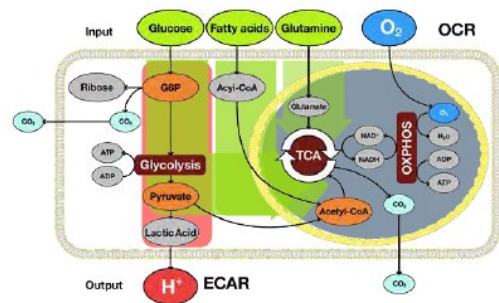
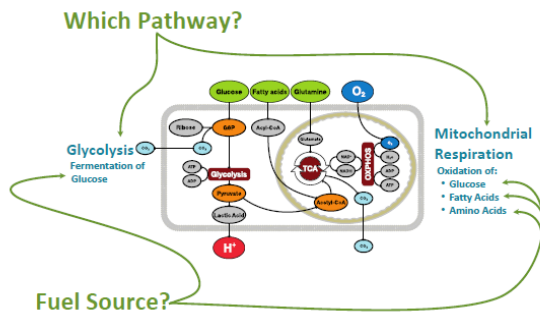


Comprehensive assessment of energy metabolism pathways

In the **Renal Physiology Sub-Core** of **Core B Pre-clinical Studies of AKI**, Dr. Prabhleen Singh's laboratory has developed novel techniques for comprehensive assessment of energy metabolism pathways as described below.

Cellular Energy Metabolism Pathway Assessment:

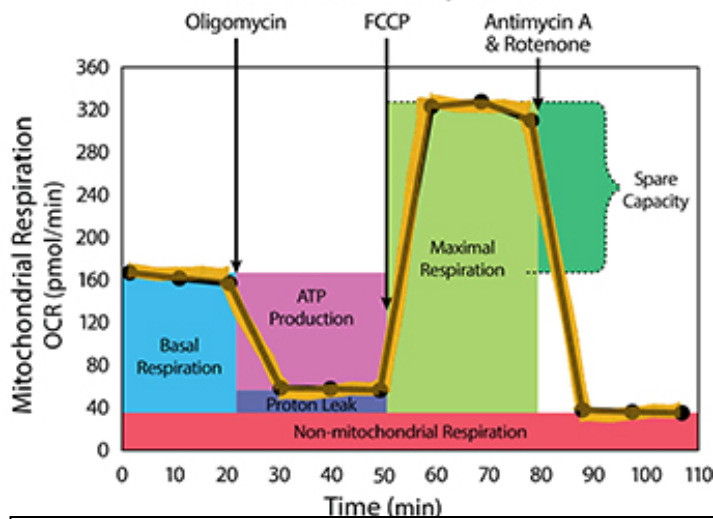
- Mitochondrial bioenergetics and respiration
- Glycolysis assessment (basal, compensatory, capacity and reserve)
- Substrate utilization for energy (ATP) generation



Cellular energy metabolism pathways (adapted from Agilent Seahorse XF96 User Guide)

Mitochondrial Bioenergetics and Respiration

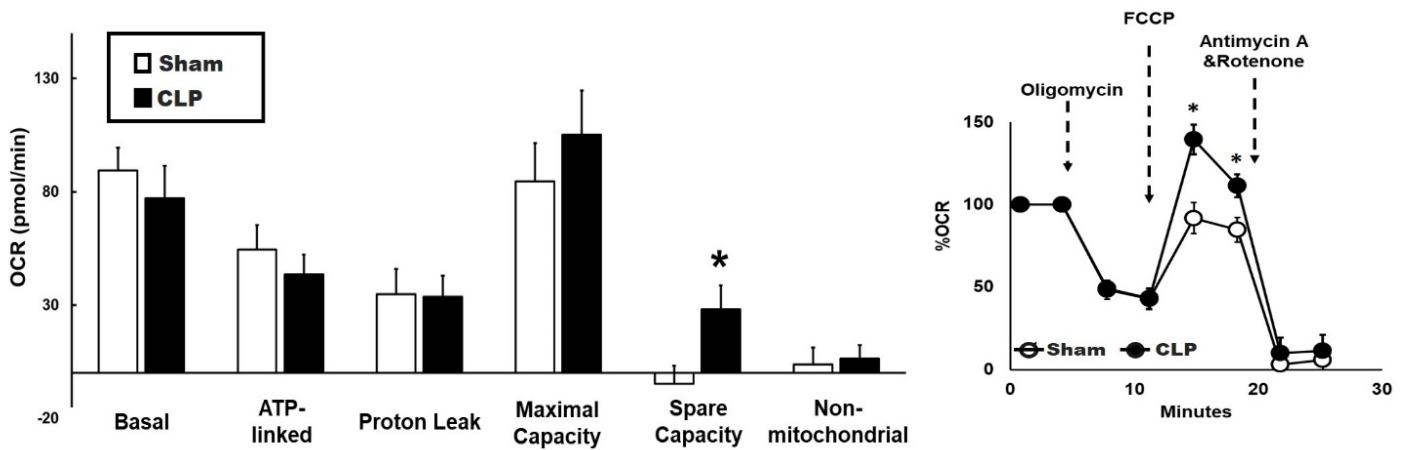
- High throughput respirometry with Agilent Seahorse XF96 extracellular flux analyzer in isolated mitochondrial and isolated cells (e.g., proximal tubules).
- Mitochondrial oxygen consumption rates (OCR) are measured sequentially utilizing oxidizable substrates and in the presence of inhibitors of complex I (Rotenone) and III (Antimycin).



