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ORIGINAL ARTICLE

Development of *ex situ* normothermic reperfusion as an innovative method to assess pancreases after preservation

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SUMMARY

Static cold storage (SCS) is the standard method for pancreas preservation prior to transplantation; however, it does not permit organ assessment. Normothermic reperfusion (NR) is utilized clinically for other organs to assess viability. Our aim was to develop NR using normothermic machine perfusion technique to simulate reperfusion at the time of transplantation, enabling evaluation of oxygenated hypothermic machine perfusion (HMPO2) as a newer strategy to optimize pancreas preservation. 13 porcine pancreases procured after circulatory death were divided into 3 groups: 4 pancreases preserved using SCS, and 2 groups preserved by HMPO2 ($n = 4$ and $n = 5$, differing by type of preservation solution). Duration of perfusion or cold storage was 6 hours before the 1-hour assessment using NR. Outcome measures were perfusion characteristics, biochemistry and change in tissue water mass as oedema assessment. During NR, the HMPO2 groups demonstrated better perfusion characteristics, normal macroscopic appearances, decreased water mass and one HMPO2 group demonstrated a response to glucose stimulation. Conversely, the SCS group showed an increased water mass and developed early macroscopic appearances of oedema, interstitial haemorrhage and minimal portal outflow. This study suggests that *ex situ* assessment of pancreases by NR is promising, and that HMPO2 may be better than SCS.

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Key words
normothermic reperfusion, organ assessment, oxygenated hypothermic machine perfusion, pancreas preservation, pancreas transplantation, porcine model

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Introduction

Pancreas transplantation (PTx) is an established therapy known to reverse the manifestations of Type 1 diabetes mellitus (T1DM). However, since the first successful procedure in 1966, the preservation method for the pancreas allograft using static cold storage (SCS) has remained unchanged.

SCS used to be the gold standard of organ preservation in transplantation, but this may be changing; the

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goal of this simple and rather effective preservation strategy for standard criteria donor organs was to cool down and maintain temperatures between 0 and 4°C in order to considerably slow down the organ's metabolic requirements up to transplantation. Some limitations of this strategy, however, are the continued exposure of the organ to ischaemia and inability to flush out built-up toxic metabolites during hypoxia that contribute to the damaging phenomenon of ischaemia reperfusion injury (IRI) at the time of transplantation [1].

It is generally accepted that SCS is not an effective method for preserving organs recovered from extended criteria or 'marginal' donors due to an increased susceptibility to IRI [2]. These suboptimal donors are defined in PTx as those with a body mass index of greater than 30kg/m^2 and outside the range of <10 to >45 years of age [3]. Donation after circulatory death (DCD) status is also regarded as risk factor for accruing ischaemic injury during preservation [4].

The native pancreas is particularly vulnerable to the effects of ischaemia, and this association has been demonstrated in a number of clinical settings specifically, with hypovolaemic shock [5] and cardiopulmonary bypass procedures [6]. The pathophysiology of the pancreas provides some explanation for its tendency towards pancreatitis after ischaemia. It is a richly vascularized organ that transmits low vascular pressures and flows [7,8], is susceptible to oedema [9] and contains a high density (> 90%) of acinar tissue that synthesizes digestive enzymes that may be inappropriately activated in ischaemia [10]. These characteristics contribute to the manifestations of IRI observed clinically as graft pancreatitis and vascular thrombosis, both leading causes of early patient morbidity and mortality after PTx [11,12].

The concern around the consequences of IRI in PTx has led to conservative pancreas allograft acceptance practices and high organ discard rates [13,14]. A further hurdle in PTx is that only a minority of deceased donors are perceived as suitable to allow successful transplantation. 2020 UK data show that only *13%* of available deceased donors will provide a pancreas that is actually transplanted [15].

It is intuitive, therefore, that the development of strategies to mitigate the impact of IRI and facilitate *ex situ* organ assessment prior to transplantation would improve pancreas allograft utilization.

Other organs, namely, the kidney [16,17], liver [18,19], lung [20] and heart [21] have seen the implementation of advanced combined assessment and preservation strategies such as hypothermic and normothermic machine perfusions (HMP and NMP) into clinical practice. Such implementation of *ex situ* machine perfusion has significantly contributed to the increased recruitment of marginal organs for transplantation that would have traditionally been declined.

Normothermic reperfusion (NR) using the NMP technique aims to replicate more physiological conditions to support the *ex situ* organ through the provision of an oxygenated perfusate solution including essential substrates. An appreciated characteristic of NMP is that it may help to assess functional assessment. Mergental and colleagues [22] in the 'VITTAL' trial utilized NMP as a means to assess high-risk livers, already declined by all UK Liver transplant centres. Out of 31 livers assessed 71% (n = 22) were transplanted, with a reported 100% 90-day patient and graft survival.

In regard to pancreas HMP, the published experiences to date are preclinical experimental models, dating back as lately as 1975 [23,24]. The few and heterogeneous collection of feasibility studies highlight real technical challenges in pancreas perfusion, with varying reported outcomes ranging from none-to-moderate oedema and few with severe necrosis [23–28]. However, the collective experience plus encouraging findings from recently published clinical trials involving machine perfusion of other solid organs [16,18] suggests that machine perfusion for pancreases is feasible, may have merit, but requires thoughtful refinements.

University of Wisconsin Cold Storage (UW-CSS) Solution® (Bridge to Life, London, UK) since its development has remained the gold standard for cold storage preservation of pancreases. UW Machine Perfusion Solution® (UW-MPS) is a similar composition to UW-CSS but contains gluconate versus lactobionate as an impermeant and has an extracellular versus an intracellular sodium-to-potassium ratio. Both solutions include a large colloid, hydroxyethyl starch (HES), to provide an oncotic pressure and minimize the development of oedema. Newer preservation solutions have replaced HES with polyethylene glycols (PEGs), a water soluble, non-toxic polymer which provides the required oncotic pressure, but with reduced viscosity [29]. Furthermore, additional benefits have been described in both *in vivo* and *in vitro* studies of oxidative stress where PEGs appear to exert anti-inflammatory and cytoprotective effects as well as being associated with up-regulation of cell-survival pathways [30–32]. IGL2® (Institut Georges Lopez 2, Lissieu France) solution is a new organ preservation solution containing a PEG concentration of 5g/L,

which we have used it in this study to evaluate the benefits of PEG.

The goal of this study was to develop NR of the pancreas using the NMP technique, as a surrogate for reperfusion at the time of transplantation in order to investigate the following two questions:

1. Does oxygenated hypothermic machine perfusion ($HMPO_2$) of pancreases provide improved organ protection over static cold storage during preservation?
2. Does polyethylene glycol, as in IGL2 solution, confer protective effects during $HMPO_2$ compared with UW-CSS or UW-MPS?

Our chosen outcome measure of effect is evidence of both exocrine and endocrine viability during machine perfusion.

