In situ lactate dehydrogenase activity-a novel renal cortical imaging biomarker of tubular injury?

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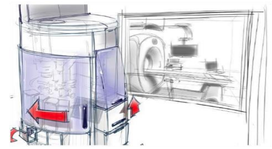
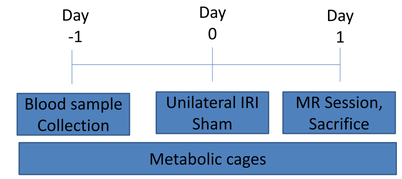


ABSTRACT: Renal ischemia/reperfusion injury (IRI) is the leading cause of acute kidney injury (AKI) in several disease states. Imbalance in energy metabolism and mitochondrial function is a hallmark in IRI which can be caused by mechanisms like oxidative stress, apoptosis and inflammation. Lactate dehydrogenase (LDH) activity has previously been suggested as a renal tubular injury marker. By the use of a hyperpolarized [1-^{13}C]pyruvate magnetic resonance imaging (MRI) approach to monitor metabolic changes, we here investigate LDH activity, renal metabolism and cortical injury after IRI. This procedure gives a novel non-invasive method for investigation renal tissue injury in concern with IRI.

Purpose: Hyperpolarized [1-^{13}C]pyruvate allows for dynamic measurements of energy metabolism and quantification of lactate to pyruvate conversion catalyzed by LDH *in situ*. This method provides an improved signal to noise ratio of more than 10,000 fold compared to classic MR spectroscopy. We therefore wish to non invasively measure single kidney energy metabolism and cortical injury

PROCEDURE: The experimental protocol was performed according the scheme below.



HYPERPOLARIZATION Temperature, arterial oxygen saturation and respiration rate were monitored throughout the experiment.

- [1-^{13}C]-pyruvate was polarized in a GE healthcare SpinLab.
- Each animal was injected with 1.5 ml hyperpolarized [1-^{13}C]-pyruvate, through a tail vein catheter
- MR scans were performed in a 3 T clinical MR system

Metabolic ratio's

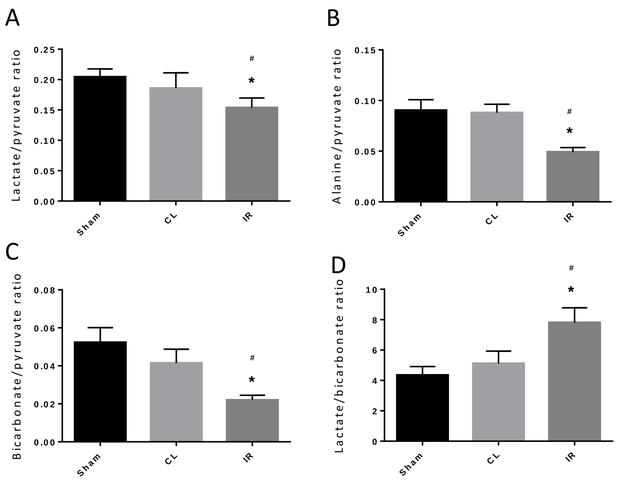


Figure 1 Metabolic parameters. (A) Lactate-to-pyruvate ratio, (B) Alanine-to-pyruvate ratio, (C) Bicarbonate-to-pyruvate ratio, (D) Lactate-to-bicarbonate ratio

Metabolic MRI maps

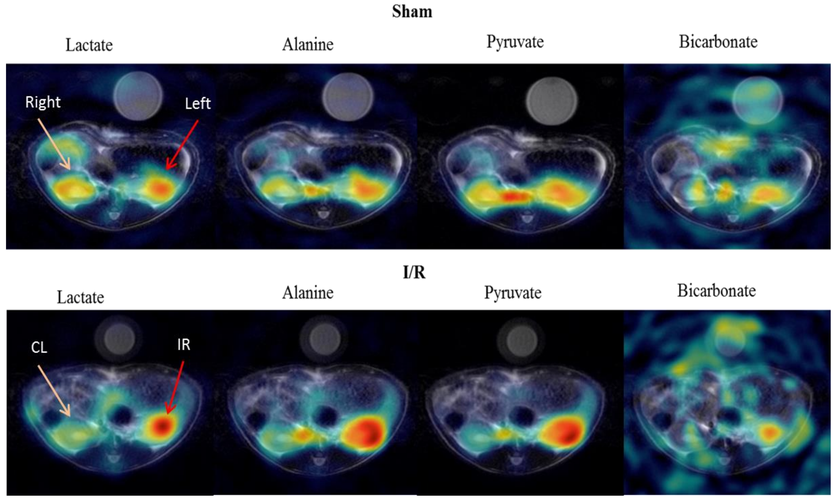


Figure 2 Metabolic MR maps
Anatomical ^{1}H magnetic resonance imaging (MRI) overlaid with metabolic maps of Lactate, Alanine, Pyruvate and Bicarbonate of sham (upper) and IRI (lower) operated rats.

