Metabolic alterations following renal ischemia reperfusion in rats

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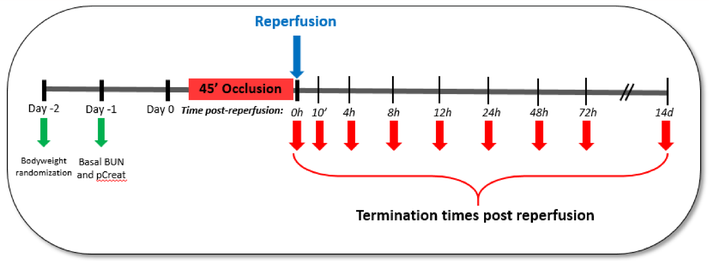
Background

Acute kidney injury (AKI) is a major health issue, associated with high morbidity, mortality, fibrosis and CKD. Ischemia reperfusion (IR) injury during surgical procedures such as coronary artery bypass grafting or transplantation can cause AKI. In this study, we sought to characterize the time course of molecular and cellular pathophysiological consequences of ischemia reperfusion kidney injury in the rat.

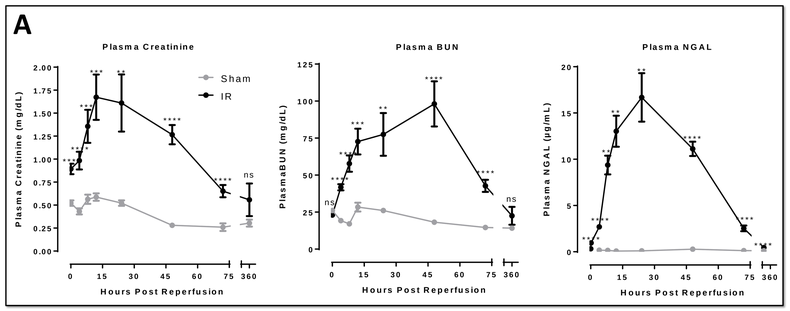
Methods

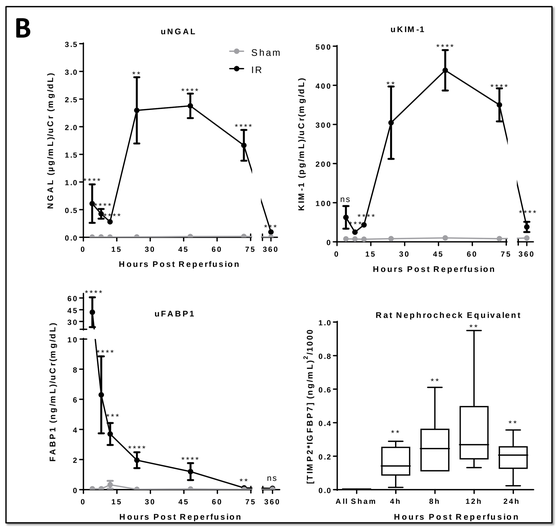
Sprague-Dawley rats underwent 45-minute bilateral renal ischemia followed by reperfusion for 10 minutes, 4, 8, 12, 24, 48, 72 hours or 14 days. Renal injury was characterized by plasma and urine biomarker measurements at all time points. Tubular injury and repair and subsequent development of renal fibrosis were evaluated by semi-quantitative scoring of H&E, Ki67 and Trichrome stained tissue sections. To delineate IR-induced metabolic changes, gene expression of metabolic pathways including pyruvate handling, fatty acid oxidation, oxidative stress, and mitochondrial homeostasis were examined. NAD⁺ utilization and biosynthesis was evaluated using mass spectrometry and ELISA based methods. Statistics obtained from student's t-test of time matched IR vs. Sham. * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001.

Study design



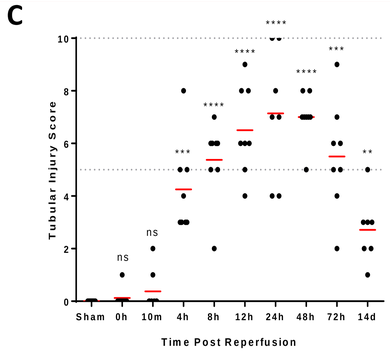
Kinetic profile of Acute Kidney Injury biomarkers