Materials and methods

13 pancreases were procured from domestic pigs within a weight range of 50 to 70 kg at a UK abattoir. Prior to animal sacrifice, the animals were randomized (sealed envelope) to prevent prediction and ensure the pancreases in each group were not treated in obvious succession to minimize bias.

Animal death is in compliance with the Welfare of Animals at the Time of Killing (England) Regulations 2015 (WATOK) and EU regulation 1099/2009; therefore, additional ethical approvals or animal licences to carry out this study were not required. After regulated stunning, the unconscious animal is exsanguinated, and two litres of whole blood was collected and anticoagulated with 20 000 IU of unfractionated heparin sodium (Wockhardt UK Ltd) as part of the NMP protocol. End of exsanguination is documented as animal death. After death, a midline thoracoabdominal incision that is extended to the perineum was made to facilitate an *en-bloc* multi-visceral excision. Once removed, the organs were moved to a backbench for the surgical team to quickly cannulate the supra-coeliac aorta with a 21Fr Argyl straight cannula^{TM} (Medtronic, UK) after cross clamping the infra-renal aorta. One litre cold perfusion solution under gravity was initiated, anterogradely via the aorta and marked the start of cold ischaemia time (CIT). Flush out was either IGL2 or UW-CSS heparinized solutions (10 000 IU) used dependent on the respective study group to be hypothermically machine perfused with oxygen thereafter and to be compared with a control group preserved with standard SCS using UW-CSS.

The perfusion pressure during flush-out did not exceed 40 cm H_20 (approximately 30 mmHg aortic pressure) to minimize the risk of pancreatic oedema.

Lumbar branches were closed with small vessel clamps to reduce leaks of the perfusion fluid.

All pancreases with a duodenal segment and attached aortic tube (including the coeliac and superior mesenteric arteries (SMA)) were excised from the surrounding intestines. After division of the portal vein at the hepaticoduodenal ligament, both the ligament and the root of the mesentery were divided by a stapling device (DST series GIA^{TM} stapler, Medtronic Ltd) to prevent fluid leaks during the subsequent machine perfusion.

The procured and flushed pancreases were then packed in 250 mls cold preservation solution in an organ bag, placed on ice in a box and transported back to the laboratory.

Preparation of porcine pancreases for preservation and machine perfusion

Following the arrival of the pancreas at the laboratory, first, a splenectomy was performed. The distal end of the aortic tube (inferior to the SMA) was tied off with a 0 Permahand^{TM} Silk suture (Johnson & Johnson Medical N.V., Belgium), and the proximal supra-coeliac aorta was cannulated with a 10 mm straight aortic cannula (Institut Georges Lopez, Lissieu France). All lumbar branches coming off the attached aortic tube segment, and any leaks demonstrated on gentle syringe flush via the arterial cannula were ligated with non-absorbable monofilament Prolene^{TM} 5/0 (Johnson & Johnson Medical N.V., Belgium). The portal vein is left open. The benched pancreases (Fig. 1a,b) according to their study groups were either submerged in 250 mls of UW-CSS and placed in an icebox at 4°C for SCS (SCS group) for 6 hours or connected to the Waves machine^{TM} (Waters Medical System, Rochester, USA) (Fig. 1c) and perfused with either oxygenated UW-MPS (UWHMP group) or IGL2 (IGL2HMP group) solutions for 6 hours (Fig. 2, study schema).

Oxygenated hypothermic machine perfusion ($HMPO_2$)

Pulsatile perfusion was delivered via the aortic cannula at a set systolic pressure of 15 mmHg at 60 beats per minute and temperature range of 4 - 7°C on the Waves machine ^{TM}. One litre of either IGL2 or UW-MPS was delivered and oxygenated via the organ cassette oxygenator by medical grade 21% O_2 (BOC, Linde group, Surrey UK) at a flow rate of 1 L/min. This provided a perfusate partial pressure of oxygen (pO_2) of greater than 150 mmHg (21 kpa). The portal vein was left open

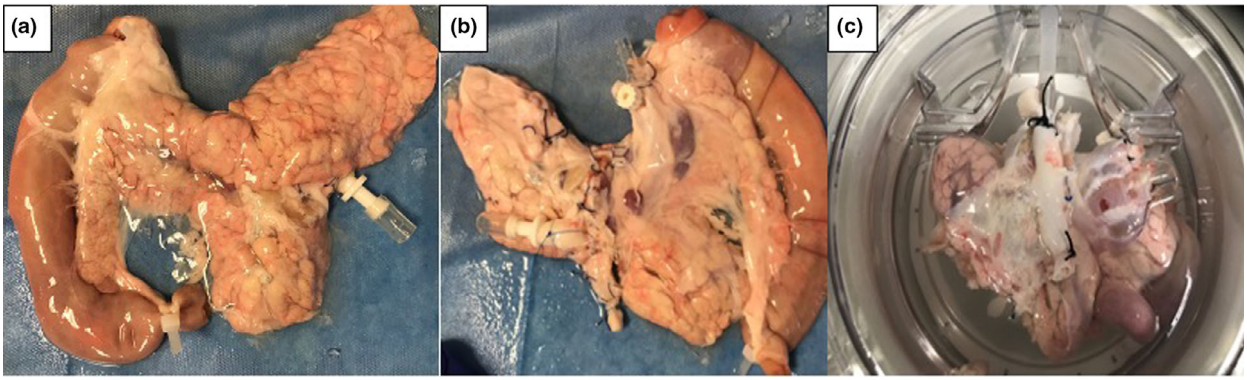

Figure 1 (a) ventral surface of benched and cannulated porcine pancreas. (b) dorsal surface of benched and cannulated pancreas. C: Oxygenated hypothermic machine perfusion of porcine pancreas.

