

Assessment of rhabdomyolysis-induced acute kidney injury (AKI) in mice using Hemodynamic Response Imaging (HRI)

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Background & Aims: The pathophysiology of rhabdomyolysis-induced acute kidney injury (AKI) is complex and incompletely understood, however hypoxia is one of the factors thought to contribute to renal injury. The use of MRI for the diagnosis and assessment of AKI includes T1-, T2- and diffusion-weighted techniques, as well as BOLD MRI, while contrast-enhanced MRI studies are limited in this setup, due to adverse effects of the contrast media. The assessment of renal oxygenation by BOLD MRI was first reported in 1996 by Prasad et al¹. While BOLD MRI enables tracking changes in organ oxygenation status, we previously demonstrated the feasibility of *Hemodynamic Response Imaging (HRI)*, an fMRI method combined with transient hypercapnia and hyperoxia challenges, for monitoring changes in liver perfusion and hemodynamics². A recent study examined the influence of carbogen and oxygen breathing on renal oxygenation in healthy kidneys as measured by BOLD MRI³. By combining CO₂ challenge, the *HRI* method adds essential information regarding blood vessels reactivity and functionality. The aim of the present study was to assess the utility of *HRI* for the evaluation of changes in renal perfusion and hemodynamics during AKI.

Methods: Mice (n=15) were scanned with a 4.7T MRI Bruker biospec spectrometer at baseline and on days 1, 4, 8, 15 and 22 after induction of rhabdomyolysis by intra-muscular (IM) glycerol injection. Axial true-FISP (True Fast Imaging with Steady-state Precession) images with T2*/T1 contrast (TR/TE=3/1.5 ms) were used to evaluate renal morphology and cortico-medullary differentiation (CMD). CMD was calculated as the ratio between the cortical and medullary signal intensities, and was normalized to the adjacent back muscle. *HRI* - T2*-weighted GE (TR/TE=147/10 ms) images were acquired during hypercapnic (5% CO₂) and hyperoxic (95% O₂) challenges as described². Data analysis was performed using IDL software. *HRI* maps are given as the percentage change of signal intensity (ΔS) induced by hypercapnia (ΔS_{CO_2}) or hyperoxia (ΔS_{O_2}). Additionally, contrast-enhanced imaging was performed using T1-weighted GE images (TR/TE=58/5 ms) following administration of Gd-DTPA for kidneys perfusion assessment. Serum urea levels were measured for renal function determination. At parallel time-points kidneys were taken for histology and were further immunostained for apoptosis (TUNEL), proliferation (BrdU), blood vessels (CD31) and hypoxia (Pimonidazole).

Results: Changes in kidney size, morphology and the difference between cortical and medullary intensities were clearly visible during AKI progression in true-FISP images (Fig.1A), and were significantly different already 6hr after glycerol injection, and at all time-points of the disease (Fig.1B). Regression analysis showed significant correlation between CMD and serum urea levels ($R=0.89$, $P<0.0001$). Cortical values correlated significantly with serum urea levels ($R=0.69$, $P<0.0001$), whereas there was no significant correlation between the medullary and urea values. The ΔS_{O_2} and ΔS_{CO_2} values of the kidney negatively correlated with serum urea ($R=-0.65$, $P=0.011$), the average ΔS values of the whole kidney were highest at baseline and were significantly attenuated on days 1, 4 and 8 post glycerol injection (Fig.1C), reflecting decreased renal perfusion or blood vessels reactivity. These findings correlated with increased renal hypoxia, as indicated by pimonidazole immunostaining (Fig.1D). In contrast, Gd-DTPA imaging experiments, while showing significant contrast-clearance delay at these time-points, corresponding to decreased renal function, did not indicate any loss of blood vessel patency. Accordingly, CD31 immunostaining excluded a decline in blood vessel density. TUNEL assay showed maximal apoptosis on day 1 and was gradually reduced on days 4 and 8, while the proliferation was highest on day 4.

Conclusions: We present the beneficial use of a non-invasive MRI method, based on BOLD fMRI, combined with hyperoxia and hypercapnia for AKI assessment. This technique provides supplementary information regarding kidney perfusion, hemodynamics and blood vessels reactivity during evolving AKI in mice. Moreover, these findings are complementary to the findings from contrast-enhanced imaging and from immunostaining, emphasizing different aspects of renal disease pathophysiology.