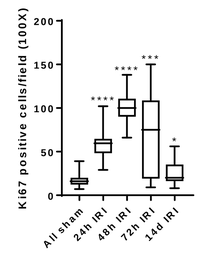


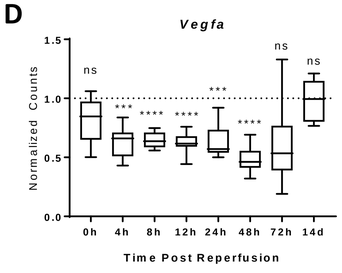


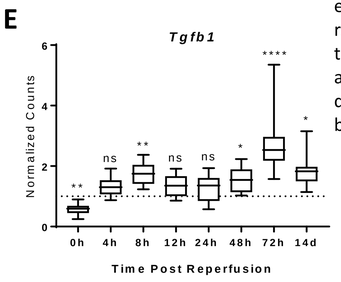
(A) Plasma biomarkers creatinine and NGAL were peaked at 24 hours, whereas the peak of BUN was at 48 hours. **(B)** Urinary biomarkers NGAL and KIM-1 had similar time-dependent profiles peaking at 24-48 hours, whereas urinary FABP-1 excretion peaked at 4 hours followed by rapid decline. The rat Nephrocheck equivalent was consistent with human data, with an increase from 4-24 hours (Kashani, et al., Crit Care. 2013)

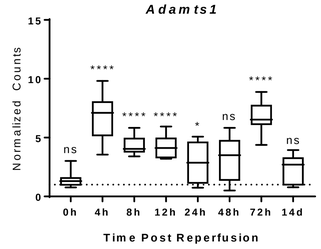
Early induction of tubular and endothelial injury leads to a late fibrotic response

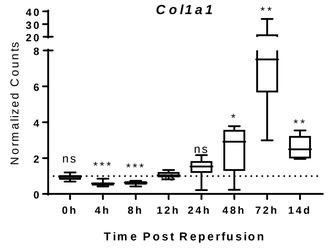




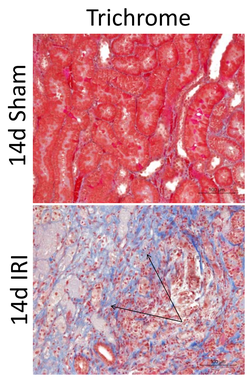




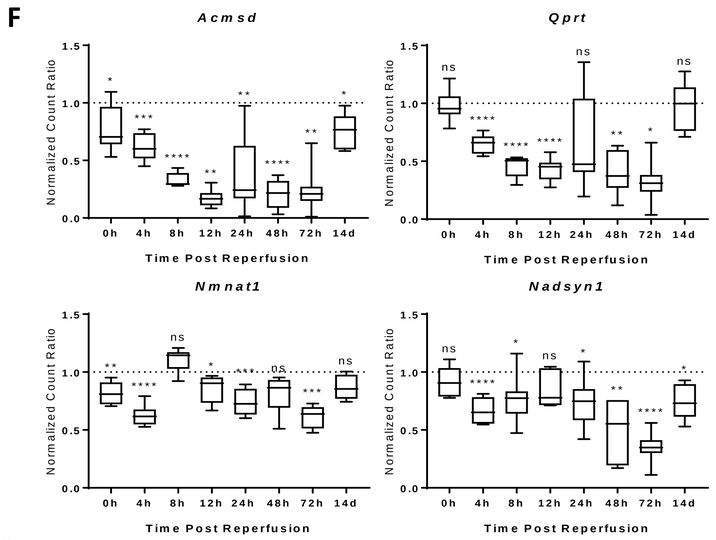


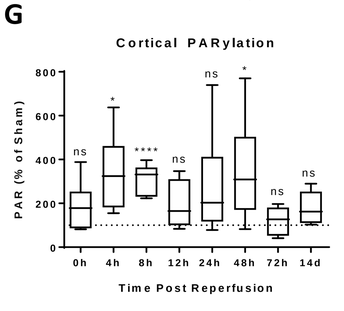


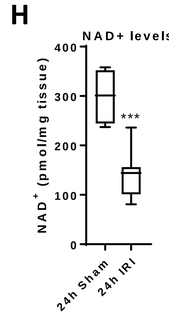
(C) Tubular injury denoted by presence of tubular necrosis and casts in parallel to initiation of repair mechanisms denoted by Ki67⁺ proximal tubular cells. Early injury occurred within 10 minutes of reperfusion for some rats, progressing through 24-72 hours, and was sustained at 14 days. **(D)** Endothelial injury is evident by reduced *Vegfa* with a concomitant increase in VEGFA inhibitor *Adamts1* as early as 4 hours post reperfusion. **(E)** Fibrosis was established by 14 days post reperfusion, mediated by early transcriptional increases in *Tgfb1* and *Col1a1* as well as collagen deposition (Trichrome staining, black arrows) in the kidney.