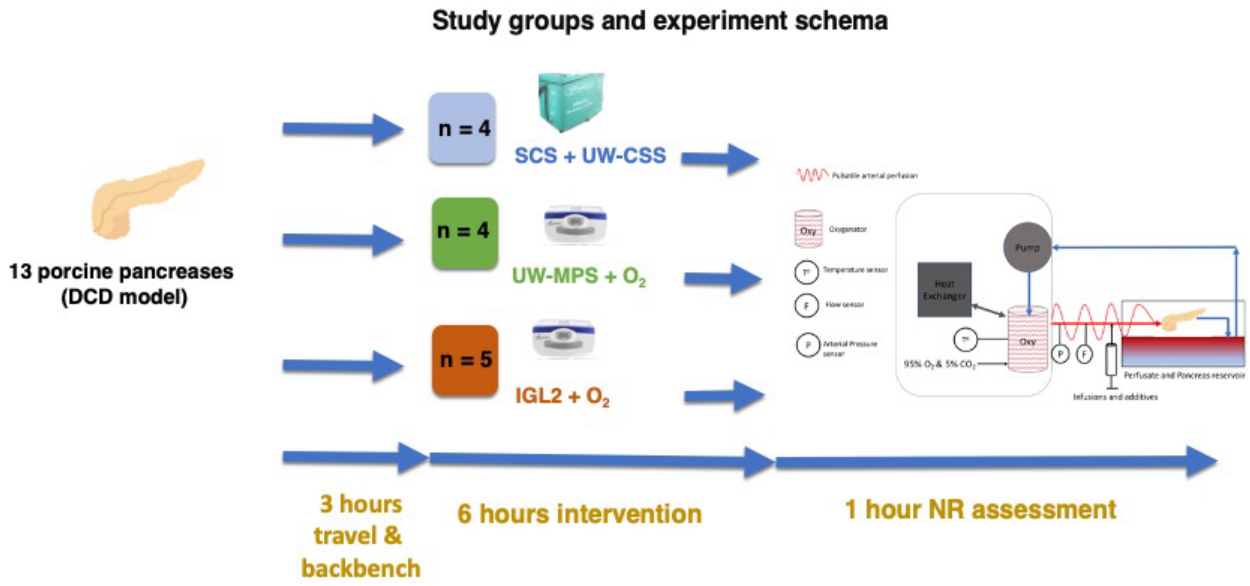

Figure 2 Study groups and experiment scheme. Abbreviations: donation after circulatory death (DCD), static cold storage (SCS), Institut Georges Lopez (IGL2), normothermic reperfusion (NR), oxygen (O2), University of Wisconsin Machine Perfusion Solution (UW-MPS), University of Wisconsin Cold Storage Solution (UW-CSS).

to passively drain effluent into the reservoir and recirculate back to the perfusion circuit.

Normothermic reperfusion (NR)

To simulate reperfusion conditions at the time of transplantation, we used autologous blood and plasma but without leucocytes as the perfusate during normothermic reperfusion. For this, the collected heparinized autologous blood was centrifuged at 3000 g at 20°C (68°F) for 20 minutes to separate the red cells from plasma. The packaged red cells were then reconstituted with enough plasma to achieve a haematocrit of 25% before leukodepletion by a Macopharma FQE6284LA^{TM} filter. Leukodepletion was confirmed on a haematology analyser, Sysmex XN-1000^{TM}, to confirm the complete absence of leucocytes and target haematocrit prior for NR.

The NR circuit was primed with 800mls of the leukodepleted blood above with additives of an antimicrobial, 1.2grams of co-amoxiclav ® (Sandoz Ltd, Surrey UK) and additional 10,000 IU of heparin as anticoagulant.

The semi-closed circuit (Fig. 3, schema) provided pulsatile perfusion at 60 beats per minute, set mean pressure of 40 mmHg with a pulse amplitude of ±8 mmHg by a Deltastream DP3 diagonal pump (MEDOS Medizintechnik AG, Stolberg, Germany).

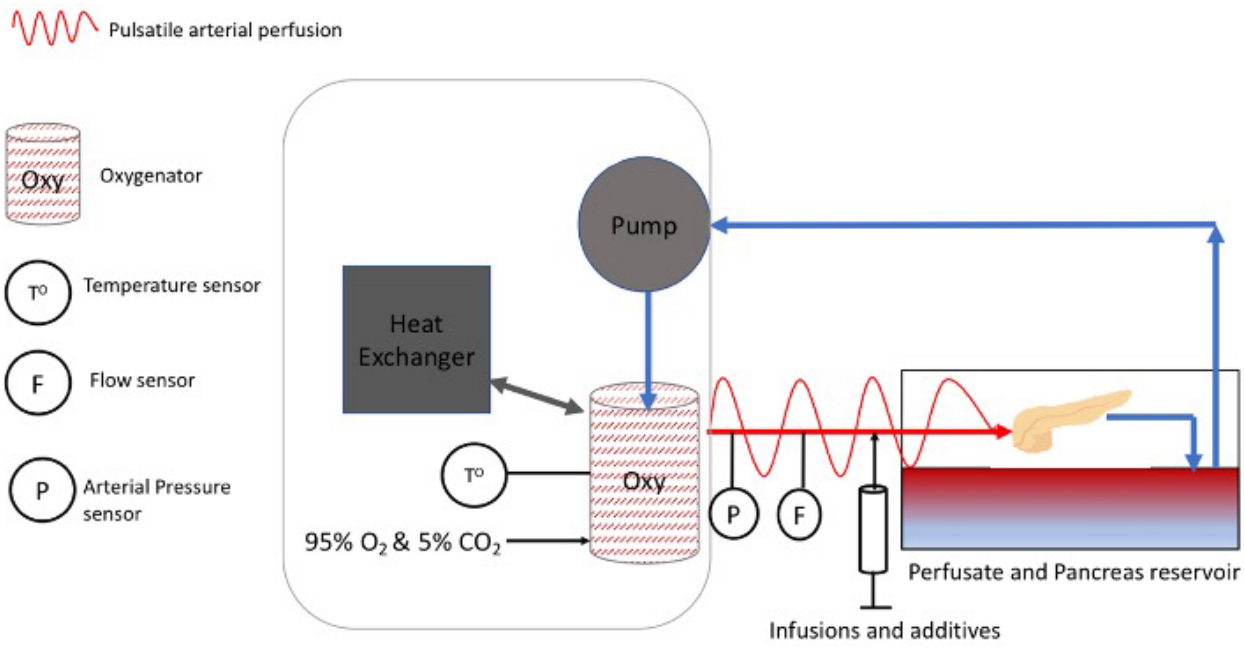

Figure 3 Schematic diagram of the pancreas normothermic reperfusion circuit.

Oxygenation with 95% oxygen plus 5% carbon dioxide at a flow rate of 0.5 litres/minute was delivered to the polymethylpentene membrane oxygenator (MEDOS HILITE 2800 LT ^{TM}), which is integrated with a bubble trap to enable gas exchange with the perfusate and provides the surface area for warming of the perfusate at 37°C by connection to a heat exchanger unit. A small piece of ¼ inch silicon tubing was placed in the portal vein (Fig. 4) to splint it open to allow easy perfusate sampling.

Sampling and data collection

Perfusate samples were sequentially collected during hypothermic preservation (SCS and $HMPO_2$) at baseline, (before start of $HMPO_2$ perfusion or at 3 hours CIT in SCS group), then hourly for 6 hours.

During NR, 5 ml perfusate samples were collected at baseline prior to attaching the pancreas and its reperfusion, then at 15, 30, 45 and 60 minutes. 0.5 ml of the perfusate samples was analysed for arterial blood gases (ABL 90 Flex Blood gas analyser, Radiometer Medical, Denmark) with the read-out including haematocrit, electrolytes, glucose, lactate, oxygen partial pressures and pH. The remaining perfusate sample was spun down to collect the supernatant, which was stored at –80°C for later biochemical analysis of lipase, amylase, lactate and lactate dehydrogenase (LDH).

