point of each intervention phase and baseline.

### Figure 4:

Time courses of physiological and MR parameters throughout baseline, hyperoxia and recovery: relative changes of RPP (n=15), RBF (n=10), renal conductance (n=10), kidney size (n=15), cortical/medullary Laser-flux (n=11/13), cortical/medullary pO<sub>2</sub> (n=12/10), cortical/outer medullary/inner medullary T<sub>2</sub>\* (n=15/15/15), and cortical/outer medullary/inner medullary T<sub>2</sub> (n=14/15/14). Hyperoxia started at t = 0. Its duration is indicated by the grey shading. Absolute parameter values at baseline are used to provide quantitative guidance.

**Figure 5:**

Time courses of physiological and MR parameters throughout baseline, aortic occlusion and recovery: relative changes of RPP (n=15), RBF (n=10), renal conductance (n=10), kidney size (n=15), cortical/medullary Laser-flux (n=12/13), cortical/medullary pO<sub>2</sub> (n=12/9), cortical/outer medullary/inner medullary T<sub>2</sub>\* (n=15/15/15), and cortical/outer medullary/inner medullary T<sub>2</sub> (n=14/15/14). Aortic occlusion started at t = 0. Its duration is indicated by the grey shading. Absolute parameter values at baseline are used to provide quantitative guidance.

**Figure 6:**

Time courses of physiological and MR parameters throughout baseline, hypoxia and recovery: relative changes of RPP (n=13), RBF (n=8), renal conductance (n=8), kidney size (n=13), cortical/medullary Laser-flux (n=11/13), cortical/medullary pO<sub>2</sub> (n=9/8), cortical/outer medullary/inner medullary T<sub>2</sub>\* (n=13/13/13), and cortical/outer medullary/inner medullary T<sub>2</sub> (n=12/13/12). Hypoxia started at t = 0. Its duration is indicated by the grey shading. In two rats the hypotensive response was so pronounced that hypoxia had to be stopped prematurely, therefore their data are not included here. Absolute parameter values at baseline are used to provide quantitative guidance.

**Figure 7:**

Parametric correlation analysis (Pearson's analysis) between relative changes of cortical T<sub>2</sub>\* and relative changes of cortical pO<sub>2</sub> (**left**) or renal blood flow (**right**) for aortic occlusion & recovery (**top**), hypoxia & recovery (**center**) and hyperoxia & recovery (**bottom**). The coefficient of determination for Pearson ( $R_p^2$ ) is given together with the Spearman coefficient

( $R_s^2$ ); \*\* denotes  $p < 0.01$  The linear regression curve is shown only for significant Pearson's correlations of  $p < 0.05$ .

**Figure 8:**

Parametric correlation analysis (Pearson's analysis) between relative changes of outer medullary  $T_2^*$  and relative changes of medullary  $pO_2$  (**left**) or renal blood flow (**right**) for aortic occlusion & recovery (**top**), hypoxia & recovery (**center**) and hyperoxia & recovery (**bottom**). The coefficient of determination for Pearson ( $R_p^2$ ) is given together with the Spearman coefficient ( $R_s^2$ ); \*\* denotes  $p < 0.01$ .

**Figure 9:**

Parametric correlation analysis (Pearson's analysis) between relative changes of inner medullary  $T_2^*$  and relative changes of medullary  $pO_2$  (**left**) or renal blood flow (**right**) for aortic occlusion & recovery (**top**), hypoxia & recovery (**center**) and hyperoxia & recovery (**bottom**). The coefficient of determination for Pearson ( $R_p^2$ ) is given together with the Spearman coefficient ( $R_s^2$ ); \* denotes  $p < 0.05$ , \*\* denotes  $p < 0.01$ . The linear regression curve is shown only for significant Pearson's correlations of  $p < 0.05$ .

**Table 1****Hyperoxia & Recovery**

|                        |    | $R^2_s$ | $R^2_p$ | $f(x) = m x + n$ |       |
|------------------------|----|---------|---------|------------------|-------|
|                        |    |         |         | m                | n     |
| $T_2^*$ vs $pO_2$      | C  | 0.14**  | 0.01    |                  |       |
|                        | OM | 0.19**  | 0.16**  | 0.16             | 0.88  |
|                        | IM | 0.21**  | 0.13**  | 0.16             | 0.88  |
| $T_2^*$ vs RBF         | C  | 0.43**  | 0.50**  | 0.84             | 0.24  |
|                        | OM | 0.43**  | 0.50**  | 0.92             | 0.18  |
|                        | IM | 0.17**  | 0.18**  | 0.51             | 0.53  |
| $T_2^*$ vs RPP         | C  | 0.50**  | 0.49**  | 0.92             | 0.16  |
|                        | OM | 0.40**  | 0.43**  | 1.01             | 0.1   |
|                        | IM | 0.10**  | 0.06*   | 0.36             | 0.69  |
| $T_2^*$ vs Laser-flux  | C  | 0.28**  | 0.28**  | -0.39            | 1.45  |
|                        | OM | 0.02    | 0.01    |                  |       |
|                        | IM | 0.05*   | 0.03    |                  |       |
| $T_2^*$ vs kidney size | C  | 0.24**  | 0.26**  | 4.87             | -3.81 |
|                        | OM | 0.24**  | 0.27**  | 5.72             | -4.64 |
|                        | IM | 0.11**  | 0.09**  | 3.12             | -2.08 |
| $T_2^*$ vs conductance | C  | 0.00    | 0.00    |                  |       |
|                        | OM | 0.01    | 0.00    |                  |       |
|                        | IM | 0.00    | 0.00    |                  |       |