The key parameters measured:

- basal OCR,
- ATP-linked OCR,
- maximal OCR,
- spare or reserve capacity,
- nonmitochondrial OCR

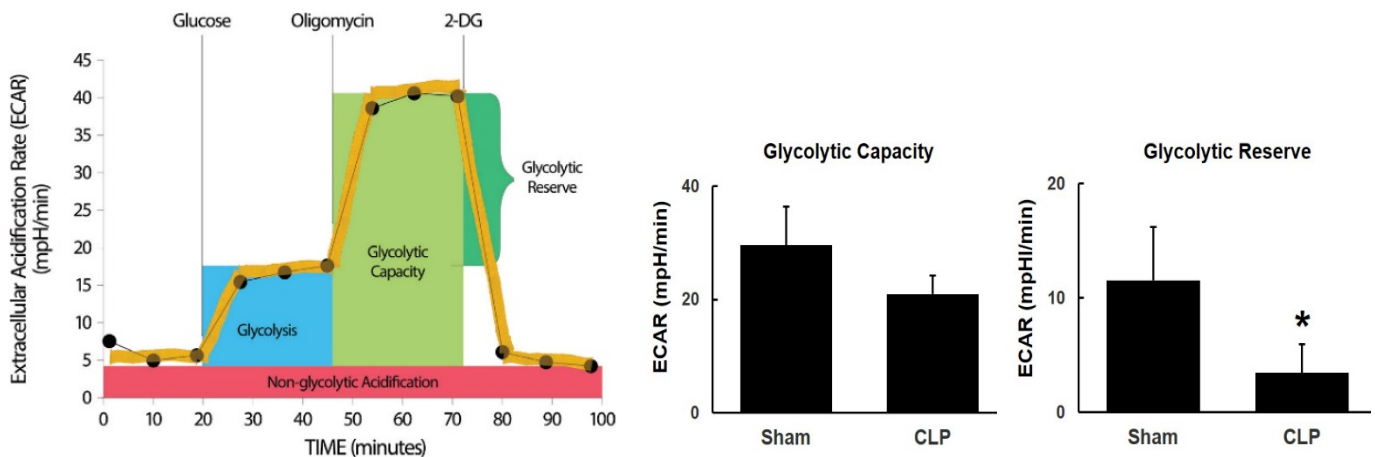
Mitochondrial Respiration profile (adapted from Agilent Seahorse XF96 User Guide)



Proximal Tubular Mitochondrial Bioenergetics in Sepsis-AKI. Mitochondrial respiration in isolated tubules from sham and mice with cecal ligation and puncture (CLP). Significant increase in spare capacity in the CLP compared to sham tubules. After normalization to basal respiration, significant increase in FCCP-induced maximal OCR observed in CLP compared to sham tubules. *P<0.05 vs. sham. (1)

Cellular Glycolysis Assessment: Extracellular acidification rate (ECAR) measurement

- Measurement of extracellular acidification from the generation of protons during conversion of glucose to lactate in glycolysis.
- ECAR measured sequentially at basal level, with glucose and in the presence of ATP synthase inhibitor (Oligomycin) and glycolysis inhibitor (2DG) with XF96 Seahorse extracellular flux analyzer and Glycolysis Stress Assay
- The key parameters measured sequentially:
 - Basal ECAR
 - Glycolysis rate
 - Maximum glycolytic capacity
 - Glycolytic reserve