NAD⁺ metabolism is dysregulated during IR-AKI

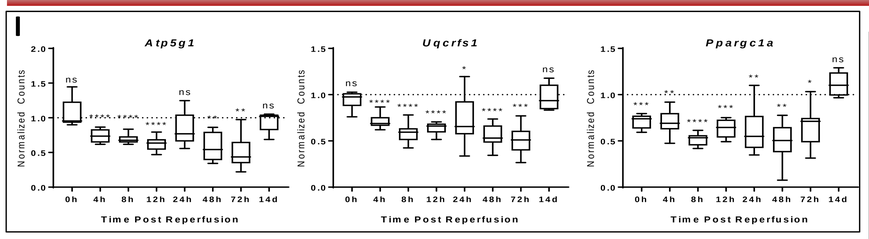


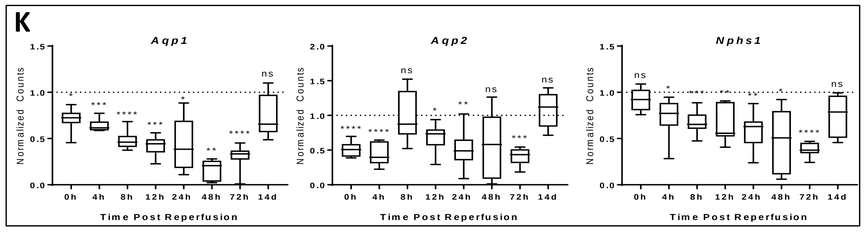


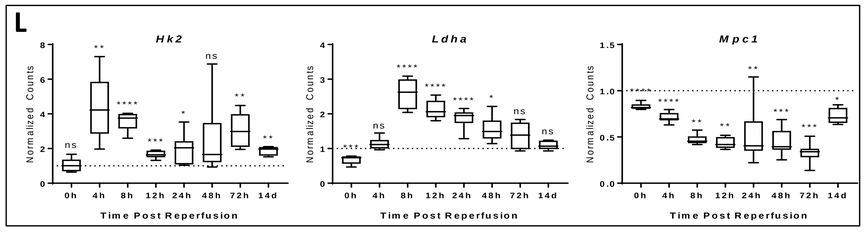


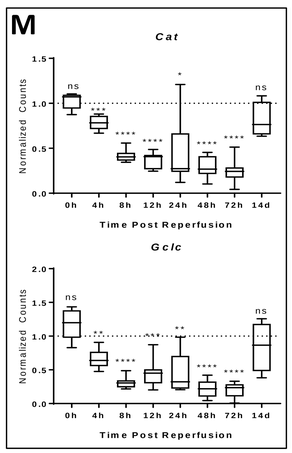
(F) Multiple genes encoding NAD⁺ biosynthetic enzymes are reduced during IRI. **(G)** Increased PARylation is evident as early as 4 hours post-IR suggesting increased NAD⁺ utilization. (H) Reduced synthesis and increased consumption lead to a significant reduction of NAD⁺ at the peak of renal injury (24h).

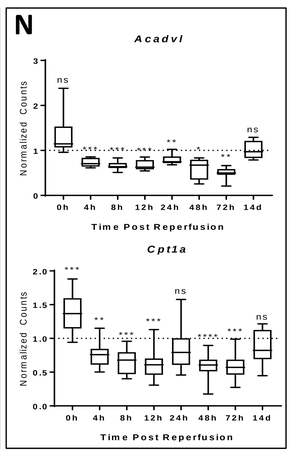
Gene expression analysis reveals persistent dysregulation of mitochondrial homeostasis, fatty acid oxidation, glucose utilization and anti-oxidant handling in IR-AKI











In the renal cortex, reduced gene expression of (**I**) *Atp5g1*, *Uqcrfs1* & *Ppargc1a* indicate sustained reduction of mitochondrial homeostasis. Decreased expression of renal cell type specific genes (**K**) *Aqp1*, *Aqp2* & *Nphs1* suggest loss of proximal, distal tubular cells, and podocytes, respectively. An increase in glycolytic flux is indicated by upregulation of (**L**) *Hk2* and *Ldha,* leading to reduced *Mpc1* expression, suggesting reduced transport of Acetyl-CoA into the mitochondria. (**M**) *Cpt1a*, a fatty acid transport mediator, and the gene responsible for fatty acid oxidation *Acadvl*, are reduced, as are key genes involved in the regulation of the antioxidant response, (**N**) *Cat* and *Gclc.*

Acknowledgments

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