**Occlusion & Recovery**

|                        |    | $R^2_s$ | $R^2_p$ | $f(x) = m x + n$ |       |
|------------------------|----|---------|---------|------------------|-------|
|                        |    |         |         | m                | n     |
| $T_2^*$ vs $pO_2$      | C  | 0.24**  | 0.23**  | 0.13             | 0.83  |
|                        | OM | 0.54**  | 0.59**  | 0.4              | 0.56  |
|                        | IM | 0.28**  | 0.24**  | 0.23             | 0.71  |
| $T_2^*$ vs RBF         | C  | 0.33**  | 0.18**  | 0.16             | 0.78  |
|                        | OM | 0.66**  | 0.58**  | 0.41             | 0.49  |
|                        | IM | 0.09*   | 0.06    |                  |       |
| $T_2^*$ vs RPP         | C  | 0.32**  | 0.14**  | 0.15             | 0.78  |
|                        | OM | 0.60**  | 0.53**  | 0.45             | 0.41  |
|                        | IM | 0.10**  | 0.07*   | 0.15             | 0.73  |
| $T_2^*$ vs Laser-flux  | C  | 0.02    | 0.03    |                  |       |
|                        | OM | 0.44**  | 0.45**  | 0.32             | 0.54  |
|                        | IM | 0.03    | 0.02    |                  |       |
| $T_2^*$ vs kidney size | C  | 0.45**  | 0.38**  | 3.68             | -2.71 |
|                        | OM | 0.54**  | 0.45**  | 6.17             | -5.28 |
|                        | IM | 0.13**  | 0.12**  | 2.83             | -1.92 |
| $T_2^*$ vs conductance | C  | 0.55**  | 0.35**  | 0.58             | 0.42  |
|                        | OM | 0.45**  | 0.10*   | 0.85             | 0.11  |
|                        | IM | 0.17*   | 0.34**  | 0.50             | 0.46  |

Hypoxia &  
Recovery

|                        |           | $R^2_s$ | $R^2_p$ | $f(x) = m x + n$ |       |
|------------------------|-----------|---------|---------|------------------|-------|
|                        |           |         |         | m                | n     |
| $T_2^*$ vs $pO_2$      | <b>C</b>  | 0.25**  | 0.03    |                  |       |
|                        | <b>OM</b> | 0.67**  | 0.67**  | 0.43             | 0.44  |
|                        | <b>IM</b> | 0.60**  | 0.63**  | 0.33             | 0.59  |
| $T_2^*$ vs RBF         | <b>C</b>  | 0.74**  | 0.74**  | 0.59             | 0.38  |
|                        | <b>OM</b> | 0.84**  | 0.81**  | 0.74             | 0.21  |
|                        | <b>IM</b> | 0.53**  | 0.49**  | 0.44             | 0.51  |
| $T_2^*$ vs RPP         | <b>C</b>  | 0.77**  | 0.74**  | 0.98             | -0.02 |
|                        | <b>OM</b> | 0.64**  | 0.68**  | 1.14             | -0.23 |
|                        | <b>IM</b> | 0.41**  | 0.36**  | 0.62             | 0.33  |
| $T_2^*$ vs Laser-flux  | <b>C</b>  | 0.03    | 0.01    |                  |       |
|                        | <b>OM</b> | 0.05    | 0.07    |                  |       |
|                        | <b>IM</b> | 0.01    | 0.00    |                  |       |
| $T_2^*$ vs kidney size | <b>C</b>  | 0.55**  | 0.43**  | 4.65             | -3.75 |
|                        | <b>OM</b> | 0.57**  | 0.40**  | 5.48             | -4.63 |
|                        | <b>IM</b> | 0.39**  | 0.31**  | 3.57             | -2.64 |
| $T_2^*$ vs conductance | <b>C</b>  | 0.27**  | 0.34**  | 0.51             | 0.39  |
|                        | <b>OM</b> | 0.44**  | 0.47**  | 0.71             | 0.16  |
|                        | <b>IM</b> | 0.27**  | 0.27**  | 0.42             | 0.49  |

**Table 1:**

Summary of the coefficients of determination for correlation analyses of MR versus physiological parameters according to Spearman ( $R^2_s$ ) and Pearson ( $R^2_p$ ) for three interventions (hyperoxia & recovery, aortic occlusion & recovery, hypoxia & recovery) and three kidney layers (C = cortex, OM = outer medulla, IM = inner medulla); \*: significant correlations with  $p < 0.05$ , \*\*: significant correlations with  $p < 0.01$ . The slope (m) and intercept (n) of the linear regression equations are shown for significant Pearson's correlations at  $p < 0.05$ .

**Table 2**

|                                     |                                       | $R^2_s$ | $R^2_p$ | $f(x) = m x + n$ |       |
|-------------------------------------|---------------------------------------|---------|---------|------------------|-------|
|                                     |                                       |         |         | m                | n     |
| <b>Hyperoxia &amp; Recovery</b>     | <b>RBF vs pO<sub>2</sub></b> <b>C</b> | 0.15**  | 0.04*   | 2.56             | -0.25 |
|                                     | <b>M</b>                              | 0.26**  | 0.12**  | 1.27             | -0.1  |
| <b>RBF vs Laser-flux</b>            | <b>C</b>                              | 0.20**  | 0.28**  | -0.68            | 1.74  |
|                                     | <b>M</b>                              | 0.01    | 0.00    |                  |       |
| <b>Laser-flux vs pO<sub>2</sub></b> | <b>C</b>                              | 0.00    | 0.01    |                  |       |
|                                     | <b>M</b>                              | 0.04*   | 0.02    |                  |       |
| <b>Kidney size vs Conductance</b>   |                                       | 0.03    | 0.06    |                  |       |

|                                     |                                       | $R^2_s$ | $R^2_p$ | $f(x) = m x + n$ |      |
|-------------------------------------|---------------------------------------|---------|---------|------------------|------|
|                                     |                                       |         |         | m                | n    |
| <b>Occlusion &amp; Recovery</b>     | <b>RBF vs pO<sub>2</sub></b> <b>C</b> | 0.46**  | 0.43**  | 0.78             | 0.08 |
|                                     | <b>M</b>                              | 0.68**  | 0.58**  | 0.8              | 0.11 |
| <b>RBF vs Laser-flux</b>            | <b>C</b>                              | 0.10**  | 0.20**  | 0.68             | 0.45 |
|                                     | <b>M</b>                              | 0.62**  | 0.69**  | 1.04             | 0.09 |
| <b>Laser-flux vs pO<sub>2</sub></b> | <b>C</b>                              | 0.05**  | 0.08**  | 0.26             | 0.46 |
|                                     | <b>M</b>                              | 0.27**  | 0.32**  | 0.5              | 0.25 |
| <b>Kidney size vs Conductance</b>   |                                       | 0.46**  | 0.37**  | 0.08             | 0.92 |

