

Controlled oxygenated rewarming of the liver prior to normothermic machine perfusion to attenuate ischaemia reperfusion injury in discarded human livers

Background

Liver transplantation is limited by donor organ shortage and there is a high waiting list mortality. Increasing utilisation therefore depends on using livers from higher-risk or "extended criteria" donors, which are particularly vulnerable to ischaemia-reperfusion injury (IRI) and to primary non-function, rejection and biliary complications.

Ex situ machine perfusion can reduce this injury and allow viability assessment before implantation. In the UK, normothermic machine perfusion (NMP) is almost exclusively used in a "back-to-base" model, in which the liver is transported under static cold storage (SCS) and perfusion is initiated at the transplant centre. Experimental evidence suggests that rapid rewarming after prolonged hypothermia is itself injurious, causing hepatocellular and sinusoidal endothelial cell injury and accumulation of inflammatory mediators and damage-associated molecular patterns in the perfusate. Hypothermic oxygenated perfusion (HMP-O₂) before NMP enhances ATP synthesis and reduces oxidative stress injury, and controlled oxygenated rewarming (COR) has been proposed as a means of avoiding the abrupt cold-to-warm transition altogether.

All previously described COR protocols require continuous manual control of temperature and vascular pressures by an operator, and an exchange of perfusate in red blood cell-based protocols. This level of intervention makes routine clinical adoption difficult. We had previously developed an automated protocol delivering HMP-O₂, COR and NMP as a single continuous sequence from 10°C to 37°C using a red cell containing perfusate. The purpose of this project was to test the translatability of that protocol in human livers declined for transplantation.

Objectives

The primary objective was to examine the safety and feasibility of the automated red cell based HMP-O₂-COR-NMP protocol in human livers declined for transplantation. The secondary objective was to compare the function of these livers with historic declined human livers perfused using standard NMP to inform the design of a subsequent clinical trial.

Methods

Between May and September 2025, four human livers declined for transplantation by all UK liver transplant centres were recruited through the UK national organ research platform and transported to the research laboratory under static cold storage. The study was approved by the London Riverside Research Ethics Committee (REC reference 20/PR/0111; IRAS project ID 280477; Substantial Amendment 3, 14 January 2025). Four livers were perfused rather than the six originally proposed, reflecting the availability of declined organs meeting the study criteria within the funded period.

Livers were back-benched and perfused using the automated protocol on the OrganOx metra device. Perfusion began with highly oxygenated (>60kPa) albumin-based acellular perfusate at a portal pressure of 4mmHg and hepatic arterial pressure of 25mmHg for one hour. Livers were then rewarmed over 90 minutes following a pre-defined non-linear temperature rise, with portal and arterial pressures increasing according to pre-defined profiles. Simultaneous, equal-rate packed red cell infusion and haemofiltrate removal achieved a continuous transition from acellular to red cell containing perfusate. Livers were then assessed over 10-12 hours of normothermia.

Perfusate, bile and liver tissue were sampled at predefined time points. Blood gas analysis was performed on an ABL90 Flex analyser and biochemistry (ALT, AST, ALP, LDH, GGT, bilirubin) on

an Abbott Architect c8000. Biopsies taken before and at the end of perfusion were reported by a blinded specialist liver histopathologist. Outcomes were compared with the 31 declined human livers perfused using standard NMP in the VITTAL study as a historic control cohort.

Results

The livers perfused were of higher risk than the historic cohort. Median donor age was 66 years, three of four were from donation after circulatory death, and reasons for decline were hepatic artery dissection, poor in situ perfusion, prolonged time to asystole and severe steatosis. Donor risk scores were higher than the VITTAL cohort (median UK-DLI 2.5 vs 1.4; DRI 3.1 vs 2.2), and cold ischaemia time was significantly longer (846 vs 453 minutes, $p = 0.014$). These livers therefore represent a more marginal group than the historic comparator, with a longer preservation interval before perfusion.

The automated protocol was delivered reproducibly. Vascular flows were consistent across all four livers, with the expected progressive increase in hepatic arterial and portal venous flow during rewarming and a stable plateau on reaching normothermia (Figure 1). Temperature followed the predefined profile in every perfusion and total haemoglobin was stable after the red cell exchange. Perfusate pH stabilised within one hour of reaching normothermia without additional bicarbonate supplementation, potassium remained stable throughout, and methaemoglobin remained within acceptable limits. This demonstrates that a fully automated and continuous hypothermic-to-normothermic transition can be achieved reliably in human grafts without operator intervention.

