Identification of Urine Metabolic Biomarkers for Hypoxia-Induced Acute Kidney Injury Mouse Model

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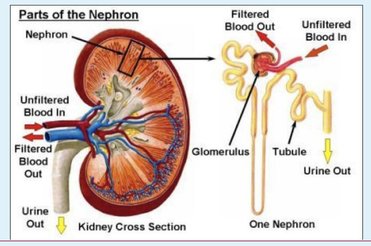
Background

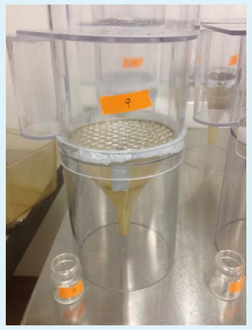
Acute Kidney Injury (AKI) refers to the rapid decline of renal filtration and urine production due to functional and/or structural damage. Kidney damage is often due to sepsis, respiratory failure, heart failure, trauma, major surgery, burns, or toxic insult caused by medications. AKI can lead to severe complications, such an increased risk of chronic kidney disease and damage to other organ systems. Mortality associated with AKI is estimated at 45-70% in intensive care unit patients requiring renal replacement therapy, and more than 2 million people die from AKI each year. Currently available protein biomarkers for AKI do not permit diagnosis prior to significant loss of kidney function, and the most promising new diagnostic tests measure these biomarkers in both urine and serum. The aims of this study are to metabolically profile both urine and serum for biomarkers that allow for earlier detection of AKI and examine the effects of hypoxic-induced AKI on the structure of the kidney in mouse models.



Methods

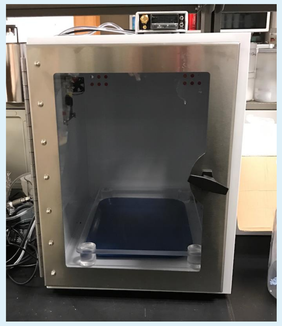
Control mice were placed in metabolism cages[A], and then sacrificed for collection of their urine, feces, and serum. Experimental mice were separated into four groups of twelve at their respective time intervals of 24, 48, 72, and 168 hours, which correspond to the time spent in hypoxic environment[B]. The hypoxic environment consisted of a constant flow of 6.5% oxygen. After the exposure period, the mice were anesthetized for sacrifice. Urine samples were collected before and after the exposure period and analyzed using Nuclear Magnetic Resonance (NMR). Kidney tissue collection occurred for Transmission Electron Microscopy, Scanning Electron Microscopy, and histological analysis. Additionally, serum was collected and analyzed using NMR.