|                                     |                                       | $R^2_s$ | $R^2_p$ | $f(x) = m x + n$ |      |
|-------------------------------------|---------------------------------------|---------|---------|------------------|------|
|                                     |                                       |         |         | m                | n    |
| <b>Hypoxia &amp; Recovery</b>       | <b>RBF vs pO<sub>2</sub></b> <b>C</b> | 0.27**  | 0.02    |                  |      |
|                                     | <b>M</b>                              | 0.86**  | 0.79**  | 1.34             | -0.3 |
| <b>RBF vs Laser-flux</b>            | <b>C</b>                              | 0.14**  | 0.12**  | 0.4              | 0.44 |
|                                     | <b>M</b>                              | 0.07*   | 0.18**  | 0.42             | 0.44 |
| <b>Laser-flux vs pO<sub>2</sub></b> | <b>C</b>                              | 0.02    | 0.01    |                  |      |
|                                     | <b>M</b>                              | 0.06*   | 0.03    |                  |      |
| <b>Kidney size vs Conductance</b>   |                                       | 0.45**  | 0.43**  | 0.10             | 0.90 |

**Table 2:**

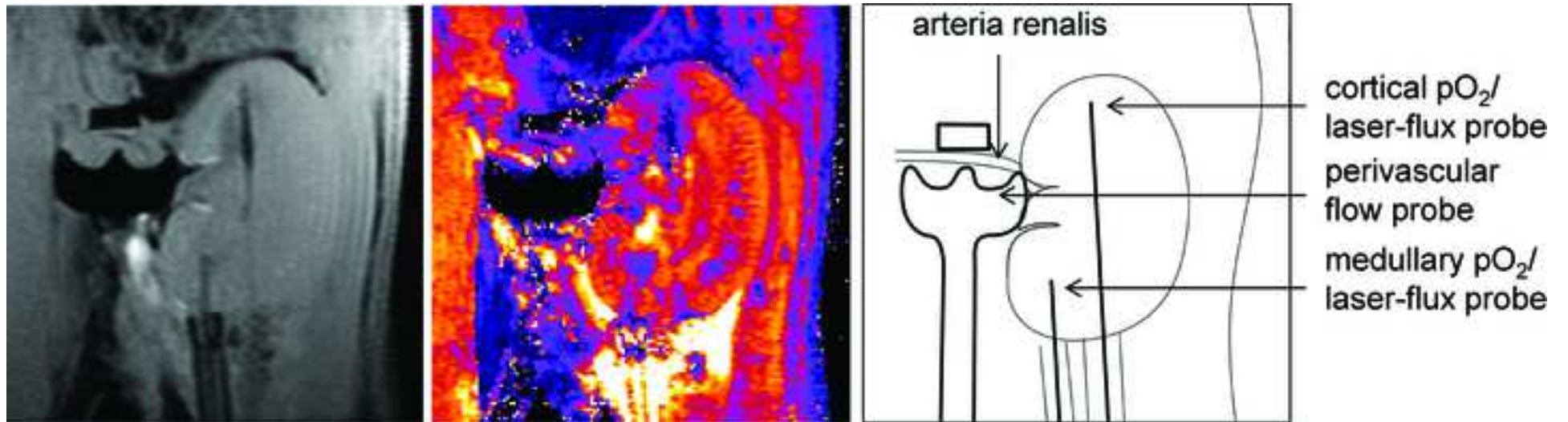
Summary of the coefficients of determination for correlation analyses of physiological parameters according to Spearman ( $R^2_s$ ) and Pearson ( $R^2_p$ ) for three interventions (hyperoxia & recovery, aortic occlusion & recovery, hypoxia & recovery) and two kidney layers (C=cortex, M=medulla); \*: significant correlations with  $p < 0.05$ , \*\*: significant correlations

with  $p < 0.01$ . The slope (m) and intercept (n) of the linear regression equations are shown for significant Pearson's correlations at  $p < 0.05$ .



