

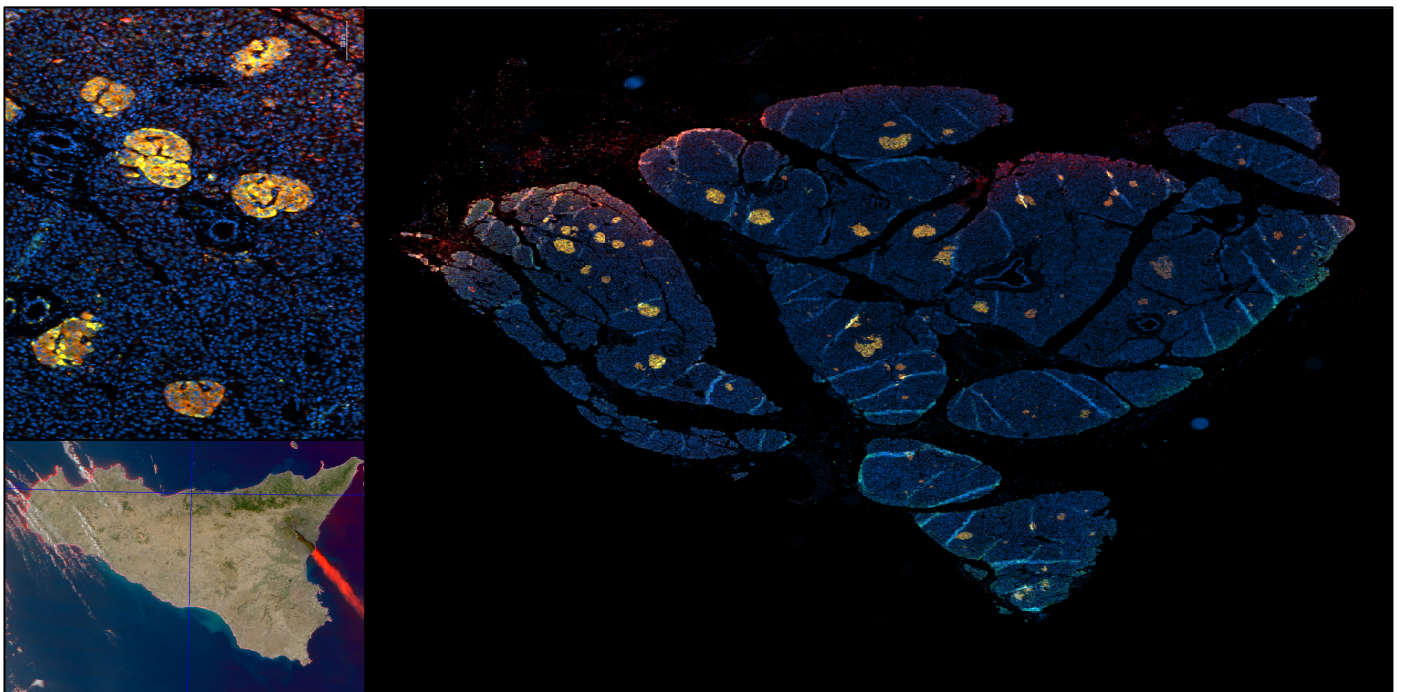
Exploring preservation modalities in a split human pancreas model to investigate the effect on the islet isolation outcomes.

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Secteur des sciences de la santé



Cette thèse de doctorat, avant même d'être l'accomplissement d'un parcours professionnel, est surtout le résumé d'un voyage dans lequel, malgré moi, j'ai été le protagoniste.

Le monde de la recherche est un monde difficile, que seuls ceux qui l'ont vécu savent ce qu'il implique vraiment. Il est fait d'incertitudes, de grandes déceptions, souvent de découragement, mais aussi d'immenses joies, parfois incompréhensibles tant les causes qui les ont déclenchées peuvent sembler petites et insignifiantes.

La recherche scientifique est un monde solitaire. Une lumière allumée dans la nuit d'un laboratoire en attendant qu'une alarme retentisse. Un rayon d'espoir quand tout le monde pense que ce que vous faites n'est pas suffisant.

La recherche est aussi un moment de partage et de rencontres. Elle crée cet état de dépendance, ce moment où vous réalisez que c'est seulement à travers les autres que l'on peut atteindre le meilleur pour la cause.

Mon parcours a été difficile, solitaire mais aussi rempli de rencontres, comme la recherche le demande.

Et ce fut un voyage magnifique.

Ma première reconnaissance va donc à elle.

À cette incroyable force qui pousse les hommes à cette frénétique envie d'ajouter, même petit, un élément à la vérité.

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Vous êtes mon île. Mon point de départ et mon point d'arrivée.

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...Garde sans cesse Ithaque présente dans ton esprit. Ton but final est d'y parvenir, mais n'écourte pas ton voyage: mieux vaut qu'il dure de longues années et que tu abordes enfin dans ton île aux jours de ta vieillesse, riche de tout ce que tu as gagné en chemin, sans attendre qu'Ithaque t'enrichisse.

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Table 1. Product release criteria for clinical islet preparation

Abbreviation list

- **ADP**, Adenosin-Diphosphate
- **ATP**, Adenosin-Triphosphate
- **BMI**, body mass index
- **CCL**, chemokine ligand
- **CITR**, Collaborative Islet Transplantation Registry
- **DAMPs**, damage-associated molecular patterns
- **DBD**, donor after brain death
- **DC**, Dendritic cells
- **DCD**, donor after circulatory death
- **ECS**, Euro-Collins solution
- **eGFR**, estimated glomerular filtration rate
- **ESRD**, End-stage renal disease
- **EU**, endotoxin units
- **FDA**, fluorescein diacetate
- **GSIS**, glucose-stimulated insulin secretion
- **HES**, Hydroxyethyl Starch
- **HLA**, Human leucocyte antigen
- **HMP**, hypothermic machine perfusion
- **HTK**, Histidine Tryptophan Ketoglutarate
- **IAK**, islet-after-kidney
- **IEQ**, islet equivalents
- **IGL-1**, Institut Georges Lopez-1
- **IL**, Interleukin
- **IRI**, ischemia-reperfusion injuries
- **ITA**, Islet transplantation alone
- **KPS-1**, Kidney Perfusion Solution
- **MCP**, monocyte chemoattractant protein
- **MODY**, Maturity-onset diabetes of the young
- **MRI**, magnetic resonance imaging
- **NEM**, novel enzyme mixture
- **NETs**, neutrophil extracellular traps
- **NMP**, normothermic machine perfusion
- **NRP**, normothermic regional perfusion
- **O₂ HMP**, oxygenated hypothermic machine perfusion
- **OCR**, Oxygen consumption rate
- **PAK**, Pancreas after kidney
- **PFCs**, Perfluorocarbons
- **PI**, Propidium iodide
- **PSF**, Oxygen persufflation
- **PTA**, Pancreatic transplantation alone
- **SCS**, Static cold storage
- **SPK**, Simultaneous pancreas kidney
- **T1D**, Type 1 Diabetes
- **T2D**, Type 2 Diabetes
- **TLM**, Two-Layer Method
- **TLR**, Toll-like receptor
- **TNF- α** , Tumor Necrosis Factor- α
- **UW**, University of Wisconsin

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1.1 Pancreas transplantation: State of the art

The discovery of insulin almost a century ago by Banting and Best marked a pivotal moment, rendering diabetes a treatable disease. Subsequent advancements, such as the development of synthetic short- and long-acting insulin, portable blood sugar monitoring, and insulin pumps, have significantly enhanced diabetes care. Nevertheless, many patients continue to grapple with the secondary complications of diabetes, encompassing retinopathy, neuropathy, nephropathy, and premature cardiovascular disease. Pancreas transplantation emerges as a solution offering the potential to restore normoglycemia in individuals with type 1 and selected type 2 diabetes. Its objectives include replacing beta-cell function, mitigating short- and long-term diabetic complications and improving long-term survival rates. Since the inaugural pancreas transplant in 1966, over 50,000 such procedures have

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been conducted worldwide¹. Advancements in induction and maintenance immunosuppression alongside surgical techniques have contributed to favorable outcomes post-transplant. Nevertheless, given the complexity of transplantation and the prevalence of comorbidities among recipients, meticulous patient selection remains imperative to optimize benefits.

Worldwide, approximately 90% of pancreas transplants are performed concurrently with kidney transplants (SPK), especially for patients with type 1 diabetes and diabetic nephropathy whose estimated glomerular filtration rate falls below 20mL/min/1.73m².²

Type 2 diabetics with renal failure and a Body Mass Index (BMI) below 30 kg/m² and low insulin requirements may also be considered for transplantation³.

Individuals who have undergone kidney transplantation, whether from living or deceased donors, may be candidates for pancreas transplantation following kidney transplantation.

Pancreas transplant alone is reserved for those experiencing severe metabolic complications of diabetes despite optimal insulin therapy, characterized by severe hypoglycemic unawareness but preserved renal function. These patients must have a documented history of at least two severe hypoglycemic episodes within the last 24 months, confirmed by a diabetologist⁴. The pancreas transplant procedure involves meticulous preparation of the

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graft, utilizing the donor iliac vessels to form a 'Y-graft' connecting the recipient's common iliac artery with the pancreas's splenic and superior mesenteric arteries. The portal vein is typically anastomosed to the iliac vein, while the duodenum is connected to a segment of the small bowel to facilitate drainage of exocrine secretions (Figure 1).

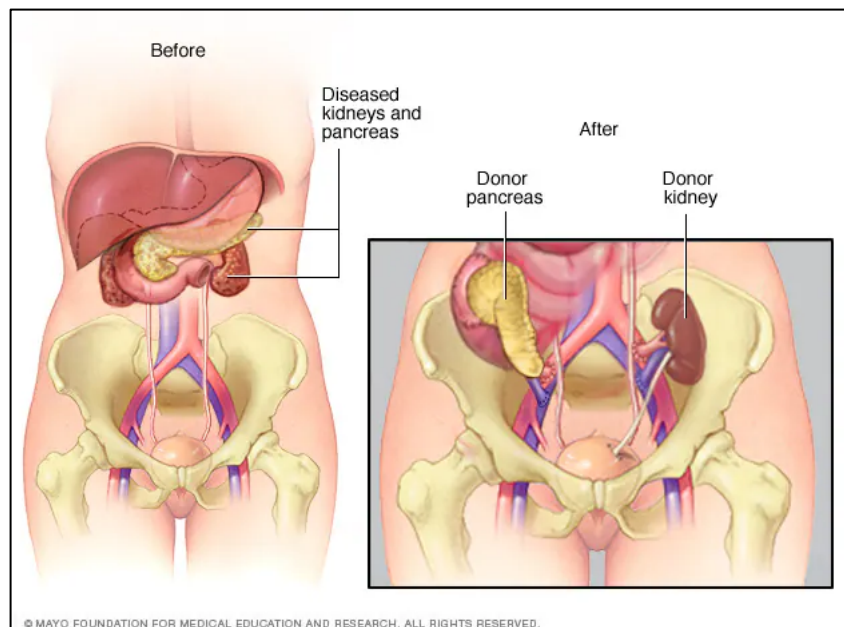


Figure 1: Anatomical diagram showing the placement of pancreas and kidney for a SPK transplant. (<https://www.mayoclinic.org/pac-20384783>.)

In cases of pancreas transplant alone, the duodenum may alternatively be connected to the bladder for urinary amylase monitoring⁵.

Following transplantation, recipients require maintenance immunosuppression, which typically includes a calcineurin inhibitor like tacrolimus and an

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anti-metabolite such as mycophenolate. Some may receive low-dose steroids, though steroid-sparing regimens are preferred. Immunosuppression increases the risk of infection and malignancy compared to the general population, particularly concerning pancreas transplant alone, where the risks of hypoglycemic coma must be carefully balanced against those of surgery and immunosuppression.

Potential risks and complications associated with pancreas transplantation include pancreatitis due to poor tolerance of cold ischemia, bleeding post-implantation, leakage from the duodenal–jejunal anastomosis, and thrombosis in the arterial Y-graft or the portal vein. Complications may necessitate relaparotomy, with graft pancreatectomy required in some cases. The 1-year mortality rate following pancreas transplant is approximately 3%².

In the UK, pancreatic graft survival rates at 1 and 5 years stands at 90% and 81%, respectively⁶. In individuals with renal failure, undergoing a simultaneous pancreas-kidney transplant presents a distinct advantage in terms of survival. In Scotland, diabetic patients aged 45–64 years undergoing dialysis exhibit a mere 10% survival rate over a span of 10 years⁷. Meanwhile, for those in the younger age bracket of 18–45 years, the 10-year survival rate remains at a mere 50%. Analogous survival figures can be observed in other regions of Europe and the United States^{8,9}.

Research indicates that a functional pancreatic graft can impede the progression of complications, offering improvements in retinopathy and diabetic

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neuropathy symptoms. These positive outcomes are contingent on the sustained functionality of the transplanted pancreas.

Take Home message

1. Historical Context: The discovery of insulin by Banting and Best nearly a century ago revolutionized diabetes treatment. Subsequent advancements, such as synthetic insulin development and portable blood sugar monitoring, have improved diabetes care. However, many patients still face secondary complications.

2. Pancreas Transplantation: Offers potential to restore normoglycemia and mitigate diabetic complications. Over 50,000 procedures performed worldwide since 1966. Advancements in immunosuppression and surgical techniques have improved post-transplant outcomes.

3. Patient Selection: Meticulous patient selection crucial due to transplantation complexity and recipient comorbidities. Approximately 90% of pancreas transplants performed concurrently with kidney transplants (SPK). Specific criteria for pancreas transplant alone recipients.

4. Procedure: Involves meticulous preparation of graft, connecting donor iliac vessels to recipient's arteries. Portal vein anastomosed to iliac vein, duodenum connected to small bowel for exocrine drainage. Maintenance immunosuppression required post-transplant.

5. Risks and Complications: Potential complications include pancreatitis, bleeding, anastomotic leakage, and thrombosis. Mortality rate around 3% at 1 year post-transplant. Graft survival rates vary but can significantly improve survival in diabetic patients, especially when combined with kidney transplant.

6. Outcomes: Functional pancreatic grafts can impede progression of complications and improve symptoms of retinopathy and neuropathy. Long-term success depends on sustained graft functionality.

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1.2 From Pancreas to Islet Transplantation: Competitors or Allies?

For individuals with Type 1 Diabetes (T1D), a lifelong regimen of exogenous insulin is essential to regulate blood glucose levels. However, some patients face the risks of Impaired Awareness of hypoglycemia and severe hypoglycemic events, significantly impacting their quality of life¹⁰. Additionally, unstable blood glucose levels can lead to various complications, including retinopathy and neuropathy¹¹.

To address the issue of blood glucose instability in T1D patients, therapies such as β -cell replacement become crucial. This includes procedures like whole pancreas transplantation and pancreatic islet transplantation. Pancreas transplantation, even from a single donor, holds the promise of achieving long-term insulin independence. The advancement of immunosuppressive regimens, organ preservation methods, and operative techniques has led to a rise in successful transplantations over time.¹² Despite the proven efficacy of pancreas transplantation, some physicians hesitate to recommend this procedure due to its complexity and associated risks, particularly when performed as a standalone pancreas transplant¹³. Islet transplantation, involving the intraportal infusion of isolated pancreatic islets, emerged as a promising alternative (Figure 2). This procedure was expected to be an ideal transplant therapy for patients with severe glycemic instability, and development aiming to improve this procedure has been undertaken worldwide. Ballinger and Lacy demonstrated a method for isolating pancreatic islets

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from rodents and confirmed in vivo function in 1972¹⁴. Early successes in islet transplantation, such as achieving clinical insulin independence in 1990, spurred further research making this therapeutic approach a viable alternative¹⁵⁻²². Although apparently competing, pancreas and islet transplantation should ideally be viewed as complementary approaches, providing optimal clinical benefit to risk balance for each candidate and maximizing the use of quality donor pancreases.

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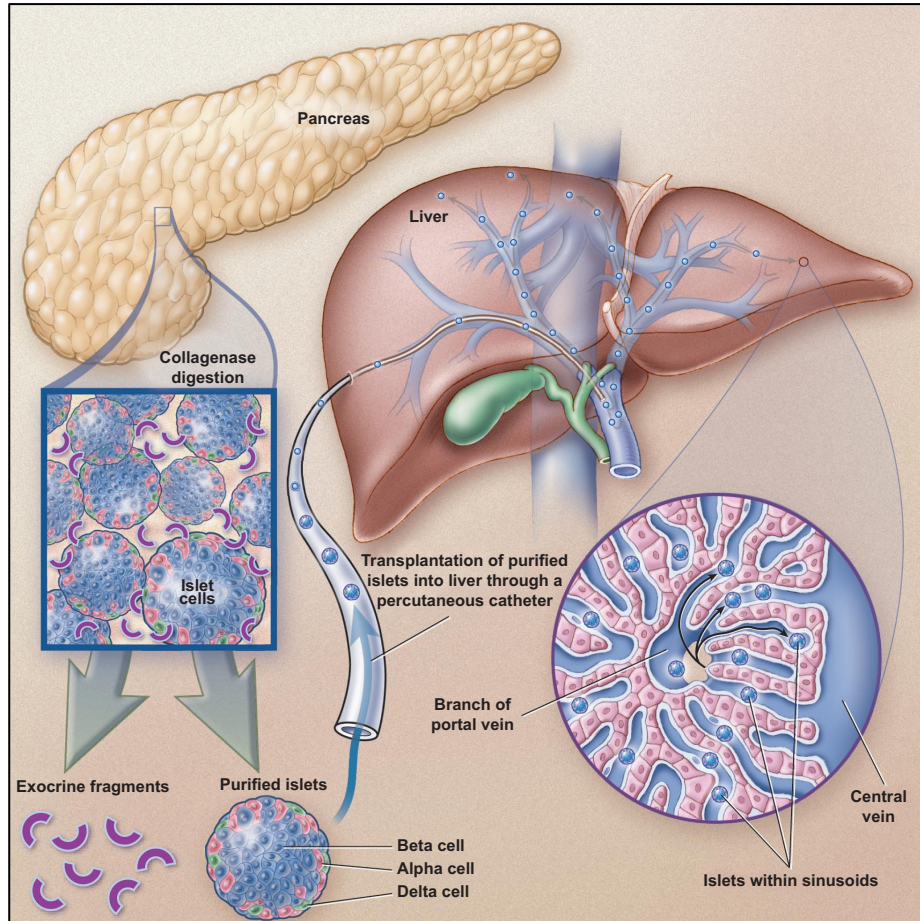


Figure 2. General method of intrahepatic islet transplantation.

The process of isolating and transplanting islets begins with the extraction of islets from a donated pancreas through collagenase digestion. Subsequently, a purification step separates the islets from exocrine tissue. These purified islets are then introduced into a catheter positioned within the hepatic portal vein, where they are infused by gravity. As blood flows through the vein, it carries the islets into the liver tributaries. Within these tributaries, the islets settle within the sinusoids and establish vascular connections, facilitating their integration into the liver tissue. (Robertson RP. Islet transplantation a decade later and strategies for filling a half-full glass. *Diabetes*. 2010 Jun;59(6):1285-91. doi: 10.2337/db09-1846. PMID: 20508222; PMCID: PMC2874688.)

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1.2.1 Pancreas vs Islet: Donor Selection

Organ sharing groups face the challenge of allocating a finite supply of resources between islet and pancreas transplantation. While pancreas transplantation often takes precedence, potential biases may impact the allocation of islet grafts, raising concerns about discrimination and its effects on clinical outcomes.

One proposed solution is establishing a shared waiting list for all individuals needing β -cell replacement treatment, aiming to mitigate biases²³. However, variations in organ requirements for the two treatments pose challenges, necessitating careful evaluation of organ suitability and the development of efficient allocation algorithms.

Organ quality scores, considering risk variables, have been explored to assess organ appropriateness for islet or pancreatic transplantation^{24,25}. Donor age, weight, BMI, hemodynamic stability, and ischemia times are reliable factors influencing outcomes²⁶⁻²⁹. Grading methods, such as the pancreas Donor Risk Index, aid in evaluating organs for whole pancreas transplantation, with technical failure being a significant cause of graft loss³⁰. The overlap between optimal donors for pancreatic and islet transplantation depends on factors like BMI and age (Figure 3). Higher donor BMI is associated with increased risks and complications in pancreas transplantation³¹, impacting graft thrombosis, infections, and technical failure. In contrast, overweight or

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obese donors are considered to yield good islet results³²⁻³³. In a comprehensive analysis of how donor BMI affects the outcome of pancreas transplantation, it was revealed that certain complications significantly escalate with BMI. The occurrence of graft thrombosis rose to 4.8% in the BMI less than 25 kg/m² group, 12.2% in the BMI 25 to 30 kg/m² group, and 14.8% in the BMI more than 30 kg/m² group (P= 0.03). Likewise, the likelihood of deep infections nearly doubled with moderately to severely obese donors (21.0%, BMI >30 kg/m² vs. 11.7%, BMI < 25 kg/m², P=0.07). This notably impacted the incidence of technical graft failure, which stood at 9.7% in the BMI less than 25 kg/m² group, 16.3% in the BMI 25 to 30 kg/m² group, and 21.0% in the BMI more than 30 kg/m² group³¹. Similar findings were echoed in another pancreas transplant study, wherein heavier donors (>200 lbs) exhibited lower 1-year graft survival rates compared to lighter donors (88% vs. 84%). Though statistically insignificant, weight might be considered less discerning than BMI as an obesity indicator²⁴. The heightened fat content in pancreases from obese donors is poorly vascularized and more prone to ischemia-reperfusion injury, potentially leading to thrombosis, pancreatitis, and infection³⁴. Establishing a cutoff BMI value for rejecting a pancreas for whole organ transplantation presents a challenge (it could be speculated that the maximum acceptable BMI lies between 25 and 30 kg/m²) and hinges not only on other unfavorable donor factors but also on a patient's risk-benefit analysis between procedure morbidity and the hazards of extended wait times³⁵.

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Conversely, overweight and obese donors have been viewed as excellent sources for islet yields^{32,33}.

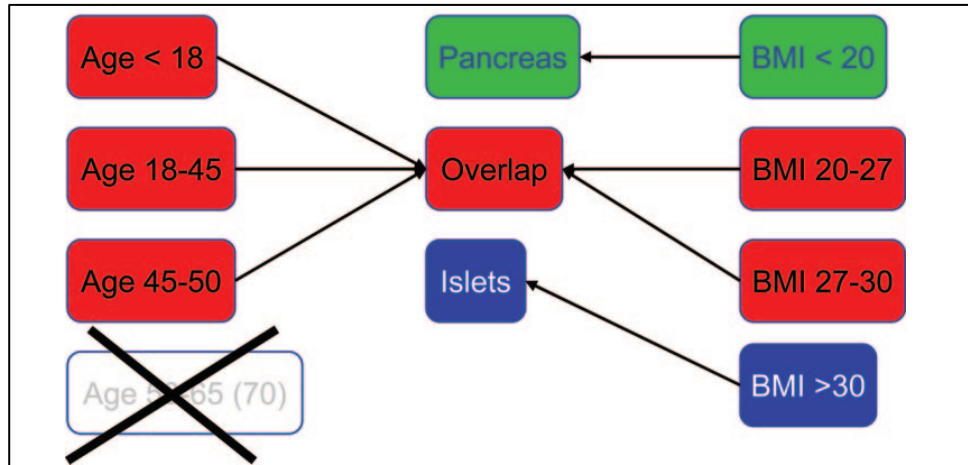


Figure 3. Criteria for ideal pancreas and islet donors and the resulting overlap

(Berney T, Johnson PR. Donor pancreata: evolving approaches to organ allocation for whole pancreas versus islet transplantation. Transplantation. 2010 Aug 15;90(3):238-43. doi: 10.1097/TP.0b013e3181e25a40. PMID: 20463635.)

Obese nondiabetic individuals have exhibited increased beta-cell mass compared to controls, facilitating a response to elevated metabolic demands. Conversely, lean donors (BMI <21 kg/m²) demonstrated notably lower islet yields³⁶. However, the functionality of islets from obese donors must be evaluated. In a study³³, islets isolated from obese donors (BMI >30 kg/m²) displayed nearly identical viability compared to non-obese donors, in vitro function assessed by insulin response to glucose in static incubation assays,

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and in vivo function evaluated by the time required to reverse chemical diabetes in a nude mouse bioassay. While islets from nondiabetic obese donors appear functionally sound, caution is warranted regarding identifying type 2 diabetes in such donors. A comparative analysis of islets isolated from type 2 diabetic versus control donors of similar BMI unveiled significantly lower recovered islet mass and markedly abnormal endocrine function both in vitro (assessed by glucose-stimulated insulin release in perfusion assays) and in vivo (in a nude mouse bioassay)³⁷. Utilization of pediatric donors for pancreas transplantation, either as whole organs or for islet transplantation, has been limited. Concerns regarding islet cell mass, technical complexities, and potential risks of vascular thrombosis have deterred their widespread use³⁸. However, a recent retrospective analysis revealed significantly improved 10-year kidney and pancreas graft survivals in recipients of simultaneous pancreas-kidney transplants from pediatric donors, provided donors were older than 3 years or weighed at least 25 kg³⁸. The recommendation was to allocate pancreases from pediatric donors preferably to recipients with lower BMI. Regarding islet isolation, the challenge with pancreases from pediatric donors lies in the technical intricacies of separating islets from exocrine cells. Researchers noted that in younger human pancreata, ducts are collapsed towards the islet cells, hindering the proper distribution of exogenous enzyme solution through the pancreatic ductal system³⁹. Consequently, islet yields more than 40% lower than those from adult donors have been reported²⁹, with less than 15% of preparations from pediatric donors deemed

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suitable for transplantation based on islet count²⁷. Nonetheless, it was acknowledged that pediatric islets exhibited better *in vitro* function²⁷. Recent findings suggest that optimizing the enzymatic digestion of the pancreatic extracellular matrix could greatly enhance islet yields from pediatric donors, leading to superior functionality confirmed through immunodeficient mouse bioassays³⁹. Nevertheless, obtaining sufficient islets from pediatric pancreases remains challenging.

