

Ex situ Normothermic Kidney Perfusion: setting up of a long-term protocol

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Introduction

Normothermic machine perfusion (NMP) is increasingly used to recondition donor kidneys, particularly marginal organs, prior to transplantation. It provides near-physiological conditions that help limit ischemia-reperfusion injury (IRI) and allows functional assessment. Long-term NMP—extending to days—opens new possibilities for repair and regeneration, though standardized protocols are lacking. This study establishes 3-days NMP protocol using a porcine model and evaluates metabolic performance and perfusate management.

Methods

A porcine model was developed for heart-beating kidney procurement for functional and metabolic *ex vivo* assessment of perfused kidneys using the PerLife system. As the organs were transported under cold static storage, a rewarming phase was performed to gradually bring the organ from hypothermic to normothermic conditions without sudden perfusion condition changes (temperature, pressures, liquids). To guarantee the proper oxygenation of the organ, an oxygen concentrator was used to infuse 93% oxygen in the oxygenator module. After reaching NMP, arterial and venous electrolytes, lactate, glucose, hematocrit, pH, pO₂ and pCO₂ were monitored every 30 minutes until stabilization and then every 4 hours. Oxygen consumption, lactate and glucose clearance were used as markers of metabolic status. Urine was recirculated in the perfusate.

Results

Kidneys were metabolic active after reaching normothermia, with oxygen consumption and CO₂ production confirming active respiration. Venous pO₂ of 25 mmHg maintained oxidative phosphorylation and stabilized lactate levels. Early trials showed that CO₂ infusion was necessary for pH buffering and consistent oxygenation, which was validated in a pilot experiment. A glycolytic shift emerged after 12 hours, evidenced by glucose use, lactate production, and increased renal resistance. When CO₂ was added, the shift remained oxidative without lactate buildup. Principal component analysis identified two metabolic profiles: one supporting diverse energy production through fatty acid oxidation and lactate synthesis, and another emphasizing oxidative phosphorylation and lactate clearance.

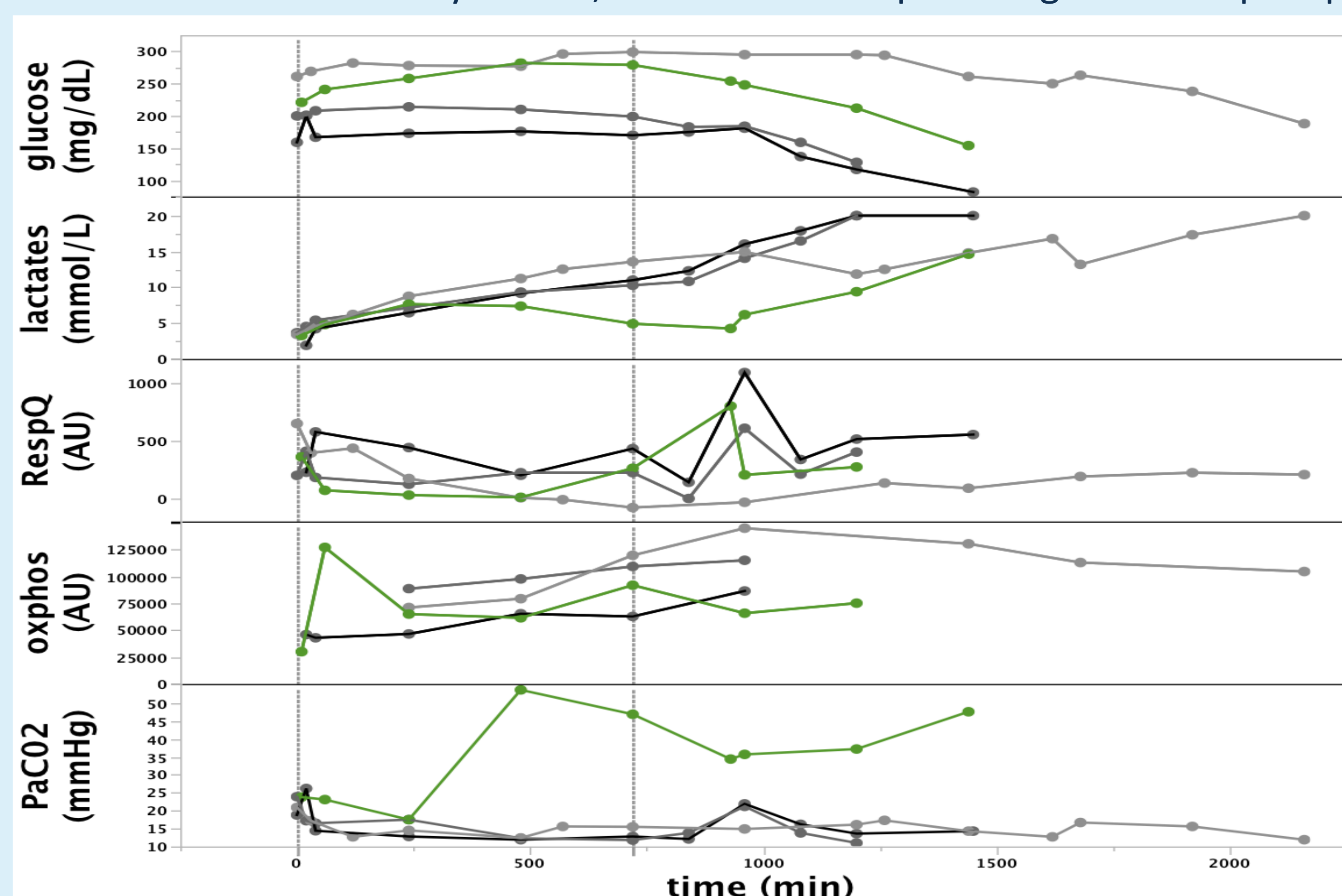


Figure: Metabolic course of the normothermic kidney perfusion

Perfusions without CO₂ are shown in different shades of grey.

Perfusion with CO₂ infusion is shown in green. RespQ: respiratory quotient calculated as the ratio between CO₂ production and O₂ consumption.

oxphos: oxidative phosphorylation estimated by O₂ consumption.

Conclusion

This extended NMP protocol represents a viable model for long-term *ex vivo* kidney perfusion. Controlled oxygenation and acid-base balance are achievable through targeted O₂ and CO₂ infusion. The observed glycolytic switch marks a limiting factor in long-term perfusion, and the data suggest that introducing metabolic substrates for fatty acid oxidation could enhance stability and function.