Figure 1

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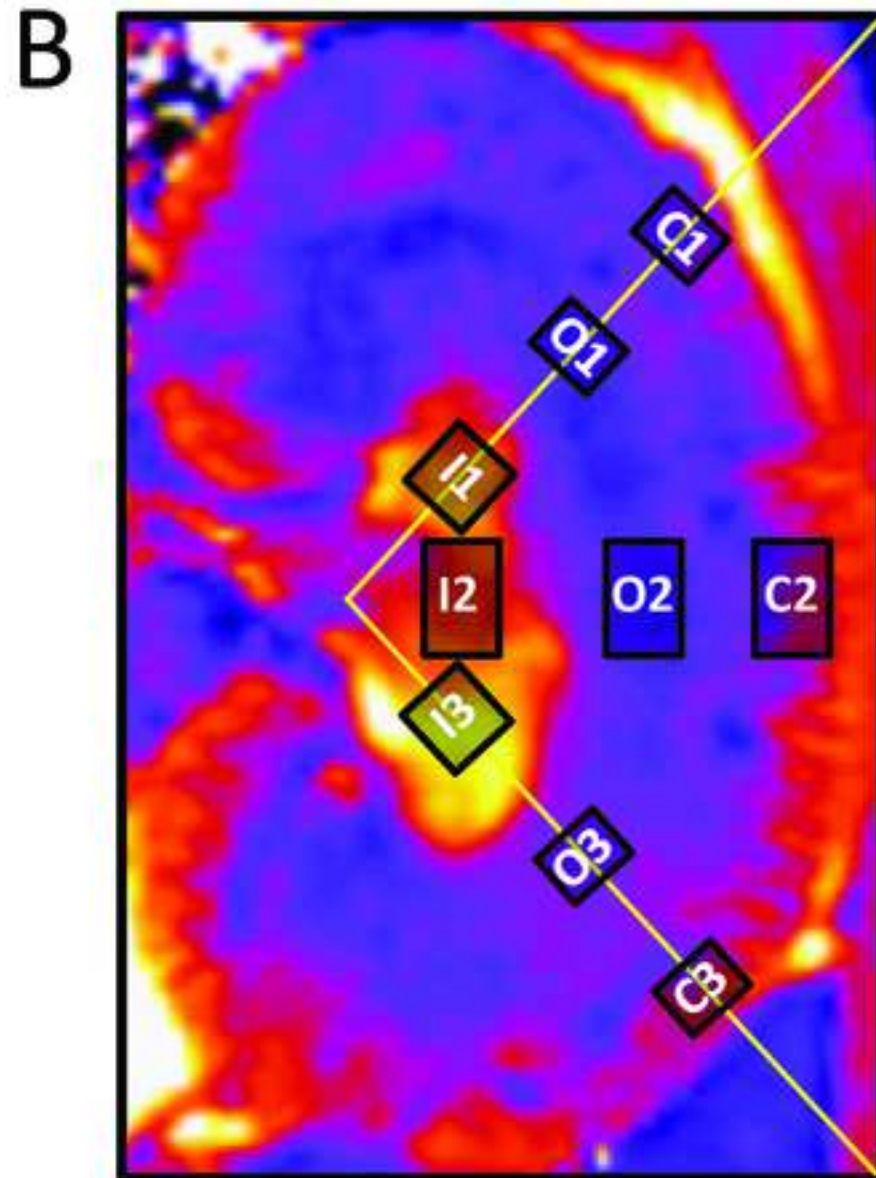
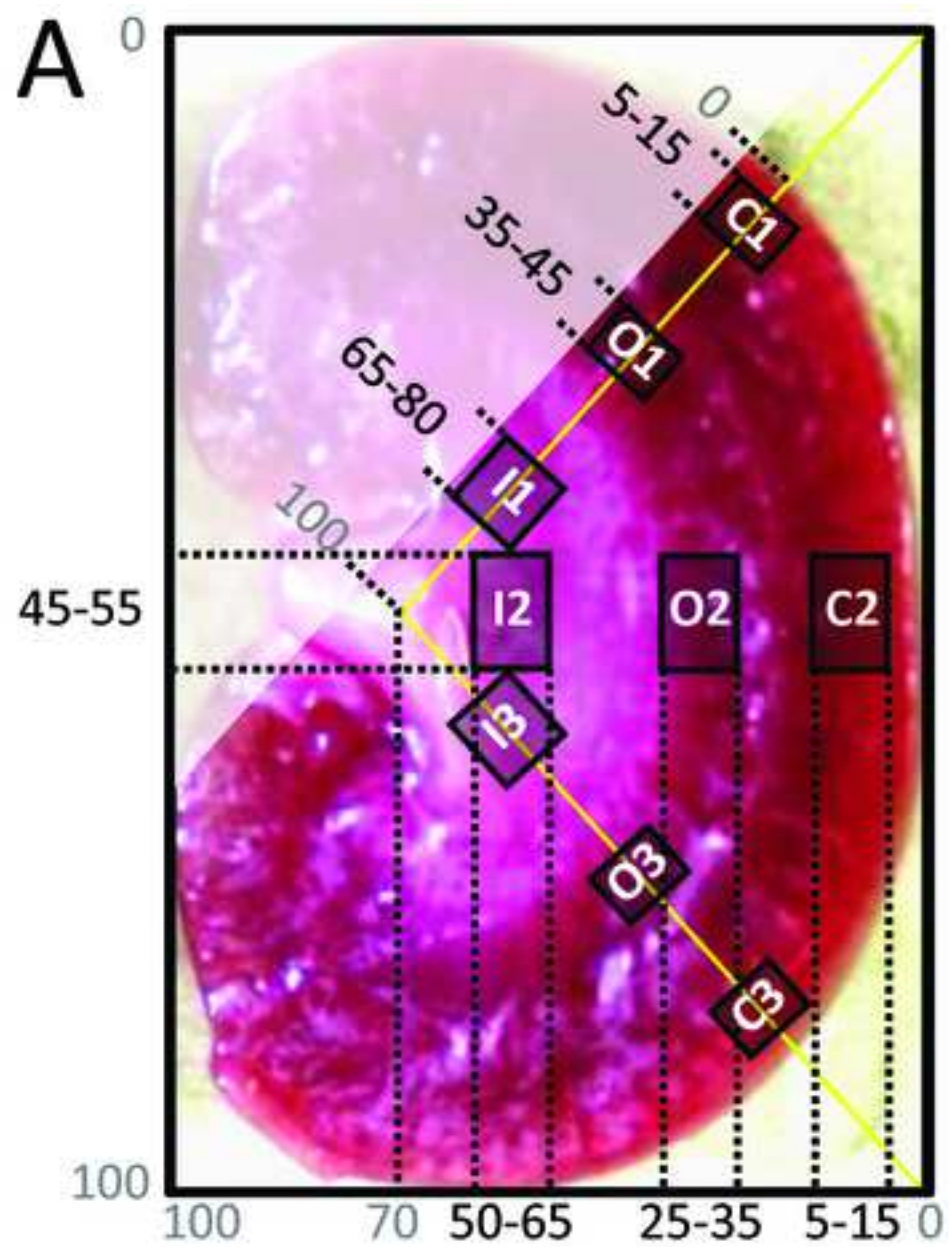