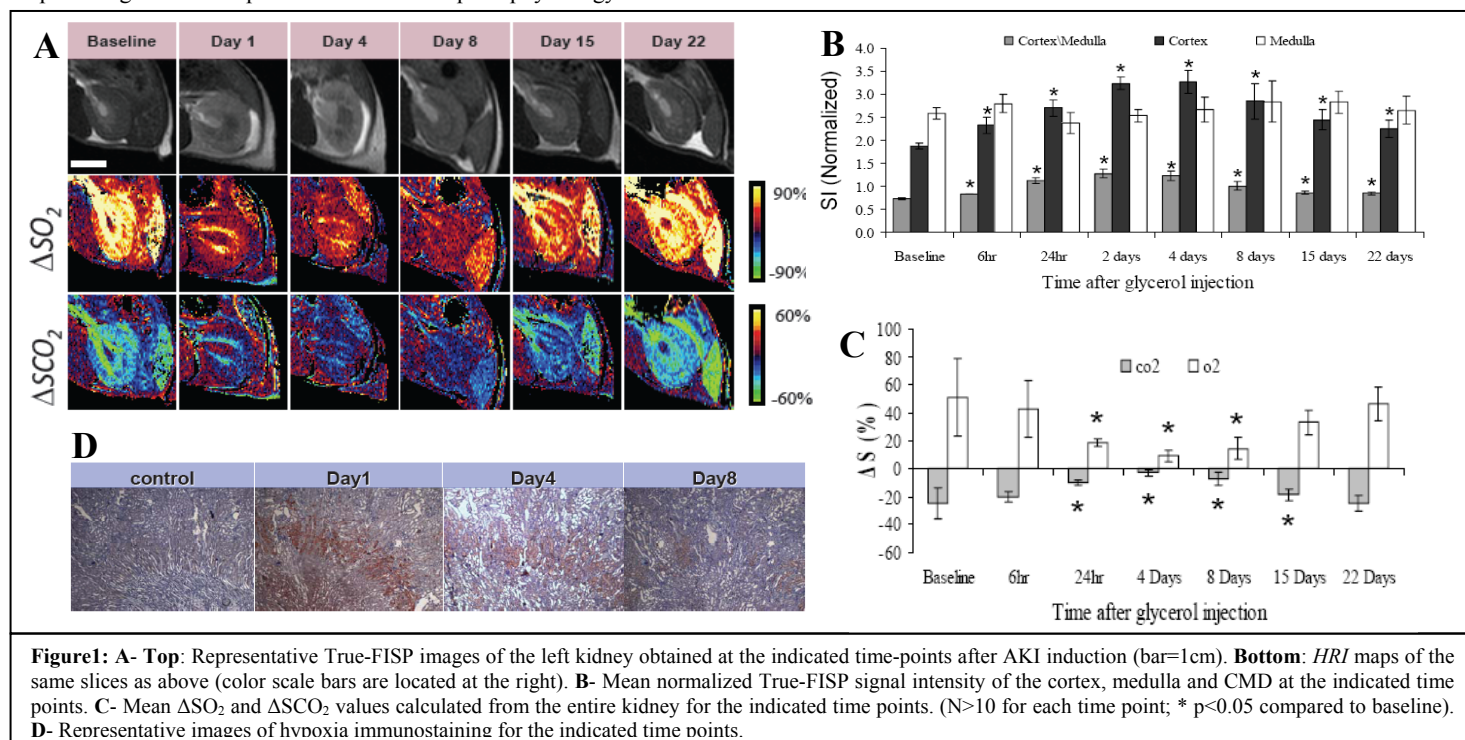


Figure 1: A- Top: Representative True-FISP images of the left kidney obtained at the indicated time-points after AKI induction (bar=1cm). Bottom: *HRI* maps of the same slices as above (color scale bars are located at the right). B- Mean normalized True-FISP signal intensity of the cortex, medulla and CMD at the indicated time points. C- Mean ΔS_{O_2} and ΔS_{CO_2} values calculated from the entire kidney for the indicated time points. (N>10 for each time point; * $p<0.05$ compared to baseline). D- Representative images of hypoxia immunostaining for the indicated time points.

References: ¹Prasad PV, Circulation 94: 3271, 1996. ²Barash H, Radiology 243(3):727, 2007. ³Boss A, NMR Biomed, 22: 638, 2009.