Pump-Priming Grant

Final Report – Richard Dumbill

Title: Effect of the age of blood in ex-vivo normothermic machine perfusion.

Objective

To determine whether the storage lesion (a collective term for various abnormalities that develop as blood donated for transfusion is stored for increasing lengths of time) has an effect on ex-vivo normothermic machine perfusion of the kidney. To assess whether biochemical reversal of the storage lesion has a measurable impact on the perfused kidney in this setting.

Methods

In order to conduct the project a collaboration has been established between the groups of Professor Friend (work led by Richard Dumbill), and Professor Swietach (DPAG, University of Oxford). The key methods involved are 1. ex-vivo normothermic perfusion of the kidney, and 2. single-cell saturation imaging of red cells used during perfusion. Kidney perfusions have been performed in both a clinical trial setting, and the laboratory. Clinical perfusions were all conducted in accordance with the NKP1 protocol (https://doi.org/10.1186/ISRCTN13292277). Separate ethics and NHSBT approval was sought for work perfusing human kidneys retrieved with the intention of transplantation, but subsequently discarded ('Optimising normothermic perfusion of the kidney', London – Fulham Research Ethics Committee, ref. 21/PR/1546).

The original study intent was to use a xenoperfusion model combining porcine kidneys with expired units of human blood, to study the effect of blood rejuvenation. However, this proved challenging to establish due to concerns held by NHSBT about the use of donated blood in the context of an animal model. Donated human kidneys unsuitable for transplantation are available for such research, however are challenging to work with due to sporadic availability, heterogeneity, and borderline viability. We therefore secured the necessary approvals to do this work with a human/human perfusion system, and developed a novel experimental platform to test the effect of different blood preparations on the same kidney. This involves construction of a dual circuit with two perfusion machines and separate blood loops for red cells of different conditions, connected by a dialysis system ensuring that the non-cellular components of the perfusate are equilibrated between the loops. Supply of perfusate to a single organ positioned in the centre of the set-up can be switched instantaneously from one machine to the other and back again (Fig. 1). This permits detailed study of oxygen offloading in the ex-vivo perfused kidney with red cells of two different experimental conditions, with all other variables held constant.

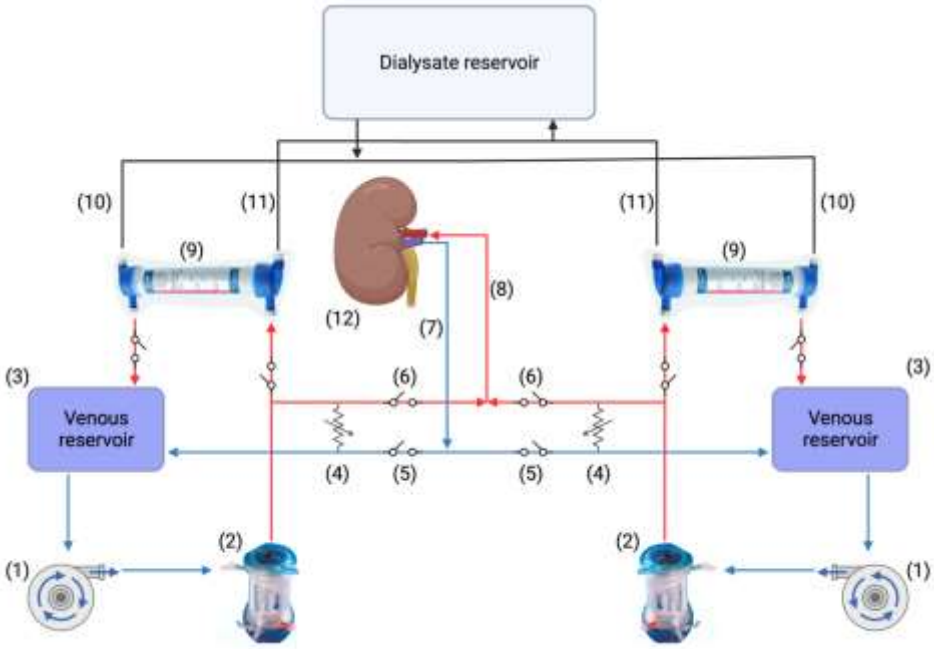


Fig. 1: *Design of a dual-loop perfusion system to allow sequential and alternating exposure of a single human kidney to red cells of two difference conditions, whilst holding all other parameters constant*

This work has therefore consisted of two major components: 1. an observational study conducted during a phase 1 clinical trial (Normothermic Kidney Perfusion Phase 1; NKP1) examining associations between ex-vivo oxygen consumption and blood kinetic properties (mean oxygen dissociation time, 'tau'); and 2. an interventional study investigating the effect of reversal or prevention of the storage lesion on the perfused kidney.