Figure 3  
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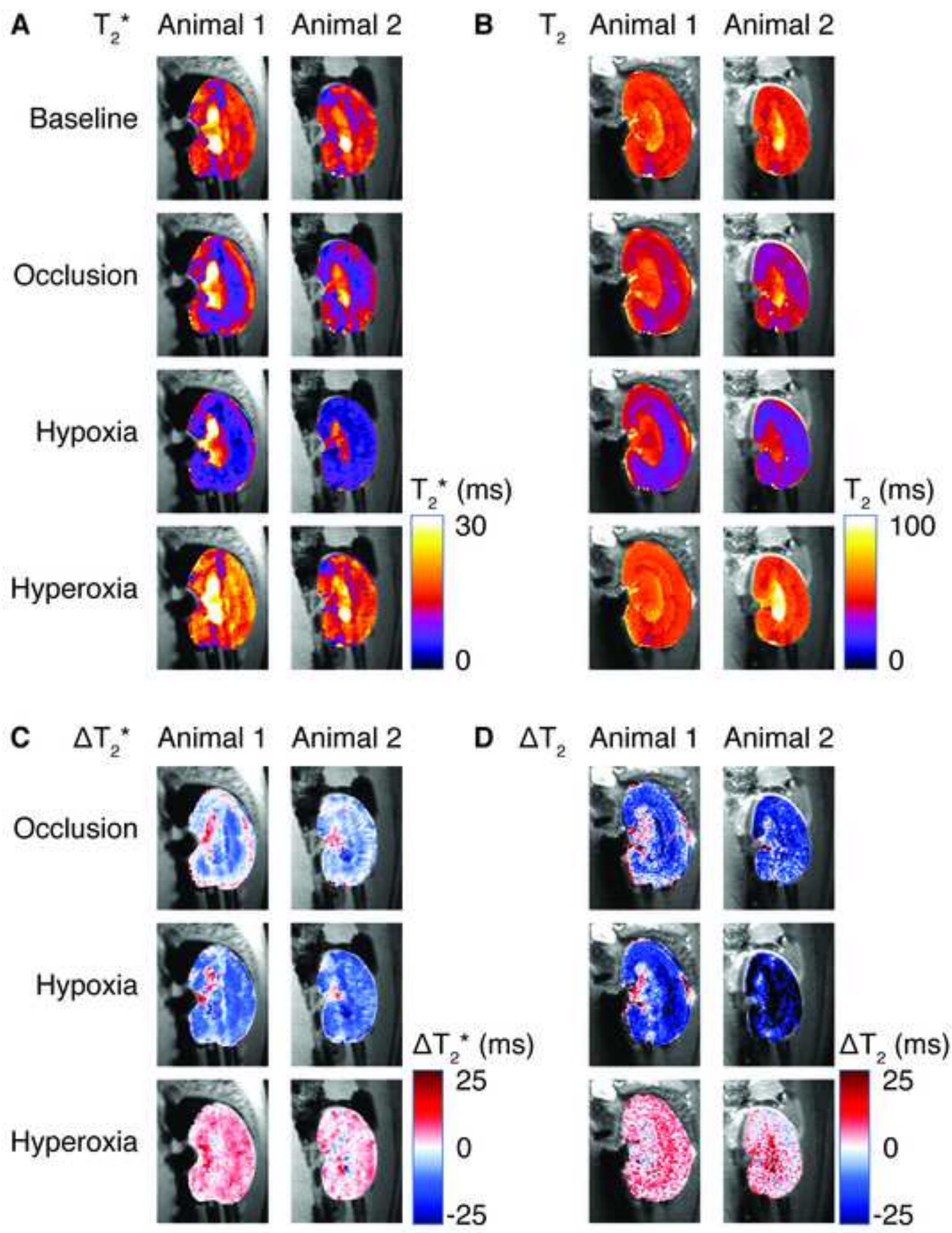


Figure 4

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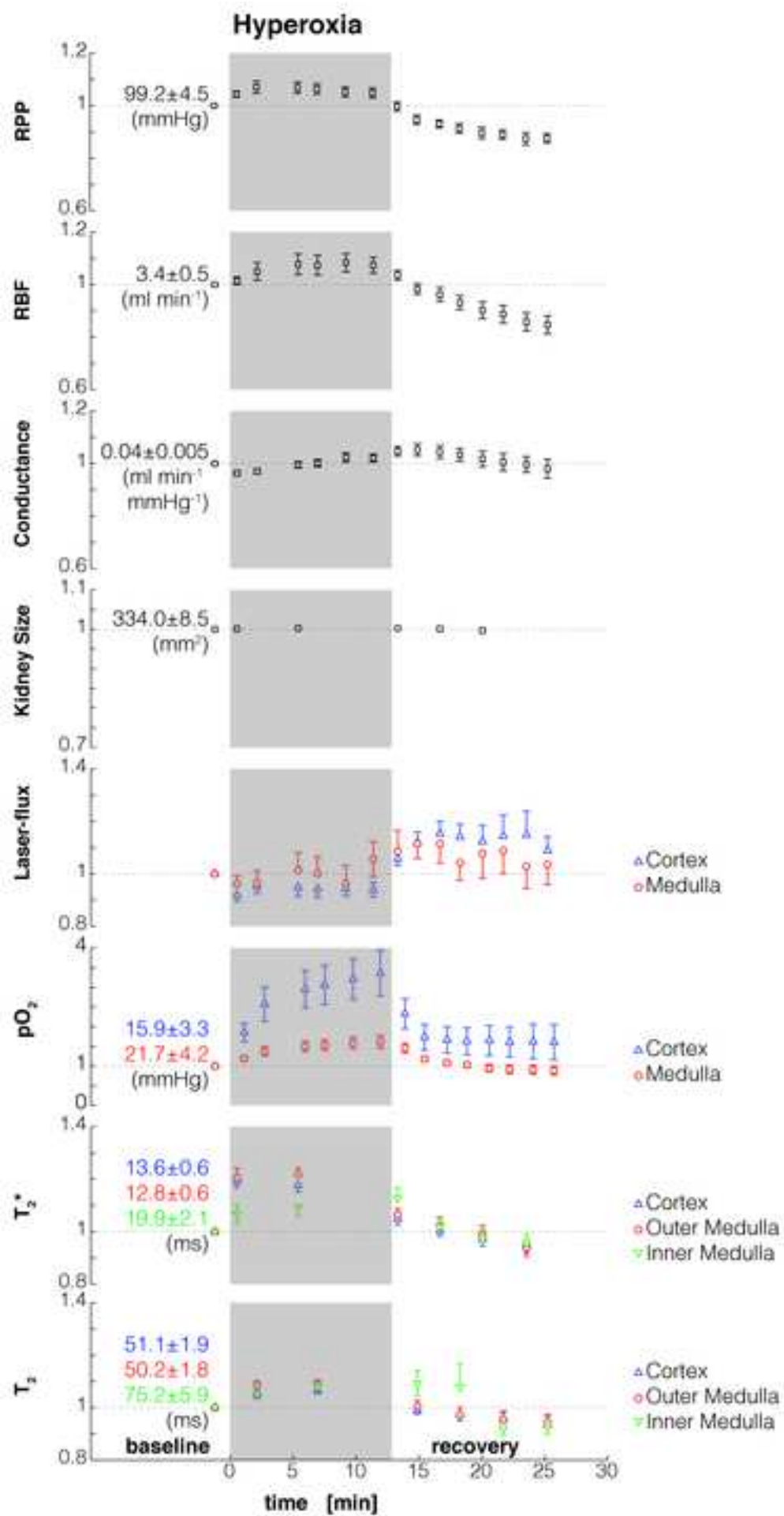


