ECR First Contact Initiative Grant Report

Dear ESC Council Members,

I want to say a huge thank you for supporting the proposed project and providing me with the opportunity to stay in Pavia for three weeks to build a new collaboration with Prof Simone Porcelli.

Background

The microvascular bed is (in my opinion) the most important tissue in the human body. It is involved in the supply of oxygen and nutrients, the removal of waste products, and delivery of cells crucial to tissue maintenance and repair. It is especially important in skeletal muscle as a functional determinant of endurance performance (Tickle et al., 2020), for example, exercise tolerance is tightly correlated with capillary density (CD). Over the last decade there has been a pronounced increase in research into the changes in microcirculation of skeletal muscle across various disease/pathological populations. For example, heart failure (Espino-Gonzalez et al., 2021), diabetes (Dunford et al., 2017) and spinal cord injury (Warren et al., 2020) populations have all been found to have reduced vascular supply in the skeletal muscle. Though the impaired microvascular supply is thought to be a secondary response to the initial condition, it now forms a primary barrier to successful intervention as these patient populations present with high levels of exercise intolerance.

The ability to quantify changes in skeletal muscle microvascular composition is a valuable tool in the characterization of disease progression and in the development/assessment of rehabilitation therapies. Quantifying changes in skeletal muscle capillarity is typically performed on histological sections of skeletal muscle, which is an invasive procedure in humans, and not routinely available to many physiology labs. However, Pilotto et al. (2022) has developed a unique near-infrared spectroscopy (NIRS) protocol that may make it possible to estimate O_2 diffusive capacity (an indirect measure of histologically derived CD). This unique approach gives promise to a cost effective and non-invasive way to assess muscle diffusive capacity *in vivo*.

While Pilotto et al. (2022) showed there to be a reasonably strong relationship between the NIRS derived estimate of tissue diffusivity and CD, CD is not the best structural descriptor of oxygen diffusivity. Here in Figure 1, I present hypothetical data (Figure. 1A) with two identical CD profiles (homogeneous *vs.* heterogeneous), which result in two different oxygen profiles across the tissue. CD does not account for the spatial distribution of the microvasculature which is integral to oxygen diffusion. In addition to the local capillary distribution, tissues oxygen demand (i.e. muscle fibre phenotype) has a substantial bearing on O_2 diffusivity (Figure. 1B). We have recently developed a histological analysis pipeline (Al-Shammari et al., 2019) that provides measures of local capillary distribution and allows for mathematical modelling of O_2 transport kinetics.

Therefore, the proposed aims of this ECR First Contact Initiative Grant were to attend Prof Simone Porcelli's lab at the University of Pavia to (1) recieve training in the use of NIRS as a non-invasive approach to assess tissue oxygenation, and specifically their newly developed protocol for assessing tissue oxidative capacity and oxygen diffusivity. (2) Secondly, I proposed a further refinement and validation of the published methodology (Pilotto et al., 2022) by generating measures of local capillary supply using our published open access Oxygen Transport Modeller (Al-Shammari et al., 2019), we could further explore the descriptive power of the NIRS approach.