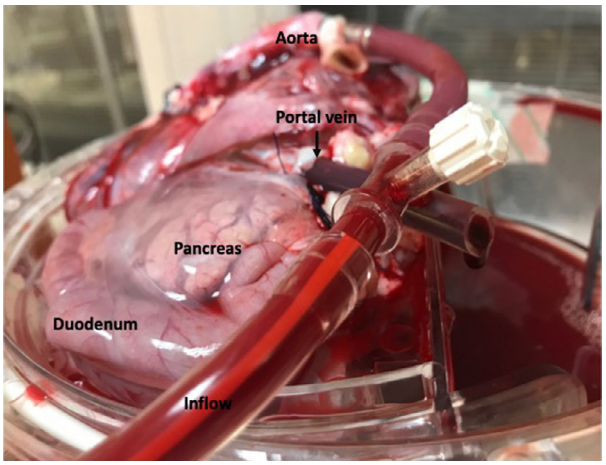

Figure 4 Cannulated (labelled) pancreas undergoing normothermic reperfusion.

Glucose-stimulated insulin secretion (GSIS) test was performed at 30 minutes into NR. 20mM of glucose in 20 ml of water was injected into the arterial line, and portal samples were collected at two minute intervals over 15 minutes. The insulin concentration was analysed by enzyme-linked immunosorbent assay (Insulin ELISA kit 10-111-01, Mercodia, Uppsala, Sweden) according to the manufacturer's instructions.

0.5 cm wedge tissue samples were taken (tail of the pancreas) at baseline and at the end NR for tissue water mass as a surrogate assessment for oedema. The weight

of the tissue biopsy is measured and document as the 'wet weight' and then subsequently dehydrated in an incubator for 24 hours at 60°C and reweighed for its 'dry weight'. The difference between the wet and dry weights constitutes the water mass expressed in grams, and the baseline and end of NR water mass are compared for differences.

Statistical analysis

Data are expressed as means ± standard error unless otherwise specified. Mean continuous variables were plotted versus time for the experimental groups. One-way analysis of variance (ANOVA) was performed to determine differences between the groups. $P < 0.05$ was considered significant. Analysis was performed using R (R Core Team, 2020).

Results

Ischaemia times

The mean warm ischaemia time for all pancreases was 25 minutes (range 15 – 30 minutes).

All pancreases, irrespective of study group, had an initial mean CIT of 3 hours that involved organ procurement, travelling and pancreas bench work prior to subsequent intervention. Therefore, the mean cold ischaemia time for all pancreases was 9 hours (± 0.17 minutes).

Perfusion parameters

Flow rates (Fig. 5a) were lowest in the SCS group ($P = 0.030$), and this group also demonstrated the highest resistance indices (Fig. 5b), ($P = 0.030$) during NR.

Mean flow rate (mL/minute) in the groups were IGL2HMP (67 ± 43), UWHMP (137 ± 86) and SCS (46 ± 32).

Mean resistance indices (ru) in the groups were IGL2HMP (0.45 ± 0.3), UWHMP (0.39 ± 0.2) and SCS (1.33 ± 1) (Fig. 6).

Water mass content

SCS pancreases had an increase in tissue weight expressed as mean water mass (gram) after NR, (0.017 ± 0.17) ($P = 0.45$). In both $HMPO_2$ groups, a decrease in mean tissue weight was observed with IGL2HMP at −0.058 ± 0.09 and UWHMP at −0.019 ± 0.58.

Macroscopic appearances

Throughout NR (Fig. 7), the $HMPO_2$ pancreases appeared homogeneously perfused throughout and maintained normal appearances up to the end of the perfusion. The SCS pancreases showed macroscopic gross appearances of interstitial haemorrhage, oedema, patchy ischaemia and minimal portal outflows as early as 15 minutes into NR.

Glucose Stimulated Insulin Secretion (GSIS)

30 minutes into NR, 20mM of glucose was delivered (Fig. 8a). A rise in perfusate glucose concentration above baseline was observed at 12 minutes after the glucose intraarterial bolus (42 minutes into NR) only in the UWHMP group. Close to this time point (45 minutes into NR), there was a corresponding rise in insulin levels only in the UWHMP group (Fig. 8b).

Biochemistry

During the 6 hours of cold preservation, amylase and lipase levels (Fig. 9a,b) were highest in the UWHMP group, $P = 0.04$ and $P = 0.23$, respectively.

Lactate (Fig. 9c) and LDH (Fig. 9d) levels were significantly higher in the SCS group $P = 0.0003$ and $P = 0.03$, respectively. During NR, there were no differences observed between the groups for LDH (Fig. 10a) $P = 0.45$, lactate (Fig. 10b) $P = 0.65$, amylase (Fig. 10c) $P = 0.71$ and lipase (Fig. 10d) $P = 0.71$.

Discussion

A better system for donor pancreas assessment is necessary to increase pancreas utilization for transplantation. Perceived unsuitability of donor pancreases obtained from older and higher-risk donors nowadays often results in discard due to clinical uncertainty of pancreas viability, especially in the presence of arteriosclerosis, fatty infiltration, oedema or procurement injuries. Axelrod [33] *et al.* have reported an index of the quality of a potential graft-to-be, the pancreas donor risk index (PDRI). PDRI is based on the characteristics of the donor estimating survival of the pancreas graft; however, this tool is limited as it was developed by using USA registry data, which predominately includes 'ideal donors' and requires further validation in other donor populations. The clinical implementation of machine perfusion either HMP and/or NMP of the pancreas graft is tools that may help to objectify and enhances