During the observational component of this project, samples of red cells used during clinical perfusions in the NKP1 trial were obtained and stored on ice until perfusion was completed. Samples were taken from the packed red cells prior to addition to the machine; following reconstitution as perfusate; at timepoints throughout perfusion, and at the end of perfusion. Following reperfusion in the recipient, samples were transferred to DPAG and analysed using single-cell saturation imaging. The time taken for red cells to unload oxygen was measured ('tau'). During perfusion, paired arterial and venous blood gases were taken at regular timepoints. Haemoglobin concentration, partial pressure of oxygen, and percentage haemoglobin-oxygen saturation were used to calculate oxygen content. An equation was developed to calculate renal oxygen consumption account, taking into account the concentrating effect of urine production on the venous blood. Models were developed to describe the association between renal oxygen consumption and speed of oxygen unloading.

Results

Data from 30 clinical perfusions is available, consisting of measurements of oxygen release time from red cells taken from the system at various points during perfusion and associated measurements of arterial and venous oxygen content, blood flow, and urine production. Renal oxygen consumption was calculated in accordance with the following equation:

$$v'_{R,O2} = Q_A \cdot \left(\alpha \cdot \left(P_{A,O2} - P_{V,O2}\right) + \beta \cdot [Hb]_A \cdot (S_A - S_V)\right) + Q_U \cdot \alpha \cdot \left(P_{V,O2} - P_{U,O2}\right)$$

which was developed specifically for this application by consideration of the mass balance of arterial, venous, urinary, and respiratory oxygen flows, following the observation that venous values for haemoglobin concentration were often higher than paired arterial values (due to the concentrating effect of urine production, Fig. 2).

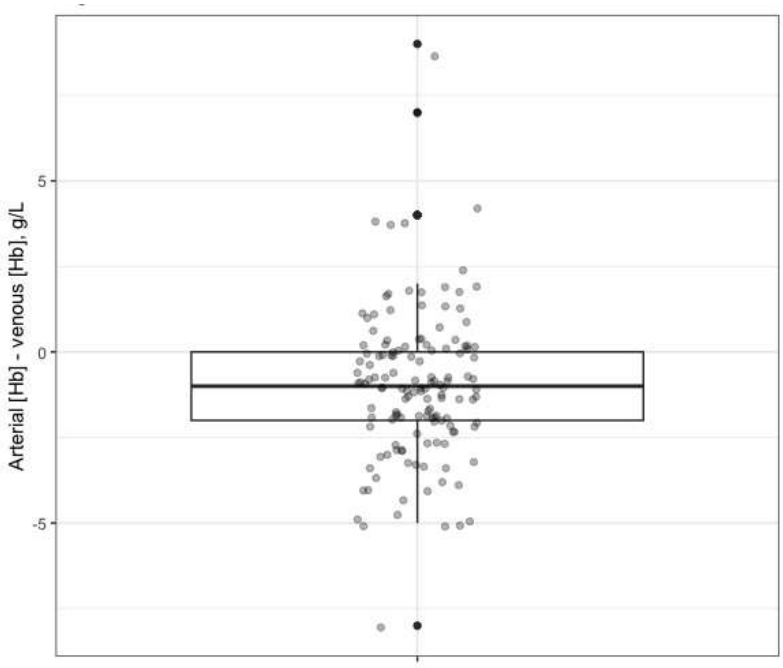


Fig. 2: *A-V difference in haemoglobin concentration observed during the trial*

It is notable that we found numerous instances in the literature where ex-vivo oxygen consumption had been calculated inaccurately, on occasion with gross errors conflating partial pressure with oxygen content. This is the first time a comprehensive equation has been developed for accurate determination of ex-vivo renal oxygen consumption, accounting for oxygen loss in the flow of urine and the concentrating effect of urine production on venous haemoglobin concentration. We observed wide variation in organ-to-organ oxygen consumption not explained by function (creatinine clearance, Fig. 2) or renal mass (Fig. 3).