Renal injury parameters

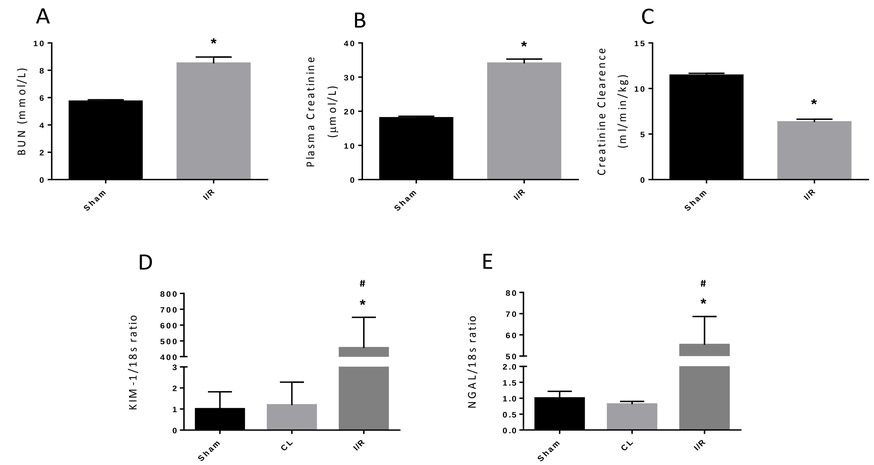


Figure 3 Evaluation of renal function. Renal function was measured by (A) BUN, (B) plasma creatinine and (C) creatinine. (D) KIM-1mRNA expression and (E) NGAL mRNA expression was analyzed using QPCR. *P < 0.05 compared to the sham group, #P < 0.05 compared to CL kidney.

SUMMARY:

- A reduced metabolite/pyruvate ratio is observed (fig 1 and 2)
- This activity reduction is caused by cellular injury (fig 3) leading to membrane disruption and loss of enzymes
- Released LDH is observed in the urine and plasma (fig 4).
- Metabolic alterations were observed (fig. 5). Lactate/bicarbonate ratio representing the ratio between anaerobic and aerobic respiration. This was supported by elevated LDH mRNA expression together with a reduced NAD^+/NADH ratio (fig. 5).
- No metabolic alteration was seen in the contralateral (CL) kidney.
- Alanine/pyruvate and bicarbonate/pyruvate ratios experience reductions lower than lactate/pyruvate as these pathways are not upregulated (fig. x)

CONCLUSION:

- Reduced metabolite/pyruvate ratio was associated with renal injury in the post-ischemic kidney.
- An upregulation of the anaerobic pathway was observed by an elevated lactate/bicarbonate ratio, elevated mRNA expression of LDHA and reduced NAD^+/NADH ratio.
- No metabolic alterations was observed in the contralateral kidney.

Biochemical analysis of LDH

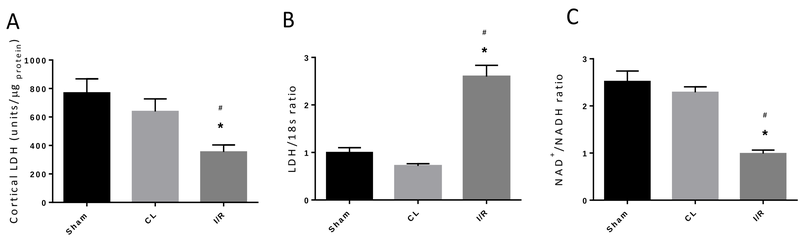


Figure 5 Biochemical analysis of LDH: (A) LDH, (B) mRNA expression of LDHA normalized to 18s, (C) NADH/NAD^+ ratios for left sham kidney, CL kidney and IRI kidney. *P<0.05 compared to Sham, #p<0.05 compared to CL.

Urine and plasma LDH activity

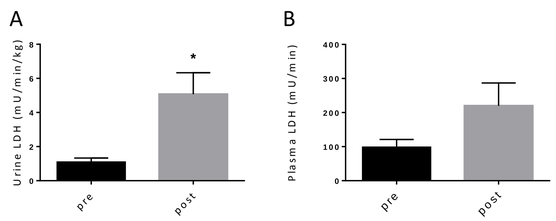


Figure 4 LDH activity analyses from a bilateral IRI rat model: (A) LDH activity in plasma, (B) LDH activity in urine normalized to urine output. *P<0.05 compared to Sham.

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