Conversely, pancreata from older donors are linked to notably lower graft survival and increased morbidity in whole organ transplantation^{24,40}. The age threshold defining an "old" donor is relatively low, typically ranging from 45 to 50 years. Studies have shown inferior pancreas graft survival rates in older donors compared to younger ones, with technical issues early post-transplantation accounting for the observed differences. Additionally, older age combined with higher body weight exacerbates the risk factors and further diminishes graft survival²⁴. Similarly, lower pancreatic graft survival rates were documented in retrospective studies⁴¹. Assessing the situation in islet transplantation is more complex. While older donors can provide similar or higher islet yields after processing⁴², a negative correlation between donor age and *in vitro* islet function has been noted, with significantly lower rates of diabetes reversal observed in mouse bioassays with islets from donors older than 50 years⁴³. This inferior *in vivo* function was further confirmed in clinical islet transplantation studies^{42,43}. Notably, successful single-

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donor insulin independence in islet transplantation series was consistently achieved through strict donor age criteria, typically limiting donor age to less than 50 years^{44,45}. Various studies have explored the mechanisms underlying the decline in beta-cell functionality with increasing age^{46,47}, including diminished proliferation capacity and increased apoptosis rates, along with decreased expression of genes like Pdx1, associated with beta-cell function^{48,49}.

Take Home message

- 1.Allocation Challenges:** Organ sharing groups must balance resources between islet and pancreas transplantation, with potential biases affecting islet graft allocation, raising concerns about discrimination and clinical outcomes.
- 2.Proposed Solutions:** Establishing a shared waiting list for all needing β -cell replacement treatment could mitigate biases, but challenges in organ suitability and allocation algorithms remain.
- 3.Assessing Organ Quality:** Organ quality scores, considering risk variables like donor age, weight, BMI, and hemodynamic stability, aid in assessing organ appropriateness for transplantation.
- 4.Donor BMI Impact:** Higher donor BMI increases risks and complications in pancreas transplantation but may yield good results for islet transplantation. Overweight or obese donors are considered excellent sources for islet yields.
- 5.Utilization of Pediatric Donors:** Utilization of pediatric donors for transplantation is limited due to technical complexities and risks. However, recent data suggest improved graft survivals in recipients of simultaneous pancreas-kidney transplants from pediatric donors.
- 6.Challenges with Older Donors:** Pancreata from older donors are associated with lower graft survival and increased morbidity in whole organ transplantation. In islet transplantation, older donors can provide similar or higher islet yields, but inferior in vitro and in vivo function is noted.
- 7.Mechanisms of Decline:** Mechanisms underlying the decline in beta-cell functionality with increasing age include diminished proliferation capacity, increased apoptosis rates, and decreased expression of genes associated with beta-cell function.

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1.2.2 Pancreas vs Islet: Recipient Selection

It is difficult to justify islet transplantation (ITA) and pancreatic transplantation alone (PTA) in individuals with well-controlled diabetes who still have renal function. These procedures have significant risks. Nonetheless, certain clinical circumstances can justify the use of either transplantation strategy⁵⁰. When it comes to creating a customized plan and modifying treatment for people with T1D, early patient referral is essential. The prognosis for T1D patients may be greatly affected by options including ITA, early kidney transplantation from a living donor followed by PTA or ITA, and preemptive SPK transplantation. Sensitization, cardiovascular diseases, diabetic lability, and progressive end-stage renal disease (ESRD) all influence the choice of strategy. Several approaches to these considerations are described⁵⁰.

The main indication for T1D patients with renal failure is SPK transplantation (Figure 4 a), accounting for approximately 80% of pancreatic surgeries performed in Europe⁵¹. It should be offered to individuals with imminent or established ESRD who are considering a kidney transplant in the near future. In some circumstances, preemptive SPK transplantation may be thought of even before dialysis. The estimated glomerular filtration rate (eGFR), kidney function loss slope, proteinuria, albumin/creatinine ratio, and surgical risk related to a double graft are among the considerations that go into the decision-making process. A minimum of two vascular sites free of significant

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atheroma are needed for iliac anastomosis⁵². Moreover, patients under the age of 55 are often given priority.

Depending on the patient's physiological age, cardiovascular status, and surgical risk, Simultaneous islet and kidney transplantation or a kidney transplantation alone followed by IAK transplantation may be favored. Regardless, a BMI of less than 30 kg/m² is necessary.

There are two methods available for T1D patients with functional kidney grafts: islet-after-kidney (IAK) or pancreas-after-kidney (PAK) transplantation (Figure 4,b). These choices are limited to those with stable renal transplant function who, even with the best possible diabetic care, are unable to maintain adequate glucose control. Patients without cardiovascular contraindications or iliac artery atherosclerosis may be recommended for PAK. However, opting for this approach will result in the patient spending a prolonged duration on the waiting list.

Any patient receiving a kidney transplant should be evaluated for IAK, particularly if they do not have insulin resistance, hypertension, proteinuria, hypersensitization, or episodes of rejection⁵⁰. Patients with non-functioning kidneys or pancreas from living donors can benefit from SPK transplantation; nevertheless, there is a small risk of hypersensitization, which could restrict access to future transplants.

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PTA or islet transplantation ITA may be proposed to individuals with T1D who do not have renal failure or need a kidney transplant but who frequently experience severe hypoglycemia or significant glycemic lability despite receiving adequate diabetic treatment (Figure 4c). ITA is considered a less risky procedure. Thanks to the quick advancements in insulin delivery and glucose monitoring, defining 'optimal' insulin therapy has become more difficult. Four daily insulin shots and at least four capillary blood tests are necessary, nevertheless⁵³. According to clinical practice guidelines, insulin pumps and sensors are the current standard modality for beta-cell replacement therapy. The metabolic criteria for considering beta-cell replacement treatment are defined by the EPITA-IPITA consensus⁵⁰. The risk of long-term immunosuppressive regimens, the patient's incapacity to control hypo- or hyperglycemia, and degenerative consequences from persistent hyperglycemia are among the indications.

The patient's characteristics and the available resources in the area will determine whether PTA or ITA is best. PTA is recommended for younger individuals who are at a higher risk of developing complications from their diabetes, whereas ITA is recommended for people with unstable diabetes who are unaware of hypoglycemia, do not have insulin resistance, are not overweight, and are not candidates for whole pancreas transplants. Evaluation of instability is facilitated by quantitative measurements of lability and hypoglycemia. Because of its better benefit/risk ratio, ITA is frequently chosen

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even in cases when insulin independence is not attained in order to reestablish glycemic stability⁵⁰.

Specific considerations include:

Brittle diabetes and desire for pregnancy: PTA is the recommended indication for glycemic lability; pregnancy post-transplantation may be explored 1 or 2 years after switching immunosuppressants. It is important to discuss any possible immunosuppressive dangers to the newborn⁵⁴.

T1D with insulin resistance or overweight: These conditions make surgery more difficult and can affect the outcome, especially in ITA situations involving questionable islet mass transplants⁵¹.

Maturity-onset diabetes of the young (MODY): After SPK transplantation, a study shows excellent metabolic results, particularly in MODY types 1, 3, and 5⁵⁵.

Type 2 diabetes (T2D): While kidney transplantation is suggested for patients with T2D who have ESRD, there is ongoing debate on the appropriate timing and indications for pancreas transplantation in T2D patients. For T2D patients who do not have significant insulin resistance, intermediate forms of diabetes, trouble managing several daily insulin infusions, or diabetic instability, SPK may be taken into consideration⁵⁰.

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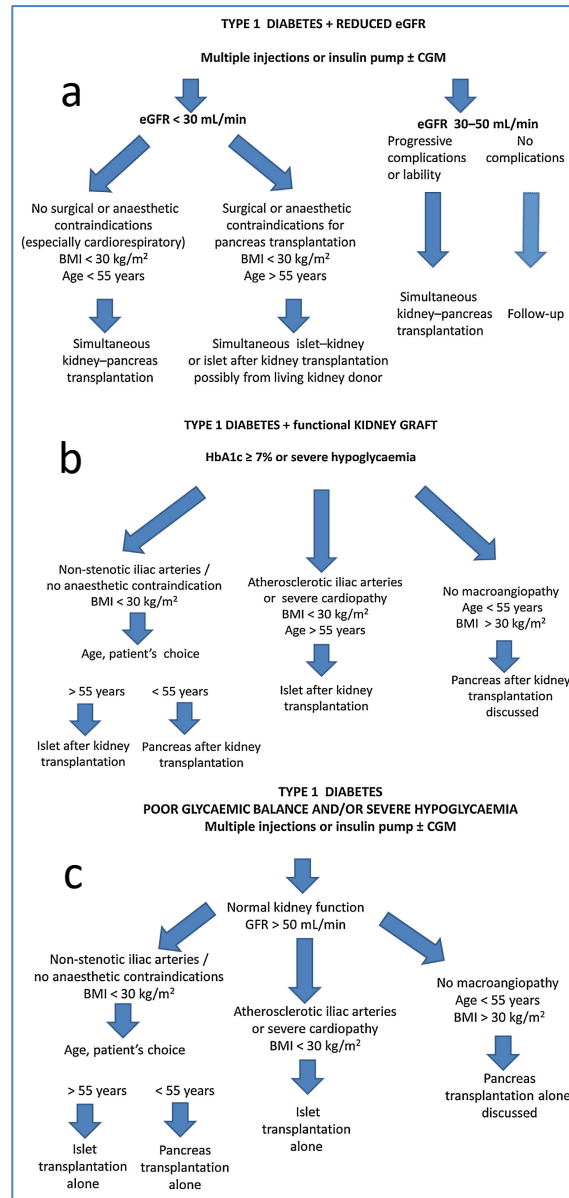


Figure 4. Indications for islet and pancreas transplantation.

(A) Patients with insulinopenic type 1 diabetes (T1D) and renal failure; and (B) T1D patients with functioning kidney graft; (C) patients with labile type 1 diabetes. (Rickels MR, Stock PG, de Koning EJP, Piemonti L, Pratschke J, Defining Outcomes for β -cell Replacement Therapy in the Treatment of Diabetes: A Consensus Report on the Igls Criteria From the IPITA/EPITA Opinion Leaders Workshop. Transplantation. 2018 Sep;102(9):1479-1486.)

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The indications for whole pancreas and islet transplantation can be altered by several factors, including age, BMI, smoking, blood group, sensitization, kidney function, macro- and micro-angiopathy, patient preference, local resources, living kidney donor, related autoimmune diseases, related grafts, preemptive transplantation, and consequences. In cases of hyper-immunization, long-term evaluations are required for potential risks related to immunosuppression and graft results.

Take Home message

1. Customized Treatment Plans: Early patient referral is crucial for creating customized treatment plans for individuals with Type 1 Diabetes (T1D). Various options, including preemptive simultaneous pancreas-kidney (SPK) transplantation, early kidney transplantation followed by PTA or ITA, and preemptive SPK transplantation, should be considered based on sensitization, cardiovascular diseases, diabetic lability, and progressive end-stage renal disease (ESRD).

2. Gold Standard for T1D patients with ESRD: SPK Transplantation: SPK transplantation is the main indication for T1D patients with renal failure, accounting for approximately 90% of pancreatic surgeries in Europe. It should be offered to individuals with imminent or established ESRD, with considerations such as kidney function, surgical risk, and vascular suitability.

3. Alternative Approaches: Depending on physiological age, cardiovascular status, and surgical risk, simultaneous islet and kidney transplantation or kidney transplantation alone followed by ITA may be favored. However, a BMI of less than 30 kg/m² is necessary.

4. Options for Patients with Functional Kidney Grafts: Islet-after-kidney (IAK) or pancreas-after-kidney (PAK) transplantation may be considered for stable renal transplant recipients unable to maintain adequate glucose control despite optimal diabetic care.

5. Considerations for Kidney Transplant Recipients: Patients receiving kidney transplants should be evaluated for IAK, particularly if they do not have insulin resistance, hypertension, proteinuria, hypersensitization, or rejection episodes. However, there is a small risk of hypersensitization, limiting access to future transplants.

6. PTA or ITA for Stable Diabetes: PTA or ITA may be proposed for T1D patients experiencing severe hypoglycemia or glycemic lability despite adequate diabetic treatment. ITA is considered less risky and may be chosen to reestablish glycemic stability, even if insulin independence is not achieved.

7. Specific Considerations: Various factors, including brittle diabetes, desire for pregnancy, insulin resistance, maturity-onset diabetes of the young (MODY), and type 2 diabetes (T2D), influence the choice of transplantation strategy and timing.

8. Factors Affecting Indications: Age, BMI, smoking, blood group, sensitization, kidney function, macro- and micro-angiopathy, patient preference, local resources, living kidney donor, related autoimmune diseases, related grafts, preemptive transplantation, and consequences, all impact the indications for whole pancreas and islet transplantation. Long-term evaluations are required, especially in cases of hyper-immunization, to assess risks related to immunosuppression and graft outcomes.

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1.2.3 Pancreas vs Islet: Comparative Outcomes

The Edmonton protocol initially showed promise in rendering recipients with type 1 diabetes insulin-independent through glucocorticoid-free immunosuppression and subsequent islet infusion¹⁶. However, disappointing long-term outcomes, as indicated by the return to insulin therapy in most patients after a median of 2 years, were observed⁵⁶. A multicenter clinical trial confirmed these results, emphasizing improved glycemic control without hypoglycemia in over 70% of recipients experiencing refractory hypoglycemia⁵⁷. The trial also revealed a center effect, with better outcomes in sites with extensive experience in pancreatic processing and immunosuppression management. Withdrawal of immunosuppression for failed islet grafts led to a high rate of sensitization to HLAs, prompting a shift in emphasis towards stabilizing metabolic control using the least number of donor pancreases. This approach necessitates a focus on the peri transplant period to ensure the survival of a larger number of high-quality islets during engraftment.

The focus of islet allotransplantation has shifted towards establishing metabolic control without hypoglycemia. Programs for islet alone transplantation have established selection criteria based on patients experiencing severe hypoglycemia, hypoglycemia unawareness, or marked glycemic lability, with a primary outcome of achieving an HbA1c <7.0% without severe hypoglycemia^{20,21}. Prospective single-arm clinical trials indicate that over 80% of

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recipients achieve this primary outcome at 1 year, and this effect can be sustained in over 80% of recipients at 2 years¹⁸. Notably, in the phase 3 CIT₀₇ trial, over 90% of recipients were protected from severe hypoglycemic events during 2 years of follow-up. The study demonstrated significant improvements in diabetes distress, fear of hypoglycemia, and patient well-being, with health-related quality-of-life measures showing significant improvement¹⁹. Despite concerns about chronic immunosuppressive drug treatment, the results indicate that appropriately selected candidates experience clinically significant and personally meaningful improvement in glycemic control and quality of life.

Recent studies have validated positive outcomes of islet allotransplantation compared to intensive insulin therapy⁵⁸. In a multicenter Australian consortium study, continuous subcutaneous insulin infusion (pump) modestly reduced glucose variability and lowered severe hypoglycemia events, while islet transplantation significantly reduced HbA1c from 8.2% to 6.4%, with marked reduction in glucose variability and elimination of hypoglycemia²⁰.

The TRIMECO study, the first randomized clinical trial assessing a transplant intervention for diabetes, showed that islet allotransplantation significantly lowered HbA1c compared to intensive insulin therapy (5.6% vs. 8.2%), with improved health-related quality of life. Achieving HbA1c <7.0% in the absence of severe hypoglycemia was significantly higher with islet

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transplantation²¹. These results substantiate the positive outcomes reported from the single cohort phase 3 CIT₀₇ trial^{18,19}.

Recent Igls criteria⁵⁰ have been established to define outcomes for β -cell replacement therapy, applicable to both islet and pancreas transplants. These criteria focus on achieving HbA1c targets, avoiding severe hypoglycemia events, reducing insulin requirements, and restoring clinically significant C-peptide production. Consistent application of these criteria is crucial for assessing and comparing the effectiveness of various forms of β -cell replacement therapy.

Longer-term outcomes for graft function in islet allotransplantation have significantly improved, with some recipients maintaining insulin independence for over 10 years. Recent protocols, utilizing T cell depletion and TNF- α inhibition for induction therapy, achieve 5-year insulin independence rates of up to 50%⁵⁷. As this approaches the 5-year insulin independence rates for pancreas transplantation, consideration should be given to islet transplantation as an alternative, offering fewer risks associated with major abdominal surgery. Simultaneous pancreas/kidney transplantation provides over 70% 5-year insulin independence, supporting its preference for long-term graft function and glycemic control⁵⁷. The superiority is attributed to less risk for immunologic graft loss, allowing earlier detection of rejection episodes and targeted treatment. While insulin independence rates are lower with simultaneous islet/kidney transplantation, it offers significant improvements in

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HbA1c without hypoglycemia compared to kidney transplant alone, with less morbidity than simultaneous pancreas/kidney transplantation⁵⁹.

Take Home message

1. The Edmonton protocol initially showed promise in achieving insulin independence in type 1 diabetes patients through islet infusion without glucocorticoid immunosuppression. However, long-term outcomes revealed a return to insulin therapy in most patients after about two years, indicating the need for improvement in islet transplantation strategies.
2. Islet allotransplantation has shifted its focus towards achieving metabolic control without hypoglycemia. Programs prioritize selecting patients with severe hypoglycemia events, hypoglycemia unawareness, or glycemic lability, aiming for an HbA1c <7.0% without severe hypoglycemia events. Clinical trials have shown that over 80% of recipients achieve this primary outcome at 1 year, with sustained effects in over 80% of recipients at 2 years.
3. Simultaneous pancreas/kidney transplantation provides high rates of insulin independence and superior long-term graft function compared to kidney transplant alone. While insulin independence rates are lower with simultaneous islet/kidney transplantation, it offers significant improvements in HbA1c without hypoglycemia events, making it a valuable option for diabetic patients.

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1.3 Islet Isolation process step by step

1.3.1 Pancreas dissection

In a clinical laboratory, the organ isolation process follows organ donor procurement, organ perfusion, and cold shipping. The primary objective is to optimize outcomes, focusing on factors such as the quantity and quality of isolated islets, pancreatic islet equivalents per gram (IEQ/g), and the functional capacity of the islets. Given the heightened infection risk for diabetic and immunocompromised recipients, stringent safety measures are crucial to prevent the introduction of pathogens during the islet transplant.

Stringent procedures are employed, encompassing sample collection, multi-step surveillance, and microbiological evaluation throughout the isolation phase. A rigorous release criteria protocol, addressing endotoxin and microbial burden, is adhered to before transplantation. Decontamination and prevention form the initial steps to minimize the risk of introducing germs. During pancreas retrieval, the "No touch technique" is employed, and the pancreas is carefully transported in sterile bags with cold preservation solution, still enveloped by connective tissue, fat, blood arteries, and occasionally duodenum or spleen segments. Upon arrival, a sample of the transport medium is taken for microbiological evaluation.

To expose the head, body, and tail of the pancreas, excess tissue is removed, and the pancreas is cannulated (Figure 5).

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Figure 5. Pancreas dissection for islet isolation.

(a) displays a pancreas within the islet isolation laboratory. Note the presence of connective tissue, blood vessels, fat and a segment of duodenum. In contrast, image (b,c) showcases the same pancreas as depicted in (a), subsequent to the elimination of extraneous connective tissue, blood vessels and fat deposits.

Decontamination is achieved by rinsing in cold media like M199 after a series of 4 °C solutions, including a Povidone-Iodine bath and a Cephazolin/M199 solution. The pancreas is then transported for enzyme distension following this cleaning procedure. These meticulous steps ensure the safety, quality, and organ viability for subsequent transplantation.

1.3.2 Pancreas distention

The difficulty of islet release from surrounding tissues impedes the effectiveness of islet isolation and transplantation for patients with T1D. The intricacy of the isolation process is increased by variations in the types of enzymes used, the injection and the shaking techniques^{61,62}. Although crude enzyme

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mixes caused problems in the earliest trials⁶³, Liberase HI's release first appeared promising⁶⁴. However, because of the possible risk of spreading Bovine Spongiform Encephalopathy, its use was stopped in 2007⁶⁵⁻⁶⁸. After that, other enzymes were examined, such as Liberase MTF (mammalian tissue-free) from Roche and Collagenase NB1 and Neutral protease NB from SERVA⁶⁹. SERVA enzymes proved to be effective and safe, which allowed for the successful transplantation of islets⁷⁰. More alternatives were made available with the creation of VitaCyte collagenase HA and a novel enzyme mixture (NEM)⁷⁰⁻⁷¹. Research contrasting several enzyme combinations showed how challenging it is to find the right mixture for a high number of good-quality islets⁷². According to recent studies evaluating various combinations of enzymes, the NEM consistently met transplanting criteria and produced greater islet yields. The selection of collagenase blends and combinations is still unclear despite continued research, with different studies showing diverse results⁷³⁻⁷⁵. In islet isolation, adjusting enzyme concentrations—especially with SERVA GMP enzymes—has become standard procedure. In most cases, concentrations of 1-2 DMC U/g of neutral protease and 18-24 U/g of pancreas collagenase demonstrate adequate levels⁷⁶.

1.3.3 Pancreas digestion

Digestive enzymes are infused into the major pancreatic duct, which subsequently divides into smaller channels that enter the exocrine portion of the pancreas, releasing islets from the human pancreas. A uniform distribution

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of enzymes throughout the organ is essential for achieving the best islet release. Hand distension and pressure-controlled pumping using a mechanical perfusion device are the two ways to achieve this objective. Since 2002, an islet isolation unit has effectively used hand distension^{20,67}, which entails cannulating the pancreatic ducts of decontaminated and trimmed pancreas halves and pumping an enzyme solution with a pulsatile syringe. This technique enables modification according to the physical characteristics of the pancreas. Although some groups have experimented with hand-held manometry to track pressure while pumping, the device was found to be laborious and did not improve results. As an alternative, some groups use a pump and recirculating perfusion device system for controlled-pressure enzyme infusion. Although there was no discernible difference between the two approaches in terms of *in vitro* islet function as determined by glucose perfusion, a comparative investigation indicated a higher post-purification islet yield using the perfusion device⁷⁴.

The pancreas is distended completely with an enzyme solution, then sliced into pieces about 2 cm³ in size and put in the Ricordi Chamber, a metal dissociation chamber. This chamber is a component of a closed circulation tubing system that circulates the collagenase and media containing the pancreas. It has stainless steel ball bearings. The fluid and chamber are heated to 37 °C, and the pancreas cannot obstruct the tubing because of a 500 µm mesh in the chamber cover. When the chamber is gently shaken, sterile ball

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bearings help break up the tissues of the pancreas that are being digested. The islets flow through the mesh, and the release of islets from the acinar and other tissues is determined by continuous biopsy, which marks the point at which the isolation has advanced to that degree.

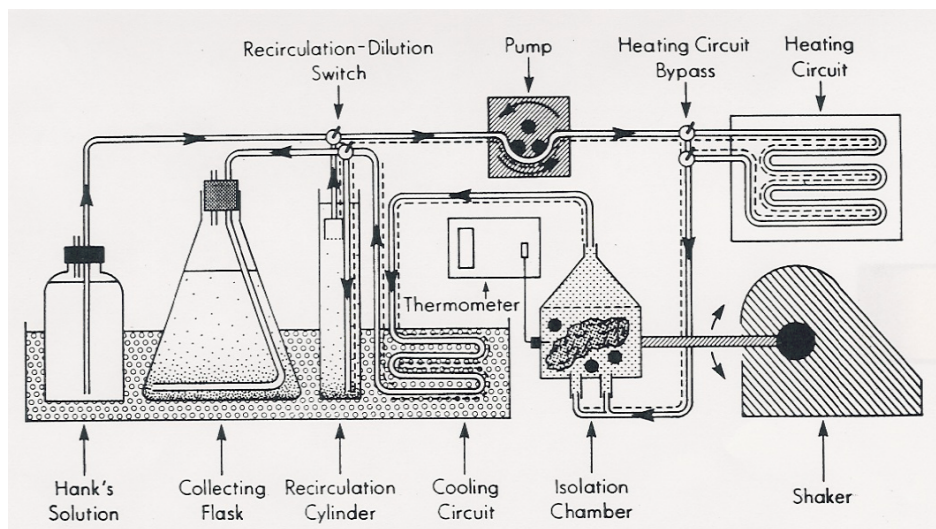


Figure 6. Automated method for isolation of human pancreatic islets.

(Ricordi C, Lacy P, Finke E, Olack B, Scharp D. Automated method for isolation of human pancreatic islets. *Diabetes*. 1988;37(4):413–20.)

Part of the "automated method", the Ricordi chamber was first launched in 1988 and is coupled to a tubing system⁶² (Figure 6). In order to facilitate the enzymatic breakdown and cause mechanical disruption of the pancreatic tissue by the ball bearings, the enzyme solution is recirculated through the tubing system and the chamber is shaken. Two options for shaking are described: manually or mechanically with a 'shaker.' In a study comparing

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mechanical and manual shaking, hand shaking produced more islets with higher integrity, but at the cost of longer digestion durations⁷⁰.

Based on the breakdown of pancreatic tissue seen during digestion, this approach offers more control over the intensity of shaking. The agitation intensity is changed depending upon the amount of tissue disruption that has occurred. Shaking intensity is decreased as the gland pulls apart as observed as biopsies are collected during the digestion process as can be seen being collected from a 3 way tap in the closed tubing circuit⁷⁶.

1.3.4 Switching from digestion to collection

Selecting the ideal time to move from pancreatic digestion to tissue collection is a critical step in isolating human islets for transplantation. The task at hand involves a careful balancing act to ensure that islets are separated from exocrine tissue without going overboard and potentially causing fragmentation and destruction. Islet function depends on maintaining cell-cell contact during digestion since any disruption may affect insulin secretory responses⁷⁸. By taking biopsies, the release of islets during digestion is continuously tracked in order to achieve this balance. Dithizone is used to dye these biopsies so that islet tissue may be seen under a microscope. Biopsies are taken at regular intervals, and once free islets are seen, the collection process begins. This conversion entails switching from a closed system, in which the enzyme solution is continuously circulated via the tubing and Ricordi chamber, to an open system (Figure 7,8). A new, enzyme-free solution is pumped

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through the open system, collecting the digested tissue in a chilled medium that contains human albumin.