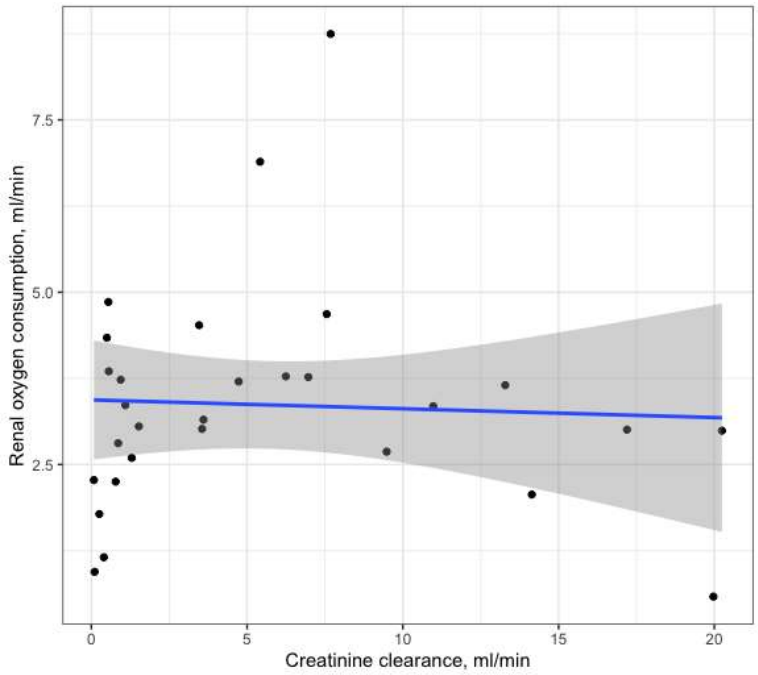


Fig. 3: *Renal function does not explain variability in measured oxygen consumption.*

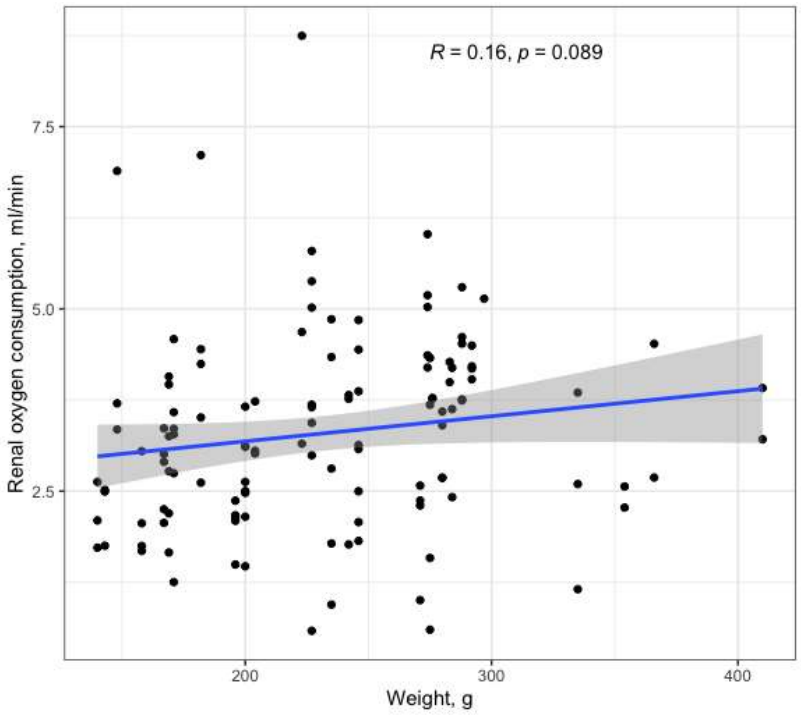


Fig. 4: *Renal mass does not explain variability in measured oxygen consumption.*

The most important finding from this study is that there was a strong correlation between oxygen consumption and speed of oxygen unloading by the stored red cells (Fig. 4). This suggests that oxygen supply to tissue by blood exhibiting a typical storage lesion is diffusion-limited rather than perfusion-limited. This finding has substantial implications for both transfusion medicine and ex-vivo normothermic machine perfusion using red cell based perfusate.

