RCoA Research, Education & Travel Grants 2018

Award: Ernest Leach Fund

Applicant: Dr Jia Stevens

Project Title: *Investigating variations in platelet mitochondrial function in healthy subjects*

Project Description:

Mitochondria are small organelles found in almost every cell within our body. It is a vital component of our cellular machinery, involved in energy production, reactive oxygen species synthesis, calcium homeostasis and even the control of the life span of a cell itself. The process of mitochondrial respiration uses carbon rich compounds consumed from our diet to produce electrons, this subsequently drives the production of ATP we use to function, through the use of oxygen as the final electron acceptor (1). To probe the capacity of mitochondrial respiration, a technique called high resolution respirometry (HRR) can be used (Oxygraph, Oroboros instruments, Austria) (2). Different types of samples can be tested, however this frequently involves invasive biopsies. Our laboratory currently uses the HRR technique to characterise the changes in mitochondrial function during critical illness and major surgery, using human skeletal muscle biopsies and peripheral blood mononuclear cells, to understand the differences in metabolic phenotypes that lead to survival and recovery. Changes in mitochondrial function have been linked to survival in critical illness, however very little is known regarding the changes during the perioperative period, and its longitudinal effects. From our pilot data we have been able to demonstrate alterations in tissue oxygen consumption during the operative period, as a reflection of changes in mitochondrial respiration (3).

We would like to further develop techniques to study cellular alterations in platelet respiration, which have been found to reflect overall disease burden, and effects of aging within the body. Thus, may serve as a potential biomarker of systemic mitochondrial function (4). Using the technique of graded centrifugation through easily accessible blood extractions, platelet isolation can be performed. The samples are tested ex-vivo within one-hour of extraction, which will offer a rapid physiological reflection of mitochondrial function. A few clinical studies have demonstrated the success of using this technique, to characterise changes in mitochondrial respiration in diseased states (5,6). We propose to develop a robust protocol using blood from healthy volunteers, to form a basis to study the metabolic responses during the perioperative period, and develop a further understanding into the responses generated during pathological stress.

The aims of our study

- To explore the use of HRR to probe platelet respiration.

• To use flow cytometry to validate our platelet isolation technique.
• To compare physiological inter-individual differences in platelet mitochondrial respiration.
• To develop a minimally invasive, ex-vivo technique to investigate human mitochondrial function, forming the basis of metabolic phenotyping.

Methodology

• 15 healthy adult human subjects will be recruited from the Royal Free Hospital Campus. We will approach hospital staff and students. Full consent will be obtained (Ethical approval is currently being sought from UCL).
• Basic demographic information will be collected.
• 18mls of blood will be collected by phlebotomy.
• Platelets will be isolated using graded centrifugation.
• Platelets will be isolated and tested immediately for HRR.
• Samples of isolated platelets will be further characterised using flow cytometry.
• Samples will be assayed to ascertain mitochondrial content.

References

1. Muravchick, S. et al. Clinical Implications of Mitochondrial Dysfunction. Anesthesiology 2006;105(4):819-837.
2. Pesta D. et al. High-resolution respirometry: OXPHOS protocols for human cells and permeabilized fibers from small biopsies of human muscle. Methods Mol Biol. 2012;810:25-58. doi: 10.1007/978-1-61779-382-0_3.
3. Stevens, J.L. et al. Effects of major hepato-pancreatico-biliary surgery and general anaesthesia on skeletal-muscle mitochondrial respiration: a pilot study
British Journal of Anaesthesia, Volume 121, Issue 2, e18
4. Sjövall, F. et al. Mitochondrial respiration in human viable platelets-methodology and influence of gender, age and storage. Mitochondrion. 2013 Jan;13(1):7-14. 1.
5. Sjövall, F et al. Temporal increase of platelet mitochondrial respiration is negatively associated with clinical outcome in patients with sepsis. Critical Care, 2010, 14(6), R214. http://doi.org/10.1186/cc9337
6. Puskarich, M.A. et al. Early alterations in platelet mitochondrial function are associated with survival and organ failure in patients with septic shock. Journal of critical care. 2016;31(1):63-67. doi:10.1016/j.jcrc.2015.10.005.