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

Ann Etohan Ogbemudia¹,², Gabriella Hakim¹, Fungai Dengu¹,², Faysal El-Gilani¹,², Richard Dumbill¹,², John Mulvey¹, Karen Sayal¹,², Thomas Prudhomme⁴, Benoit Mesnard⁵, Kaithlyn Rozenberg¹, Letizia Lo Faro¹, Timothy James⁵, Joshua Oliver², Edward Sharples¹,², Shruti Mittal¹,², Paul Johnson¹,⁶, Peter J. Friend¹,², Rutger Ploeg¹,², James Hunter¹,²,⁷, & Julien Branchereau¹,⁵,⁸

1 Nuffield Department of Surgical Sciences, Oxford Transplant Centre, University of Oxford, Oxford, UK
2 Oxford University Hospitals NHS Foundation Trust, Oxford, UK
3 CRUK, Oxford Cancer Centre, University of Oxford, Oxford, UK
4 Department Urology, Kidney Transplantation and Andrology, Toulouse Rangueil University, Toulouse, France
5 Institut de Transplantation Urologie Néphrologie (ITUN), CHU Nantes, Nantes, France
6 DRWF Human Islet Isolation Facility, Oxford, UK
7 University Hospitals Coventry and Warwickshire NHS Trust, Oxford, UK
8 Centre de Recherche en Transplantation Et Immunologie (CRTI), UMR1064, INSERM, Université de Nantes, Nantes, France

Correspondence
Ann Etohan Ogbemudia, Nuffield Department of Surgical Sciences, Oxford Transplant Centre, Churchill Hospital, Oxford OX3 7LE, UK.
Tel.: +44 (0) 1865 223872; e-mail: etohan.ogbemudia@ouh.nhs.uk

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.

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

Received: 28 June 2021; Accepted: 7 July 2021

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
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\textsubscript{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\textsubscript{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\textsuperscript{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\textsubscript{2}O (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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsubscript{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\textsuperscript{TM}. One litre of either IGL2 or UW-MPS was delivered and oxygenated via the organ cassette oxygenator by medical grade 21% O\textsubscript{2} (BOC, Linde group, Surrey UK) at a flow rate of 1 L/min. This provided a perfusate partial pressure of oxygen (pO\textsubscript{2}) of greater than 150 mmHg (21 kpa). The portal vein was left open...
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™ filter. Leukodepletion was confirmed on a haematology analyser, Sysmex XN-1000™, 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).
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™), 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 HMPO₂) at baseline, (before start of HMPO₂ 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).

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
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 HMPO₂ 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 HMPO₂ 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-HMPO₂ and observed no induction of oedema or apoptosis.

![Figure 5](image-url)

**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.
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).
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 HMPO2 compared with SCS. The HMPO2 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 HMPO2 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,
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 HMPO2 groups. Leemkuil et al. [27] quantified the effect of oxygenation in their work observing that 6 hours of HMPO2 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 HMPO2 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 HMPO2, 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
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 HMPO2 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 HMPO2 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.

Funding

The authors have declared no funding.

Conflicts of interest

The authors have declared no Conflicts of Interest.