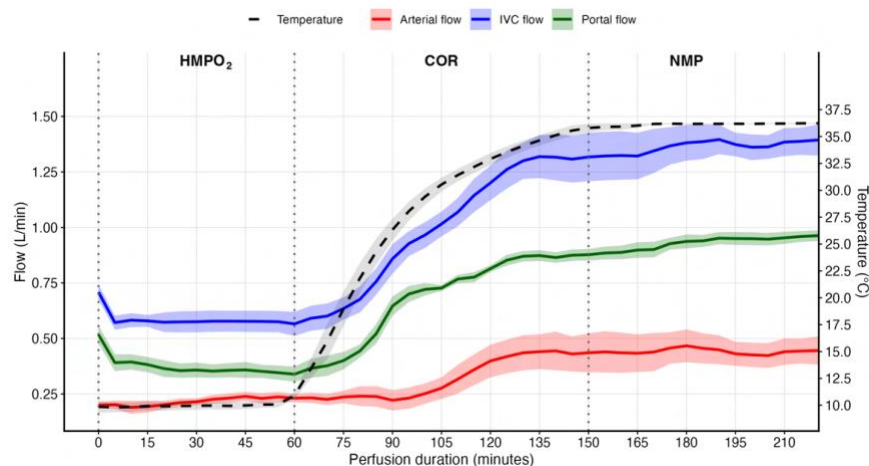


Figure 1. Vascular flows and perfusate temperature (mean $\pm$ SEM) during the HMP-O₂, COR and NMP phases of four declined human livers.

Injury markers and histology. Perfusate liver enzymes increased over time in all grafts, with the greatest rise in ALT, ALP and LDH occurring between pre-perfusion and the end of the hypothermic phase, followed by a slower rate of increase once normothermia was established. Pre-perfusion biopsies showed preserved lobular architecture with variable steatosis and glycogen depletion. Following perfusion, appearances varied between grafts, from preserved architecture with only focal necrosis to extensive confluent hepatocellular necrosis. Where biliary structures were present, cholangiocellular architecture was preserved with only mild ductular change and no histological evidence of biliary necrosis.

Function was restored in very high-risk livers. All four livers cleared lactate to less than 2.5mmol/L within one hour of normothermia. As demonstrated in Figure 2, the lactate profile of the perfused livers closely matched that of the VITTAL livers which were subsequently transplanted and all four livers metabolised glucose. Two of four met biliary glucose viability criteria ($< 3\text{mmol/L}$) and all four met biliary pH criteria (> 7.50).

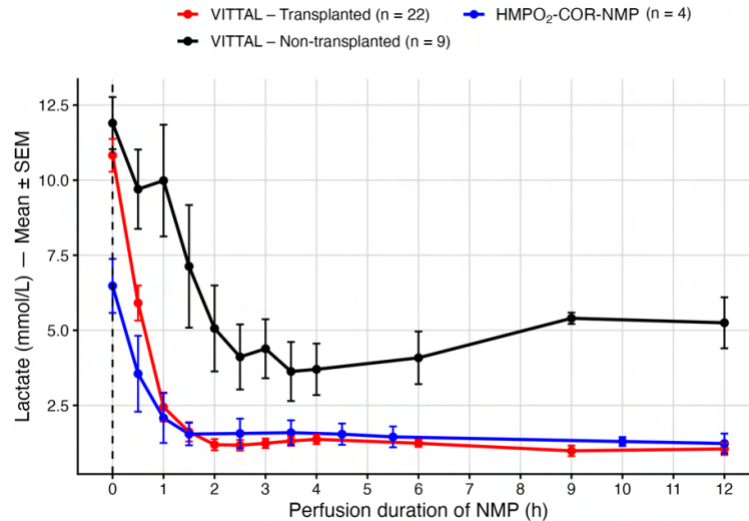


Figure 2. Perfusate lactate concentration (mean $\pm$ SEM) during NMP comparing the four perfused livers with transplanted (n=22) and non-transplanted (n=9) declined livers from the VITTAL study.

Next steps

This work demonstrates that a fully automated, continuous hypothermic-to-normothermic perfusion protocol can be delivered reliably in human livers, and that it supports recovery of function in grafts declined by every UK transplant centre despite prolonged cold ischaemia and high donor risk. The use of a non-randomised historic control cohort limits direct comparison, and no livers in this study were transplanted; the study was designed to establish safety and feasibility as the necessary step before seeking ethical approval for clinical transplantation.

The next step is a phase I safety and feasibility clinical trial, followed by a randomised comparison of HMP-O₂-COR-NMP against standard NMP. This work was presented as an oral presentation at the American Transplant Congress 2026 in Boston, where it was recognised with a Young Investigator Award, and a manuscript is in preparation. I am very grateful to the Oxford Transplant Foundation for supporting this project.

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