Mitochondria, Sepsis and Organ Dysfunction

John F Fraser
Critical Care Research Group, Brisbane, Australia
First Time in Canada

How Canadians Are Born
Intensive Care Unit

Common ICU presentations:

- Sepsis
- Respiratory
  - ARDS
- Cardiovascular
  - Ischaemia/Infarct, arrhythmias
- Neurologic
  - Stroke/trauma
- Renal
  - Renal failure
- Post-operative Care

- Both inflammation and mitochondrial dysfunction play a significant roles in all common conditions, exacerbating pathophysiology and impeding treatment

Critical Care Research Group current focus

- Fluid resuscitation
- ARDS
- Heart Failure/Transplantation
- ECMO
- TAVI
- Acute kidney injury

Griffith University
Metro North Hospital and Health Service
QUT
Queensland University of Technology

criticalcare
research group
Mitochondria

• Powerhouse of the cell

• Roles in energy production, Ca\(^{2+}\) homeostasis, ROS elimination, cell death, thermoregulation

• Tissue injury → rupture of cell membranes → release of mitochondrial contents (mtDNA, DAMPs, ROS, cytochrome c etc) → widespread tissue damage

Heart failure
Ageing
I-R injury
Arrhythmias
Neurodegeneration
Sepsis
ARDS
ECMO
Transplantation
Chronic kidney disease
Cancer
Mitochondrial diseases
Mitochondria and Inflammation

Mitochondria damaged in response to sepsis

ROS from mitochondria may worsen tissue damage

May facilitate hibernation
Mitochondria and Inflammation

- Mitochondrial dysfunction promotes inflammation
  - *Decreased ATP production*
  - *Poor Ca\(^{2+}\) handling* – apoptosis and necrosis
  - *Release of DAMP’s*
    - mtDNA
    - ATP
    - Succinate
    - mtROS production
Mitochondria and Inflammation

- Inflammation alters mitochondrial function
  - IL-1β, TNF, IL-6 – known to induce fragmentation – mitophagy
  - ROS mediates inflammasome activation – mtDNA release

Protti & Singer, Critical Care (2006)
How are mitochondria affected during sepsis

Bioenergetic function and Oxygen-dependent oxidative phosphorylation

Biosynthetic functions

Immune functions

Oxidative stress

Cell death
Fission and Fusion

Fission – damaged Mit isolated and removed to minimize negative effect on network

Fusion – Mildly damaged Mit brought into the network and assists function

Imbalance of these two can result in cell injury
Self Cleaning Mechanism

- Auto/mitphagy – Debris isolated within phagosome and taken to lysosome for safe destruction
- Failure of this mechanism in rats worsens outcome
- Enhancing autophagy conversely improves organ function
Biogenesis

• Production of new mitochondria for ATP, calcium homeostasis, maintenance of cellular redox state, cell signalling

• Septic pts with higher levels of PGC-1α (co-activator) have higher survival, more ATP

• Pre Clinical - more biogenesis correlates with improved mito respiration
Sepsis

- Initiated by PAMP’s and DAMP’s leading to “cytokine storm”
- **Simultaneous** immunosuppression and hyperinflammation
- Persistent inflammation

Sepsis - Pathophysiology

- PRR Activation
- Pro- & Anti-Inflammation
- Immunosuppression

Active Research Areas

Mitochondrial Dysfunction

Multiple Organ Failure

Tissue ischaemia

Nanez-Verona et al, 2016
Sepsis - Mitochondria

1. Impaired Perfusion → Hypoxia → depletion of ATP
   Excessive NO, CO, H₂S → Inhibit respiration & damage mito proteins particularly Complex I

2. Generation of ROS → Decreased T3 “sick euthyroid” → modulates mito respiration

3. Hormonal Alterations → Downregulated early in inflammatory response

4. Mitochondrial Gene Alterations

Singer M., Virulence, 2018
ARDS

Mitochondrial Dysfunction

- Disrupted membrane potential
- $\uparrow$ Intracellular $\text{Ca}^{2+}$
- $\uparrow$ Bax expression
- $\uparrow$ Caspase 3
- $\uparrow$ Cytochrome c
- $\uparrow$ ROS
- $\downarrow$ ATP
- $\uparrow$ NO