Figure 5

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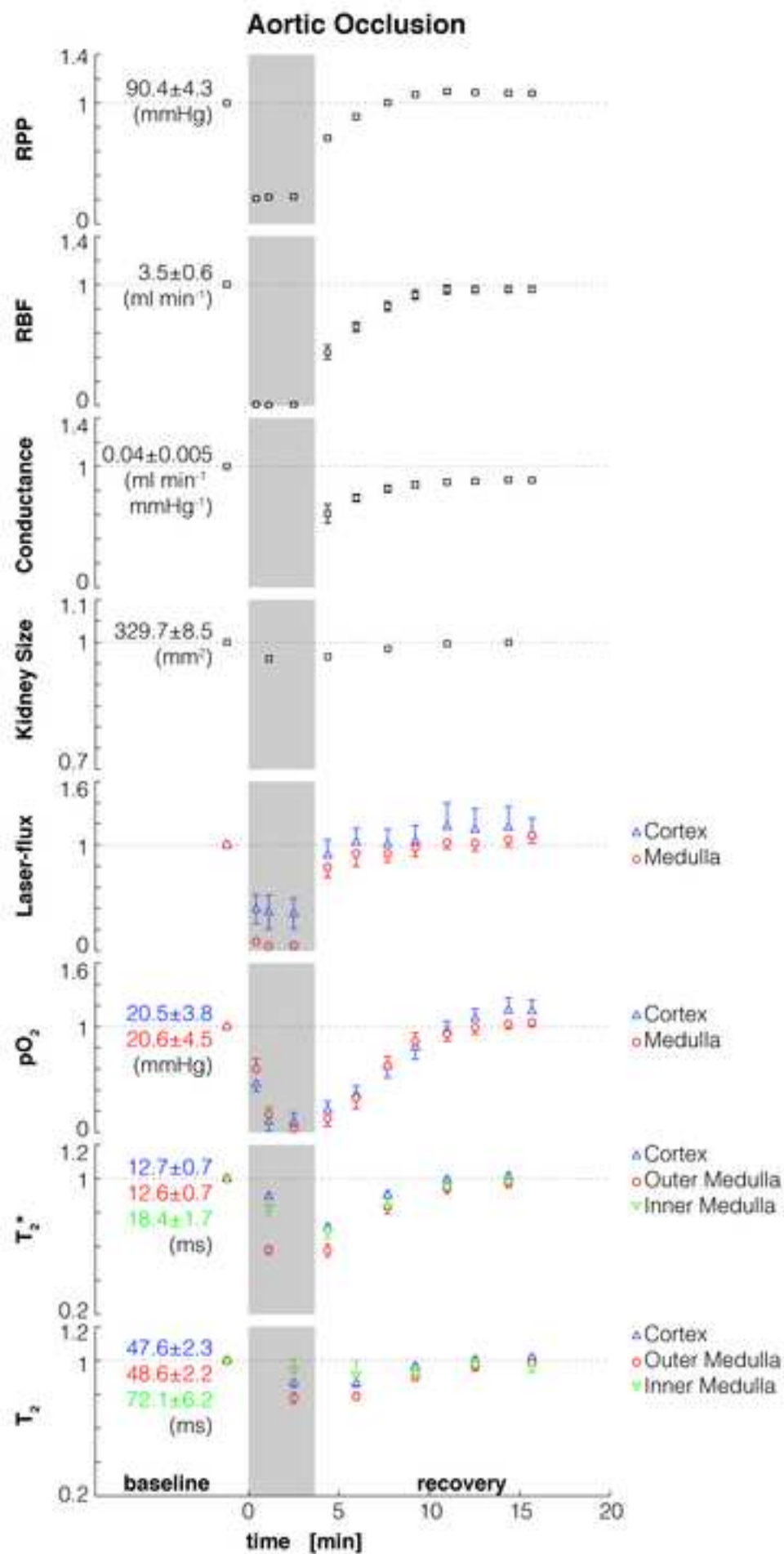
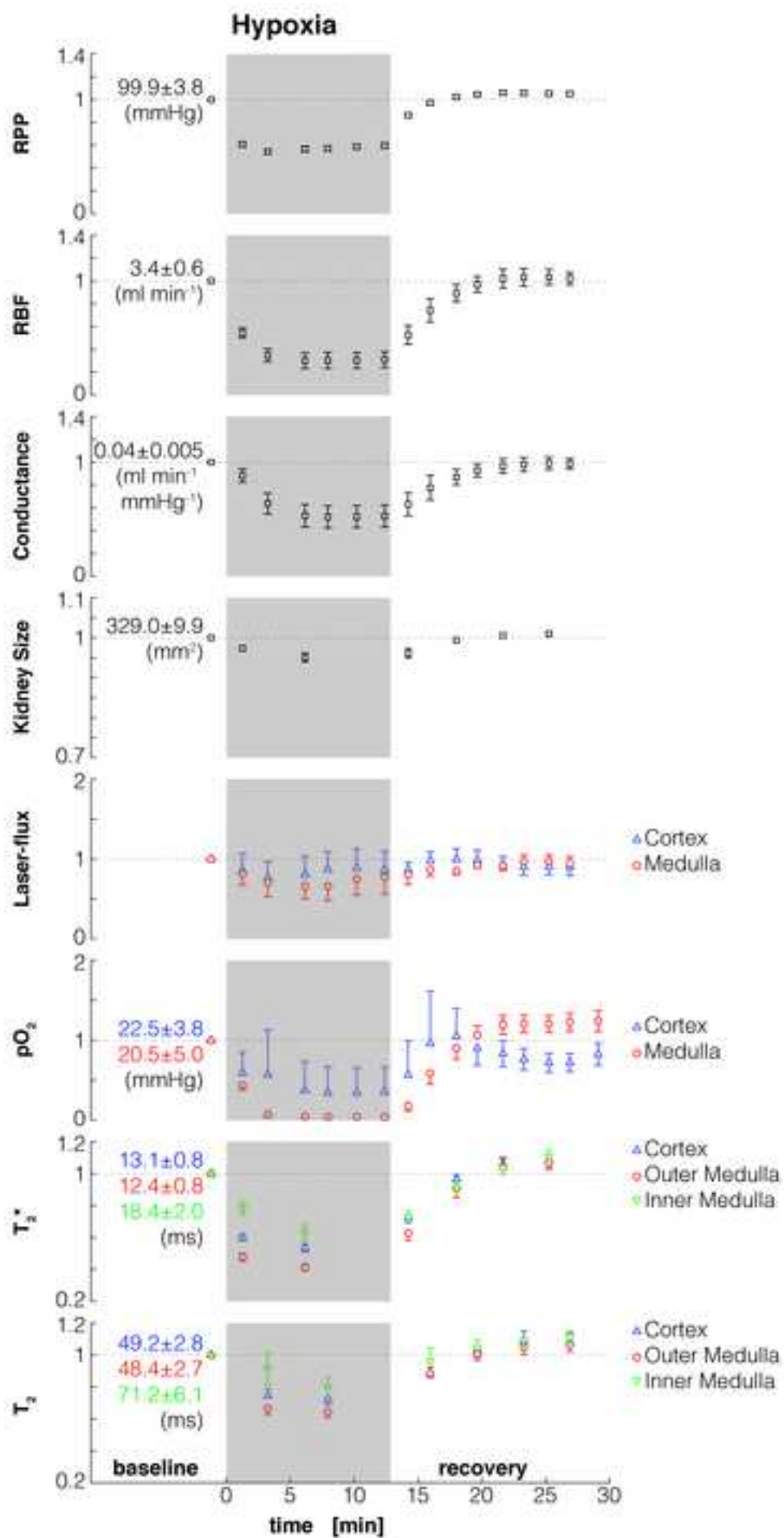