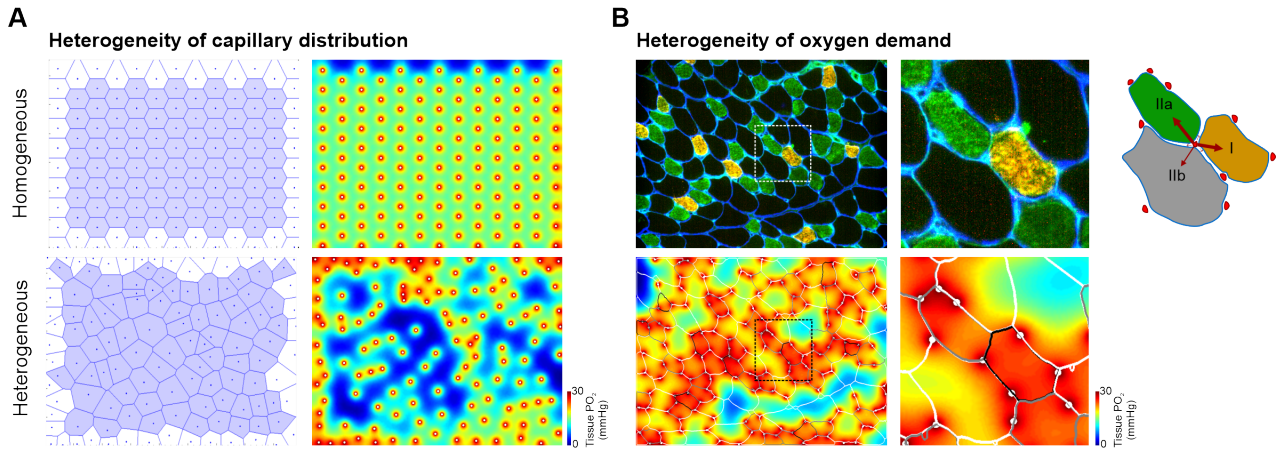

Figure 1. (A) The implication of capillary heterogeneity on tissue PO_2. (B) In addition to heterogeneous capillary supply distribution, muscle fibre type and thus oxygen demand differs across a muscle.

Knowledge Exchange (i.e. my time in Pavia)

1. Learning NIRS

The main aim of my ESC First contact Initiative Grant was to learn how to use the near-infrared spectroscopy (NIRS) method to indirectly assess the oxidative capacity and diffusivity potential of skeletal muscle. Over the three weeks I was in Prof Porcelli's lab we managed to recruit and perform the full experimental protocol (VO_2 Max ramp test, mVO_2 measures using NIRS and a muscle biopsy of the vastus lateralis, Figure 2A-B) from four individuals (one of those being me!). Each of these protocols are themselves not time consuming, but allowing sufficient time between the maximum ramp test, recording days and the muscle biopsy means that this approach is complete over several days. I have always felt that the use of NIRS, effectively and meaningfully was a dark art not utilised by many labs, but the expertise of those in the lab using this technique made the whole learning experience feel incredibly 'simple'. During my three weeks visit I was fortunate enough to also shadow two of the lab members at Parma Hospital where Prof Porcelli currently has a longitudinal ageing study taking place where they are using NIRS (in conjunction with a host of other cardio-respiratory measures). This exposure and training opportunity has provided me the ability to establish and undertake these measures back at my host institution (University of Liverpool).

2. Provide training in histology processing and Modelling of tissue PO2.

Over the course of my three-week trip, I provided training in histological staining, processing and analysis of the microvascular bed in human skeletal muscle samples. During this time, we compared our own histological protocols on our newly sampled biopsies. One of the main differences in our approach was the marker used to identify capillaries. We have shown previously that in rodent skeletal muscle the use of alkaline phosphatase, platelet endothelial cell adhesion molecule (PECAM-1, aka CD31) and biotinylated Griffonia Simplicifolia Lectin I can lead to differences in quantitative global measures of microvascular supply (Kissane & Egginton, 2019). However, to our knowledge this has not been tested in humans. Therefore, across the four subjects we have recruited and completed muscle biopsies on, we will be comparing the two different capillary markers, to see if any such variation in global and local capillary indices exist.

A B C

Figure 2. (A) I completed the three-part experiment with a VO_2 Max ramp test and the NIRS recordings followed by a muscle biopsy (B). I also delivered a departmental talk to those in the Institute of Physiology (C) in their beautiful Physiology-Library. This is also the where I provided the training seminar on histological processing of global and local capillary indices, and the use of the Oxygen Transport Modeller (Al-Shammari et al., 2019).

Future Direction

During my time in Pavia, I processed 13 previously sampled histological sections and have now 4 new samples to add to this data set. The next step in our new collaboration is to explore if there exists any further correlation of NIRS estimates of diffusivity with the new measures of local capillary supply. This work we hope will form the basis of a scientific manuscript for submission into a high impact, peer reviewed physiology journal.

All that is left for me to say is a huge thank you to Simone, and his lab who were unbelievably kind made me feel incredibly welcome. I need to say a particular thank you to Simone's most senior lab member, Andrea, who has been a fountain of knowledge, providing both academic support and outside of the office, making my time in Pavia a fun and social experience.

Roger Kissane

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