REFERENCES

1. Martin JL, Costa ASH, Gruszczky AV, et al. Succinate accumulation drives ischaemia-reperfusion injury during organ transplantation. Nature Metabolism. 2019; 1: 966.
2. Proneth A, Schnitzbauer AA, Schenker P, et al. Extended pancreas donor program - The EXPAND Study: A prospective multicenter trial testing the use of pancreas donors older than 50 years. Transplantation 2018; 102: 1330.
3. Fridell JA, Rogers J, Stratta RJ. The pancreas allotransplant donor: current status, controversies, and challenges for the future. Clin Transplant 2010; 24: 433.
4. Leemkuil M, Leuvenink HGD, Pol RA. Pancreas transplantation from donors after circulatory death: an irrational reluctance? Curr Diab Rep 2019; 19: 129.
5. Warshaw AL, O’Hara PJ. Susceptibility of the pancreas to ischemic injury in shock. Ann Surg 1978; 188: 197.
6. Fernandez-del Castillo C, Harringer W, Warshaw AL, et al. Risk factors for pancreatic cellular injury after cardiopulmonary bypass. N Engl J Med 1991; 325: 382.
7. Schaser KD, Puhl G, Vollmar B, et al. In vivo imaging of human pancreatic microcirculation and pancreatic tissue injury in clinical pancreas transplantation. Am J Transplant 2005; 5: 341.
8. Sunamura M, Yamauchi J, Shibuya K, et al. Pancreatic microcirculation in acute pancreatitis. J Hepatobiliary Pancreat Surg 1998; 5: 62.
9. Sweiry JH, Mann GE. Pancreatic microvascular permeability in caerulein-induced acute pancreatitis. Am J Physiol 1991; 261(Pr 1): G689-G692.
10. Sakorafas GH, Tsiotos GG, Sarr MG. Ischemia/Reperfusion-Induced pancreatitis. Dig Surg. 2000; 17: 3.
11. Nadalin S, Girotti P, Königsrainer A. Risk factors for and management of graft pancreatitis. Current Opinion in Organ Transplantation. 2013; 18: 89.
12. Troppmann C. Complications after pancreas transplantation. Current Opinion in Organ Transplantation. 2010; 15: 112.
13. NHS Blood and Transplant. Annual report on pancreas and islet transplantation 2018. Available from: https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/12251/nhsbtt-pancreas-and-islet-transplantation-annual-report-2017-2018.pdf. [Accessed 2020 4 May]
14. Annual Report of the U.S. Organ Procurement and Transplantation Network and the Scientific Registry of Transplant Recipients: 1994-2020. [Internet]. Department of Health and Human Services, Health Resources and Services Administration, Healthcare Systems Bureau, Division of Transplantation, Rockville, MD; United Network for Organ Sharing, Richmond, VA; University Renal Research and Education Association, Ann Arbor, MI: UNOS. 2021. Available from: http://optn.transplant.hrsa.gov.
15. Transplant Nba. Organ Donation and Transplantation Activity Report 2018/19. 2019. Available from: https://nhsbttde.blob.core.windows.net/umbraco-assets-corp/16537/organ-donation-and-transplantation-activity-report-2018-2019.pdf. [Accessed 2020 01 June 2020]
16. Jochmans I, Brut A, Davies L, et al. Oxygenated versus standard cold preservation in kidney transplantation (COMPARE): a randomised, double-blind, paired, phase 3 trial. The Lancet 2020; 396: 1653.
17. Nicholson ML, Hosgood SA. Renal transplantation after ex vivo normothermic perfusion: the first clinical study. Am J Transplant 2013; 13: 1246.
18. van Rijn R, Schurink IJ, de Vries Y, et al. Hypothermic machine perfusion in liver transplantation — a randomized trial. N Engl J Med 2021; 384: 1391.
19. Nasralla D, Coussios CC, Mergental H, et al. A randomized trial of normothermic preservation in liver transplantation. Nature 2018; 557: 50.
20. Cypel M, Yeung JC, Liu M, et al. Normothermic ex vivo lung perfusion in clinical lung transplantation. N Engl J Med 2011; 364: 1431.
21. Ardehali A, Esmailian F, Deng M, et al. Ex-vivo perfusion of donor hearts for human heart transplantation (PROCEED II): a prospective, open-label, multicentre, randomised non-inferiority trial. Lancet 2015; 385: 2577.
22. Mergental H, Laing RW, Kirkham AJ, et al. Transplantation of discarded livers following viability testing with normothermic machine perfusion. Nat Commun 2020; 11: 2939.
23. Tersigni R, Toledo-Pereyra LH, Pinkham J, et al. Pancreaticoduodenal preservation by hypothermic pulsatile perfusion for twenty-four hours. Ann Surg 1975; 182: 743.
24. Brynger H. Twenty-four-hour preservation of the duct-ligated canine pancreatic allograft. Eur Surg Res 1975; 7: 341.
25. Florack G, Sutherland DE, Heil J, et al. Preservation of canine segmental pancreatic autografts: cold storage versus pulsatile machine perfusion. J Surg Res 1983; 34: 493.
26. Karcz M, Cook HT, Sibbons P, et al. An ex-vivo model for hypothermic pulsatile perfusion of porcine pancreata:
hemodynamic and morphologic characteristics. Exp Clin Transplant 2010; 8: 55.