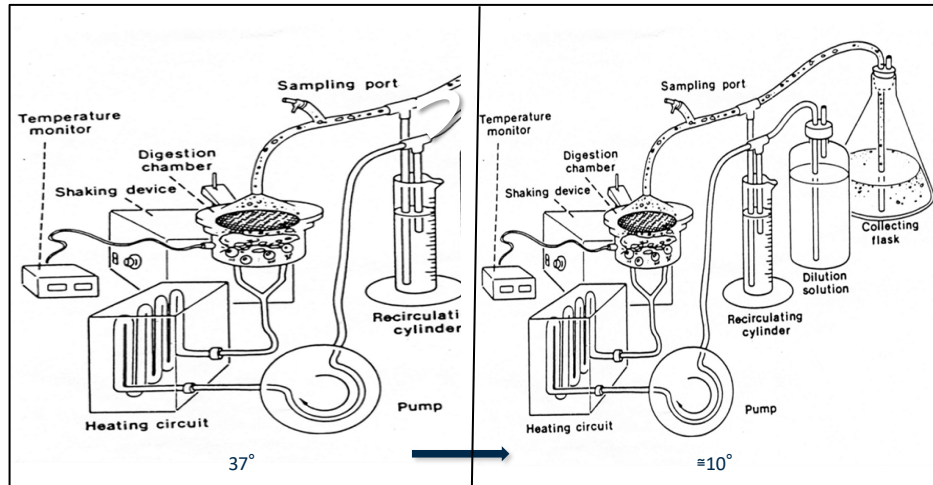


Figure 7. Switching from digestion to collection

The pancreatic digestion in the “Ricordi” chamber occurs through the synergy of enzymatic activity, which requires a 37-degree environment, and mechanical action by the ball bearings breakdown. By continuously assessing the digestion process through the examination of biopsies extracted from the circuit, the optimal time to halt digestion can be determined. Enzyme deactivation is achieved by rapidly lowering the temperature and diluting the digested tissue.

After collection, the digest is subjected to two washes in a cold medium containing heparin, insulin, and human albumin in order to neutralize and remove any remaining digestive enzymes. It has been demonstrated that collagenase does not remain in the islets upon washing guaranteeing patient safety^{79,80}.

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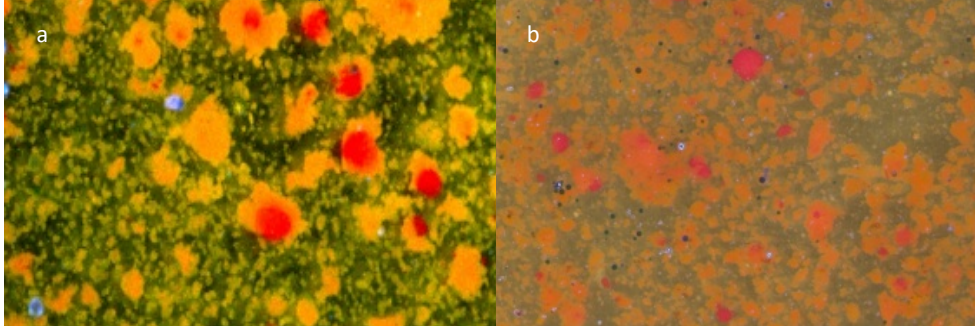


Figure 8. Digestion process

(a) depicts embedded islets during the digestion phase, while (b) illustrates islets fully detached from the surrounding exocrine tissue.

After the washing process, the digest is left for 30 minutes in UW solution that has been fortified with a high concentration of insulin, heparin, and human albumin before it is purified. This "quenching" stage helps the acinar tissue absorb starch, changing its density and facilitating the succeeding purification process's islet separation from exocrine tissue.

1.3.5 Islet purification

Despite differences in size and design, all islet isolation laboratories use the same equipment and follow the same guidelines for procedures. The asepsis in the clean room, together with necessary equipment, greatly aids in the procedure as a whole. Equipment and separation phases—which involve specific protocols described in multiple studies—remain essential⁸¹⁻⁸³. Experiments using several density gradients, such as Ficoll-sodium diatrizoate,

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Dextran, Iodixanol, and organ preservation solutions UW and Euro-Collins, were conducted in the early stages of optimizing post-digestion islet purification⁸⁴⁻⁸⁸. The goal of these studies was to improve the fundamental density separation procedure (Figure 9). Newer density gradients, such as Ficoll, Dextran, Biocoll, and Iodixanol, developed in spite of these research and are being used in major units across the globe.

Ficoll, also known as Ficoll-Paque, is a highly effective method for isolating viable islet cells with a high yield and purity⁸⁵. It is made up of high molecular weight sucrose polymers and sodium diatrizoate. Although it is used less, dextran, a complex branched glucan, was employed in early density gradients⁸⁴. Hydrophilic polymer biocoll, which is usually manufactured in advance for islet purification, enables density adjustment for ideal pH and osmolality²⁰. Various doses of iso-osmolar contrast agent iodixanol are utilized as a density gradient⁸⁹.

Density gradient advances coincided with advancements in equipment, most notably the development of automated systems. The preparation of islets became more economical and efficient with the use of the IBM 2991 COBE cell separator, which greatly shortened the purification process. The COBE® 2991™ Cell Processor's cooling adjustments guaranteed gradient temperature maintenance, reducing ischemic cell damage and promoting density gradient stability during purification^{88,90}.

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The temperature gradients produced by two cooling techniques—a "Clean Room Cold Room" and machine modification—were comparable.

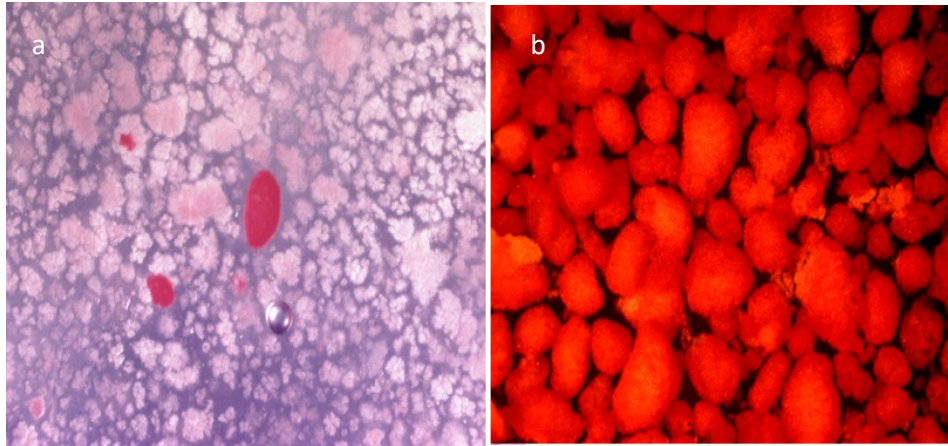


Figure 9. Islet purification

(a) displays pre-purified islets while (b) presents fully purified islets.

In order to operate the COBE® 2991™ Cell Processor, density gradients must first be loaded and then pre-cooled to the bottom of the COBE bag and pancreatic milieu⁹⁰. Two distinct density gradients can be continuously loaded using chilled-density mixer devices. Following processing, the separated fractions undergo biopsies, dithizone staining for islet evaluation, and prompt washing. To ensure that only the purest fractions are mixed for transplantation, pure fractions are kept apart from impure ones. Dithizone labeling is a rapid and easy way to estimate the quantity and purity of islet cells,

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which is important for figuring out the success of isolation and the density of seeding for culture.

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1.4 Islet Quality Assessment

Numerous methods have been developed to assess the quality of islet preparations post-isolation, crucial for ensuring safe and effective transplantation. Key criteria for release include evaluating cell mass, purity, viability, sterility, and functional capacity (Table 1). Quality assessment becomes particularly critical when preparing islets for clinical transplantation, as poor-quality preparations may impact the efficacy of islet transplants in reversing diabetes⁹¹. In Europe, islets for transplant therapy adhere to the European Committee on Organ Transplantation regulations for biological products and human cells, necessitating demonstrated product stability, consistency, and compliance with identity, safety, purity, and potency standards⁹². However, applying regulations designed for therapeutics in controlled settings to islet preparation criteria may be challenging due to donor variability and the isolation process. Global centers employ methods to provide rapid, sensitive, and reproducible quality assessments while ensuring the release of safe and efficacious preparations. The Collaborative Islet Transplantation Registry (CITR) program's release criteria, based on islet count per recipient weight, purity, viability, endotoxin concentration, and glucose stimulation index, serve as guidelines to exclude subpar preparations but may not necessarily correlate with in vivo functional outcomes⁹³.

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Table 1. Product release criteria for clinical islet preparation

TYPE OF TEST	PRODUCT TEST	SPECIFICATION	TYPE OF SAMPLE
Safety	Endotoxin	< 5 EU/kg	Supernatant of islet suspension in transplant media
	Gram stain	No organisms detected within limits of assay	
Identity	Islet count (IE/kg)	5,000–20,000 (1 st transplant)	Islets in transplant media
		3,000–20,000 (Re-transplants)	
	Purity	≥ 30%	
Viability	Dye exclusion (FDA/PI)	≥ 70%	Islets after overnight culture and in transplant media
Potency	Glucose stimulated insulin release (ELISA)	Stimulation Index >1	Islets after overnight culture

(Papas KK, Suszynski TM, Colton CK. Islet assessment for transplantation. *Curr Opin Organ Transplant.* 2009 Dec;14(6):674-82. doi: 10.1097/MOT.0b013e328332a489. PMID: 19812494; PMCID: PMC2859186.)

At the National Pancreas Transplant Unit in Australia, formal criteria include predetermined thresholds for islet number / mass, viability, purity, and sterility before release for transplantation^{20,67}.

This section outlines commonly assessed indicators of islet quality used as release criteria, emphasizing their role in predicting islet survival and function in recipients. Additionally, it introduces several useful methods providing functional insights that are not part of formal release criteria.

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1.4.1 Islet Mass and Purity

Evaluating islet cell mass is crucial in determining the suitability of islet preparations for transplantation⁹⁴⁻⁹⁶. To address potential losses due to engraftment failure or immune responses, a minimum of 4,000 IEQ per kilogram of recipient weight is required for clinical islet transplantation in Australia²⁰. Studies highlight the importance of critical islet mass for normalizing glucose responses post-transplantation, and a positive correlation exists between sufficient islet mass and graft function in human islet transplantation⁹⁵.

Insulin content in porcine and rat islet grafts is closely correlated with beta cell mass, indicating the significance of considering beta cell mass rather than total islet cell mass alone⁹⁶. Dithizone staining, commonly used to assess islet numbers, mass, and purity, aids in estimating islet cell numbers and purity quickly⁹⁷. Islet numbers are categorized by diameter, converted to IEQ, and summed to obtain total IEQ⁹⁸. Modifications to this method have been proposed to better represent islet size distribution⁹⁹. Islet size distribution also influences graft function, with smaller islets demonstrating better function in both rat models and human islet transplants. Smaller islets tend to have higher viability and survival rates compared to larger islets^{100,101}. Purity is crucial, as acinar tissue remaining attached to islets post-isolation releases proteases, contributing to islet cell death¹⁰²⁻¹⁰⁴. Low-purity preparations show reduced viability and functional capacity, potentially impacting survival and

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engraftment post-transplant. Islet purity, assessed through dithizone staining, is considered acceptable for clinical transplantation if it exceeds 30%, with a total packed cell volume of less than 10 ml^{20,67}.

1.4.2 Islet viability

Since live cells are essential for a successful transplant, evaluating the mass of islet cells alone is insufficient to assess the quality of an islet preparation. Large-scale non-viable cell transplantation not only results in subpar functional outcomes but also puts the patient at risk of developing transplant-related hypersensitivity. Therefore, evaluating islet viability is a key factor in quality assessment, and various methods are employed for this purpose. Staining with DNA-binding dyes, usually propidium iodide (PI) and fluorescein diacetate (FDA), is the standard method for determining islet viability^{105,106}(Figure 10). A minimum of 70% viability, determined by FDA/PI staining, is required for the release of the product for transplantation, according to criteria similar to those used by the CITR program^{20,67}. FDA fluoresces green in live cells, while PI fluoresces red in damaged or dying cells with compromised membrane integrity. However, because dye concentration, incubation durations, and imaging parameters are variable, using FDA/PI can be arbitrary. Furthermore, membrane integrity does not always correlate with cell viability, as viable cells judged based on nucleic staining may not necessarily be functioning cells and this has been shown in a number of stud-

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ies including transplantation into mouse models, likely due to the fact that DNA-binding dye exclusion does not identify apoptotic cells¹⁰⁷.

Apoptosis and necrosis indicators are combined to improve accuracy. Tetramethylrhodamine ethyl ester, 7-aminoactinomycin D, and Newport Green can be used in a method that allows for the simultaneous measurement of beta cell composition, viability, and apoptotic cell percentage. This method presents the beta cell viability index as a predictor of graft survival and possible function after transplantation, which has been verified in a mouse model¹⁰⁸. The beta cell viability index is calculated using flow cytometric analysis on islet cells that have been post-cultured at Westmead Hospital in Australia^{20,67}. An index of 0.5 or greater is deemed satisfactory. Although not currently part of product release criteria for transplantation, ongoing studies are being conducted to assess its inclusion. But this approach needs more time, a bigger sample of the islet, technical know-how, and specialized tools¹⁰⁹.

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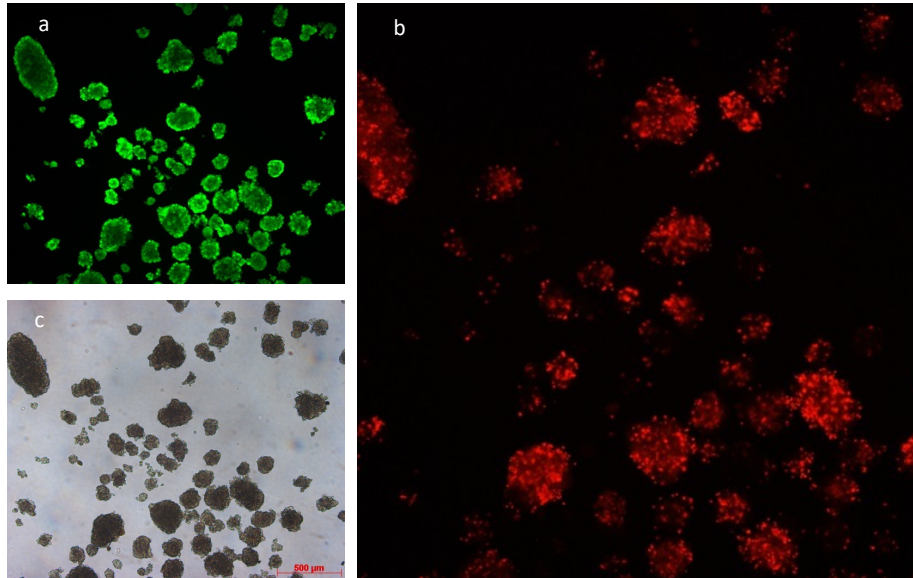


Figure 10. Islet viability

(a) displays FDA-labeled cells (b) presents PI labeled cells (c) Brightfield.

1.4.3 Islet sterility

Ensuring the sterility of the final islet product is crucial for clinical islet isolation, especially since recipients are immunosuppressed, increasing their susceptibility to infections from contaminants in the preparation. One major danger is endotoxin contamination, which has been linked to islet cell injury and early graft loss¹¹⁰⁻¹¹².

The islet isolation and culture procedure may involve microbial contamination at different phases. Testing solutions used in pancreas preservation suggest that the donor duodenum during pancreas retrieval is a possible

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source¹¹³⁻¹¹⁵. Most of the contamination is eliminated during the isolation process, even if it occurs often during recovery.

Measures are used during isolation and culture to evaluate the sterility of the product. These include adding antibiotics (usually ciprofloxacin) to the culture media and using Gram staining, endotoxin content assessment, and microbiological culture^{116,117}. A negative Gram stain and an endotoxin level of less than 5 endotoxin units (EU)/kg of recipient weight are prerequisites for product release¹¹⁶. Many investigations have shown that following these guidelines prevents recipients from developing clinical infections and has no impact on long-term graft survival^{114,116}.

Sample aliquots are taken during transport, post-decontamination, post-purification, and post-culture to demonstrate the microbiological sterility. For both aerobic and anaerobic cultures, these samples are injected in culture vials. Furthermore, samples are cultured for mycoplasma, mycobacteria, and fungus^{114,116}. Although the time needed for results means that culture assessment is not employed as a release criterion, positive cultures lead to the prescription of antibiotic prophylaxis with little side effects seen in recipients¹¹⁴.

1.4.4 Islet function

Various techniques are employed to describe islet function, with a particular emphasis on dynamic glucose-stimulated insulin secretion (GSIS)^{98,118}. These

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methods play a crucial role in understanding the intricacies of pancreatic islet function. Here is an overview of some commonly utilized techniques:

1. Perifusion Systems⁹⁸:

- *Description*: Perifusion systems involve exposing isolated islets to dynamic changes in glucose levels to simulate physiological conditions.
- *Advantages*: Mimics the in vivo environment, allowing for the measurement of dynamic GSIS.
- *Considerations*: Lack of standardized, inexpensive apparatus and protocols across laboratories.

2. Static Incubation Assays⁹⁸:

- *Description*: Islets are incubated at static glucose concentrations, and insulin release is measured.
- *Advantages*: Simplicity and ease of implementation.
- *Considerations*: Limited in capturing dynamic aspects of GSIS.

3. Microfluidic Platforms⁹⁸:

- *Description*: Microfluidic devices allow precise control of the microenvironment around islets, enabling dynamic GSIS measurements.
- *Advantages*: High precision, ability to mimic physiological conditions.

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- *Considerations:* Technical expertise required for fabrication and operation.
4. Patch-Clamp Electrophysiology⁹⁸:
 - *Description:* Measures electrical currents across the cell membrane to assess ion channel activity.
 - *Advantages:* Provides insights into the underlying mechanisms of insulin secretion.
 - *Considerations:* Invasive, requires specialized skills.
 5. Imaging Techniques (e.g., Confocal Microscopy)⁹⁸:
 - *Description:* Visualizes changes in calcium dynamics or insulin granule exocytosis.
 - *Advantages:* Real-time visualization, potential for subcellular resolution.
 - *Considerations:* Limited by the need for fluorescent markers and potential phototoxicity.
 6. Genetic and Molecular Approaches⁹⁸:
 - *Description:* Involves manipulating gene expression or using knockout models to study specific proteins involved in insulin secretion.
 - *Advantages:* Targeted investigations into molecular pathways.
 - *Considerations:* May not fully replicate complex physiological interactions.
 7. Islet Perfusion Systems⁹⁸:

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- *Description:* Involves perfusing pancreas with solutions containing various stimulants to assess insulin secretion.
- *Advantages:* Allows controlled exposure to different substances.
- *Considerations:* Less dynamic than perfusion systems.

In summary, a combination of these techniques is often employed to gain a comprehensive understanding of islet function, especially dynamic GSIS¹¹⁸. The choice of method depends on the specific research question, the desired level of detail, and the available resources and expertise in the laboratory.

Islet Perfusion

The primary objective steering the islet perfusion system is to replicate, with utmost precision, the physiological conditions governing the pancreas within the controlled confines of a laboratory environment¹¹⁹⁻¹²¹.

The experimental design involves the placement of isolated islets within a chamber, subjecting them to a continuous perfusion of a solution that varies in glucose concentrations. Also, this method is useful for determining regulation of hormone release in response to various drugs and agents.

In contrast to static incubation, perfusion permits evaluation of the dynamics of insulin release in a situation where feedback inhibitory/stimulatory signals of released hormone(s) are avoided.

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The usual biphasic pattern of insulin secretion, marked by a sharp and intense surge in insulin release (first phase) succeeded by a lower and constant secretion rate (second phase), serves as a hallmark of a healthy insulin response when islets encounter a transition from low to high glucose levels.

Nonetheless, this characteristic pattern disappears in perfused islets from pancreatic donors with pre-diabetes or type 2 diabetes¹²².

Furthermore, dynamic analysis of insulin secretion enables the discernment of which parameters associated with the donor and inherent organ characteristics may have favorable or adverse effects on the physiological responses of the islets¹²³.

As a result, within the domain of pancreas or islet transplantation, it has the potential to distinguish between optimal and suboptimal donors.

Researchers employ various parameters to quantify and characterize insulin secretion during perfusion experiments. These include metrics like the perfusion index, the area under the curve (AUC), and the stimulation index (SI= ratio of insulin secretion in high and low glucose). However, quantitative comparisons of insulin secretion data reported by distinct laboratories are complicated by inconsistent modes of expression¹²². The only useable common unit is SI that is either published or can be recalculated from data presented otherwise.

CHAPTER 1 ~ Background

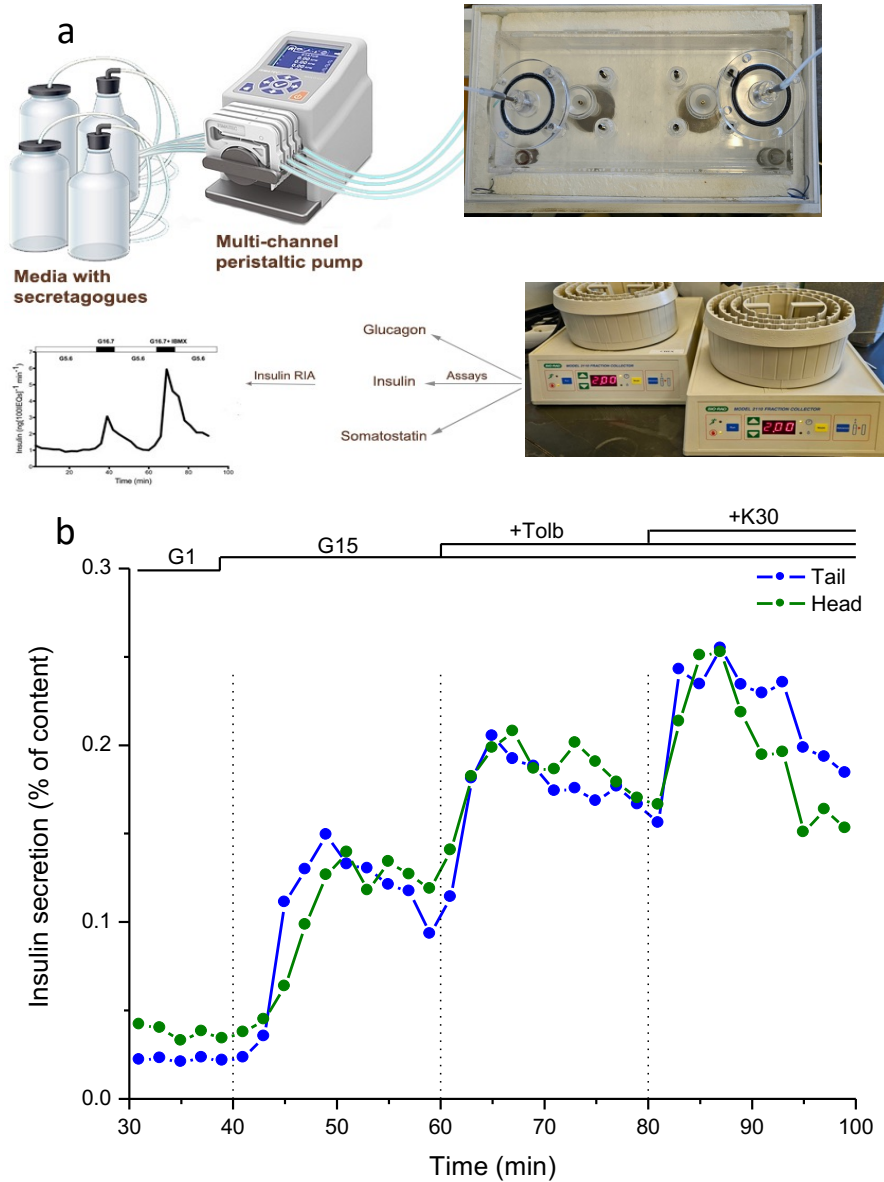


Figure 11. Example of dynamic perfusion to assess human islets function.

a) Photograph of the perfusion system b) Biphasic dynamics and concentration-dependency of glucose-induced insulin secretion in perfused human head and tail islets.

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Moreover, the absence of standardized perfusion protocols creates challenges when attempting to compare data obtained from different laboratories. Consequently, establishing standardized protocols for interpreting perfusion data could prove invaluable, particularly when perfusion experiments are conducted as quality control tests for human islet preparations. Such standardized protocols would enhance the reliability and comparability of findings across research studies, fostering greater collaboration and advancement in the field of islet research.

In vivo islet functionality

The conventional benchmark for assessing islet viability and function involves determining the ability of an islet preparation to reverse diabetes upon transplantation into immunodeficient mice^{98,124}. This mouse bioassay, while reliable, has limitations, particularly due to its time-consuming nature (up to a week post-transplant), making it unsuitable for immediate clinical transplantation release criteria. Various methods, such as Islet Oxygen consumption rate (OCR) and ADP:ATP ratio assessments, are often evaluated by correlating assay results with the achievement of normoglycemia in vivo. To evaluate the capacity of islets to reverse diabetes, a small number of islets are transplanted into athymic mice rendered diabetic, and blood sugar levels are monitored to gauge normoglycemia achievement. Studies with different islet preparations have shown that higher transplant numbers increase the likelihood of successful diabetes reversal¹⁰². However, extended time in

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culture may compromise islet viability and function, impacting transplant outcomes¹²⁴. Factors influencing transplant success, independent of islet quality, include variations in starting body weights, surgical complications, streptozotocin-induced diabetes effects, and the time between induction and transplant.