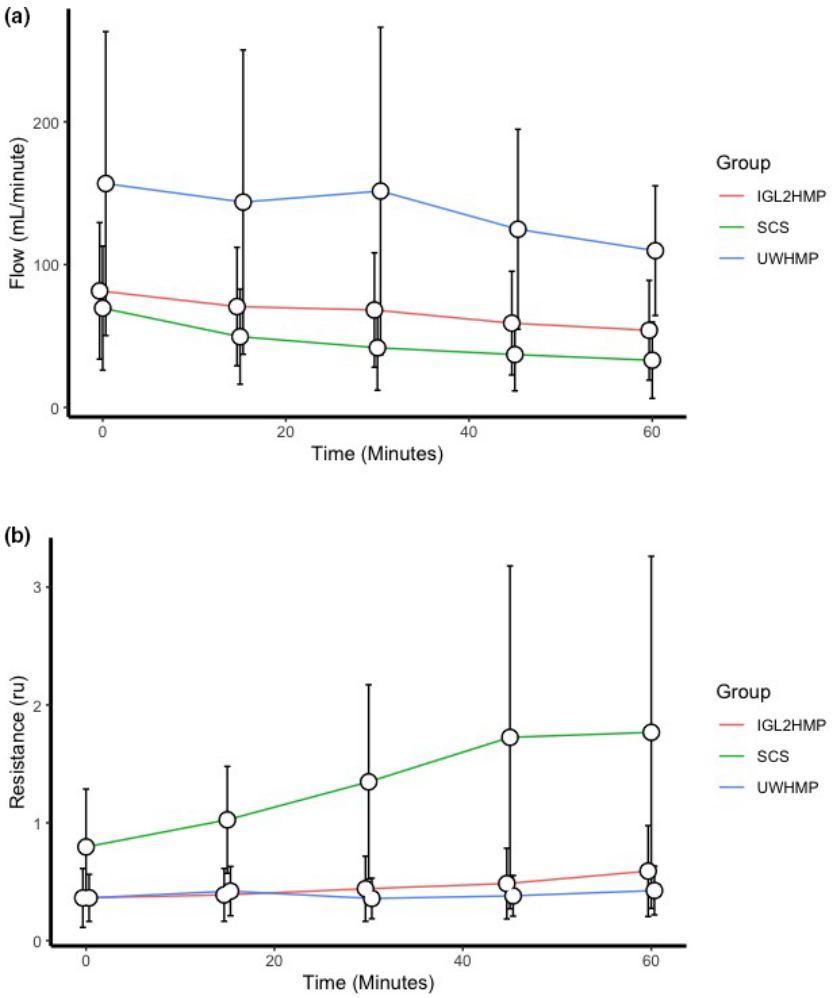

Figure 5 (a) Flow rate (ml/minute) in the 3 study groups during normothermic reperfusion: Line graph showing error bars and standard error of the mean. (B) Resistance (ru) in the 3 study groups during normothermic reperfusion: Line graph showing error bars and standard error of the mean.

the quality of many potential pancreas grafts to be previously deemed untransplantable.

Majority of the evidence supporting the benefits of HMP comes from kidney transplantation where it is associated with reduced risks of delayed graft function particularly for non-standard criteria donor kidneys [16, 34–36]. However, unlike the kidney, pancreas preservation has not changed since its early development in the 80s.

Recently, our collaborative group established non-oxygenated HMP specifically for pancreases using non-human primates [37] and non-transplanted human grafts [38], comparing the benefits of HMP over a period of 24 hours to SCS preserved pancreases in the same time frame. The main findings were that HMP in the non-human primate series up to 24 hours was not injurious; with no histological evidence of necrosis and apoptosis (assessed as < 1% cleaved caspase 3 activity on immunostaining) and following 12 hours of HMP in the human pancreases, there was an observed absence of duodenal and pancreatic oedema on histological assessment.

We also reported a controlled study of HMP to SCS in the first description of pancreas allotransplantation after graft preservation in a porcine diabetic model and found no significant differences between in recipient and graft survival between the groups [39]. The donor pancreatectomy was performed in conditions similar to a 'living donation' (i.e. minimally injured graft), which may explain the equivalence between groups.

In this study, we report our first experience of establishing $HMPO_2$ of the pancreas followed by a read-out model of NR simulating the reperfusion period in transplantation. Similar to our work, Leemkuil *et al* in their recently published (2021) study [40] investigated the impact of $HMPO_2$ in human non-transplanted DCD pancreases, compared with DBD SCS preserved pancreases, and used islet isolation to assess effect, evaluating the quality of the intervention *in vitro* and *in vivo* in an immunodeficient diabetic mouse model. They achieved successful islet isolation post-$HMPO_2$ and observed no induction of oedema or apoptosis.

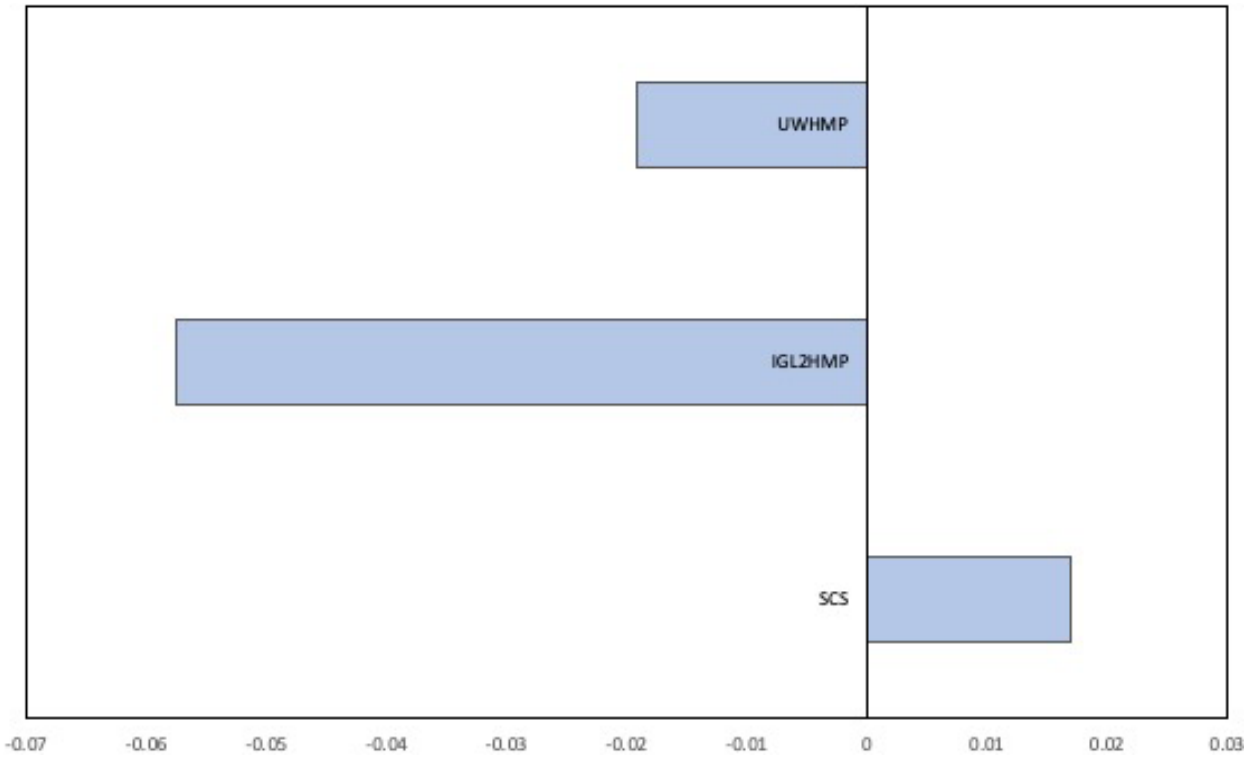

Figure 6 Mean water mass difference per study group (grams). Abbreviations: IGL2HMP (Institut Georges Lopez 2 solution oxygenated hypothermic machine perfusion group), SCS (static cold storage group), UWHMP (University of Wisconsin-MPS oxygenated hypothermic machine perfusion group).