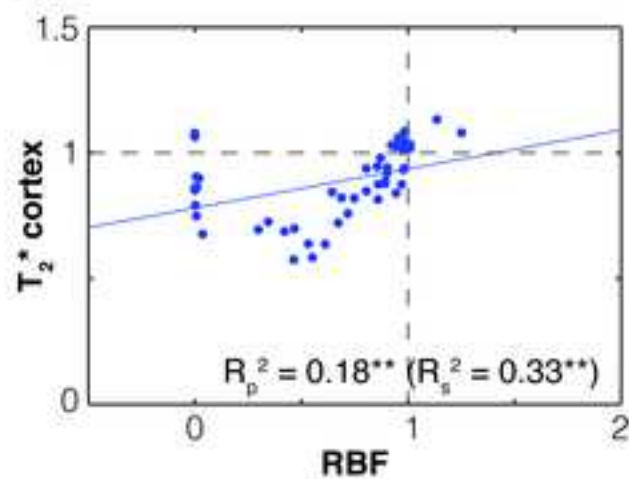
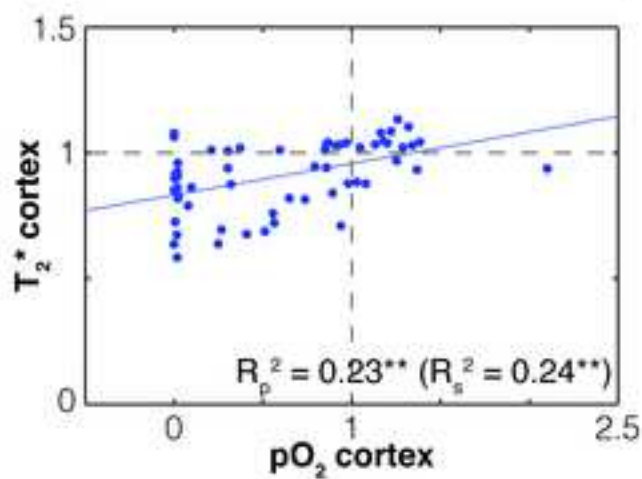
Figure 6

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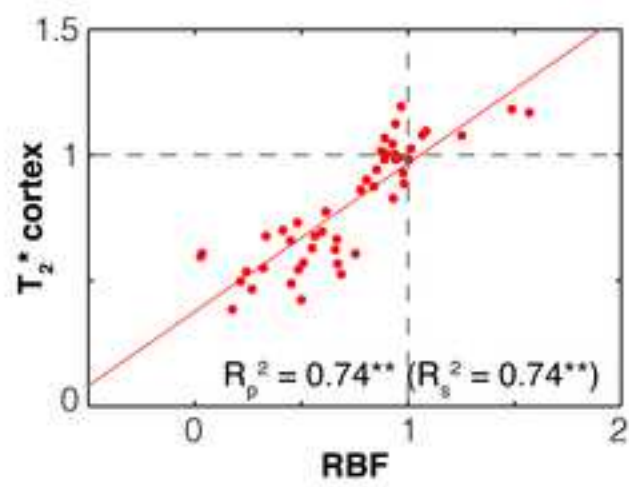
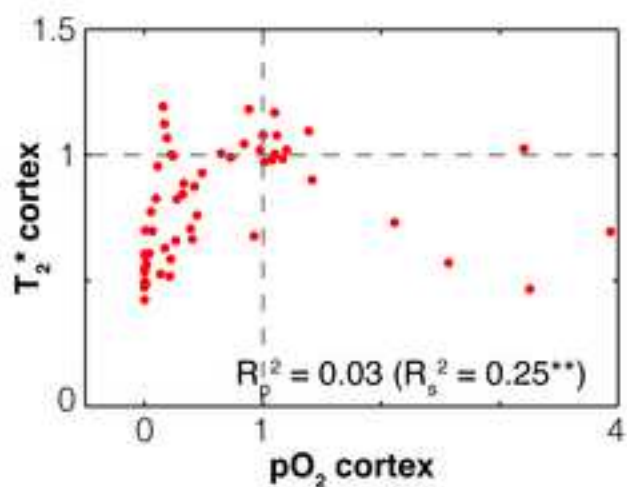


