Ex-vivo Subnormothermic Oxygenated Machine Perfusion of Swine Forelimbs Enables Prolonged Graft Preservation Prior to Transplantation

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Background
The current gold standard in tissue preservation is static cold storage (SCS) on ice-cold (0-4°C) UW solution. While SCS slows down graft deterioration, it does not have restorative capabilities. We previously developed an ex-vivo perfusion system for subnomothermic oxygenated machine perfusion (SNMP) to resuscitate cadaveric organs. Recovered livers were perfused for 3 hours and transplanted successfully into recipient rats in 5/6 cases[1]; when scaled up to DCD human livers, SNMP demonstrated sustained and enhanced viability of liver grafts. To expand the donor pool in VCA, we investigated the utility of SNMP on preservation time and resuscitation of ischemic limbs in a swine model.

Methods
2 porcine forelimbs were procured and flushed with ice-cold UW on the back table through the cannulated axillary artery and veins. Warm ischemia was 45 mins and SCS was 2 hours. Before starting SNMP, the forelimbs were flushed with 1500 mL of cold Lactated Ringers. During SNMP (3 hours), the amputated forelimbs were perfused by a pressure-controlled system through the axillary artery. The perfusion solution consisted of William's E medium, which was enriched with dexamethasone, insulin and heparin. A venous outflow was prepared for sample collection. Hemodynamics of the limbs was monitored by evaluation of arterial flow and vascular resistance. Perfusion samples were collected at 30 min intervals for biochemical analysis. Lactate clearance was monitored as a marker of muscle injury. Muscle biopsies were collected at 60 min intervals for measurement of ATP production.

Results
Arterial outflow and vascular resistance remained stable throughout the perfusion, between 270 and 320 mL/min and 0.23 and 0.26 mmHg/mL/min, respectively. Despite the initial increase in lactate levels from 0.2 mmol/L to > 6 mmol/L, this value remained stable during the final hour of perfusion. The increase in ATP production reflects successful resuscitation of the forelimb, increasing from a baseline of 5500 before perfusion to 7500 nmol/g protein during SNMP.

Conclusions
SNMP has the potential to both actively preserve and enhance overall preservation of forelimbs in a swine model. It may provide the crucial enabling technology for tissue preservation, transport, and eventual transplantation of VCAs.

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Reference:

1. Tolboom, H., Izamis, M.L., Sharma, N.et al Subnormothermic machine perfusion at both 20 degrees C and 30 degrees C recovers ischemic rat livers for successful transplantation. *The Journal of surgical research* **175**, 149-156 (2012).