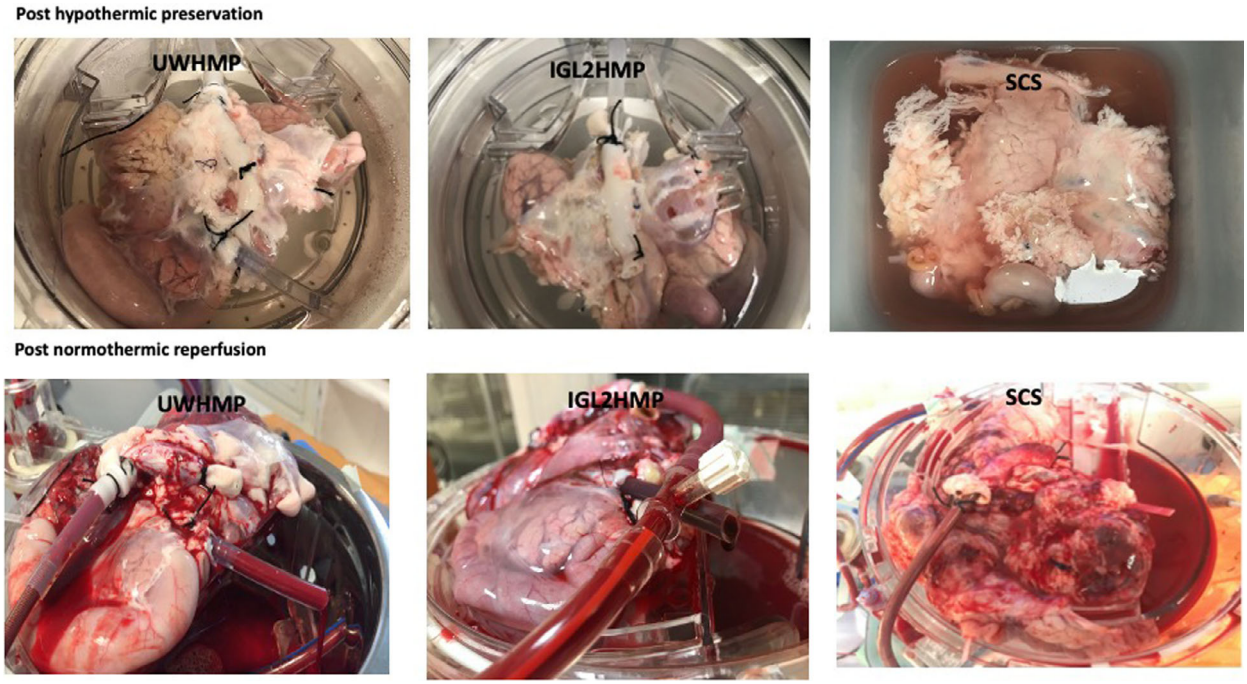

Figure 7 Macroscopic appearances of the study group pancreases. Top row shows appearances after hypothermic preservation in each group. Bottom row shows appearances after 60 minutes of normothermic reperfusion assessment. Abbreviations: IGL2HMP (Institut Georges Lopez 2 solution oxygenated hypothermic machine perfusion group), SCS (static cold storage group), UWHMP (University of Wisconsin-MPS oxygenated hypothermic machine perfusion group).

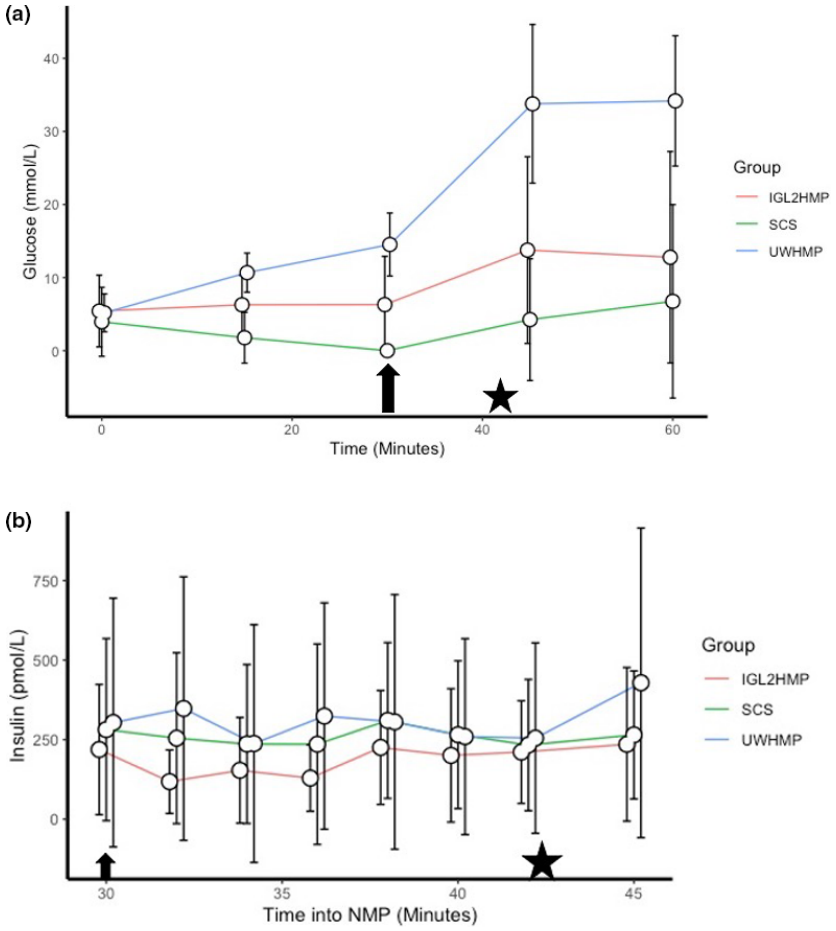

Figure 8 (a) Glucose-stimulated insulin secretion (GSIS) study in the 3 study groups. Glucose levels during normothermic reperfusion (NR). The black arrow shows the point of delivery of 20mM glucose, at 30 minutes of NR. The black star shows the point in time when the mean insulin levels in the UWHMP group rose above baseline in parallel to glucose concentration rise in the perfusate. Line graph showing error bars and standard error of the mean. Abbreviations: GSIS (glucose-stimulated insulin secretion), IGL2HMP (Institut Georges Lopez 2 solution oxygenated hypothermic machine perfusion group), NR (normothermic reperfusion), SCS (static cold storage group), UWHMP (University of Wisconsin-MPS oxygenated hypothermic machine perfusion group). (b) Glucose-stimulated insulin secretion (GSIS) study in the 3 study groups. Insulin levels during normothermic reperfusion (NR). The black arrow points out point of delivery of 20mM glucose and the black star is when an insulin response was observed in the UWHMP group in parallel to increased glucose concentration. Line graph showing error bars and standard error of the mean. Abbreviations: GSIS (glucose-stimulated insulin secretion), IGL2HMP (Institut Georges Lopez 2 solution oxygenated hypothermic machine perfusion group), NR (normothermic reperfusion), SCS (static cold storage group), UWHMP (University of Wisconsin-MPS oxygenated hypothermic machine perfusion group).