Despite similar viability, glucose response, and endotoxin content, only 54% of mice transplanted with different islet preparations achieved normoglycemia, highlighting additional factors affecting transplant outcomes¹⁰². Rodents' lower sensitivity to porcine and human insulin, susceptibility to glucotoxicity post-transplant, and delayed assessment of graft outcomes make them less than ideal for evaluating transplant success¹²⁵. Currently, clinical islet transplant centers rely on islet yield, viability, purity, endotoxin content, and Gram stain results for release criteria^{20,126}. Transplanted mass consistently correlates with in vivo islet graft function, but advancements in technology and understanding of islet physiology prompt the ongoing development of new, efficient methods for assessing cell viability and function in islet preparations for clinical transplantation.

CHAPTER 1 ~ Background

Take Home message

1. Islet Function Assessment Techniques: Various techniques are employed to assess islet function, with a focus on dynamic glucose-stimulated insulin secretion (GSIS). These methods include perfusion systems, static incubation assays, microfluidic platforms, patch-clamp electrophysiology, imaging techniques, and genetic and molecular approaches. Each technique offers unique advantages and considerations, and their choice depends on the specific research question and available resources.

2. Islet Perfusion System: The islet perfusion system replicates physiological conditions to investigate dynamic responses of islets to varying glucose levels. It allows for precise control of the extracellular environment and mimics natural regulatory mechanisms, facilitating the assessment of insulin responses. Despite its advantages in providing physiologically relevant data, challenges such as lack of standardization and technical expertise exist.

3. In Vivo Islet Functionality: Assessing islet viability and function involves transplanting islets into diabetic mice and monitoring blood sugar levels to gauge normoglycemia achievement. Factors influencing transplant success include islet quality, variations in starting body weights, surgical complications, and the time between induction and transplant. While the mouse bioassay remains a reliable method, advancements are ongoing to develop efficient methods for assessing islet viability and function for clinical transplantation.

CHAPTER 1 ~ Background

1.5 Ischemia-Reperfusion Injuries During Pancreas Preservation

The knowledge about ischemia-reperfusion injuries (IRI) in whole pancreas and islet transplantation is limited compared to kidney transplantation^{127,128}, attributed to the infrequency of pancreatic and islet transplants and challenges in obtaining biopsy samples (Figure 12). Brain death is associated with reduced graft survival in various transplants, causing inflammation and endothelial activation even before organ procurement^{129,130}. Pancreatic microcirculation is compromised in a rat model of brain death, predicting the severity of graft pancreatitis¹³¹. Brain death induces a systemic inflammatory response, releasing proinflammatory cytokines.

Elevated donor chemokine ligand 2/ monocyte chemoattractant protein 1 (CCL2/MCP-1) is a predictive biomarker for pancreatic transplant outcomes¹³², correlating with lower graft survival, especially in simultaneous kidney-pancreas transplants. Moreover brain death likely triggers CCL2 release due to ischemia, causing peripheral vasoconstriction. Studies show increased TNF- α and IL-6 in brain-dead patients, indicating inflammation in general and pancreatic tissues¹³³.

It's probable that pancreatic damage involves complement C3 activation, Toll-like receptor (TLR)-4 upregulation, and innate immunity activation.

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Similar processes occur in kidneys from deceased donors¹³⁴. Brain death initiates an inflammatory cascade affecting organ endothelium and microvascularization.

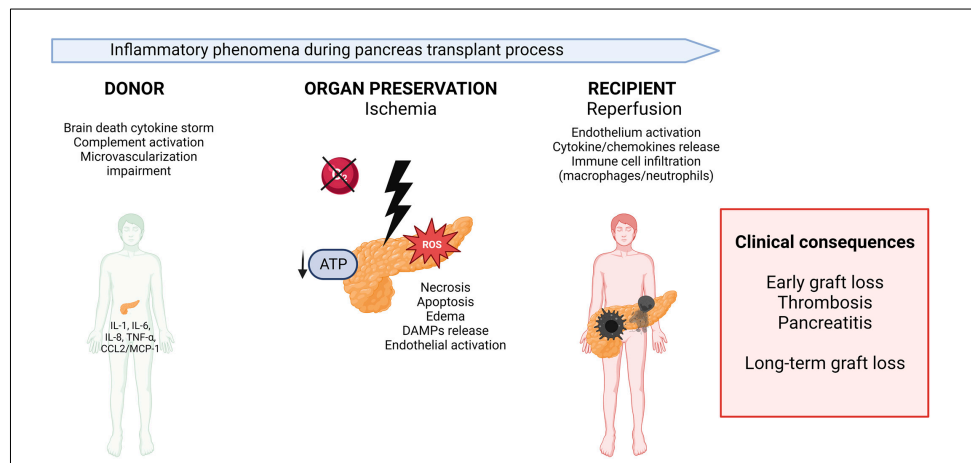


Figure 12. Inflammatory phenomena during pancreas transplantation.

(Prudhomme T, Mulvey JF, Young LAJ, Mesnard B, Lo Faro ML, Ogbemudia AE, Dengu F, Friend PJ, Ploeg R, Hunter JP, Branchereau J. Ischemia-Reperfusion Injuries Assessment during Pancreas Preservation. *Int J Mol Sci.* 2021 May 13;22(10):5172. doi: 10.3390/ijms22105172. PMID: 34068301; PMCID: PMC8153272.)

Cold organ preservation worsens ischemic damages, leading to cellular edema, oxidative stress, and ATP depletion¹³⁵⁻¹³⁷.

Severe ischemia can cause cellular death through necrosis or apoptosis, intensified by oxygen delivery during reperfusion¹³⁸. Renal ischemia-reperfusion-induced lesions exacerbate tubulointerstitial fibrosis¹³⁹. Innate immune system-mediated inflammation starts during ischemia, escalating during

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reperfusion, and recruits adaptive immunity¹⁴⁰. Endothelial lesions enhance vascular permeability, facilitating immune cell infiltration¹⁴¹. Macrophages, dynamic in polarization, contribute to IR lesions and influence allorecognition¹⁴².

Neutrophils obstruct microvessels and release damaging substances during IRI, while activated neutrophils intensify damage by releasing neutrophil extracellular traps (NETs)¹⁴³. Complement cascade molecules negatively influence IR lesions¹⁴⁴. Pancreas susceptibility to IRI impacts organ quality, particularly islet cells^{145,146}. Extended ischemia associates with decreased islet yield¹⁴⁷. Pancreatic ischemia-reperfusion induces membrane breakdown, oxidative stress, necrosis, apoptosis, and leukocyte infiltration¹⁴⁸.

The inflammatory milieu during ischemia-reperfusion amplifies transplant immunogenicity, with macrophages bridging innate and adaptive immunity¹⁴⁹. Dendritic cells (DC) activated by DAMPs recruit donor and recipient DCs, promoting alloimmune responses¹⁵⁰. Neutrophils and macrophages can modulate the adaptive immune response, aiding tolerance and tissue repair^{151,152}. Auto-reactive T and B lymphocytes in pancreas transplant recipients pose challenges, but there is no direct evidence linking ischemia-reperfusion to type 1 diabetes recurrence¹⁵³.

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Take Home message

1.Limited Understanding of Ischemia-Reperfusion Injuries (IRI) in Pancreatic and Islet Transplantation: Compared to kidney transplantation, knowledge about IRI in whole pancreas and islet transplantation is limited. Challenges in obtaining biopsy samples and the infrequency of pancreatic and islet transplants contribute to this knowledge gap.

2.Impact of Brain Death on Graft Survival: Brain death is associated with reduced graft survival in various transplants, including pancreatic transplants. It triggers inflammation and endothelial activation even before organ procurement, compromising pancreatic microcirculation and predicting the severity of graft pancreatitis. Brain death induces a systemic inflammatory response, releasing proinflammatory cytokines and biomarkers like CCL2/MCP-1, which correlate with lower graft survival, especially in simultaneous kidney-pancreas transplants.

3.Inflammatory Cascade and Ischemia-Reperfusion Injury: Brain death initiates an inflammatory cascade affecting organ endothelium and microvascularization. Cold organ preservation exacerbates ischemic damages, leading to cellular edema, oxidative stress, and ATP depletion. Ischemia can cause cellular death through necrosis or apoptosis, intensified by oxygen delivery during reperfusion. Innate immune system-mediated inflammation starts during ischemia, escalating during reperfusion, and recruits adaptive immunity. Endothelial lesions enhance vascular permeability, facilitating immune cell infiltration.

4.Role of Immune Cells in Ischemia-Reperfusion Injury: Neutrophils, macrophages, and dendritic cells play crucial roles in exacerbating ischemia-reperfusion injury and influencing alloimmune responses. Neutrophils obstruct microvessels and release damaging substances, while macrophages bridge innate and adaptive immunity. Dendritic cells recruit donor and recipient dendritic cells, promoting alloimmune responses. Neutrophils and macrophages can modulate the adaptive immune response, aiding tolerance and tissue repair.

5.Impact on Islet Quality and Transplant Immunogenicity: Pancreatic susceptibility to IRI impacts organ quality, particularly islet cells. Extended ischemia associates with decreased islet yield, and pancreatic ischemia-reperfusion induces membrane breakdown, oxidative stress, necrosis, apoptosis, and leukocyte infiltration. The inflammatory milieu during ischemia-reperfusion amplifies transplant immunogenicity, posing challenges for tolerance induction and tissue repair. Auto-reactive T and B lymphocytes in pancreas transplant recipients present additional challenges, although there is no direct evidence linking ischemia-reperfusion to type 1 diabetes recurrence.

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1.6 Pancreas preservation: clinical practice and future developments

Maintaining organ viability during the period between donation and transplantation is essential for the best possible outcome and longevity of the graft. For organ preservation in pancreatic and islet transplantation, static cold storage (SCS) continues to be the most commonly used technique. Microvascular dysfunction combined with the pancreas's susceptibility to ischemia-reperfusion damage and edema during retrieval and preservation presents difficulties, though. SCS contributes to cell death during ischemia by denying cells of oxygen, which prevents aerobic respiration and the production of ATP. Graft injury is made worse by reperfusion, which especially affects the delicate Langerhans islets. This susceptibility, which is essential for better glucose regulation, is linked to unfavorable clinical results following islet and whole pancreas transplantation. Compared to other abdominal organs, pancreas transplantation presents a unique challenge due to microvascular dysfunction, which exacerbates graft thrombosis, pancreatitis, and ultimately graft failure.

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1.6.1 Static cold storage

SCS stands for standard organ retrieval and international organ transportation. It is thought that keeping the temperature between 2 and 4 °C hypothermic can lessen the effects of ischemia injury by slowing down cellular metabolism, which in turn lowers the amount of ATP and oxygen used. For every 10 °C drop in temperature, there is an observed decrease in metabolic activity of up to two times¹⁵⁴.

In 1982, preliminary studies comparing machine perfusion versus cold storage in canine models showed that machine perfusion resulted in graft failure rates of 30% at 24 hours and 40% at 48 hours¹⁵⁵. On the other hand, with cold storage, no malfunctions were seen at 24 or 48 hours. Over the past three decades, SCS has become the most popular and recommended approach of pancreatic preservation because to these results as well as the difficulties associated with machine perfusion¹⁵⁶.

Using materials that are metabolically inert for organ preservation, Belzer and Southard created the UW in the late 1980s¹⁵⁷. The two main components are lactobionate and raffinose. A hydroxyethyl starch (HES) called raffinose has free radical scavengers and reduces edema. UW is the most widely utilized preservation solution since it has been found to produce better results than SCS when used in whole pancreatic organ preservation¹⁵⁸.

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Histidine Tryptophan Ketoglutarate solution (HTK) is an alternative to UW. HTK is a low-potassium, low-viscosity solution designed to remove extracellular calcium and sodium in order to inactivate organ activity. Its mechanism of action is based on this as well as the strong buffering of the extracellular space by histidine hydrochloride. HTK's electrolyte content and osmolality are comparable to extracellular fluid and plasma, respectively. Comparing UW versus HTK in 77 consecutive pancreatic transplants, Englesbe et al. conducted a multi-center research in 2006 and found similar results for the two groups¹⁵⁹.

Similar to this, Becker et al.'s 2007 study found no appreciable variations in patient or graft survival after SCS using HTK or UW¹⁶⁰. HTK-flushed grafts seemed more edematous, although this did not appear to affect early graft function. Based on some research, HTK may be preferred to UW due to early transplant rejection, graft pancreatitis, and graft loss¹⁶¹.

An extracellular low-viscosity preservation solution is called Celsior. Baldan et al. used a pig auto-transplant model to show how effective it is as a pancreatic procurement substitute for UW¹⁶². In contrast to these results, Uhlmann et al. reported in a swine model that using Celsior was linked to more ischemia-reperfusion injury when compared to UW¹⁶³. Lipid peroxidation upon reperfusion of pancreata kept in Celsior and UW was shown to be identical by Garcia-Gil et al¹⁶⁴. Comparing UW with Celsior in human models, Boggi et al.'s first prospective randomized research of SCS revealed

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comparable safety profiles for pancreatic preservation¹⁶⁵. Similar 2-year recipient and graft survival rates, leak rates, and rates of clinical graft pancreatitis were found in a related study by Manrique et al. contrasting Celsior with UW¹⁶⁶.

Despite having a substantially different composition, the Institut Georges Lopez-1 solution (IGL-1) is another low-viscosity solution that has been demonstrated to be comparable to UWS and HTK in terms of feasibility and safety. It is believed that the inverted Na⁺ and K⁺ concentrations in IGL-1 relative to UW lessen edema. In IGL-1, polyethylene glycol PEG takes the place of the HES component of UW. This substitution lowers viscosity and promotes nitric oxide, which is thought to prevent ischemia. Garcia-Gil et al. discovered that IGL-1 was equally as efficacious as UW for pancreatic FCS in a swine pilot investigation¹⁶⁷. Subsequently, Chedid et al. reported on five kidney and pancreas transplants performed concurrently using IGL-1 during organ procurement and fixed cold storage¹⁶⁸. With all five pancreas performing primary function and resulting in insulin independence and no graft thrombosis, the median cold ischemia period was 13 hours.

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1.6.2 Two-Layer Method

For the purpose of cold storing pancreatic grafts, the Two-Layer Method (TLM) combines Perfluorocarbons (PFCs) with standard SCS perfusion fluid (Figure 13). PFCs have the amazing ability to reversibly bind enormous amounts of oxygen, giving them a concentration of oxygen higher than that of human blood¹⁶⁹. In order to take use of the advantages of SCS while using PFCs as a source of oxygen, the TLM forms a bottom layer of PFCs and a top layer of UW or (ECS)^{170,171}.

According to early research on small animals, the TLM could maintain pancreatic viability for up to 96 hours¹⁷². Large-scale animal experiments, however, have not provided clear evidence of the efficiency of oxygen penetration into deep tissue. There is no conclusive evidence that PFCs are superior to conventional SCS, and their therapeutic effects seem to be restricted to the surface layer of cells in solid organs¹⁷³.

Promising outcomes were observed in a study comparing TLM with UW in the cold storage of pancreatic grafts. When reperfusion occurred, none of the grafts in the TLM group showed signs of edema, while 23.3% of the grafts in the UW group did. Grafts with the highest overall quality score were more common in the TLM group (70% vs. 57.1% in the UWS group). Furthermore, during hospitalization, a higher percentage of recipients in the TLM group (90%) than in the UW group (70.5%) attained insulin independence; nevertheless, there was no statistically significant difference

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in the duration required for insulin independence between the two groups¹⁷⁴.

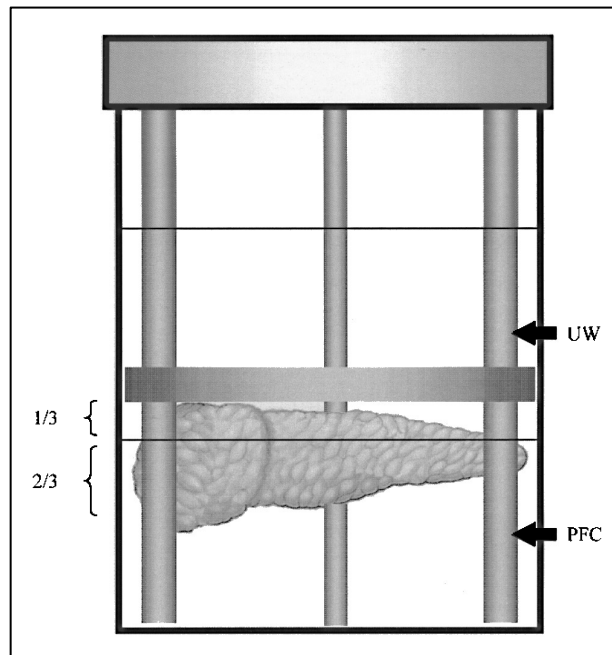


Figure 13. Two-layer technique for pancreas preservation.

Following spleen and excess fat removal (and preferably the duodenum), the pancreas is positioned beneath the carriage, and the threaded legs are adjusted to optimize its placement at the preoxygenated PFC/UW interface. Ensuring adequate coverage, approximately two-thirds of the organ is immersed in PFC.

(Noguchi H, Levy MF, Kobayashi N, Matsumoto S. Pancreas preservation by the two-layer method: does it have a beneficial effect compared with simple preservation in University of Wisconsin solution? *Cell Transplant*. 2009;18(5):497-503. doi: 10.1177/096368970901805-603. PMID: 19775509.)

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1.6.3 Oxygen Persufflation

During SCS, oxygen is simply infused into an organ using a process called oxygen persufflation (PSF), in which bubbling introduces oxygen into the organ's vasculature (Figure 14). It has been shown that directly introducing filtered and humidified oxygen into the graft's vasculature can increase ATP levels and reduce metabolic stress.

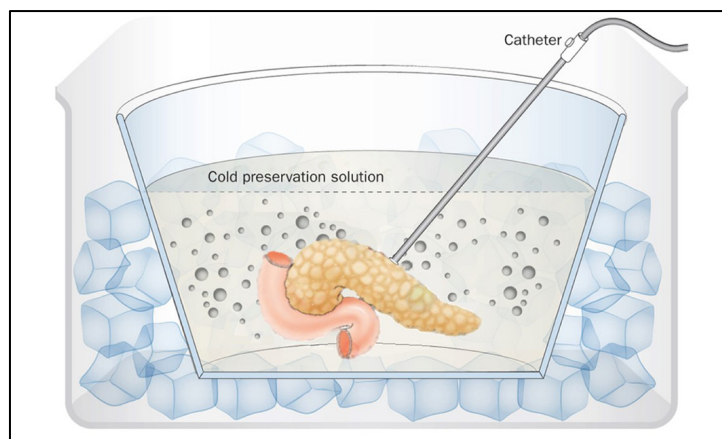


Figure 14. Oxygen persufflation

The entire pancreas organ is maintained in University of Wisconsin solution (UW), cooled via an ice bath, and oxygenated through direct cannulation. Cannulation of either the arteries or portal vein allows for anterograde or retrograde persufflation. Integrating this approach with the two-layer technique would necessitate a transition to a different preservation medium from UW solution. (Dholakia S, Royston E, Sharples EJ, Sankaran V, Ploeg RJ, Friend PJ. Preserving and perfusing the allograft pancreas: Past, present, and future. *Transplant Rev (Orlando)*. 2018 Jul;32(3):127-131. doi: 10.1016/j.trre.2018.02.001. Epub 2018 Mar 6. PMID: 29566990.)

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PSF is not a novel idea; it was accidentally discovered in 1902 when Rudolf Magnus noticed it happening during the perfusion of a cat's heart¹⁷⁶. Here, compressed oxygen was accidentally given to the heart perfusion circuit rather than blood, but the heart's contractility remained. Bunzl et al. examined the benefits of PSF versus liquid perfusion in a spinal model of frogs in 1954¹⁷⁷. According to the model, PSF can maintain muscular contractions and peripheral nerve reflexes for up to 6–8 hours while also lowering edema levels and enhancing tissue oxygenation.

The positive benefits of PSF are supported by several experimental investigations. Reddy et al. showed that raising ATP levels by PSF improved the preservation of a donor pancreas that wasn't beating heartily in a rat model¹⁷⁸. Similar results were shown by Scott et al. in another investigation, in which paired pig or human pancreatic lobes were retained for up to 24 hours using either PSF or the TLM¹⁷⁹. PSF entailed injecting 20 cm³/min of gas humidified with 40% oxygen into the celiac trunk and superior mesenteric artery while preserving pressures between 10 and 20 mm Hg. Using magnetic resonance imaging (MRI), which shows the negative contrast linked to gas in the vasculature, the uniformity of PSF was evaluated. After 6 and 24 hours of preservation, pig islets were extracted and the ATP to inorganic phosphate ratio (ATP:Pi) was determined non-invasively. The results showed that pancreatic tissue was uniformly persufflated, with

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increased ATP levels and islet yields that were similar to those of fresh pancreatic tissue.

1.6.4 Hypothermic machine perfusion

The methods created for kidney preservation have mostly been applied to the application of hypothermic machine perfusion (HMP) to pancreatic preservation (Figure 15). HMP uses a variety of techniques to control flow and/or pressure while recirculating cold preservation solution. The removal of anaerobic metabolism byproducts, the lowering of vascular resistance—which is regarded as a viability marker—and the supply of oxygen are among the purported benefits of HMP¹⁸⁰. Devices for liver and kidney applications are now commercially accessible with HMP technology^{181,182}.

In HMP for the pancreas, a variety of perfusates are employed, most often the Kidney Perfusion Solution (KPS-1), which is a modification of the UW intended for machine perfusion. The reduction of interstitial oedema and ischemic damage is the fundamental scientific justification for the perfusion solutions utilized in HMP.

HMP has the capacity to preserve the pancreas for up to 24 hours, according to several investigations on pigs and dogs, albeit some of the cases had oedema¹⁸³.

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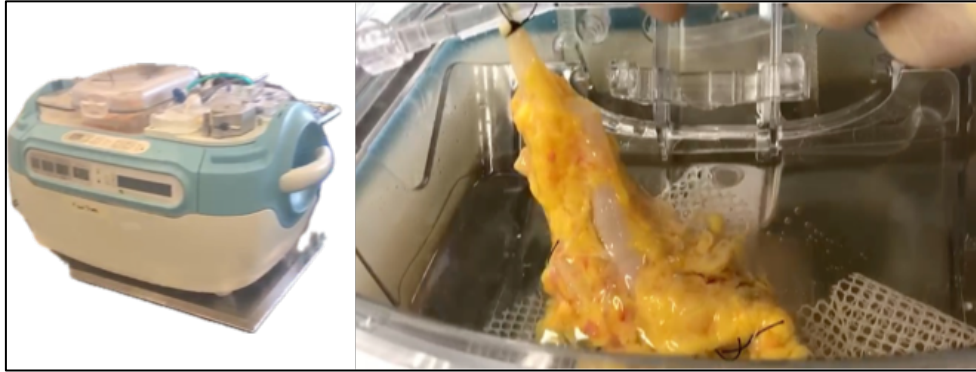


Figure 15. Hypothermic Machine Perfusion

In the hypothermic perfusion investigations (typically conducted at 4°C), pancreas perfusion is regulated by pressure, with arterial pressures upheld at standard values. Hence, contingent upon intravascular resistance—often elevated in impaired organs—arterial blood flow will vary under constant perfusion pressure. Consequently, it's imperative to evaluate and concurrently report flow, pressure, and resistance.

In a research, Karcz et al. perfused 15 pig pancreases for 315 minutes at a temperature between 4 and 10 °C and a perfusion pressure between 15 and 23 mm Hg¹⁸⁴. Although the amount of pancreatic weight gained varied, post-perfusion imaging showed a marked decrease in damage to the islets and acinar cells. It is hypothesized that even at low pressures, the extracellular space may be disrupted, leading to a greater degree of interstitial oedema with HMP as opposed to SCS. This implies that HMP could be useful in islet cell separation, as oedema and intercellular disruption could facilitate enzymatic digestion¹⁸⁵.

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Human investigations conducted recently have demonstrated the effectiveness of HMP in pancreatic preservation^{186,187}. Eight human pancreases, four donated after circulatory death (DCD) and four donated after brain death (DBD), preserved by HMP, were compared with oxygenated Belzer UW by Leemkuil et al. For six hours, the splenic and superior mesenteric arteries were dual perfused. At baseline and six hours during preservation, tissue biopsies and preservation fluid samples were taken. After HMP, ATP levels—which had been lower in the DCD groups at baseline—rose and eventually reached levels comparable to those of DBD organs prior to preservation. After five hours of HMP, the enzyme levels in the preservation fluid peaked, indicating that there was no more cellular damage accumulating¹⁸⁷.

Cantarovich et al. have demonstrated the use of the HMP system—the WAVE perfusion machine—developed by Waters Medical Systems to perfuse seven human DBD pancreases with the preservation solution IGL-1. Tissue biopsies performed at a pressure of 25 mm Hg at 6, 12, and 24 hours of perfusion revealed no signs of oedema¹⁸⁸.

1.6.5 Normothermic Perfusion

Normothermic pancreas perfusion (Figure 16), initiated in 1926 by Babkin and Starling perfusing isolated canine pancreases, showed that pancreases with shorter preservation periods exhibited higher insulin production

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compared to those preserved for over 24 hours¹⁸⁹. The concept arose from this discovery that insulin production could be correlated with organ preservation and represent the degree of organ harm.

Unlike hypothermic models, which frequently function at sub-physiological pressures, normothermic perfusion models seek to match physiological characteristics of pressure and flow. Two canine pancreases were perfused by Meyer et al. and Eckhauser et al. at pressures of 75 mm Hg and 90–110 mm Hg, respectively. This resulted in good function preservation but continued tissue edema^{190,191}.

Barlow et al. performed normothermic perfusion on five human pancreases that were discarded after they were found to be unsuitable for transplantation. DCD and DBD Organs were made by donors ranging in age from 14 to 51. Thirteen hours and nineteen minutes was the median cold ischemia time prior to initiating normothermic machine perfusion (NMP). The mean arterial flow was consistently maintained at 35 ± 2.8 ml/min/100 g by maintaining perfusion pressures between 50 and 55 mm Hg^{192,193}.