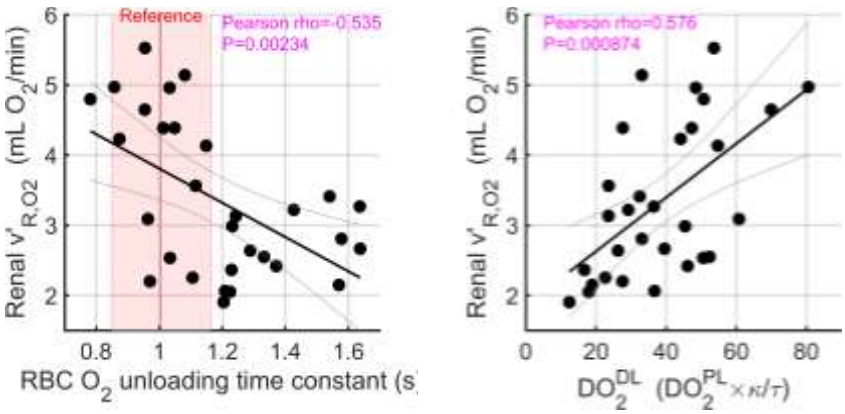
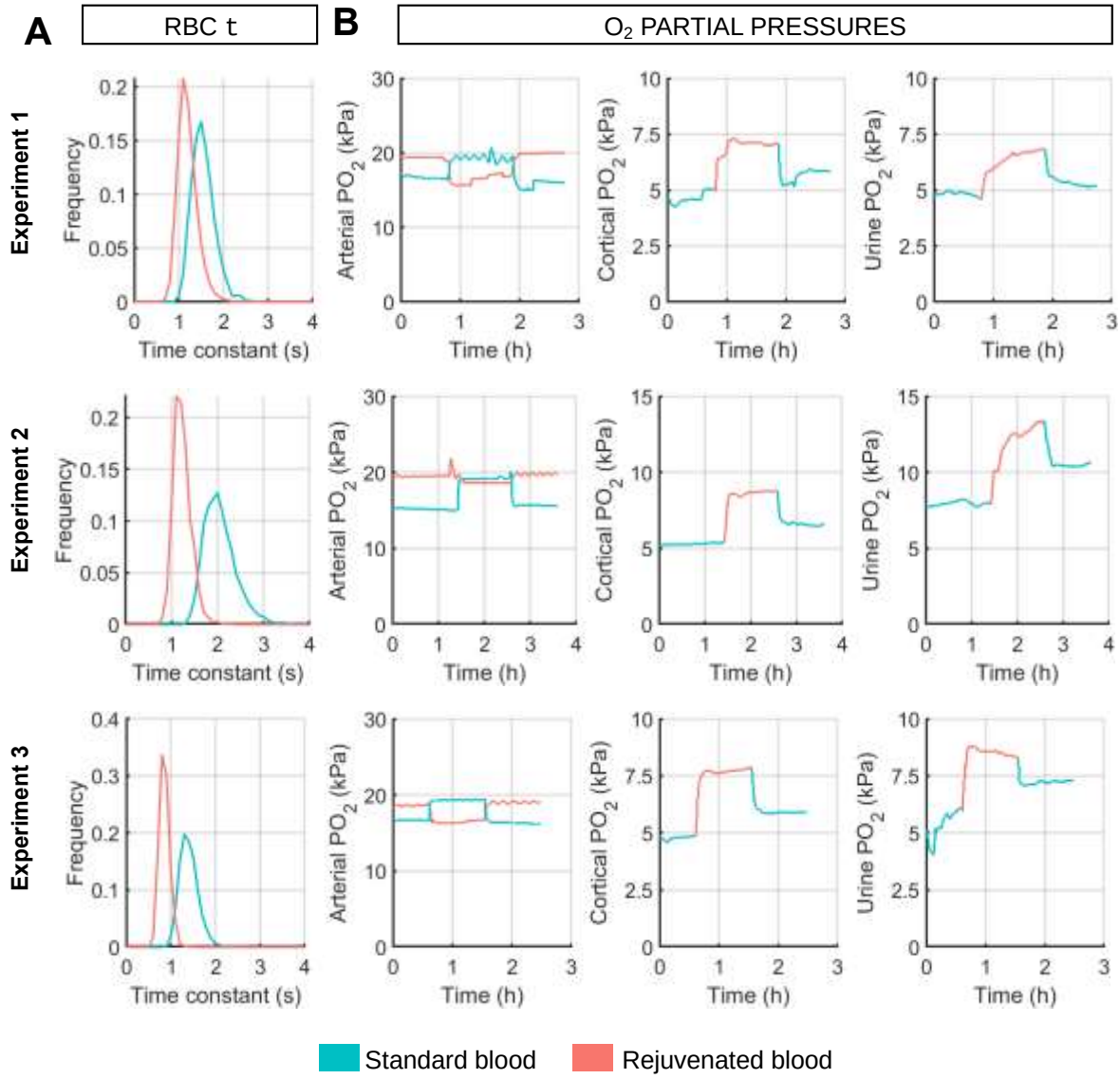