Our DCD porcine model was chosen due to its pathophysiological similarities to humans [41] By using an abattoir model, we not only achieved an ethically conscious supply of animal organs for research but also an economical, reliable and reproducible model by utilizing normally discarded viscera from livestock reared for consumption.

In this study, we observed a trend in favour of $HMPO_2$ compared with SCS. The $HMPO_2$ groups demonstrated better blood flows, lower resistance indices, consistent portal venous blood flow and overall normal macroscopic appearances compared with the SCS pancreases during NR. The poor perfusion characteristics and minimal portal venous flows observed in the SCS group could be due to vasoconstriction and interstitial oedema as there was no evidence of vascular thrombosis on postperfusion dissection of the organ for purposes of evaluation.

The better perfusion characteristics observed in the $HMPO_2$ groups during NR may reflect the benefit of pulsatile HMP opening up the microcirculation, continually flushing out microthrombi and stabilizing the endothelium. The provision of oxygenation during HMP theoretically supports the maintenance of cellular energetics (although demand is significantly reduced due to hypothermia), and cell membrane integrity,

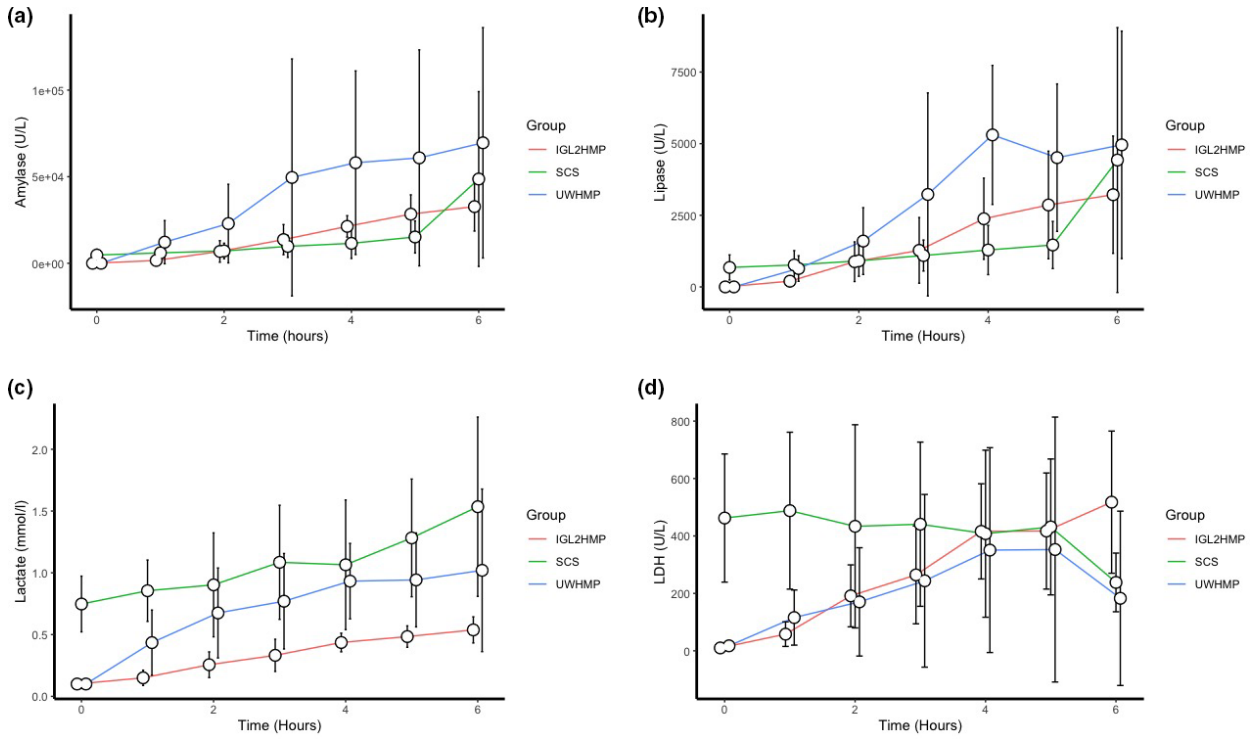

Figure 9 (a) Amylase U/l levels during hypothermic preservation. Line graph showing error bars and standard error of the mean. Abbreviations: IGL2HMP (Institut Georges Lopez 2 solution oxygenated hypothermic machine perfusion group), SCS (static cold storage group), UWHMP (University of Wisconsin-MPS oxygenated hypothermic machine perfusion group). (b) Lipase U/l levels during hypothermic preservation. Line graph showing error bars and standard error of the mean. Abbreviations: IGL2HMP (Institut Georges Lopez 2 solution oxygenated hypothermic machine perfusion group), SCS (static cold storage group), UWHMP (University of Wisconsin-MPS oxygenated hypothermic machine perfusion group). (c) Lactate mmol/l levels during hypothermic preservation. Line graph showing error bars and standard error of the mean. Abbreviations: IGL2HMP (Institut Georges Lopez 2 solution oxygenated hypothermic machine perfusion group), SCS (static cold storage group), UWHMP (University of Wisconsin-MPS oxygenated hypothermic machine perfusion group). (d) Lactate Dehydrogenase U/l (LDH) levels during hypothermic preservation. Line graph showing error bars and standard error of the mean. Abbreviations: IGL2HMP (Institut Georges Lopez 2 solution oxygenated hypothermic machine perfusion group), SCS (static cold storage group), UWHMP (University of Wisconsin-MPS oxygenated hypothermic machine perfusion group).

therefore, translating to a reduced susceptibility to IRI. The absence of oxygenation in the SCS group may explain the significantly higher levels of lactate, a marker of anaerobic respiration and LDH, a marker of cellular damage during cold preservation compared with the $HMPO_2$ groups. Leemkuil *et al.* [27] quantified the effect of oxygenation in their work observing that 6 hours of $HMPO_2$ led to a 6.8- and 2.6-fold increase in ATP concentration in DCD and DBD non-transplanted human pancreases, respectively.

Water mass content was used as a surrogate for oedema assessment. The $HMPO_2$ groups demonstrated a decrease in water mass, whilst the SCS pancreases were observed to have a mean increase. This is an encouraging finding, as there is some concern for oedema development during HMP, described in a few studies [23–26]. Our observation is similar to that of Leemkuil *et al.* [27] where after 6 hours of $HMPO_2$, there were no histological appearances of oedema. Likewise, Hamaoui *et al.* [28] observed in their work that pancreas HMP led to more stable perfusion dynamics during NR and minimal weight gain compared with SCS preserved pancreases.

The UWHMP group was the only group that demonstrated a rise in insulin levels at 42 minutes from baseline in parallel to an increase in glucose concentration in the perfusate. This response occurred 12 minutes postdelivery of the high-glucose bolus. The remaining groups (SCS and IGL2HMP) did not show a similar trend. This may be likely due to the observation that perfusate glucose levels had not yet increased in these groups during the 15 minutes poststimulation sampling interval, and perhaps, a longer duration of sampling, for example over an hour, may be enough time for the increase in glucose to equilibrate in the perfusate to evaluate insulin secretion.