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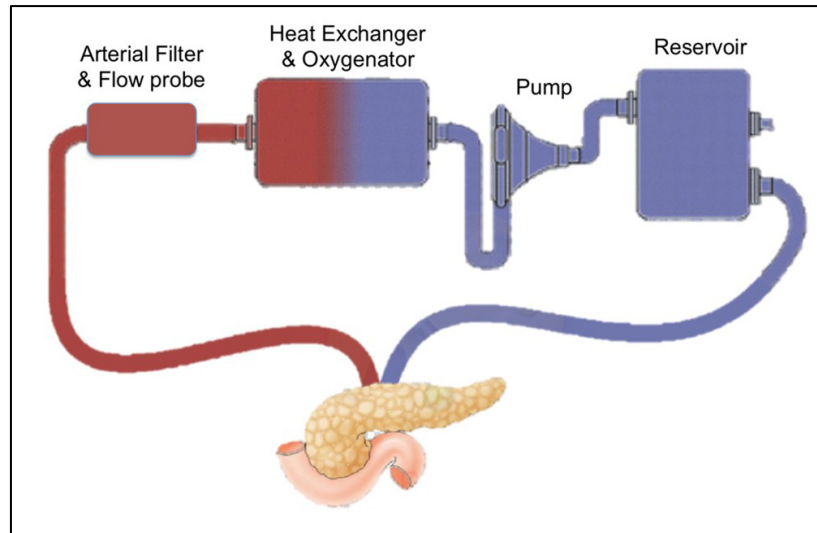


Figure 16. Normothermic perfusion.

Demonstrates the principles of closed-circuit perfusion, employing either pulsatile or continuous flow methods, with meticulous monitoring of pressure and flow rates, along with oxygenation and temperature regulation of the blood. Unlike open circuits, which lack recirculation, and hypothermic circuits utilizing preservation fluid and ice, normothermic perfusion maintains the organ within a near-physiological state without necessitating additional circuitry.

(Dholakia S, Royston E, Sharples EJ, Sankaran V, Ploeg RJ, Friend PJ. Preserving and perfusing the allograft pancreas: Past, present, and future. *Transplant Rev (Orlando)*. 2018 Jul;32(3):127-131. doi: 10.1016/j.trre.2018.02.001. Epub 2018 Mar 6. PMID: 29566990.)

Amylase levels, perfusate insulin, and other indicators were measured. In order to test lipase and amylase, exocrine secretions were drained. Tissue biopsies evaluated indicators of ischemia-reperfusion injury, tissue damage, and viability. All of the organs showed tissue edema, however there was

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significant diversity amongst them. The organs were neither islet isolated or transplanted, which made it difficult to estimate the mass of functional beta cells. The study emphasizes that it is feasible to evaluate organ viability while a machine is perfusing blood, but it also poses an important technical question of whether a normothermic perfusion device must be able to be transported to the hospital of the donor. This delicate transplanted organ is susceptible to inflammatory damage from even a few hours of cold ischemia.

1.6.6 Normothermic regional perfusion and flush

Clinical pancreatic transplantation has used normothermic regional perfusion (NRP), which makes use of extracorporeal membrane oxygenation-based technology (Figure 17). This technique is particularly useful for donation from DCD donors^{194,195}. During the process, venous and arterial cannulations are made, with the goal of particularly supplying oxygen-rich, normothermic blood to the abdominal organs.

While there are few documented successful examples of islet cell transplantation and solid organ pancreas transplantation following NRP, new research indicates encouraging results¹⁹⁶. According to histological studies, organs flushed at a flow rate of ≤ 500 mL/min with an IGL1 preservation solution did not negatively impact pancreas histology¹⁹⁷.

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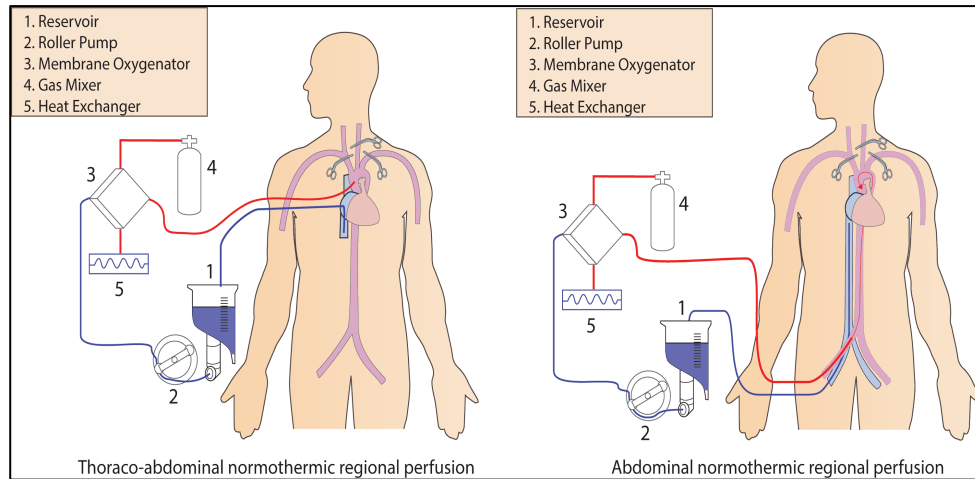


Figure 17. Cannulation and circuit for normothermic regional perfusion.

ECMO circuit is the same for both thoracoabdominal normothermic regional perfusion and abdominal-normothermic regional perfusion. In TA-NRP, the aorta and right atrium are cannulated; in A-NRP, the femoral artery and femoral vein are cannulated. In addition, the circuit is modified to accommodate two suctions for blood collection. Alamouti-Fard E, et al. Normothermic Regional Perfusion is an Emerging Cost-Effective Alternative in Donation After Circulatory Death (DCD) in Heart Transplantation. Cureus. 2022 Jun 29;14(6):e26437

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Take Home message

1.Importance of Organ Preservation: Maintaining organ viability during the period between donation and transplantation is crucial for optimal graft outcomes. For pancreatic and islet transplantation, static cold storage (SCS) remains the most commonly used technique, despite challenges associated with microvascular dysfunction and susceptibility to ischemia-reperfusion damage.

2.Static Cold Storage (SCS): SCS involves keeping the organ at temperatures between 2 and 4 °C to slow down cellular metabolism and reduce oxygen consumption. Various preservation solutions, such as University of Wisconsin (UW) solution, Histidine Tryptophan Ketoglutarate (HTK) solution, Celsior solution, and Institut Georges Lopez-1 (IGL-1) solution, are used during SCS to minimize ischemia-reperfusion injury.

3.Two-Layer Method (TLM): The Two-Layer Method combines Perfluorocarbons (PFCs) with standard SCS perfusion fluid to provide oxygen to the organ during preservation. While promising results have been observed, particularly in maintaining pancreatic viability, further research is needed to establish its efficacy.

4.Oxygen Persufflation (PSF): PSF involves infusing oxygen directly into the organ's vasculature during SCS, aiming to increase ATP levels and reduce metabolic stress. Experimental studies have shown potential benefits in improving pancreatic preservation and maintaining islet function.

5.Hypothermic Machine Perfusion (HMP): HMP uses a machine to control flow and pressure while recirculating cold preservation solution through the organ. It has shown promising results in preserving pancreatic viability for up to 24 hours and reducing ischemia-reperfusion injury in animal and human studies.

6.Normothermic Perfusion: Normothermic perfusion seeks to match physiological characteristics of pressure and flow, maintaining the organ in a near-physiological state. Studies have demonstrated its feasibility in evaluating organ viability and minimizing inflammatory damage, particularly in pancreases discarded after being deemed unsuitable for transplantation.

7.Normothermic Regional Perfusion and Flush (NRP): NRP utilizes extracorporeal membrane oxygenation-based technology to supply oxygen-rich, normothermic blood to abdominal organs, particularly beneficial for donation from Donation after Circulatory Death (DCD) donors. While there are limited documented cases, new research indicates promising results in islet cell transplantation and solid organ pancreas transplantation following NRP.

CHAPTER 2 ~ Personal Contribution

CHAPTER 2 ~ Personal Contribution

CHAPTER 2 ~ Personal Contribution

Chapter 2 ~Personal contributions

CHAPTER 2 ~ Personal Contribution

CHAPTER 2 ~ Personal Contribution

Aims of the thesis

The overall aim was first to evaluate the feasibility of active oxygenated and non-active oxygenated hypothermic machine perfusion (HMP) in human donor pancreata as a viable and potentially superior preservation technique and to compare these two preservation techniques to the traditional method of static cold storage (SCS) by a human split pancreas model from deceased donors (donation after cardiocirculatory death (DCD) and donation after brain death (DBD)) discarded from Eurotransplant centers for whole-pancreas transplantation and islet transplantation.

The first paper studies the impact of the Discontinuous purification method on the islet isolation outcome. This study aims to compare the efficacy of the COBE method vs the “bottle” method for the purification of human islets from deceased human pancreas donors.

With the cessation of distribution of COBE 2991 in Europe, there is an urgent need to investigate alternative purification methods.

The findings from this study, which are not currently documented in the existing literature, have paved the way for the integration of this purification method into subsequent research endeavors.

CHAPTER 2 ~ Personal Contribution

Paper 1: Continuous vs. discontinuous purification of isolated human islets: functional and morphological comparison. Buemi A. et al, Front Endocrinol (Lausanne). 2023 Jun 30;14:1195545.

The second paper investigates the viability and potential superiority of two preservation techniques—active oxygenated and non-active oxygenated hypothermic machine perfusion (HMP)—in human donor pancreata. A comparison is drawn between these two preservation methods and the conventional approach of static cold storage (SCS). This assessment is conducted through the utilization of a human split pancreas model sourced from deceased donors, encompassing cases of donation after cardiocirculatory death (DCD) and donation after brain death (DBD), which were previously discarded from Eurotransplant centers designated for whole-pancreas transplantation and islet transplantation.

Paper 2: Exploring preservation modalities in a split human pancreas model to investigate the effect on the islet isolation outcomes. Buemi A. et al, Transplantation Direct, Paper on press.

The focus of the third paper is an exploration into the various parameters, beyond the preservation method, that are associated with donor

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characteristics, the pancreas, and isolation results. This investigation seeks to identify factors that can either positively or negatively impact the islet secretory function. It is crucial to establish reliable parameters for selecting organs capable of yielding favorable isolation outcomes, as the ultimate objective of any transplantation procedure is to normalize blood sugar levels and alleviate diabetic symptoms in transplant recipients.

Recent studies have emphasized the importance of non-quantitative indicators in this context. However, as of now, there is a gap in the literature regarding the use of perfusions as an analytical tool for assessing islet function and predicting parameters related to successful isolation outcomes.

Paper 3: Donor- and isolation- related predictive factors of in vitro secretory function of cultured human islets. Buemi et al. Front Endocrinol (Lausanne), 2024 15:1345351.

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Results:

Paper 1

Continuous vs Discontinuous Purification of Isolated Human Islets: Functional and Morphological Comparison.

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Continuous vs. discontinuous purification of isolated human islets: functional and morphological comparison

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Background: The COBE 2991 cell processor, commonly used for pancreatic islet isolation, is no longer distributed in Europe, leading to a search for alternative purification procedures with equivalent efficacy. The aim of this study was to evaluate the efficacy of an alternative method based on the discontinuous purification of islets.

Methods: The conventional isolation procedure using a standard continuous islet purification with COBE 2991 of $n = 4$ human pancreas was compared to $n = 8$ procedures using a discontinuous purification with a "bottle" method from donors of similar characteristics. Islet equivalents, purity, and dynamic glucose-stimulated insulin secretion were evaluated.

Results: A similar islet yield was obtained using continuous vs. discontinuous purification methods ($76,292.5 \pm 40,550.44$ vs. $79,625 \pm 41,484.46$ islet equivalents, $p = 0.89$). Islets from both groups had similar purity ($78.75\% \pm 19.73\%$ vs. $55\% \pm 18.16\%$, $p = 0.08$) and functionality both in terms of stimulation index (3.31 ± 0.83 vs. 5.58 ± 3.38 , $p = 0.22$) and insulin secretion (1.26 ± 0.83 vs. 1.53 ± 1.40 mean AUC, $p = 0.73$). Moreover, the size of the islets was significantly larger in the discontinuous vs. continuous purification group ($19.2\% \pm 10.3\%$ vs. $45.4\% \pm 18.8\%$ of islets less than $100 \mu\text{m}$, $p = 0.0097$ and $23.7\% \pm 5.3\%$ vs. $15.6\% \pm 5.8\%$ of $200\text{--}250 \mu\text{m}$ islet size, $p = 0.03$).

Conclusion: Compared to the conventional purification procedure, discontinuous purification with a bottle method shows similar results with regard to isolation yield and islet secretory function. Furthermore, this alternative technique allows for obtaining larger islets.

KEYWORDS

islet isolation, islet purification, COBE 2991 cell processor, discontinuous density gradient, continuous density gradient

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Introduction

Pancreatic islet transplantation is a minimally invasive treatment option to achieve better glucose control in selected patients with type 1 diabetes (1). The techniques used for islet isolation have improved over the years, leading to better outcomes. However, many recipients still require two- or three-islet transplantation procedures to achieve insulin independence (2). The purification process is a critical step in islet isolation, and its efficiency is directly related to the success of the procedure. Thus, up to 15%–51% rates of islet loss during this step have been reported (3).

Ficoll-based density gradients have been widely used for human islet purification in most islet processing centers using a semi-automated computerized COBE 2991 cell processor (4).

However, with the cessation of the distribution of COBE 2991 in Europe, there is an urgent need to investigate alternative purification methods.

Historically, other purification techniques have been used, such as handpicking, serial sieving, and discontinuous density gradient using 50–250 ml tubes/bottles, widely referred to as the “bottle” method (2, 4, 5). To our knowledge, the efficacy of these techniques has not been compared with the conventional COBE 2991 technique.

The present study aims to compare the efficacy of the COBE method vs. the bottle method for the purification of human islets from deceased human pancreas donors.

Material and methods

Between November 2019 and May 2022, 36 human islet isolations were performed for research purposes.

Of these, $n = 12$ procedures were performed with the same functional and morphological assessment.

$N = 4$ human islet isolations were made using continuous purification, and $n = 8$ isolations were performed using discontinuous purification with the bottle method.

Human pancreases for isolation

Human deceased donor pancreases were procured through a multiorgan donor program from 2019 to 2022 following aortic cross-clamp and *in situ* flush with Institut Georges Lopez-1 preservation solution (IGL-1) and transported to our islet laboratory facility. Organs were used for research only if discarded for clinical pancreas or islet transplantation and if research consent was present, according to the guidelines of our

local medical ethical committee (Comité d'éthique hospitalo-facultaire UCL (CEHF), 2019/07/MAI/201).

The following donor characteristics at donation were collected: donor age and gender, body mass index (BMI), graft origin, cause of death, medical history, laboratory results, medications, and ischemia times.

Pancreatic islet isolation

Dissection, cannulation, enzymatic perfusion, and digestion of pancreatic tissue were identical in both groups. A blend of collagenase NB1 and neutral protease (SERVA Electrophoresis, Heidelberg, Germany) was used to perfuse the organ, aiming for pancreas distension with minimal leakage.

The enzyme was delivered *via* the pancreatic duct under controlled temperature and pressure. The perfused pancreas was then transferred to a Ricordi chamber and enzymatic and mechanical dissociation was initiated by warming the closed circuit to 37.0°C. Assessment of the tissue digest was performed under microscopy as timed samples were stained with dithizone, once a sufficient number of islets were free of exocrine tissue the digestion was halted by dilution of enzyme and washing of the tissue. The collected tissue was pooled and quantified to determine islet mass and the percentage of islets still encapsulated with acinar tissue.

Continuous purification

Islet separation from acinar tissue was achieved using a UW/Ficoll continuous gradient on a COBE 2991 cell processor. Density gradients of 1.062 and 1.072 g/ml were loaded into the COBE bag, followed by the addition of tissue digest suspended in UW solution. Following a 5-min centrifugation at 3,000 RPM, purity fractions were collected and again pooled to yield a pure layer and subsequent less pure layers.

Discontinuous purification

Digested pancreas tissue was suspended in 75 ml of 1.132 g/ml polysucrose solution (Corning, USA) and transferred into a 250-ml flat-bottom bottle. Two additional polysucrose solutions with densities of 1.096 (65 ml) and 1.06 (45 ml) g/ml, respectively, were then carefully added by slowly pipetting the solutions on top of the first layer to create a discontinuous density gradient. All media were cooled to 4°C, and centrifugation was equally performed at this temperature. Isolated islets were then collected at the 1.096/1.06 interface following centrifugation for 17 min at 55 g (Figure 1).

Islet assessment

Islet yield and purity

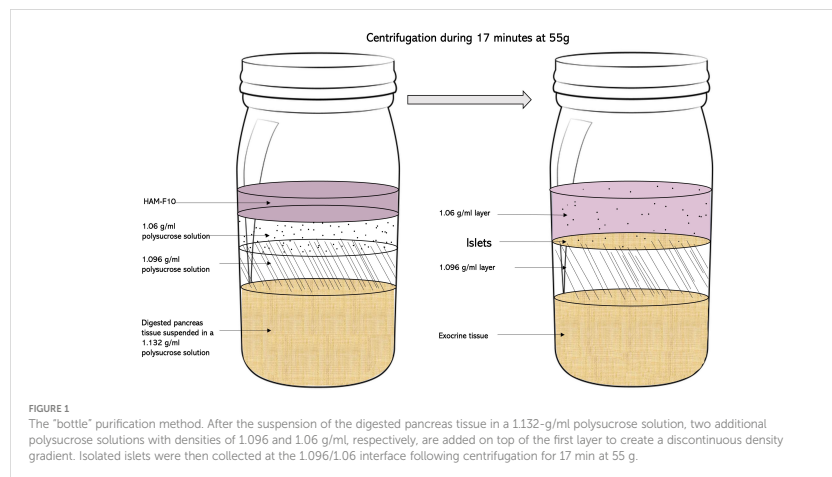
Quantification and purity of islets were performed at the end of the isolation using standard dithizone staining protocols (3). The crude number of islets in each diameter class was determined by counting islets using an optical graticule. The crude number of islets was then converted to the standard number of islet equivalents (IEQ).

Abbreviations: AUC, area under curve; BMI, body mass index; CVA, cerebrovascular accident; DBD, donation after brain death; DCD, donation after circulatory death; HBP, high blood pressure; IGL1, Institut Georges Lopez-1; IEQ, islet equivalent; RIA, radioimmunoassay; RPM, revolutions per minute; UW, University of Wisconsin.

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Dynamic glucose-stimulated insulin secretion test

The secretory function of isolated human islets was assessed by dynamic islet perfusion experiments according to our previous protocols (6). The working medium was a bicarbonate-buffered solution containing 120 mM NaCl, 4.8 mM KCl, 2.5 mM CaCl_2 , 1.2 mM MgCl_2 , 24 mM NaHCO_3 , 1 mg/ml BSA, and varying concentrations of glucose as indicated in the figures. Batches of 1,000–2,000 IEQ were placed in perfusion chambers, covered with 8 μm cellulose filters, and sealed. Test solutions kept at 37°C and gassed continuously to stabilize pH around 7.2 were pumped at a flow rate of 1 ml/min. Effluent fractions were collected at 2-min intervals and saved for insulin assays using radioimmunoassay (RIA) kits (DiaSource ImmunoAssays, Ottignies, Belgium). At the end of the experiments, islets were recovered, and their insulin content was determined after extraction in acid-ethanol (75% ethanol, 180 mM HCl from Merck, Overijse, Belgium).

Statistics

Data are presented as means $\pm$ standard deviation (SD). Differences between the two groups were analyzed using Student's *t*-test. *p*-values < 0.05 were considered to indicate statistical significance.

Results

Donor characteristics

The donor characteristics of human pancreases that were used for islet isolation and the islet isolation outcomes are summarized in Table 1.

Baseline characteristics were similar in both groups.

Isolation results

A similar islet yield was obtained using the continuous vs. discontinuous purification method ($76,292.5 \pm 40,550.44$ vs. $79,625 \pm 41,484.46$, $p = 0.89$), even after correction for pancreas mass ($1,181.68 \pm 650.5$ IEQ/g vs. 753.54 ± 397.73 IEQ/g, $p = 0.18$). The islet purity was also similar in continuous vs. discontinuous purification method groups ($78.75\% \pm 19.73\%$ vs. $55\% \pm 18.16\%$, $p = 0.08$).

Although the mean islet equivalent/g was similar in both groups ($p = 0.18$), some significant differences were found in the size of islets obtained after purification. Indeed, the percentage of islets with a size $< 100 \mu\text{m}$ was higher in the continuous vs. discontinuous purification method groups ($45.4\% \pm 18.8\%$ vs. $19.2\% \pm 10.3\%$, $p = 0.0097$). In contrast, the percentage of islets measuring between 200 and $250 \mu\text{m}$ was lower in the continuous vs. discontinuous purification method groups ($15.6\% \pm 5.8\%$ vs. $23.7\% \pm 5.3\%$ of islets size, $p = 0.03$). Case-by-case donor demographics results, ischemia times, and isolation data of both groups are available in the supplementary material (Supplementary Tables S1, S2).

After 1 day of culture, a dynamic GSIS was performed. Both islet groups were responsive to glucose stimulation, with a similar insulin secretory profile observed in islets purified by either method (Figure 2A): a sharp and rapid short-lived increase of insulin secretion (first phase) followed by a lower but constant secretion rate (second phase). The peak stimulation index was also similar in islets purified with a continuous vs. discontinuous purification method (3.31 ± 0.83 vs. 5.58 ± 3.38 , $p = 0.22$) (Figure 2B). Also, the area under the insulin curve (calculated over 20 min of high glucose stimulation) was similar (1.26 ± 0.83 vs. 1.53 ± 1.40 mean AUC, $p = 0.73$) (Figure 2C).

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TABLE 1 Donor demographics, ischemia times and isolation data of 4 islet isolations using continuous purification compared with 8 islet isolations using discontinuous purification.

	Continuous Purification	Discontinuous Purification	P
	n=4	n=8	
Donor Demographics			
Age, (years)	52.2±20.6	48±10.7	0.64
Gender, n			0.71
Male	2	4	
Female	2	4	
Weight (mean kg±SD)	77±10.13	82.5±12.08	0.45
Height (mean cm±SD)	171±7.87	175.5±6.84	0.32
BMI (mean kg/m ² ±SD)	27±1.8	26.8±3.7	0.8
Donor Organ Source, n			0.99
DBD	1	2	
DCD	3	6	
Cause of Death, n			0.76
CVA	3	5	
Trauma	0	3	
Cardiac Arrest	1	0	
Cardiac Arrest, n	1	4	0.45
Medical History			
HBP, n	1	1	0.62
Smoking, n	0	3	0.99
Drug Abuse, n	0	1	0.99
Alchol Abuse, n	0	2	0.99
Infections, n	0	4	0.09
Vasopressor use, n	2	4	0.45
Laboratory Results			
Lipase, (mean IU/L)	31±24.57	40.4±33.32	0.71
Amylase, (mean IU/L)	171±68.46	81.66±71.98	0.19
Hemoglobine, (mean g/dL)	13.37±1.37	11.01±2.20	0.07
Ischemia time			
Cold Ischemia time, h (±SD)	18.4±5.68	18.1 ±3.27	0.92
Warm Ischemia time, min (±SD)	12.1±20.2	20.37±9.07	0.48
Total Ischemia time, h (±SD)	19.23±5.53	18.1±3.29	0.72
Isolation data			
Islet yield (mean IEQ±SD)	76292.5±40550.44	79625±41484.46	0.89
Islet purity(mean %,±SD)	78.75±19.73	55±18.16	0.08
IEQ/gr (mean IEQ/gr,±SD)	1181.6±650.5	753.5±397.7	0.18
100/150 size (mean %)	45.5±18.8	19.2±10.3	0.0097
150/200 size (mean %)	20.4±7.1	26.1±6.3	0.84

(Continued)

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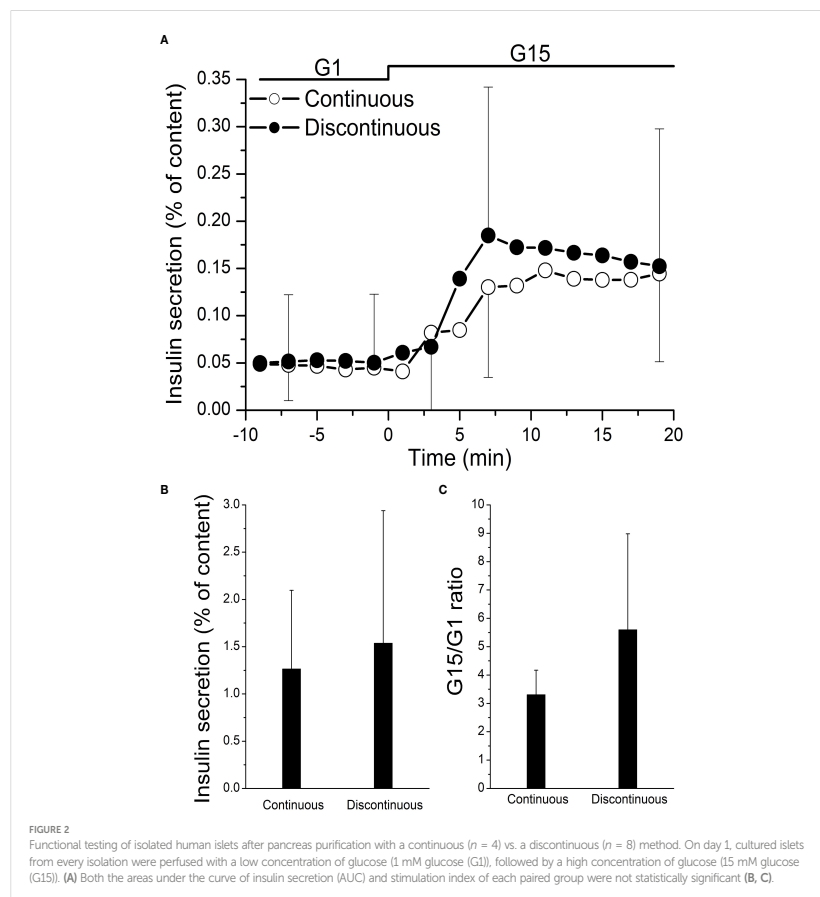
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TABLE 1 Continued

	Continuous Purification	Discontinuous Purification	P
	n=4	n=8	
200/250 size (mean %)	15.6±5.8	23.7±5.3	0.0381
250/300 size (mean %)	9.6±3.2	11.8±5.3	0.47
300/350 size (mean %)	3.6±3.5	7.8±4.6	0.14
350/ size (mean %)	5.2±2.3	9.3±8.6	0.38

BMI, body mass index; CVA, Cerebrovascular Accident; DBD, donation after brain death; DCD, donation after circulatory death; IEQ, islet equivalent; HBP, high blood pressure; SD, standard deviation.