### Cortex

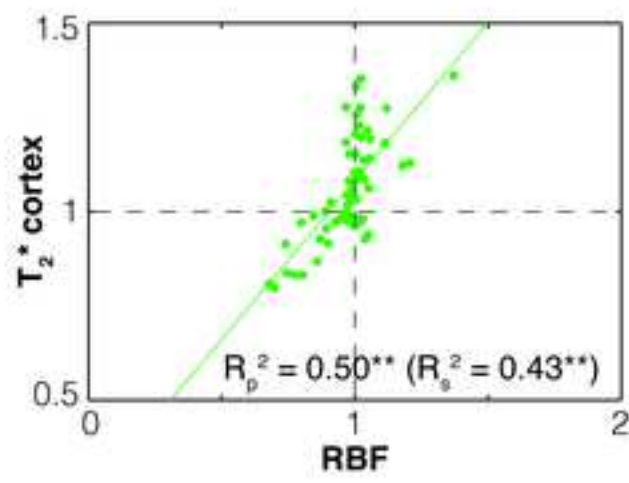
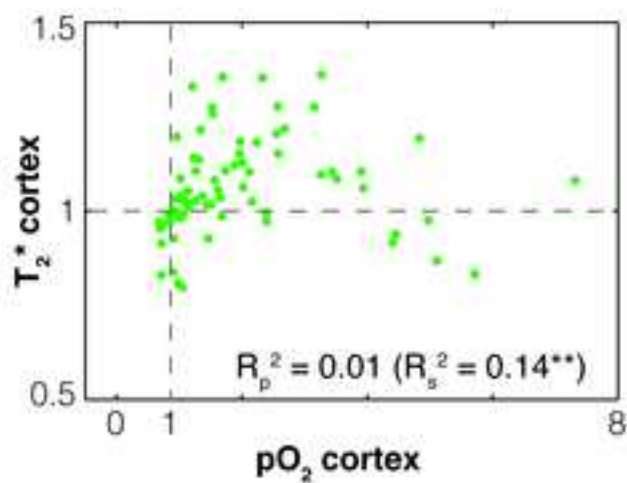
Aortic Occlusion



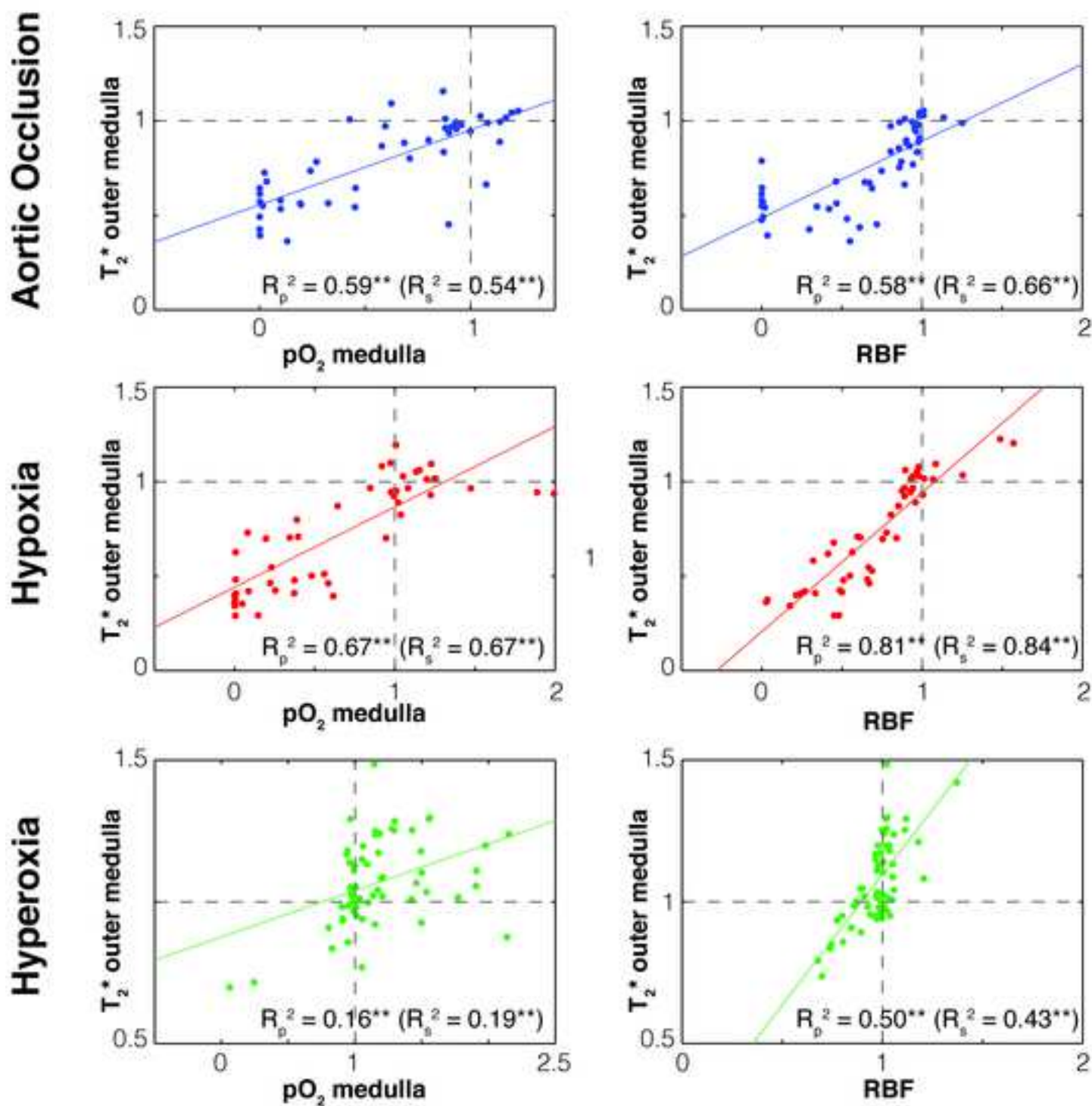
Hypoxia



Hyperoxia



## Outer Medulla





### Inner Medulla