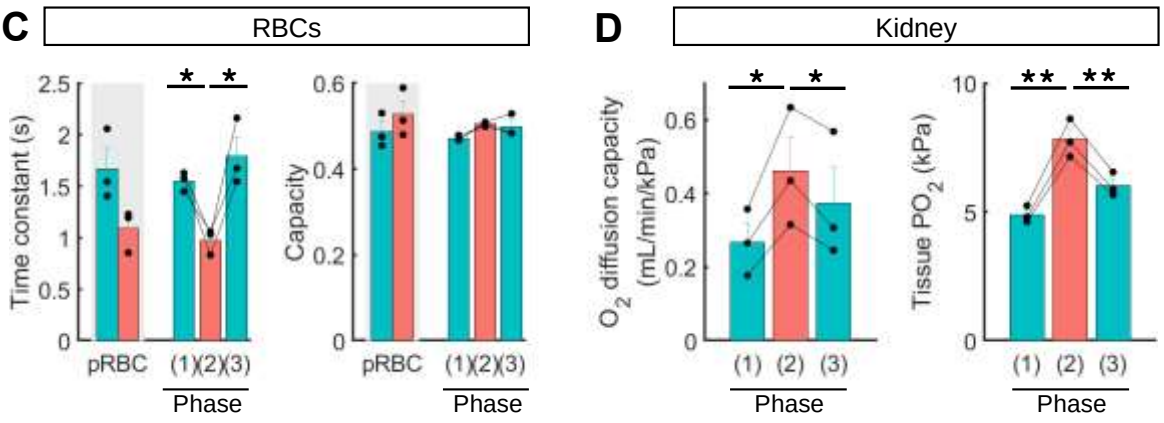
Fig. 5: *A strong negative correlation is observed between renal oxygen consumption and unloading time, and a strong positive correlation is observed between renal oxygen consumption and delivered oxygen but only once a term describing the rate of oxygen unloading is included. This provides good observational evidence that the unloading time effect is physiologically important.*

Next, we evaluated the effect of red cell rejuvenation on the ex-vivo kidney, using our newly developed dual perfusion set-up. This produced striking results, showing a substantial improvement in oxygen availability (60% increase in cortical oxygen tension) with rejuvenation, and an improvement in coefficient of oxygen diffusivity measured in the kidney (which is a direct property of red cells and their ability to unload oxygen). These results are shown in Fig. 6. This provides the first evidence 1) for diffusion-limited oxygen transfer to tissues in packed red cells manifesting a typical storage lesion, and 2) improvement with biochemical rejuvenation. These results have substantial implications for transfusion medicine, particularly in scenarios where the transfused load

represents a high proportion of the total circulating volume (e.g. ex-vivo organ perfusion, major haemorrhage, and paediatric transfusion).

FIGURE 6





Outputs (publications/presentations)

The observational work described above, pertaining to analysis of samples and data derived from NKP1, was presented at the British Transplant Society 2023 annual congress as an oral presentation in a basic science session. The observational component was published online as a preprint, available here: https://doi.org/10.1101/2023.05.07.23289584. The full manuscript, containing our observational and interventional data, has been submitted for publication and is under consideration.

Next Steps (what is it leading to)

Further funding to continue the clinical translation of this work has been applied for. If awarded, this will include a work package to optimise the rejuvenation solution, using ex-vivo organ perfusion as a model system.