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Discussion

Our results show that, compared to the conventional COBE 2991 isolation procedure, a technique using a discontinuous purification with a bottle method has similar performance in the isolation process and islet secretory function. If confirmed in larger-scale studies, these preliminary results suggest that this procedure might be considered an efficient alternative to the purification process of the human pancreas.

However, we acknowledge that even if similar, the results of the isolation process were poor in both groups, particularly in terms of the obtained islet yields.

As expressed in numerous international clinical studies, the most valuable parameters for deciding about the transplantability of isolated islets are the number of islets (generally they must correspond to more than 5,000 IEQ/kg of the recipient's body weight), purity (as a percentage of insulin-positive clusters, generally > 70%), sterility, potency expressed by a stimulation index > 1, and viability of more than 70% (7). In our study, these values are not obtained due to the long ischemia times and the nature of the donors rejected for clinical purposes. Long ischemia times, both cold and warm, and donor unfavorable characteristics affect cell life/death, thereby influencing islet and acinar cells and, subsequently, isolation outcomes (8).

However, in both groups, the islet function remains satisfactory regardless of the type of purification method performed, both in terms of stimulation index and in terms of insulin secretion (9). We can conclude that the discontinuous purification method, by not impacting islet function, can replace continuous purification and that to obtain results compatible with a transplantable product, it is necessary to respect donor selection criteria and to minimize organ ischemia time.

Moreover, as previously described by others (10), the size of islets purified by the bottle method was significantly larger than that of islets purified by COBE purification. This is more likely related to the reduced shear stress during centrifugation in the purification using the bottle method. The COBE bag has a narrow segment, and islets suffered significant shearing force during passage through this narrow segment. Since the bottle does not have such a narrow segment, the shear stress could be significantly reduced.

The impact of islet size on islet transplantation outcomes is a debated topic in the literature.

Evidence has shown that at similar islet equivalent (IE) doses, larger islets may exhibit poorer therapeutic values. This may be related to oxygen diffusion limitations that worsen proportionally with islet size (11).

However, Hughes et al. have recently shown no clear correlation between islet isolation index and graft function in recipients receiving islets that had been cultured before implantation and that large islets are equally suitable as long as they have undergone and survived a short period of postculture isolation (12).

Moreover, in our experience, the bottle purification method was relatively simple to implement and associated with lower costs than the COBE 2991 method.

Our study has limitations. The first is the relatively low number of organs used. Second, because the protocol of the study was designed for research only, our results cannot be translated for clinical use.

In conclusion, we have demonstrated that discontinuous purification using the bottle method is a viable and efficient alternative in the absence of COBE 2991 that achieves comparable results both in terms of insulin secretory function and the performance of the isolation process from discarded organs.

The use of this purification technique with better-quality organs and shorter ischemia times would make it possible to permanently replace the islet purification gold standard of the past 20 years.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

AB and NM: study design and writing of the manuscript. They had equally participated in the elaboration of the paper. All authors discussed and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fendo.2023.1195545/full#supplementary-material>

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Summary Paper 1:

Aim of the Study:

The COBE 2991 cell processor, commonly used for pancreatic islet isolation, is no longer distributed in Europe, leading to search for alternative purification procedures with equivalent efficacy.

This study aimed to evaluate the efficacy of an alternative method based on the discontinuous purification of islets.

Take Home message

1. In comparison to the traditional purification procedure, employing a discontinuous purification method with a "bottle" approach yields comparable results in terms of isolation yield and islet secretory function.
2. Notably, the islets' size was significantly larger in the discontinuous purification group when compared to continuous purification. This difference is likely attributed to the reduced shear stress experienced during centrifugation in the purification process using the "bottle" method.
3. The application of this purification technique, especially with high-quality organs and shorter ischemia times, holds the potential to establish a lasting replacement for the conventional gold standard in islet purification that has been prevalent over the past two decades.

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Paper 2: Exploring preservation modalities in a split human pancreas model to investigate the effect on the islet isolation outcomes.

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OPEN

Exploring Preservation Modalities in a Split Human Pancreas Model to Investigate the Effect on the Islet Isolation Outcomes

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Background. In islet transplantation, the use of dynamic hypothermic preservation techniques is a current challenge. This study compares the efficacy of 3 pancreas preservation methods: static cold storage, hypothermic machine perfusion (HMP), and oxygenated HMP. **Methods.** A standardized human pancreas split model was employed using discarded organs from both donation after brain death ($n = 15$) and donation after circulatory death (DCD) ($n = 9$) donors. The pancreas head was preserved using static cold storage (control group), whereas the tail was preserved using the 3 different methods (study group). Data on donor characteristics, pancreas histology, isolation outcomes, and functional tests of isolated islets were collected. **Results.** Insulin secretory function evaluated by calculating stimulation indices and total amount of secreted insulin during high glucose stimulation (area under the curve) through dynamic perfusion experiments was similar across all paired groups from both DCD and donation after brain death donors. In our hands, islet yield (IEQ/g) from the pancreas tails used as study groups was higher than that of the pancreas heads as expected although this difference did not always reach statistical significance because of great variability probably due to suboptimal quality of organs released for research purposes. Moreover, islets from DCD organs had greater purity than controls ($P \leq 0.01$) in the HMP study group. Furthermore, our investigation revealed no significant differences in pancreas histology, oxidative stress markers, and apoptosis indicators. **Conclusions.** For the first time, a comparative analysis was conducted, using a split model, to assess the effects of various preservation methods on islets derived from pancreas donors. Nevertheless, no discernible variances were observed in terms of islet functionality, histological attributes, or isolation efficacy. Further investigations are needed to validate these findings for clinical application.

(Transplantation Direct XXX;XXX: e1654; doi: 10.1097/TXD.0000000000001654.)

Patients with uncomplicated type 1 diabetes are usually treated with insulin injections. In a selected group of patients, replacement of functioning beta cells by whole pancreas transplantation or islet transplantation is the treatment of choice.¹⁻³

However, from the 2361 potential donors reported by Eurotransplant in 2019, only 157 pancreas transplants and 20 islet transplants were performed. These results are comparable in subsequent years.⁴

A key factor is the shortage of good-quality pancreas grafts.⁵ Compared with other organs, the pancreas is more vulnerable to injury related to brain death and the actual organ retrieval operation.⁶ Also, in contrast to transplantation of other organs, beta cell replacement is not an immediate life-saving procedure, and therefore, centers tend to take lower risks with regard to the quality of the donor organ when accepting the pancreas.

The current challenge is to increase the donor pool by utilization of extended criteria donor for the pancreas without

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The authors declare no conflicts of interest.

A.B. and N.I.M. were involved in study design and writing of the article. They had equally participated to the elaboration of the article. All authors discussed and reviewed the article.

Supplemental digital content (SDC) is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (www.transplantationdirect.com).

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compromising transplant outcomes. New pancreas preservation techniques could be a promising method to achieve this objective.⁷⁻¹⁷

In this study, we first evaluated the feasibility of active oxygenated and nonactive oxygenated hypothermic machine perfusion (HMP) in human donor pancreata as a viable and potentially superior preservation technique, and we compared these 2 preservation techniques to the traditional method of static cold storage (SCS) by a human split pancreas model from deceased donors (donation after cardiocirculatory death [DCD] and donation after brain death [DBD]) discarded from Eurotransplant centers for whole pancreas transplantation and islet transplantation.

MATERIALS AND METHODS

Organ Procurement

All the organ donors used in this study were procured by the transplantation team of the Cliniques Universitaires Saint Luc, UCL (Brussels, Belgium) and by the transplantation team of the University Hospital Gasthuisberg, Leuven (Leuven, Belgium) from donors of centers affiliated with both institutions and declined for clinical use. Permission for research was given by the of the donor relatives.

This study was approved by our local medical ethical committees (CEHF, Comité d'éthique hospitalo-facultaire UCL, 2019/07/MAI/201).

In all the procedures, the procurement was performed according to the no-touch technique as previously described.¹⁸

Initially, all pancreases were flushed and stored by SCS in an Institute George Lopez 1 solution to be transported to our laboratory.

Then, the pancreas was divided into 2 segments for use in paired isolation procedures.

The first segment, the head and neck portion of the pancreas, was the control group of each procedure, and it was always processed for islet isolation after a SCS preservation with the same cold ischemia time of the respective second segment.

The second segment—body and tail portion—was procured with the entire vascular network. This segment of the pancreas was prepared to ensure only one inflow (splenic artery) and one outflow (splenic vein) through ligatures of the splenic hilum and the collateral vessels of the superior and inferior border (Figure 1).

Cold ischemia time was calculated as time from the start of aortic cold flush in the donor to the initiation of ductal enzymatic perfusion in the pancreas, therefore including oxygenated or not oxygenated HMP.

In the DCD donors, warm ischemia time was calculated as the time from the cardiac arrest and the start of aortic flush.

Study Design and Perfusion Protocol

The study design is illustrated in Figure 2. After a cold storage preservation in Institute George Lopez 1 solution, the first segment—head and neck—of the pancreas was always managed with the traditional preservation method of SCS. The second section—body and tail—of the organ was managed either with SCS (SCS group), HMP (HMP group), or HMP O₂ (HMP O₂ group).



FIGURE 1. Preparation of the pancreas tail before connection to the hypothermic machine perfusion.

In the HMP and HMP O₂ groups, the splenic artery of the body and tail segment of the second (HMP group) and third groups (HMP O₂ group) was cannulated using SealRing™ cannulas (10 × 35 mm; Organ Recovery Systems, Itasca, IL) and connected to a LifePort Kidney Transporter (Organ Recovery Systems, Diegem, Belgium) in a disposable perfusion pack with optional oxygenation tubing (reference LKT200X, Organ Recovery Systems, Diegem, Belgium). The perfusion circuit and organ reservoir were primed with 1L University of Wisconsin machine perfusion solution (PumpProtect, Carnamedica, Poland).

Based on the current literature,¹⁹⁻²⁴ a systolic hypothermic pressure of 25 mm Hg was applied, delivered via the splenic artery with venous return via the splenic vein.

For the HMP O₂ group, oxygenation was performed by delivery of 100% O₂ through the hollow fiber oxygenators with a fixed flow of 100 mL/min achieving perfusate pO₂ of at least 80–100 kPa (600–750 mm Hg) as described earlier for previous organ perfusion protocols.^{25,26} Perfusate and pancreas were cooled to 2–8 °C.

Perfusate pO₂ was continuously measured during perfusion by a microfiber oxygen transmitter (OXY-4 micro, Precision Sensing GmbH, Regensburg, Germany).

Islet Isolation

All the organs were processed at the CHEX Lab (IREC) of the UCLouvain. Both head-neck and body-tail pancreas portions were simultaneously isolated using a modified automated method that has been previously described.²⁷ Briefly, a 16-G catheter was inserted into the main pancreatic duct of both pancreas specimens.

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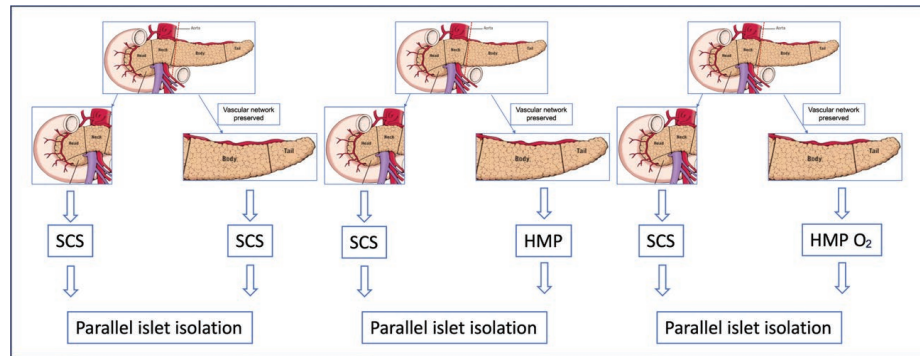


FIGURE 2. Study design of a split pancreas model to evaluate the impact of various preservation strategies on islet isolation outcome. HMP, hypothermic machine perfusion; HMP O₂, oxygenated hypothermic machine perfusion; SCS, static cold storage.

A blend of collagenase NB1 and neutral protease (SERVA Electrophoresis, Heidelberg, Germany) was used to perfuse the organ, aiming for pancreas distension with minimal leakage.

Every specimen was then transferred to 2 separate digestion chambers and processed simultaneously (Figure 3). Digested pancreatic tissues were collected and purified through a discontinuous density gradient purification method.²⁸

Islet Yield and Purity

Quantification and purity of islets was performed at the end of the isolation procedure using standard dithizone staining protocols.²⁹ The crude number of islets in each diameter class was determined by counting islets using an optical graticule. The crude number of islets was then converted to the standard number of islet equivalents (IEQ).

Islet Culture

Based on purity, after isolation the islets were cultured using RPMI supplemented with 5 mM glucose, 10% FBS, 100 U/mL penicillin, 100 µg/mL streptomycin, and 1 µg/mL mero-penem in 75 cm² culture flasks at 37 °C in 5% CO₂.

Islet yield (in IEQ) was determined after isolation (day 0), after the first medium change 1 d after isolation (day 1), and after the third medium change 7 d after isolation (day 7).

Dynamic Glucose-stimulated Insulin Secretion Test

A portion of the isolated islets from both the head-neck and body-tail segments were tested by dynamic islet perfusion experiments at days 1 and 7 of culture as previously described³⁰ to assess the in vitro functionality. The working medium was a bicarbonate-buffered solution containing 120 mM NaCl, 4.8 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgCl₂, 24 mM NaHCO₃, 1 mg/mL bovine serum albumin (BSA), and varying concentrations of glucose as indicated in the figures. Batches of 1000–2000 IEQ were placed in perfusion chambers, covered with 8 µm cellulose filters and sealed. Test solutions kept at 37 °C and gassed continuously to stabilize pH around 7.2 were pumped at a flow rate of 1 mL/min. Effluent fractions were collected at 2-min intervals and saved for insulin assays using radioimmunoassay kits (DiaSource

ImmunoAssays, Belgium). At the end of the experiments, islets were recovered and their insulin content was determined after extraction in acid-ethanol (75% ethanol, 180 mM HCl from Merck).

Islet Histology

Histological assessment was performed from biopsies obtained after the organ procurement and before the islet isolation of each group in the ventral part of both head-neck and body-tail pancreas portion.

The samples were fixed in 4% formaldehyde, subsequently embedded in paraffin, and cut into sections of 4 µm. Hematoxylin and eosin stained sections were digitalized using a Zeiss Axioscan.z1 slide scanner to evaluate changes in morphology.

All samples were examined by the same pathologist who gave blinded scores through the following variables: fibrosis, edema/dislocation, steatonecrosis, islet and exocrine autolysis.

The extent of parenchymal changing before and after preservation were scored from 0 to 3, based on the estimated percentage of the area involved: absent (0), minimal or mild (1), moderate (2), and severe (3).³¹

For the comparison, all the histologic variables of each organ were expressed as the difference of histological changes of pancreas tails and heads of the three groups before and after preservation.

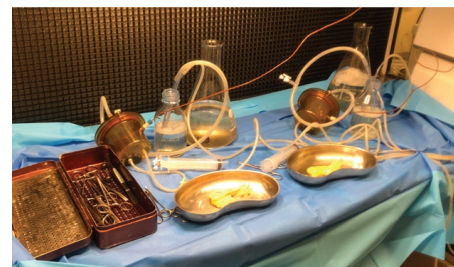


FIGURE 3. Automated procedure for paired isolations of human pancreatic islets.

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Histological Markers of Islet Oxidative Stress and Apoptosis

In this study, we have performed multiplex immunofluorescent staining for assessing islet cell composition, beta cell apoptosis, and oxidative stress using serial sections of paraffin embedded, undissociated whole pancreas samples obtained after the organ procurement and before the islet isolation. Pancreas sections were stained for specific markers: insulin, glucagon, caspase 3, TUNEL, peroxiredoxine 3, and hemoxygenase 1.

Pancreas samples were fixed in 4% formaldehyde, embedded in paraffin and sectioned. After deparaffinization, 5- μ m tissue sections were processed according to the protocol described in Aboubakar Nana et al.³²

Endogenous peroxidases were inhibited by a 20-min treatment with 3% hydrogen peroxide in methanol. Sections were then subjected to antigen retrieval in 10mM citrate buffer pH 5.7 and to blocking of specific antigen binding sites (Tris-buffered saline [TBS] supplemented with 5% BSA and 0.1% Tween20). The first primary antibody was incubated overnight at 4 °C in TBS containing 1% BSA and 0.1% Tween20, then detected by corresponding horseradish peroxidase-conjugated polymer secondary antibodies incubated for 40 min at room temperature. Horseradish peroxidase was then visualized by tyramide signal amplification using AlexaFluor-conjugated tyramide. After a new antigen retrieval in citrate buffer (this step also detached antibodies from tissue sections), the same protocol was applied with other primary antibodies and different AlexaFluor-conjugated tyramides. In this study, 2 panels of 4 sequential incubations were performed as indicated in Table S1 (SDC, <http://links.lww.com/TXD/A663>).

After a washing step in phosphate-buffered saline, nuclei were stained with Hoechst 33342 (Thermo Fisher Scientific) diluted in TBS containing 10% BSA and 0.1% Tween 20, washed in TBS containing 0.1% Tween 20, and mounted with Dako fluorescence mounting medium (Agilent). Slides were stored at 4 °C until multispectral whole slide imaging at 20 $\times$ magnification with an Axioscan.z1 slide scanner (Zeiss).

Computer-assisted quantification of the different markers within the islets was performed using Author version 2017.2 (Visiopharm). Results were expressed as % of stained area (above threshold) of each marker. For comparison, data were expressed as delta of changes in histological expression of insulin, glucagon, oxidative stress, and apoptosis markers of pancreas tails and heads samples of the 3 groups before and after preservation.

Statistics

For the paired analysis, *P* values (based on *t* test of least squares means) were reported along with a mean $\pm$ SD graph where appropriate. Differences between both groups were analyzed using Student *t* test. In case of variables without repeated measurement (eg, IEQ/g between head and tail for each preservation method) the MMRM model was reduced to an ANOVA model with factor treatment.

P values of <0.05 were considered to indicate statistical significance. GraphPad Prism (La Jolla, CA) was used in all analyses regarding donor characteristics, islet isolation function, histology, and oxidative and apoptosis markers expression.

RESULTS

Demographics and Ischemia Times

Between 2019 and 2022, 36 procedures were performed for this study from cadaveric donors declined for clinical pancreas or islet transplantation.

Of these organs, the 7 first organs were used to refine the procedure feasibility and finalize the isolation techniques and islet function procedures.

Consequently, they were not included in the analysis.

Among the next 29 organs, 24 were eligible to be included in the study and were distributed arbitrarily to one of the 3 above-described groups (SCS *n* = 8, HMP *n* = 8, HMP O₂ *n* = 8). The exclusion of the remaining organs was related to technical reasons (*n* = 1), contaminants (*n* = 2) and insufficient quantity or function of the isolated islet (*n* = 2).

Tables 1 and 2 summarize donor demographics of the 24 organs included from DBD (*n* = 15) and DCD donors (*n* = 9), respectively. There were no significant differences in both groups regarding donor age, sex, morphometrics, cause of death, donor history, donor management, and laboratory results.

Cold and warm ischemia times were also similar in both groups. The mean perfusion time in the HMP and HMP O₂ groups was not significantly different in both DBD (989 $\pm$ 253 versus 1051 $\pm$ 468 mean min, *P* = 0.80) and DCD donors (805 $\pm$ 86 versus 703 $\pm$ 17 mean min, *P* = 0.11).

Machine perfusion parameters are available in Figures S1 and S2 (SDC, <http://links.lww.com/TXD/A663>).

In Vitro Functionality

After 1 and 7 d of culture, a dynamic GSIS was performed. Both islet groups were responsive to glucose stimulation with a similar insulin secretory profile observed in islets obtained from head and tail pancreas portions of DBD donors (Figure 4A and B) and DCD donors (Figure 5A and B) of each group: a sharp and rapid short-lived increase of insulin secretion (first phase) followed by a lower but constant secretion rate (second phase).

No significant differences from both DBD and DCD donor procedures were found both in term of stimulation index and area under the insulin curve.

The peak stimulation index in pancreas head and tail isolated islets from DBD donors of the SCS, HMP, and HMP O₂ groups at day 1 of culture were 6.8 $\pm$ 2.1 versus 4.7 $\pm$ 3.2, *P* = 0.28; 5.5 $\pm$ 5.6 versus 5.9 $\pm$ 4.6, *P* = 0.79; and 3.3 $\pm$ 2.5 vs 4.5 $\pm$ 2.8, *P* = 0.41, respectively (Figure 4C). Also the area under the insulin curve calculated >20min of high glucose stimulation (G15) and at baseline (G1) were similar at day 1 for the SCS group (1.4 $\pm$ 1.2 versus 1.2 $\pm$ 0.6 mean area under curve [AUC], *P* = 0.82 and 0.17 $\pm$ 0.22 versus 0.12 $\pm$ 0.06 mean AUC, *P* = 0.89, respectively), the HMP group (0.6 $\pm$ 0.2 versus 0.7 $\pm$ 0.2 mean AUC, *P* = 0.56 and 0.1 $\pm$ 0.04 versus 0.09 $\pm$ 0.05 mean AUC, *P* = 0.06, respectively), and the HMP O₂ group (1.2 $\pm$ 0.6 versus 0.8 $\pm$ 0.07 mean AUC, *P* = 0.51 and 0.35 $\pm$ 0.38 versus 0.17 $\pm$ 0.12 mean AUC, *P* = 0.34, respectively) (Figure 4). After 7 d of culture, no statistically significant differences were found in both groups (Figure 4D, E, H). Similar results were obtained from pancreas head and tail sourced from DCD donors (Figure 5C–H).

Detailed results are contained in Table S2 (SDC, <http://links.lww.com/TXD/A663>).

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TABLE 1.
DBD donor characteristics and ischemia times of SCS, HMP, and HMP O₂ groups

	SCS	HMP	HMP O ₂	P
	n = 5	n = 5	n = 5	
Donor demographics				
Age, y	55.8 ± 17.2	67.6 ± 8.2	51.4 ± 18.1	0.25
Sex, n				
Male	2	5	4	
Female	3	0	1	
Weight, kg (mean ± SD)	75.8 ± 18.9	71.4 ± 13.3	75 ± 10.2	0.75
Height, cm (mean ± SD)	167.8 ± 9.7	176.2 ± 5.4	174 ± 8.2	0.26
BMI, kg/m ² (mean ± SD)	26.6 ± 5.4	22.8 ± 3.7	24.6 ± 3	0.38
Cause of death, n				
CVA	2	4	4	
Trauma	3	1	1	
Cardiac arrest	1	1	1	
Medical history				
HBP, n	1	1	2	
Diabetes, n	0	0	0	
Smoking, n	1	3	1	
Drug abuse, n	0	1	0	
Alcohol abuse, n	0	2	0	
Infections, n	1	1	2	
Malignancy	0	0	0	
Vasopressor use, n	2	4	4	
Laboratory results				
Lipase, U/L (mean)	32.7 ± 32.1	12.2 ± 3.5	62.7 ± 83.5	0.33
Amylase, U/L (mean)	10 ± 23.3	49.6 ± 29.2	79 ± 111.7	0.30
Hemoglobin, g/dL (mean)	9.7 ± 2.4	10.8 ± 2.1	13.3 ± 2.4	0.07
Ischemia time				
Cold ischemia time, min (±SD)	970 ± 293	1219 ± 301	1111 ± 302	0.44
Time of machine perfusion, min (±SD)	/	989 ± 253	1051 ± 468	0.80

BMI, body mass index; CVA, cerebrovascular accident; DBD, donation after brain death; HBP, high blood pressure; HMP, hypothermic machine perfusion; HMP O₂, oxygenated machine perfusion; IEQ, islet equivalent; SCS, static cold storage.

Macroscopic Morphology and Pancreas Histology

No macroscopic signs of edema or fibrosis were found in the human pancreas used in this study at the time of organ procurement and after preservation from both DBD and DCD organs.

Figure 6 shows an example of a pancreas tail obtained before and after the preservation period.

An example of the semiquantitative histologic analysis used based on a score from 0 to 3 is shown in Figure 7.

We found similar results in terms of steatonecrosis, islet and exocrine autolysis, fibrosis, and edema between the pancreas tails of the SCS, HMP, and HMP O₂ and their own control groups (the head portion) preserved using the SCS method in both DBD and DCD donors (Figures 8 and 9).

Insulin, Glucagon, Oxidative Stress, and Apoptosis Marker Expression

Two Multiplex staining of 4 markers each (insulin-glucagon-hemeoxygenase 1-peroxiredoxine 3 and insulin-glucagon-caspase 3-TUNEL) were performed on pancreas FFPE sections to identify the oxidative stress (Figure 10) and apoptosis (Figure 11) in both endocrine and exocrine component of each sample. Each staining was quantified using computer-assisted image analysis.