A



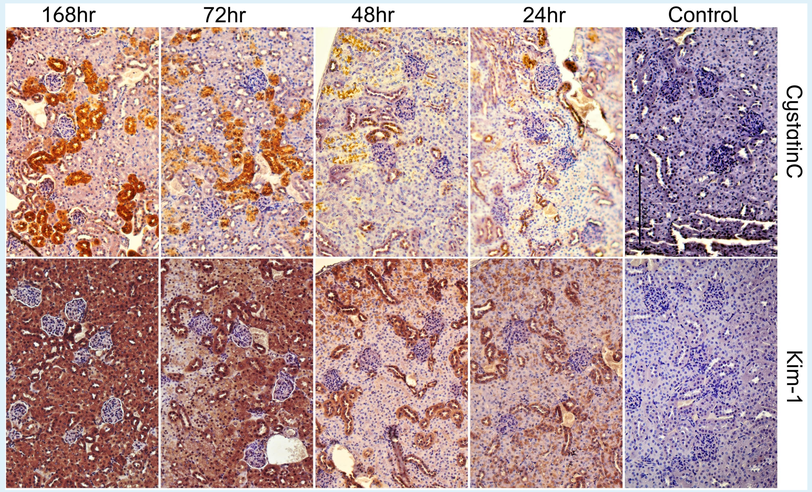
Metabolism Cage

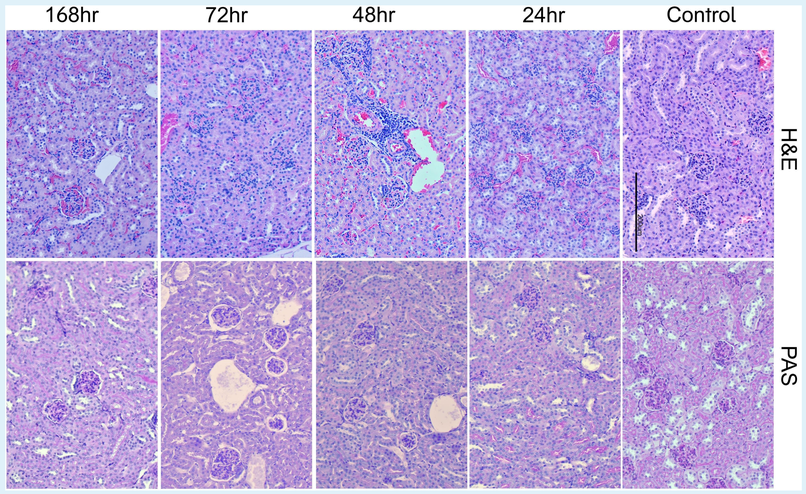
B



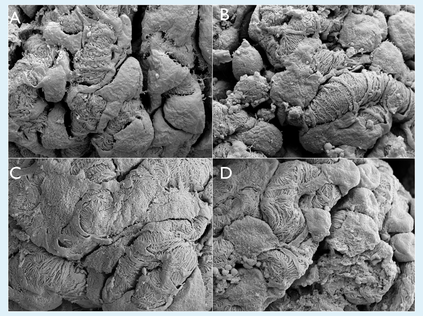
Hypoxia Chamber

Results





	Serum Creatinine	
	Average	Standard Deviation
Control	0.11	
24hrs	0.40	0.10
48hrs	0.33	0.036
72hrs	0.35	0.049
7days	0.32	0.051



Scanning Electron Microscopy Images of Glomerulus
All SEM images were taken at 10,000x, working distance 9.8 mm, and an accelerating voltage of 10 KeV. Image A is of a control mouse glomerulus. Image B is of a study mouse of 24 hours glomerulus. Image C is of a study mouse of 48 hours glomerulus. Image D is of a study mouse of 72 hours glomerulus.

Discussion

Immunohistochemical analysis of the kidney demonstrated an increase in expression of KIM-1 and Cystatin-C using known protein biomarkers. Hematoxylin and Eosin (H&E) staining showed no significant difference of kidney structure after damage, while Periodic Acid Schiff's (PAS) stain also did not show structural differences. Serum Creatinine Levels showed a decline through the progression of time increments indicating that damage in fact did occur. Scanning Electron Microscopy images showed no significant difference of damage on the exterior of the glomerulus. Although damage occurred, structural damage of the kidney tissue was insignificant; therefore, the results mimic clinical examples of Chronic Obstructive Pulmonary Disease (COPD) and sleep apnea.

References

1. Jun JC, Shin M, Yao Q, Bevans-Fonti S, Poole J, Drager LF, Polotsky VY. Acute hypoxia induces hypertriglyceridemia by decreasing plasma triglyceride clearance in mice. *Am J Physiol Endocrinol Metab* 303: E377-E388, 2012.
2. Jun J, Reinke C, Bedja D, Berkowitz D, Bevans-Fonti S, Li J, Barouch LA, Gabrielson K, Polotsky VY. Effects of intermittent hypoxia on atherosclerosis in apolopoprotein E-deficient mice. *Atherosclerosis 209.2:* 381-386, 2010.
3. Tarnavski O, McMullen JR, Schinke M, Nie Q, Kong S, Izumo S. Mouse cardiac surgery: comprehensive techniques for the generation of mouse models of human diseases and their application for genomic studies. *Physiological Genomics 16(3):* 349-360, 2004.

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