Hamaoui [28] is the only study that is most similar to our research, in that they utilized NR for viability assessment after a period of SCS (26 hours) or 26 hours SCS and subsequent HMP for 5 hours using porcine

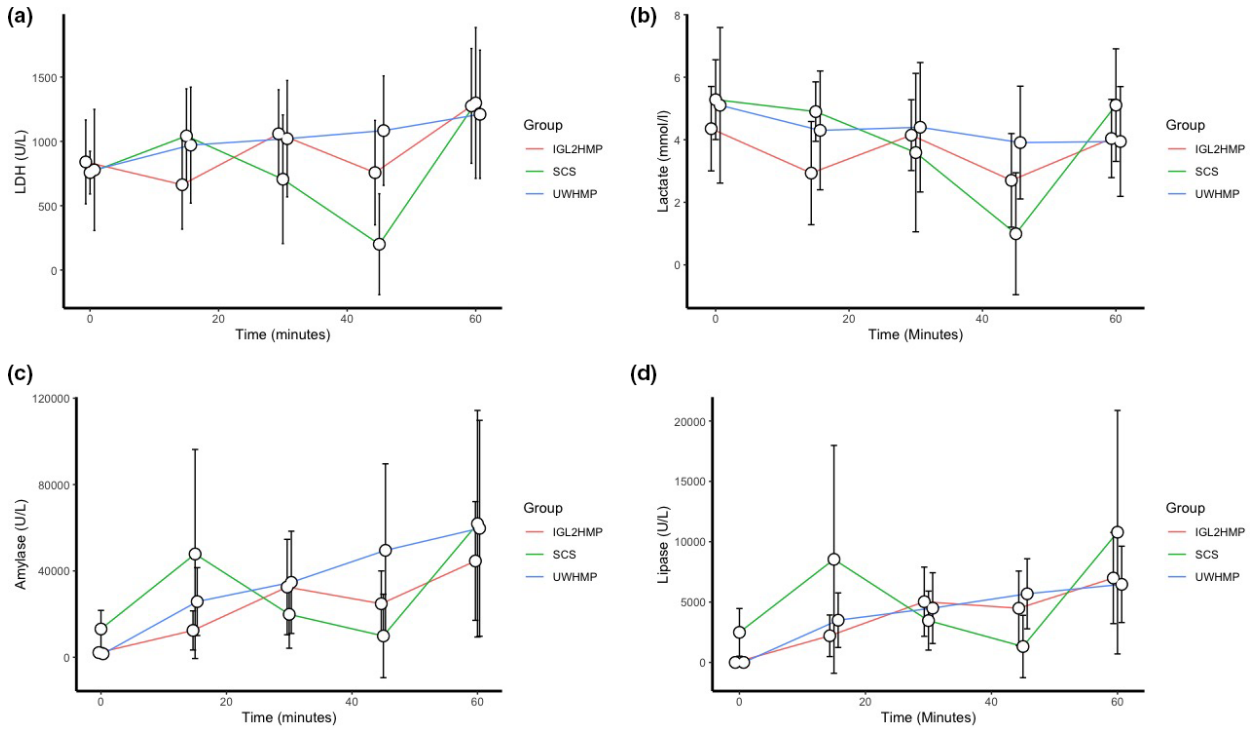

Figure 10 (a) Lactate Dehydrogenase U/L (LDH) levels during normothermic reperfusion. Line graph showing error bars and standard error of the mean. Abbreviations: IGL2HMP (Institut Georges Lopez 2 solution oxygenated hypothermic machine perfusion group), SCS (static cold storage group), UWHMP (University of Wisconsin-MPS oxygenated hypothermic machine perfusion group). (b) Lactate mmol/L levels during normothermic reperfusion. Line graph showing error bars and standard error of the mean. Abbreviations: IGL2HMP (Institut Georges Lopez 2 solution oxygenated hypothermic machine perfusion group), SCS (static cold storage group), UWHMP (University of Wisconsin-MPS oxygenated hypothermic machine perfusion group). (c) Amylase U/l levels during normothermic reperfusion. Line graph showing error bars and standard error of the mean. Abbreviations: IGL2HMP (Institut Georges Lopez 2 solution oxygenated hypothermic machine perfusion group), SCS (static cold storage group), UWHMP (University of Wisconsin-MPS oxygenated hypothermic machine perfusion group). (d) Lipase U/l levels normothermic reperfusion. Line graph showing error bars and standard error of the mean. Abbreviations: IGL2HMP (Institut Georges Lopez 2 solution oxygenated hypothermic machine perfusion group), SCS (static cold storage group), UWHMP (University of Wisconsin-MPS oxygenated hypothermic machine perfusion group).

pancreases. They reported that two-thirds of the pancreases in their SCS-HMP group demonstrated a response to GSIS versus no response observed in the SCS pancreases.

Finally, we investigated whether a newer preservation solution IGL2, containing PEG, may be beneficial compared with UW solution; although a significant elevation of both amylase and lipase levels was observed in the UWHMP group compared with the IGL2 group during HMP, no other significant differences were observed during NR, limiting any useful inferences.

Limitations

This study was primarily feasibility work involving small experimental numbers, therefore, precluding any clinical conclusions. The NR model was not entirely physiological as we use leukodepleted autologous blood. Our goal with leukodepletion was to reduce the distracting immune responses during reperfusion assessment (slightly similar to the effect of induction immunosuppression). There were high levels of exocrine enzymes observed in this study, highlighting the importance of duodenal and/or pancreatic duct drainage and clearance of accumulated exocrine proteases released during pancreas perfusion, yet to be addressed in any studies to date.

Our findings, hopefully, provide some evidence to power a larger study to investigate the effectiveness of $HMPO_2$ to optimize pancreas preservation for PTx, and the outcomes of which could be usefully translated to islet transplantation.

Conclusion

NR of pancreases is feasible and has the potential to be used as a method of assessment for preservation injury. In addition, we have also demonstrated using a porcine DCD model, that $HMPO_2$ may be a beneficial strategy

of preservation for pancreases compared with the current standard, SCS. With a number of novel organ preservation and reconditioning strategies to test in the coming years, NR as a platform for functional and viability assessment to ensure safe clinical translation will prove to be an invaluable tool.

Authorship

AEO:designed study, performed study, collected data, analysed data and wrote the paper. JB: designed study, performed study, collected data, analysed data and wrote paper. JH: designed study, wrote paper. RP and PF: designed study, paper revision. GH, FD, FE-G, RD, TP, BM, KR and JO: performed study, collected data.JM, LLF, TJ, ES and KS: data analysis. SM and PJ: designed study.

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Conflicts of interest

The authors have declared no Conflicts of Interest.

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