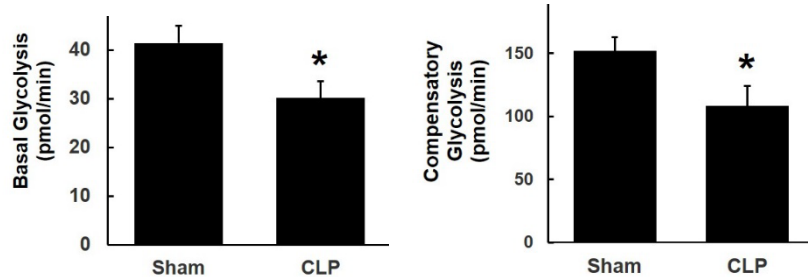
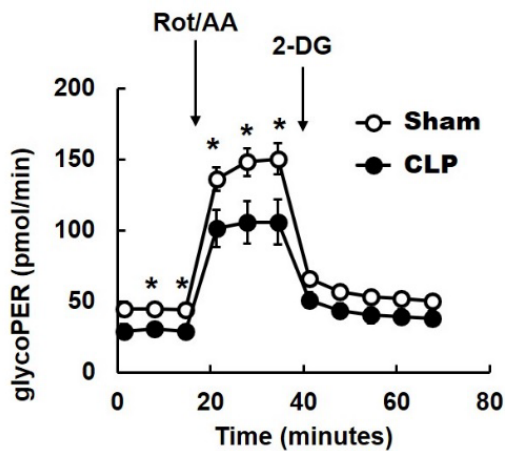
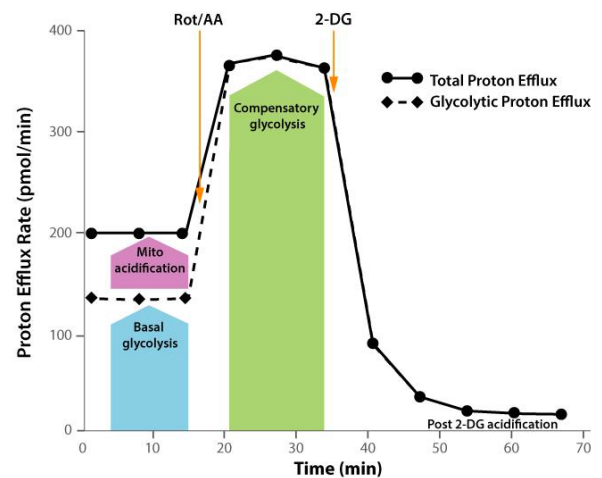
Proximal Tubular Glycolysis in Sepsis-AKI: Glycolysis measured in fresh isolated tubules from sham and mice with cecal ligation and puncture (CLP) using Seahorse XFe96 extracellular flux analyzer. Agilent Seahorse XF Glycolysis Stress Test Profile. Glycolytic capacity=Maximum ECAR after oligomycin. There was a trend for decrease glycolytic capacity in CLP proximal tubules. Glycolytic reserve= glycolytic capacity-basal glycolysis rate. Glycolytic reserve was significant reduced in CLP proximal tubules. *P<0.05 vs. sham.

Li Y, Nourbakhsh N, Pham H, Tham R, Zuckerman JE, **Singh P.** Evolution of Altered Tubular Metabolism and Mitochondrial Function in Sepsis Associated Acute Kidney Injury. *Am J Physiol Renal Physiol.* 2020 Aug 1;319(2):F229-F244

Cellular Glycolysis Assessment: Proton Efflux Rate (PER) measurement

- Measurement of protons exported by cells into the assay medium over time.
- PER measured at basal conditions and following rotenone and antimycin A (complex I and II inhibitors) and 2-DG (glycolysis inhibitor) with XFe96 Seahorse extracellular flux analyzer with Glycolytic Rate Assay

Seahorse XF Glycolytic Rate Assay Profile



Proximal Tubular Glycolysis in Sepsis-AKI: Glycolysis measured in fresh isolated proximal tubules from sham and mice with cecal ligation and puncture (CLP) using Seahorse XFe96 extracellular flux analyzer. Agilent Seahorse XF Glycolytic Rate Assay profile (adapted from Agilent Seahorse XF Glycolytic Rate Assay Kit User Guide). Glycolytic PER determined by the subtraction of mitochondrial acidification from Total PER. Both basal GlycoPER and following Rot/AA significantly reduced in CLP tubules. Basal glycolysis and compensatory glycolysis significantly reduced in CLP tubules. *P<0.05 vs. sham.

Li Y, Nourbakhsh N, Pham H, Tham R, Zuckerman JE, **Singh P.** Evolution of Altered Tubular Metabolism and Mitochondrial Function in Sepsis Associated Acute Kidney Injury. *Am J Physiol Renal Physiol.* 2020 Aug 1;319(2):F229-F244

References:

- 1) Li Y, Nourbakhsh N, Pham H, Tham R, Zuckerman JE, **Singh P**. Evolution of Altered Tubular Metabolism and Mitochondrial Function in Sepsis Associated Acute Kidney Injury. *Am J Physiol Renal Physiol*. 2020 Aug 1;319(2):F229-F244