Inflammation

ARDS
Emerging aspect in ARDS pathology: Mitochondrial damage-associate molecular patterns (mtDAMPs)

ARDS

↑ Neutrophils

mtDAMPs

mtDNA
formyl peptides
cardiolipin

↑ Lung endothelial permeability

↑ Mortality

Systemic Inflammatory Response Syndrome
Emerging aspect in ARDS pathology: Mitochondrial damage-associated molecular patterns (mtDAMPs)

Circulating cell-free mtDNA were ↑ in patients
- who died within 28 days of medical ICU admission
- in ICU patients with sepsis or ARDS
- in patients with severe trauma or sepsis in the emergency room
Renal Mitochondria

Kidneys require 10% of the body’s total energy production
- Energy required to regulate solute re-absorbance.

↑ renal energy demands = kidneys rich in mitochondria

Healthy mitochondria are essential for proper renal function
Kidney Injuries

Higher **energy demands** = renal failure due to mitochondrial conditions.

- Genetic conditions in infants

Reverse is also true, **acute kidney injuries** associated with mitochondrial dysfunction.
Kidney injuries

Kidney Injury
AKI
CKD
Kidney Tumours

Mitochondrial Dysfunction
↓ Electron Transport Chain Function
↓ Metabolite Transport

↓ Cellular Energy Production
↓ Solute Re-absorbance
↑ Apoptosis
↑ ROS Production

Renal Failure

Associated
Mitochondria have a crucial role in neutrophil biology

- Neutrophil function few mitochondria but dependent on them
- If mitochondrial MP damaged, as occurs in sepsis, neutrophils chemotaxis almost stops
- Neutrophil releases its arsenal to vessel wall
Other immune cells affected

- Macrophages – M1:M2 ratio
- Experimental ARDS diminished if Mit remain stable
- T Cells – Transition from Naïve to Memory is dependant on cellular metabolism
Energy Demand vs. Supply

- Health
- Heart failure

Contributors to energy demand:
- Synthesis, transport, phosphorylation, pumps
- Heart rate
- Relaxation
- Contraction

Contributors to energy supply:
- Glycolysis
- Mitochondria
  - Oxidative phosphorylation

Restored mitochondrial function:
- Cardiolipin
- Redox-buffering capacity
- ETC (super) complexes
- Biogenesis

Nature Reviews | Cardiology


Meta North Hospital and Health Service

The University of Queensland

Critical Care Research Group
Cardiac Issues in Sepsis

Review
The role of mitochondria in sepsis-induced cardiomyopathy

Giacomo Stanzani\textsuperscript{a}, Michael R. Duchen\textsuperscript{b}, Mervyn Singer\textsuperscript{a,\textdagger}

\textsuperscript{a}Griffith University, \textsuperscript{b}Metro North Hospital and Health Service, \textsuperscript{c}Queensland University of Technology

FEAST Maitland et al
UNINTENDED CONSEQUENCES: FLUID RESUSCITATION WORSENS SHOCK IN AN OVINE MODEL OF ENDOXEMIA

Liam Byrne¹,²,³, Nchafatso G. Obonyo¹, Sara D. Diab¹, Kimble R. Dunster¹,⁴, Margaret R. Passmore¹,⁵, Ai-Ching Boon¹,⁵, Louise See Hoe¹,⁵, Sanne Pedersen¹, Mohd Hashairi Fauzi⁶, Leticia Prett Pimenta¹, Frank Van Haren²,³,⁷, Christopher M. Anstey⁸, Louise Cullen⁵,⁹, John-Paul Tung¹,¹⁰, Kiran Shekar¹,¹¹, Kathryn Maitland¹², and John F. Fraser¹,⁵,¹¹

¹Critical Care Research Group and ¹¹Adult Intensive Care, The Prince Charles Hospital, Brisbane, Australia; ²Intensive Care, Canberra Hospital, Garran, Australia; ³Australia National University, Canberra, Australia; ⁴Queensland University of Technology, Brisbane City, Australia; ⁵University of Queensland, Brisbane, Australia; ⁶School of Medical Sciences, Universiti Sains Malaysia Health Campus, Kelantan, Malaysia; ⁷University of Canberra, Bruce, Australia; ⁸Intensive Care, Sunshine Coast University Hospital, Birtinya, Australia; ⁹Royal Brisbane and Women’s Hospital, Herston, Australia; ¹⁰Australian Red Cross Blood Service, Brisbane, Australia; and ¹²Department of Paediatrics, Faculty of Medicine, Imperial College London, London, United Kingdom