No statistical differences of insulin, glucagon, oxidative stress, and apoptosis marker expression were found in islets

of pancreas tails preserved with SCS, HMP, and HMP O₂ as compared with the respective control in both DBD (Figure 12) and DCD donor samples (Figure 13).

Islet Isolation Efficacy

Table 3 illustrates the variations in the isolation data of the pancreas head and tail among the 3 groups (SCS, HMP, HMP O₂) using organs sourced from both DBD and DCD donors.

Islets obtained from DCD pancreases preserved through the HMP method demonstrated the highest purity compared with the control (90% ± 0% versus 52% ± 10%, $P \leq 0.01$). No other differences were found between the respective pancreas heads and tails of both groups in terms of pancreas weight, islet purity, and islet size (Table S3, SDC, <http://links.lww.com/TXD/A663>).

DISCUSSION

We described for the first time through a human pancreas split model a comparative analysis between 3 preservation methods of discarded pancreas designated for islet isolation. This study first shows the feasibility to isolate viable and functional islets from DCD and DBD pancreas donors discarded by Eurotransplant centers for clinical islet and pancreas

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TABLE 2.
DCD donor characteristics and ischemia times of SCS, HMP, and HMP O₂ groups

	SCS	HMP	HMP O ₂	P
	n = 3	n = 3	n = 3	
Donor demographics				
Age, y	56.6 ± 12.3	52.3 ± 10.2	51.4 ± 18.1	0.88
Sex, n				
Male	2	1	3	
Female	1	2	0	
Weight, kg (mean ± SD)	84.3 ± 8.3	79.6 ± 18.4	91.6 ± 7.6	0.52
Height, cm (mean ± SD)	176 ± 1.5	171 ± 10.1	180 ± 5.1	0.31
BMI, kg/m ² (mean ± SD)	27.3 ± 3.2	27 ± 5.2	28.3 ± 2.5	0.91
Cause of death, n				
CVA	1	2	2	
Trauma	2	1	1	
Cardiac arrest	1	2	2	
Medical history				
HBP, n	2	0	2	
Diabetes, n	0	0	1	
Smoking, n	2	2	0	
Drug abuse, n	0	0	1	
Alcohol abuse, n	1	1	1	
Infections, n	1	1	1	
Malignancy, n	0	0	2	
Vasopressor use, n	1	2	1	
Laboratory results				
Lipase, U/L (mean)	32.7 ± 33.1	45 ± 21.2	57.3 ± 67.3	0.80
Amylase, U/L (mean)	10 ± 2.1	49.25 ± 27.3	23.02 ± 42	0.31
Hemoglobin, g/dL (mean)	11.7 ± 1.15	10.4 ± 3.9	11.7 ± 3.9	0.85
Ischemia time				
Cold ischemia time, min (±SD)	1269 ± 345	1057 ± 29	842 ± 9	0.10
Warm ischemia time, min (±SD)	25.3 ± 8.9	23.3 ± 7.6	22 ± 25.4	0.96
Total ischemia time, min (±SD)	1307 ± 360	1071 ± 50	856 ± 30	0.10
Time of machine perfusion, min (±SD)	/	805 ± 86	703 ± 17	0.11

BMI, body mass index; CVA, cerebrovascular accident; DCD, donation after circulatory death; HBP, high blood pressure; HMP, hypothermic machine perfusion; HMP O₂, oxygenated machine perfusion; IEQ, islet equivalent; SCS, static cold storage.

transplantation after preservation with HMP with or without active oxygenation.

Drawing inspiration from previous studies conducted in animal pancreas split models for the comparative analysis of preservation methods,³³ we adopted a similar approach by extending this comparative methodology to human pancreases.

Even if no differences were found in term of secretory function between head and tail pancreas portions in each groups mostly related to the high variability of the results, the split model applied in this study provided the opportunity to undertake a controlled comparison of islet function from pancreatic fragments from the same donor exposed to the same ischemia time and parallel islet isolation.³³⁻³⁵

This model required special attention to the technical issues for the preparation of the pancreas fragment (the pancreas tail) used to test the HMP.

It was imperative to prepare the pancreas tail to minimize the risk of leakage through a series of ligatures at the splenic hilum and the upper and lower pancreas tail border ensuring only 1 inflow through the splenic artery and 1 outflow through the splenic vein.

This expedient was aimed to ensure a sufficient flow once the organ fragment was connected to the machine perfusion.

Moreover, to prevent the organ fragment from barotrauma caused by the hypothermic conditions and reduce the risk of edema, HMP and HMP O₂ preservation was performed at 25 mm Hg in line with others.¹⁹

In addition, by the use of a split model, we had to take in account the heterogeneity of the endocrine component of the pancreas, particularly in the selection of outcomes.³⁶⁻³⁹

Our study underscores a significant variability in outcomes regarding IEQ/g between head and tail isolation from both DCD and DBD organs.

It is well known that the tail region contains >2-fold higher islet numbers compared with the head and body regions. Despite intersubject variability, the regional differences are consistent in everyone as confirmed by several studies.^{39,40} The regional difference in islet density reflected the yield of isolated human islets (normalized to the regional pancreas weight) that is >2-fold higher in the body-tail region compared with the head-neck region⁴¹ meaning that if a split pancreas model has the advantage to compare 2 fragments sourced from the same donor, the architectural arrangement of the endocrine component within the pancreas necessitates careful consideration. This intricacy raises concerns about the reliability of utilizing the islet yield derived from both fragments as a consistent and accurate outcome.

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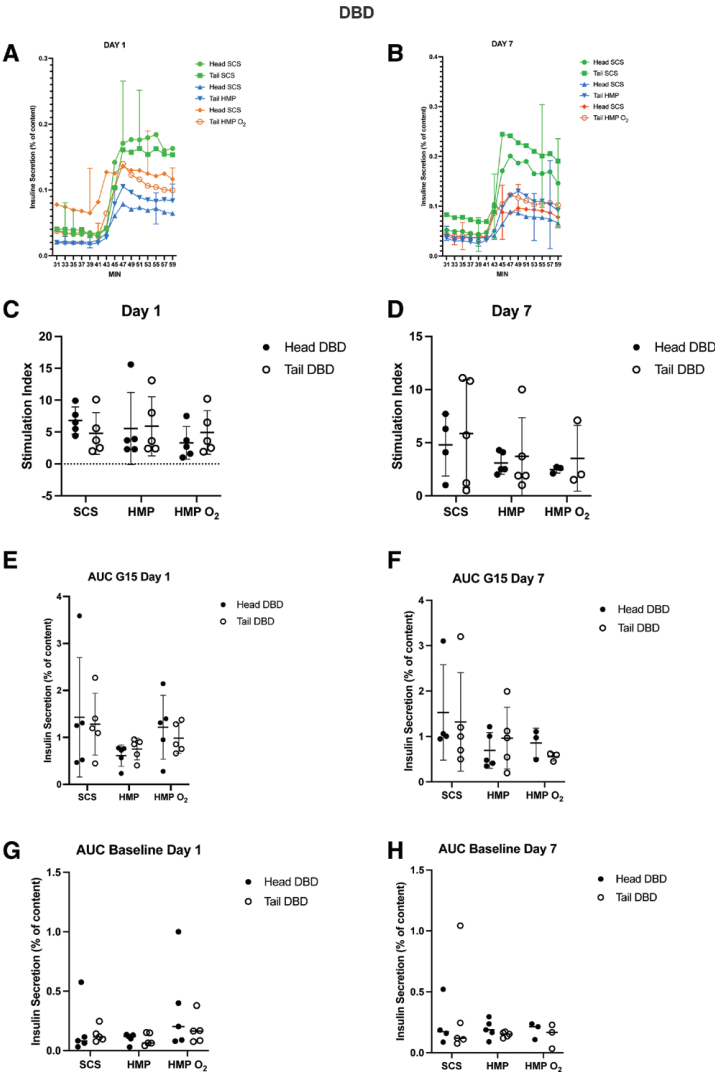


FIGURE 4. Average glucose-stimulated insulin response at days 1 and 7 of culture in DBD donor islets from SCS ($n = 5$), HMP ($n = 5$), and HMP O_2 ($n = 5$) tail pancreases compared with their respective control (the head pancreas portion) preserved through an SCS preservation method. Paired in vitro functionality results of pancreas tails preserved through an SCS method (green) $n = 5$, HMP (blue) $n = 5$, HMP O_2 (orange) $n = 5$ compared with their own control (head portion) preserved through an SCS method. At days 1 and 7, cultured islets from every isolation were perfused with a low concentration of glucose (1 mM Glucose, G1), followed by a high concentration of glucose (15 mM glucose, G15). A and B, Both AUC of insulin secretion and stimulation index (G15/G1 ratio) of each paired group were not statistically significant. C–H, Each time point is averaged per group, shown as mean $\pm$ SD. AUC, area under curve; DBD, donation after brain death; HMP, hypothermic machine perfusion; HMP O_2 , oxygenated machine perfusion; SCS, static cold storage.

Consequently, it becomes prudent to give precedence to alternative outcomes, such as assessing islet function or examining histological and physiological changes in tissues subjected to various preservation methods.

Our findings indicate that neither HMP nor HMP O_2 enhances the histological attributes of tissues, both pre- and postpreservation. Additionally, there was no notable impact on the expression of markers associated with oxidative stress

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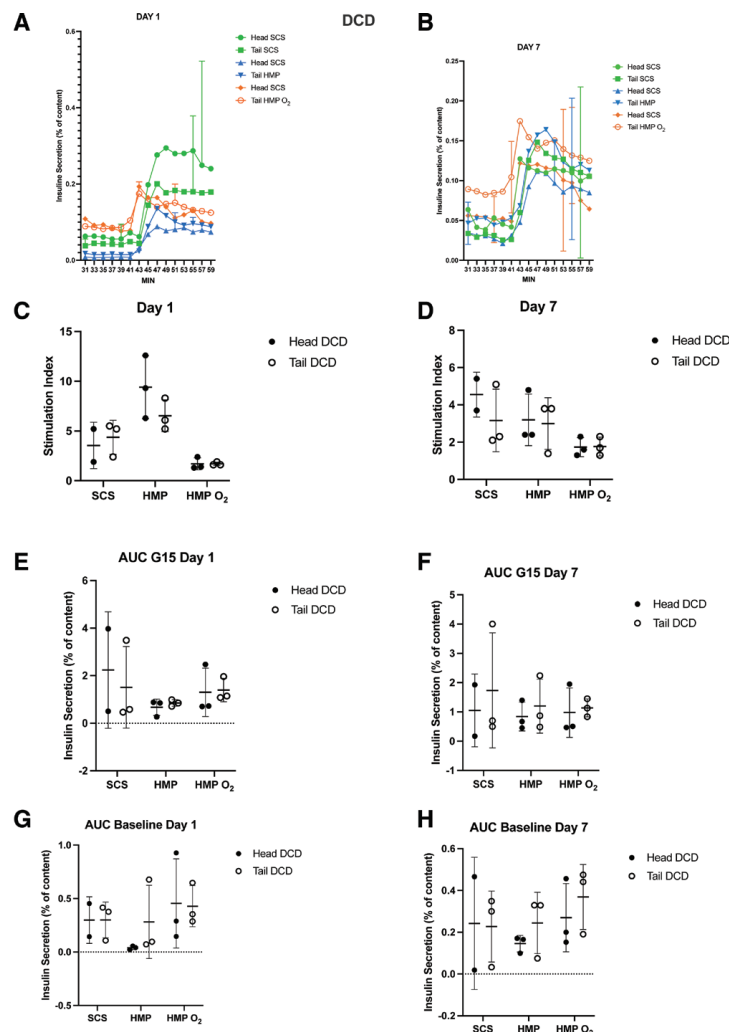


FIGURE 5. Average glucose-stimulated insulin response at days 1 and 7 of culture in DCD donor islets from SCS (n = 3), HMP (n = 3), and HMP O₂ (n = 3) tail pancreases compared with their respective control (the head pancreas portion) preserved through an SCS preservation method. Paired in vitro functionality results of pancreas tails preserved through an SCS method (green) n = 3, HMP (blue) n = 3, HMP O₂ (orange) n = 3 compared with their own control (head portion) preserved through an SCS method. At days 1 and 7 cultured islets from every isolation were perfused with a low concentration of glucose (1 mM Glucose, G1), followed by a high concentration of glucose (15 mM glucose, G15). A and B, Both AUC of insulin secretion and stimulation index (G15/G1 ratio) of each paired group were not statistically significant. C–H, Each time point is averaged per group, shown as mean ± SD. AUC, area under curve; DCD, donation after circulatory death; HMP, hypothermic machine perfusion; HMP O₂, oxygenated machine perfusion; SCS, static cold storage.

and apoptosis. These outcomes appear to be associated, in part, with the inherent characteristics of the studied organs. Because these organs were initially rejected for clinical use, their distinct features may introduce variations that influence the observed results. Additionally, the prolonged ischemia times and the utilization of continuous oxygenation in the HMP O₂ group may contribute to the observed outcomes.

Hyperoxic conditions have been shown to deteriorate cells through the production of reactive oxygen species (ROS).^{42,43} Under normal physiological conditions, cells have antioxidant defenses to neutralize ROS. However, under hyperoxic conditions, there is an overproduction of ROS that can overwhelm these defenses, leading to oxidative stress and apoptosis.⁴⁴

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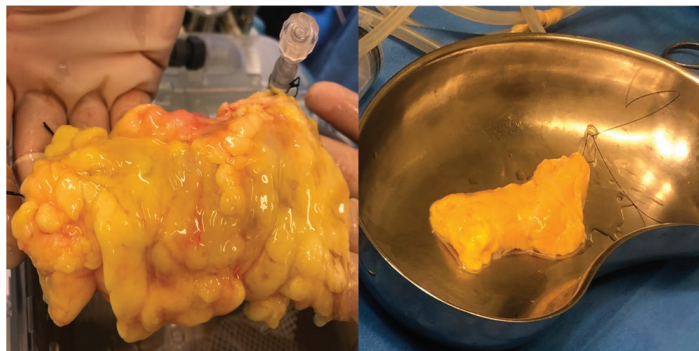


FIGURE 6. Macroscopic aspect of the pancreas directly before and after hypothermic machine perfusion. No visible indication of edema formation before and after machine perfusion was seen.

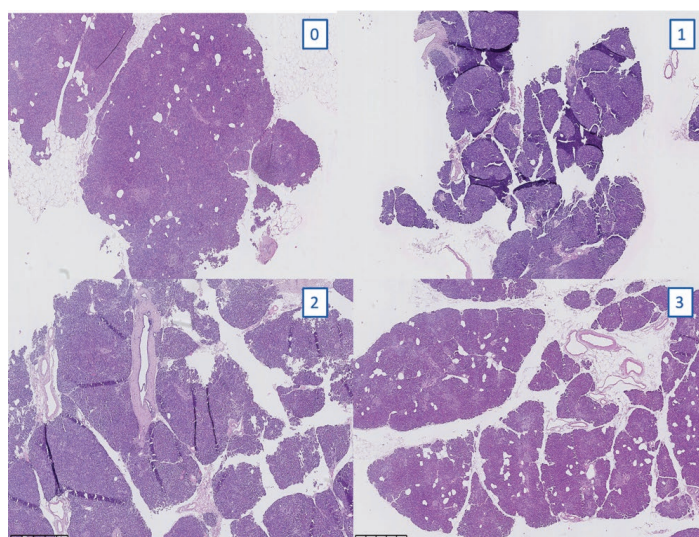


FIGURE 7. Pancreas histology of 4 samples with different degrees of edema. The histologic analysis was based on the estimated percentage of the area involved: absent (0), minimal or mild (1), moderate (2), and severe (3).

Even if in human and animal kidney transplant models the use of the HMP O_2 with 100% pO_2 has been shown improved transplant outcomes,^{25,45-47} pancreas preservation models with other methods, such as the organ preservation and the “two-layer method,” have shown that high oxygen levels are toxic to islets and a variety of other pancreatic cells and tissues leading to the reduction of the oxygen use at a nontoxic level similarly to what we reported in this study.⁴⁸⁻⁵⁰

Leemkuil et al⁵¹ shown a significant increase of ATP concentration in the tissue after addition of 100% oxygen to the perfusion system in DCD and DBD for reduced times and that the effects of oxygenated HMP persist in pancreatic

islets several days after isolation, during culturing, and after xenotransplantation in diabetic mice leading to a close correlation between short preservation times with HMP O_2 and the beneficial impact of oxygenation.¹⁹

Moreover, our study did not find any difference in terms of marker expression of apoptosis oxidative stress or histological changes in HMP O_2 compared with the SCS preservation.

Probably because in nonoxygenated preservation environments (SCS and HMP) for prolonged periods, also the effects of hypoxia, as shown in several studies,⁴³ can increase the generation of ROS within the islet mitochondria, contributing to oxidative stress and apoptosis.

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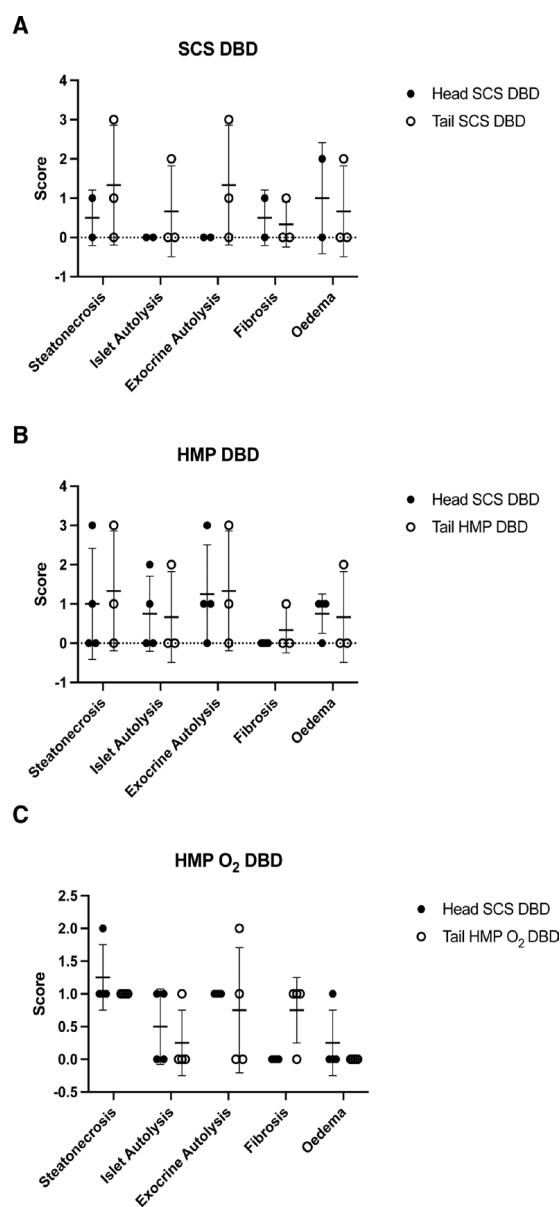


FIGURE 8. Paired histological results of human pancreas tail samples of DBD pancreas obtained before and after preservation with SCS ($n = 5$), HMP ($n = 5$), and HMP O₂ ($n = 5$) compared with their own control (the head pancreas portion) preserved through the SCS method. The extent of parenchymal changing before and after preservation were scored from 0 to 3, based on the estimated percentage of the area involved: absent (0), minimal or mild (1), moderate (2), and severe (3). Data are expressed as Δ of histological changes of samples obtained at the end of the preservation time and those obtained after the organ procurement. DBD, donation after brain death; HMP, hypothermic machine perfusion; HMP O₂, oxygenated machine perfusion; SCS, static cold storage.

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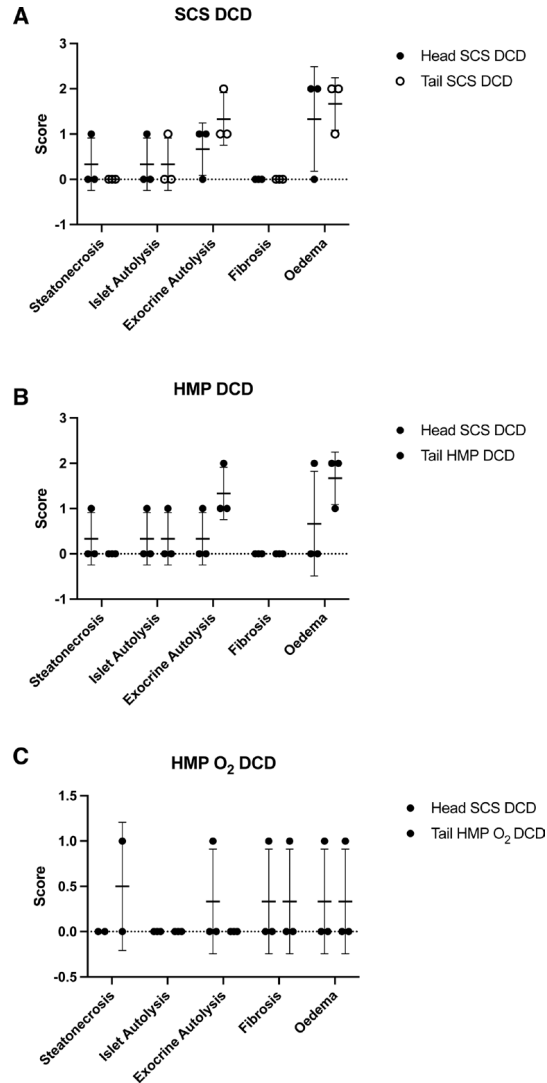


FIGURE 9. Paired histological results of human pancreas tail samples of DCD pancreas obtained before and after preservation with SCS (n = 3), HMP (n = 3), and HMP O₂ (n = 3) compared with their own control (the head pancreas portion) preserved through the SCS method. The extent of parenchymal changing before and after preservation were scored from 0 to 3, based on the estimated percentage of the area involved: absent (0), minimal or mild (1), moderate (2), and severe (3). Data are expressed as the Δ of histological changes of samples obtained at the end of the preservation time and those obtained after the organ procurement. DCD, donation after circulatory death; HMP, hypothermic machine perfusion; HMP O₂, oxygenated machine perfusion; SCS, static cold storage.

Limitations

Our study has limitations. First is the relatively low number of organs used in each group from both DCD and DBD donors leading to important variabilities of results between procedures.

Second is the low quality of the organs used which is not comparable to organs commonly accepted for clinical purpose leading to lower results both in terms of islet yield and in vitro islet functionality.

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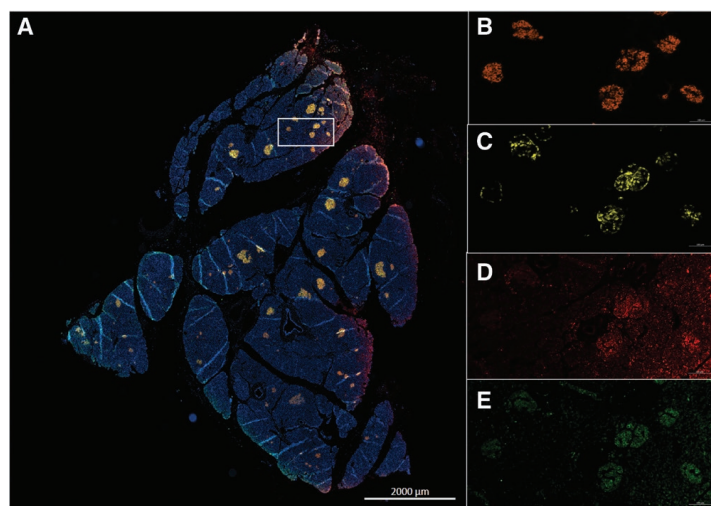


FIGURE 10. A, Illustrations of oxidative stress markers evaluated by multiplex IF in pancreas samples obtained after HMP O_2 . B, Insulin, glucagon (C), PRDX 3 (E), and HMOX 1 (F) staining. HMOX 1, hemoxygenase 1; HMP O_2 , oxygenated machine perfusion; IF, immunofluorescence; PRDX3, peroxiredoxine 3.

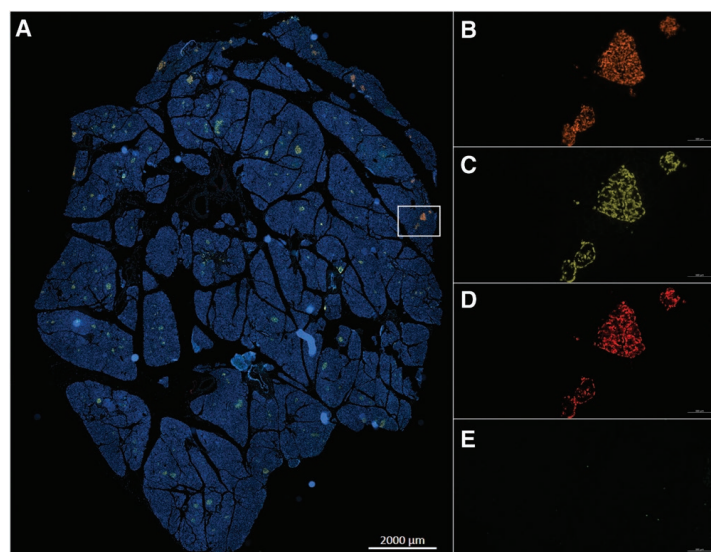


FIGURE 11. A, Illustrations of apoptosis markers evaluated by multiplex IF in pancreas samples obtained after HMP O_2 . B, Insulin, glucagon (C), caspase 3 (E), and TUNEL (F) staining. HMP O_2 , oxygenated machine perfusion; IF, immunofluorescence; TUNEL.

Third are the long ischemia times of organs in the 3 paired groups. Although the study was designed to obtain a reliable control group in each procedure, the long ischemia time could be a confounding factor.

Fourth, we did not perform in vivo function tests in this study.