27. Leemkuil M, Lier G, Engelse MA, et al. Hypothermic oxygenated machine perfusion of the human donor pancreas. Transplant Direct. 2018; 4: e388.

28. Hamaoui K, Gowers S, Sandhu B, et al. Development of pancreatic machine perfusion: translational steps from porcine to human models. J Surg Res 2018; 223: 263.

29. Hessheimer AJ, Fondevila C, García-Valdecasas JC. Chapter 106 - Extracorporeal perfusion for resuscitation of marginal grafts. In: Busuttil RW, Klintmalm GBG, eds. Transplantation of the Liver, 3rd ed. Philadelphia: W.B. Saunders, 2015: 1452–1462.

30. Bejaoui M, Pantazi E, Calvo M, et al. Polyethylene glycol preconditioning: an effective strategy to prevent liver ischemia reperfusion injury. Oxid Med Cell Longev. 2016; 2016: 9096549.

31. Ferrero-Andres A, Panisello-Rosello A, Serafin A, et al. Polyethylene glycol 35 (PEG35) protects against inflammation in experimental acute necrotizing pancreatitis and associated lung injury. Int J Mol Sci 2020; 21: 917.

32. Panisello Rosello A, Teixeira da Silva R, Castro C, et al. Polyethylene glycol 35 as a perfusate additive for mitochondrial and glycocalyx protection in HOPE liver preservation. Int J Mol Sci 2020; 21: 5703.

33. Axelrod DA, Sung RS, Meyer KH, et al. Systematic evaluation of pancreas allograft quality, outcomes and geographic variation in utilization. Am J Transplant 2010; 10: 837.

34. Moers C, Smits JM, Maathuis M-HJ, et al. Machine perfusion or cold storage in deceased-donor kidney transplantation. N Engl J Med 2009; 360: 7.

35. Treckmann J, Moers C, Smits JM, et al. Machine perfusion versus cold storage for preservation of kidneys from expanded criteria donors after brain death. Transplant Int 2011; 24: 548.

36. Jochmans I, Moers C, Smits JM, et al. Machine perfusion versus cold storage for the preservation of kidneys donated after cardiac death: a multicenter, randomized, controlled trial. Ann Surg 2010; 252: 756.

37. Prudhomme T, Renaudin K, Lo Faro ML, et al. Ex situ hypothermic perfusion of nonhuman primate pancreas: A feasibility study. Artif Organs 2020; 44: 736.

38. Branchereau J, Renaudin K, Kervella D, et al. Hypothermic pulsatile perfusion of human pancreas: Preliminary technical feasibility study based on histology. Cryobiology 2018; 85: 56.

39. Prudhomme T, Kervella D, Ogbemudia AE, et al. Successful pancreas allotransplantations after hypothermic machine perfusion in a novel diabetic porcine model: a controlled study. Transpl Int 2021; 34: 353.

40. Doppenberg JB, Leemkuil M, Engelse MA, et al. Hypothermic oxygenated machine perfusion of the human pancreas for clinical islet isolation: a prospective feasibility study. Transpl Int 2021. https://doi.org/10.1111/tri.13927

41. Roura E, Koopmans S-J, Lallès J-P, et al. Critical review evaluating the pig as a model for human nutritional physiology. Nutr Res Rev 2016; 29: 60.