At A Glance Commentary

**Scientific Knowledge on the Subject:** Fluid resuscitation is a common therapy used for the treatment of septic shock.

**What This Study Adds to the Field:** This study tests the effectiveness of the therapy in an animal model of sepsis and finds that it is actually harmful.
Microcirculation

Perfused vessel density, PVD (mm/m²)

Fraser et al. (RESUS trial)
Vasopressor dose (noradrenaline)

Less vasopressor needed, no saline group

4 hrs

Fraser et al. (RESUS trial)
Brain death and HTx

Brain Death

Systemic Inflammatory Response

Neurohormonal Dysfunction + Haemodynamic Instability

Microvascular Damage

Cardiac / Vascular Damage

↓ Heart Transplant Outcomes


RESEARCH
Open Access

Novel 24-h ovine model of brain death to study the profile of the endothelin axis during cardiopulmonary injury

Ryan P. Watts1,3,4,5, Izabela Bilska1,2, Sara Diab1, Kimble R. Dunster1,3, Andrew C. Bulmer2, Adrian G. Barnett3 and John F. Fraser1,3,4
Ovine model of BSD-HTx

Animals

Female sheep (45-55 kg)
- 6 x BSD-HTx
- 4 x Sham-HTx

Ischaemic Times:

Cold ischaemia: 100 mins
Total ischaemia: 170 mins

Sampling (progressive)

- Blood (arterial and coronary sinus) and urine
- Echocardiography
- Microcirculation (SDF)
- Tissue analyses (*mitochondrial assessment*)
- Hearts collected at end 24 hrs BSD/SHAM OR ≥ 6 hrs off CPB
Assessing Mitochondrial Function

Sample Collection: 3 x Needle Biopsies

Basal  
Lateral  
Apical  

Pooled tissue homogenisation

Functional Assays:

1. Respiration utilising carbohydrates (CHO)
2. Respiration utilising fatty acids
Cardiac mitochondrial respiration in BSD and HTx

- Post-HTx BD hearts:
  - Significantly higher LEAK for CHO substrates

Elevated oxygen consumption that does not contribute to energy production (ROS production)

Preliminary data courtesy of Matthew Wells
Cardiac mitochondrial respiration in BSD and HTx

- **Post-Tx BD hearts:**
  Significantly lower ETS efficiency for CHO substrates

**Right Ventricular ETS Coupling Efficiency**

LEAK + Poor ETS Capacity = **Mitochondrial Dysfunction** = impaired cardiac performance

Preliminary data courtesy of Matthew Wells
Peptide **Hi1a** from funnel-web spider venom is the most potent known inhibitor of ASIC1a

**Hi1a** is highly stable in blood and cerebrospinal fluid

**Hi1a** known to protect the brain - 60% reduction in Stroke size given upto 6 hrs

We have now shown that it also protects cardiac tissue

Preliminary data courtesy of Matthew Wells
Hi1a protects whole organ viability after ischemia reperfusion injury in Langendorff retrograde perfused mouse hearts

Improved left ventricular developed pressure

Reactive hyperemia

Decreased end-diastolic pressure

Preliminary data courtesy of Meredith Redd
ASIC1a inhibition prevents hiPSC death during ischemic/acidosis *in vitro*

Preliminary data courtesy of Dr Meredith Redd
ASIC1a inhibition prevents pH induced elevated intracellular calcium

[schematic adapted from Hu et. al.]

High-throughput calcium imaging with real time drug addition and pH stimulus

Preliminary data courtesy of Dr Meredith Redd
Conclusions

• Mit importance seems biphasic – cause and effect

• Adaptive vs Maladaptive

• Improved mitochondrial function generally associated with better outcomes

• Has general and organ specific effects

• Potential game changers may include cardiac Rx
Thanks

Donald and Joan Wilson Foundation

Metro North Hospital and Health Service

Queensland University of Technology

Griffith University

Critical Care Research Group