Finally, a notable limitation of this study is the absence of pancreatic head preservation using HMP and HMP O_2 . This decision was influenced by the frequent unavailability of the intricate vascular network associated with the head of the pancreas in organs designated for islet transplantation. This

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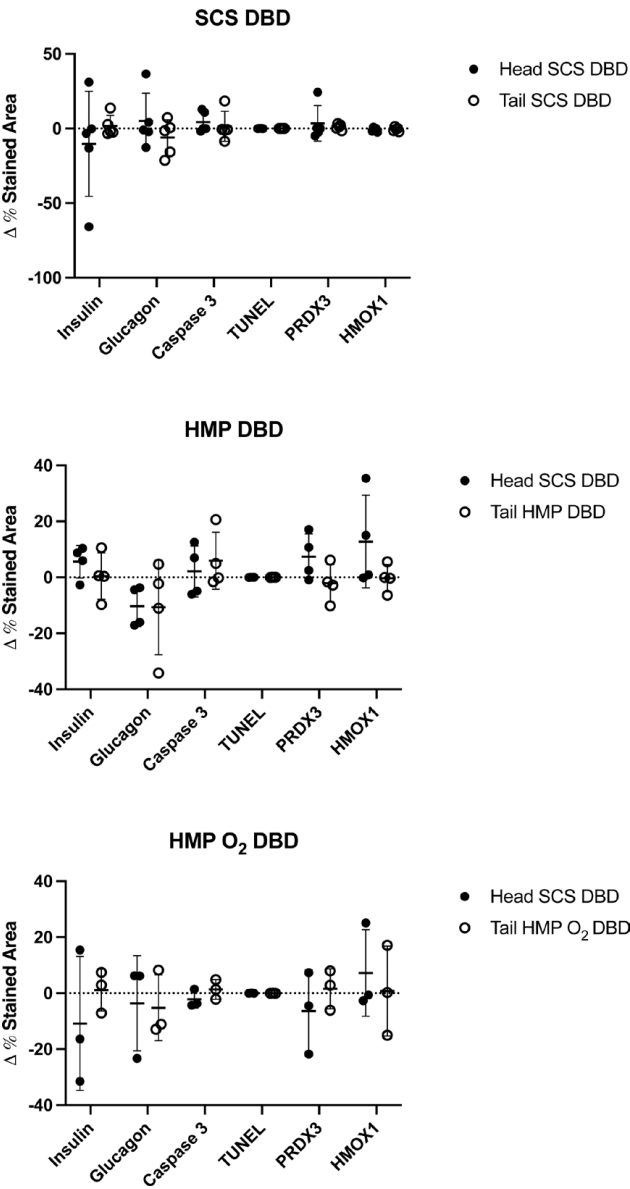


FIGURE 12. Markers of insulin, glucagon, apoptosis and oxidative stress of human pancreas tail samples from DBD donors obtained before and after preservation with SCS (n = 5), HMP (n = 5), and HMP O₂ (n = 5) compared with their own control (the head pancreas portion) preserved through the SCS method. % of stained area of markers of insulin, glucagon, HMOX1, PRX3, caspase 3, and TUNEL from pancreas tail and head samples obtained before and after preservation in the SCS, HMP, and HMP O₂ group. Data are expressed as the Δ of the marker expression of samples obtained at the end of the preservation time (after) and those obtained after the organ procurement (before). Each value is averaged per group, shown as mean ± SD. DBD, donation after brain death; HMOX1, hemoxygenase 1; HMP, hypothermic machine perfusion; HMP O₂, oxygenated machine perfusion; PRDX3, peroxiredoxine 3; SCS, static cold storage; TUNEL.

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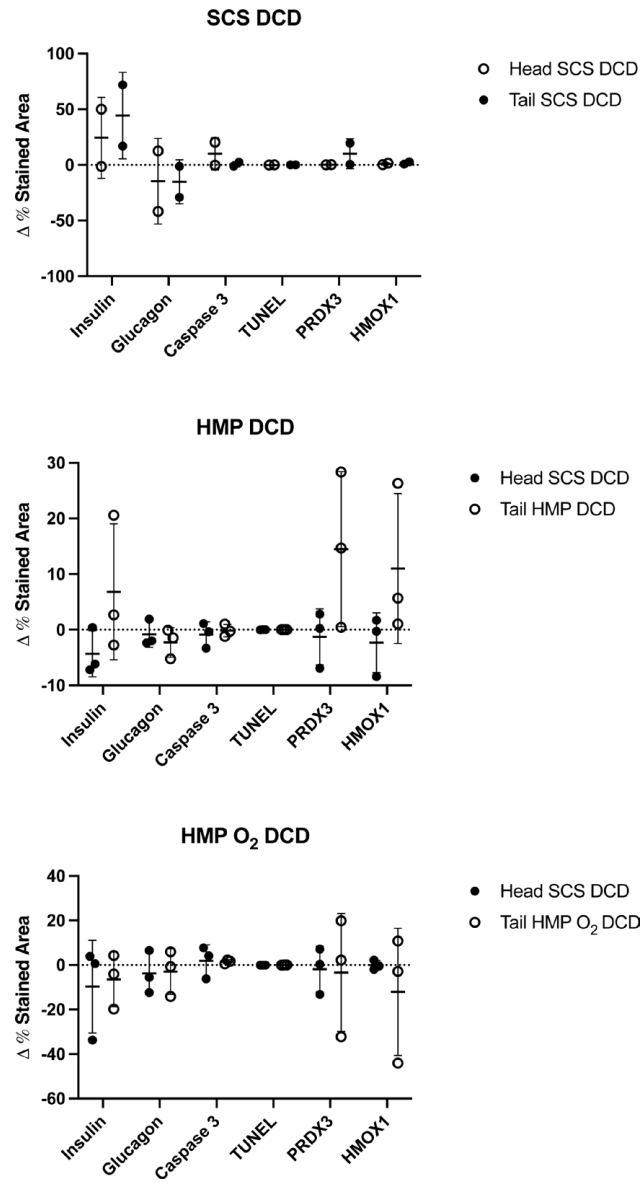


FIGURE 13. Markers of insulin, glucagon, apoptosis, and oxidative stress of human pancreas tail samples from DCD donors obtained before and after preservation with SCS (n = 3), HMP (n = 3), and HMP O₂ (n = 3) compared with their own control (the head pancreas portion) preserved through the SCS method. % of stained area of markers of insulin, glucagon, HMOX1, PRX3, caspase 3, and TUNEL from pancreas tail and head samples obtained before and after preservation in the SCS group, HMP group and HMP O₂. Data are expressed as the Δ of the marker expression of samples obtained at the end of the preservation time (after) and those obtained after the organ procurement (before). Each value is averaged per group, shown as mean $\pm$ SD. DCD, donation after circulatory death; HMOX1, hemeoxygenase 1; HMP, hypothermic machine perfusion; HMP O₂, oxygenated machine perfusion; PRDX3, peroxiredoxin 3; SCS, static cold storage; TUNEL.

TABLE 3.
Isolation data of SCS, HMP, and HMP O₂ groups from n = 15 DBD donors and n = 9 DCD donors

DBD	SCS group, n = 5			HMP group, n = 5			HMP O ₂ group, n = 5		
	Head (SCS)	Tail (SCS)	P	Head (SCS)	Tail (HMP)	P	Head (SCS)	Tail (HMP O ₂)	P
Islet yield, IEQ (mean ± SD)	10 624 ± 8654	38 436 ± 20 196	0.02	14 244 ± 17 393	51 132 ± 45 240	0.12	35 860 ± 42 404	63 181 ± 85 259	0.53
Islet purity, % (mean ± SD)	53 ± 5	53 ± 5	0.99	42 ± 19	63 ± 26	0.18	73 ± 16	63.3 ± 14.71	0.34
IEQ/g (mean ± SD)	243 ± 36	999 ± 505	0.01	181 ± 172	1171 ± 1132	0.08	725 ± 557	1313 ± 1610	0.46
Weight, g (mean ± SD)	46.8 ± 10	39.6 ± 14.8	0.39	67 ± 21.2	44.6 ± 7.4	0.06	65.4 ± 14	49.25 ± 14.61	0.11
DCD	SCS group, n = 3			HMP group, n = 3			HMP O ₂ group, n = 3		
	Head (SCS)	Tail (SCS)	P	Head (SCS)	Tail (HMP)	P	Head (SCS)	Tail (HMP O ₂)	P
Islet yield, IEQ (mean ± SD)	12 210 ± 10 649	58 366 ± 500 637	0.88	20 700 ± 11 711	82 540 ± 30 700	0.03	5820 ± 7624	16 350 ± 13 023	0.29
Islet purity (mean %, ±SD)	75 ± 5	75 ± 5	0.99	52 ± 10	90 ± 0	<0.01	60 ± 15	53 ± 5	0.48
IEQ/g (mean ± SD)	196 ± 127	1587 ± 1288	0.13	368 ± 205	1984 ± 511	<0.01	94 ± 122	388 ± 18	0.01
Weight, g (mean ± SD)	56.5 ± 17	41.6 ± 2	0.08	57.3 ± 6.6	41.3 ± 11	0.09	65.3 ± 5.7	50 ± 19	0.08

Following a split model, each organ was divided into head control group preserved through an SCS method, and tail study group preserved through SCS, HMP, and HMP O₂ respectively. BMI, body mass index; CVA, cerebrovascular accident; DBD, donation after brain death; DCD, donation after circulatory death; HBP, high blood pressure; HMP, hypothermic machine perfusion; HMP O₂, oxygenated machine perfusion; IEQ, islet equivalent; SCS, static cold storage.

limitation extends to research organs as well, as the arterio-venous vessels are more commonly directed toward the liver in clinical settings.

CONCLUSIONS

For the first time, a split model was implemented using human discarded pancreases intended for clinical purposes, revealing consistent outcomes across 3 different organ preservation methods. Moreover, islets from DCD organs had greater purity than controls ($P \leq 0.01$) in the HMP study group compared with the SCS and HMPO₂ groups. However, it is noteworthy that neither HMP nor HMP O₂ demonstrated improvements in the islet secretory function, histological changes, or the expression of markers associated with oxidative stress and apoptosis. To enhance the applicability of these preservation methods for transplantation, further endeavors should focus on testing them under conditions involving shorter ischemia times. Additionally, exploring new donor oxygenation protocols could potentially confirm and enhance the satisfactory results observed, thereby advancing the prospects for successful transplantation outcomes. Finally, normalization of isolation outcomes through islet quantitative analysis from pancreas biopsies could prove beneficial in mitigating inherent variability among organs procured for research purposes.⁵²

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Summary Paper 2

Aim of the study:

This study aims to assess the viability of active oxygenated and non-active oxygenated hypothermic machine perfusion (HMP) as potential and superior preservation techniques for human donor pancreata. A comparative analysis was then conducted between these two preservation methods and the conventional static cold storage (SCS) approach. The evaluation utilized a human split pancreas model sourced from deceased donors, encompassing cases of donation after cardiocirculatory death (DCD) and donation after brain death (DBD). The pancreases were sourced from Eurotransplant centers, originally intended for whole-pancreas transplantation and islet transplantation but ultimately discarded.

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Take Home message

1. For the first time to the best of our knowledge, a split model was performed in human discarded pancreas for clinical purpose demonstrating reliable results between three organ preservation methods.
2. The implementation of a split model using human discarded pancreases intended for clinical purposes has yielded consistent outcomes across three different organ preservation methods. Specifically, islets from donation after circulatory death (DCD) organs exhibited greater purity compared to controls in the hypothermic machine perfusion (HMP) study group, as opposed to static cold storage (SCS) and HMP with oxygenation (HMPO2) groups.
3. However, despite these promising findings, neither HMP nor HMP O2 demonstrated improvements in islet secretory function, histological changes, or the expression of markers associated with oxidative stress and apoptosis. To make these preservation methods more applicable for transplantation, further efforts should be directed towards testing them under conditions involving shorter ischemia times. Additionally, exploring new donor oxygenation protocols could potentially confirm and enhance the satisfactory results observed, thereby advancing the prospects for successful transplantation outcomes.
4. Furthermore, the normalization of isolation outcomes through islet quantitative analysis from pancreas biopsies could prove beneficial in mitigating inherent variability among organs procured for research purposes. This suggests a need for standardization and refinement of techniques to improve the reliability and reproducibility of islet isolation procedures.

CHAPTER 2 ~ Personal Contribution

Paper 3: Donor and isolation-related predictive factors of in vitro secretory function of cultured human islets.

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Donor- and isolation-related predictive factors of *in vitro* secretory function of cultured human islets

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Background and aims: Human islet preparations designated for research exhibit diverse insulin-secretory profiles. This study aims to assess the impact of donor- and isolation-related factors on *in vitro* islet secretory function.

Methods: A retrospective analysis of 46 isolations from 23 pancreata discarded for clinical transplantation was conducted. *In vitro* islet secretory function tests were performed on Day 1 and Day 7 of culture. Linear mixed-effects models (LMMs) were employed to investigate the relationships between various predictors characterizing the patient and donor characteristics as well as the isolation effectiveness and two functional outcomes including the islet stimulation index (SI) and area under the insulin curve (AUC). Fixed effects were introduced to represent the main effects of each predictor, and backward elimination was utilized to select the most significant fixed effects for the final model. Interaction effects between the timepoint (Day 7 vs. Day 1) and the predictors were also evaluated to assess whether predictors were associated with the temporal evolution of SI and AUC. Fold-change (Fc) values associated with each predictor were obtained by exponentiating the corresponding coefficients of the models, which were built on log-transformed outcomes.

Results: Analysis using LMMs revealed that donor body mass index (BMI) (Fc = 0.961, 95% CI = 0.927–0.996, p = 0.05), donor gender (female vs. male, Fc = 0.702, 95% CI = 0.524–0.942, p = 0.04), and donor hypertension (Fc = 0.623, 95% CI = 0.466–0.832, p = <0.01) were significantly and independently associated with SI. Moreover, donor gender (Fc = 0.512, 95% CI = 0.302–0.864, p = 0.02), donor cause of death (cerebrovascular accident vs. cardiac arrest, Fc = 2.129, 95% CI = 0.915–4.946, p = 0.09; trauma vs. cardiac arrest, Fc = 2.129, 95% CI = 1.112–7.106, p = 0.04), pancreas weight (Fc = 1.01, 95% CI = 1.001–1.019, p = 0.03), and islet equivalent (IEQ)/mg (Fc = 1.277, 95% CI = 1.088–1.510, p ≤ 0.01) were significantly and independently associated with AUC. There

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was no predictor significantly associated with the temporal evolution between Day 1 and Day 7 for both SI and AUC outcomes.

Conclusion: This study identified donor- and isolation-related factors influencing *in vitro* islet secretory function. Further investigations are essential to validate the applicability of these results in clinical practice.

KEYWORDS

islet isolation, islet perfusion, islet function, donor predictive variables, pancreas histology

Introduction

Successful islet transplantation holds the potential to significantly enhance the quality of life for individuals with type 1 diabetes (1, 2). Nonetheless, the scarcity of suitable donor pancreata poses a significant hurdle to the widespread adoption of this therapeutic approach (3).

Donor characteristics and the effectiveness of the islet isolation process are highly related to the success of the procedure (4, 5).

The decision to accept or decline an organ for islet isolation remains challenging, as it relies on various factors predominantly grounded in donor characteristics, coupled with subsequent macroscopic evaluations of the organ (6–10). The capability to reliably reject poor-quality donor pancreata not only improves overall isolation success but also reduces the costs associated with failed isolations (11, 12).

Several donor variables, including donor age, body mass index (BMI), body surface area (BSA), and hemodynamic stability without the use of vasopressors during donor management, have been demonstrated to be associated with higher islet yields, leading to the development of several scoring systems for predicting the suitability of potential pancreatic donors before organ processing (11, 13). However, effective isolation, as assessed by islet yields, may not necessarily translate to good islet graft function after transplantation (14, 15).

Recent investigations have shed light on the significance of non-quantitative indicators in predicting the ultimate goal of any islet transplantation procedure: the normalization of blood sugar levels and relief from diabetic symptoms in transplant recipients (16, 17). Perfusion systems, employed for over 35 years (18–20), challenge islets with glucose and measure dynamic insulin release. They remain the gold standard procedure used by many research laboratories to test islet function in a more physiologically relevant way and attempt to predict islet transplantation outcomes.

Abbreviations: AUC, area under curve; BMI, body mass index; BSA, body surface area; CVA, cerebrovascular accident; DBD, donation after brain death; DCD, donation after circulatory death; HBP, high blood pressure; IEQ, islet equivalent; LMMs, linear mixed-effects models; IGL1, Institut Georges Lopez-1; RIA, radioimmunoassay; RPM, revolution per minute; SI, stimulation index.

However, relatively little attention has been given to donor and preparation characteristics that might influence the insulin-secreting properties of human islets *in vitro* (21, 22). This study aims to retrospectively evaluate, using organs discarded from clinical pancreas and islet transplantation, how features of the preparation and donor attributes impact islet secretory function.

Research design and methods

From June 2020 to November 2022, a single team at our institution processed 58 consecutive islet isolation procedures from 29 human donor pancreata.

All the organs were divided into two segments, the head-neck portion and the body-tail portion, for use in paired isolation outcomes for other research purposes.

A total of six pancreata were excluded from our analysis for the following reasons: incomplete data ($n = 2$), technical failure in islet isolation ($n = 1$), contaminants ($n = 1$), and islet isolation from partial pancreata ($n = 2$). The remaining 46 islet isolations from 23 donors were analyzed.

Donor predictor variables used in the analysis were age, gender, body weight, height, BMI, BSA, cold ischemia time (CIT), warm ischemia time (WIT), length of cardiac arrest, cause of death, vasopressor requirement, medical history, and blood test values including peak levels of amylase, lipase, and hemoglobin at the time of procurement. Cause of death was categorized into cerebrovascular accident (CVA), trauma, and cardiac arrest.

Regarding medical history, the following information was collected: alcohol abuse, hypertension (high blood pressure (HBP)), drug abuse, malignancies, smoking, diabetes mellitus, infections, and cardiac arrest.

Islet isolation variables used in the analysis were the islet yield, islet purity, islet size, and pancreas weight.

Islet isolation

Following the acquisition of informed consent as outlined by the guidelines established by our local medical ethical committee

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(CEHF, Comité d'éthique hospitalo-facultaire UCL, 2019/07MAI/201), cadaveric pancreata discarded for clinical purposes were obtained from brain-dead donors (DBD) or circulatory-dead donors (DCD) during multiorgan procurement and preserved in Institut Georges Lopez-1 solution.

Upon arrival at our laboratory, each organ was divided into two portions, which were concurrently isolated using a modified automated method (23). The preserved pancreas was perfused through the pancreatic duct with a blend of collagenase NB1 and neutral protease (SERVA Electrophoresis, Heidelberg, Germany), aiming for pancreas distension with minimal leakage.

The distended pancreas underwent dissociation in two separate digestion chambers, a process necessitated by other studies. Islets were subsequently purified through a discontinuous purification method following established protocols (24). The quantification and assessment of islet purity were carried out at the conclusion of the isolation procedure, following established dithizone staining protocols (25). The raw count of islets in each diameter class was determined using an optical graticule. Subsequently, the raw count of islets was converted to the standard number of islet equivalent (IEQ).

Purified islets were cultured in Roswell Park Memorial Institute (RPMI) supplemented with 5 mM glucose, 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin, and 1 µg/ml meropenem in 75-cm² culture flasks at 37°C in 5% CO₂.

Dynamic glucose-stimulated insulin secretion test

A subset of isolated islets underwent dynamic islet perfusion experiments on Days 1 and 7 of culture, as previously described, to assess *in vitro* functionality (26). The working medium comprised a bicarbonate-buffered solution containing 120 mM NaCl, 4.8 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgCl₂, 24 mM NaHCO₃, 1 mg/ml BSA, and varying glucose concentrations as indicated in the figures. Batches of 1,000–2,000 IEQ were placed in perfusion chambers, covered with 8-µm cellulose filters, and sealed. Test solutions, maintained at 37°C and continuously gassed to stabilize pH ~ 7.2, were pumped at a flow rate of 1 ml/min. Effluent fractions were collected at 2-min intervals and stored for insulin assays using radioimmunoassay (RIA) kits (DiaSource ImmunoAssays, Ottignies-Louvain-la-Neuve, Belgium). At the conclusion of the experiments, islets were recovered, and their insulin content was determined after extraction in acid-ethanol (75% ethanol, 180 mM HCl from Merck, Darmstadt, Germany).

Statistical analysis

Continuous variables are presented as mean ± SD. Categorical variables are shown as the percentage of the sample.

For the paired analysis, p-values (based on the t-test of least squares means) were reported along with a mean ± SD graph, where appropriate.

p-Values <0.05 were considered to indicate statistical significance. GraphPad Prism (La Jolla, CA, USA) was used for

the computation of the descriptive statistics of donor characteristics, islet isolation, islet function, islet size, and histology.

The islet stimulation index (SI) and the area under the insulin curve (AUC) outcomes were modeled using two separate multiple linear mixed-effects models (LMMs), which incorporate all data generated per patient, including both timepoints (Days 1 and 7) and both specimens (head and tail). In each model, fixed effects included various predictors including patients' characteristics (BMI, age, etc.) as well as isolation characteristics (IEQ/mg). In addition to fixed effects, random intercepts and slopes were introduced to model inter-subject variations. Moreover, interaction effects between the timepoint (i.e., Day 7 vs. Day 1) and the predictors were also tested in order to assess whether predictors were associated with the temporal evolution. Backward elimination was used for selecting fixed effects in the final model based on their significance. For both models, a logarithmic transformation was applied to the outcome (i.e., SI and AUC) in order to meet the assumptions of the statistical model (i.e., residuals with normal distribution and homogeneity of variance). Accordingly, coefficients of the corresponding models were exponentiated to obtain the fold changes associated with the predictors in the final models. It is worth noting that multicollinearity issues were mitigated by carefully selecting variables for the model to avoid including highly correlated pairs, such as BMI and BSA.

LMM analyses were performed using the R.4.2.1, as well as the lme4.1.1.33 and lmerTest.3.1.3 packages, while graphical representations of the significant associations between predictors and the outcomes were generated using the ggplot2.3.4.2 package.

Results

Donor characteristics, ischemia times, and isolation data

Tables 1, 2 present the donor characteristics. The average age of donors was 57.6 years, ranging from 30 to 85 years, with 73.9% being male. The mean BMI and BSA were 26 kg/m² (ranging from 19 kg/m² to 33 kg/m²) and 1.96 m² (ranging from 1.63 m² to 2.23 m²), respectively. Cerebrovascular accidents were the most frequent cause of death. A majority of donors were DBD (60.8%), and 60.8% of donors received vasopressor therapy during their hospital stay. Smoking and HBP were common. Mean amylase and lipase peak levels were 71 and 38.5 U/L, respectively. The mean lowest hemoglobin level was 11.3 mg/dl. The mean CIT was 940 min, ranging from 704 to 1,765 min, and for DCD donors, the mean WIT was 12.3 min, ranging from 0 to 40 min.

The mean values of IEQ and IEQ/g of the pancreas from these 23 donors were 67,753 ± 44,513 IEQ and 698 ± 470 IEQ/g, respectively. The mean islet purity was 59.8%, ranging from 30% to 90%. Figure 1 depicts the islet size distribution of the isolation performed in this study.

The proportions of islet average sizes were 25.5% ± 27.5% (100/150 µm), 23.8% ± 8.1% (150/200 µm), 21.9% ± 7.7% (200/250 µm), 13.7% ± 7.2% (250/300 µm), 8.6% ± 4.9% (300/350 µm), and 7% ± 6% (above 350 µm).

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TABLE 1 Donor characteristics, ischemia times, and isolation data from 23 discarded organs for clinical purposes: continuous variables.

Variables	Mean ± SD	Range
Demographics		
Age, (year)	57.6 ± 14.5	30–85
Height (cm)	175 ± 7.2	160–185
Body weight (kg)	79.8 ± 12.6	57–100
BMI (kg/m ²)	26 ± 3.8	19–33
BSA (m ²)	1.96 ± 0.17	1.63–2.23
Laboratory results		
Lipase (U/L)	38.5 ± 41.9	7–186
Amylase (U/L)	71 ± 54.4	15–158
Hemoglobin (mg/dl)	11.4 ± 3	6–18.6
Ischemia time		
Cold ischemia time (min)	940 ± 301	704–1,765
Warm ischemia time (min)	12.3 ± 14.6	0–40
Length of cardiac arrest (min)	23.2 ± 13.4	0–40
Isolation data		
Islet yield (IEQ)	67,753 ± 44,513	9,000–141,960
Islet purity (%)	59.8 ± 14	30–90
IEQ/g (IEQ/g)	698.5 ± 470	67–1,549
Pancreas weight (g)	107.8 ± 22.6	84–164

BMI, body mass index; BSA, body surface area; IEQ, islet equivalent; SD, standard deviation.

Islet secretory function

Dynamic glucose-stimulated insulin secretion tests were performed after 1 day and 7 days of culture (Figure 2). In both experiments, islets exhibited a responsive insulin-secretory profile to glucose stimulation, characterized by a sharp, rapid, short-lived increase in insulin secretion (first phase) followed by a lower but constant secretion rate (second phase). The peak stimulation index and the area under the insulin curve were similar on Day 1 and Day 7 (4.36 ± 2.99 vs. 4.99 ± 4.60, $p = 0.58$; 1.41 ± 1.26 vs. 1.28 ± 0.98, $p = 0.68$, respectively) (Figures 3A, B).

Multivariate linear mixed-effect analyses

LMMs followed by predictor selection identified significant positive and negative associations between various predictors describing the isolation effectiveness as well as the donor characteristics and both outcomes of interest (SI and AUC) characterizing the islet secretory function (Table 3).

Predictors displaying significant associations with SI included donor BMI ($F_c = 0.961$, 95% CI = 0.927–0.996, $p = 0.05$), donor gender (female vs. male, $F_c = 0.702$, 95% CI = 0.524–0.942, $p = 0.04$), and donor hypertension ($F_c = 0.623$, 95% CI = 0.466–0.832, $p < 0.01$) (Figure 4).

TABLE 2 Donor characteristics of 23 organs discarded for clinical purposes: categorical variables.

Variables	N (%)
Gender	
Male	17 (73.9)
Female	6 (26.1)
Donor organ source	
DBD	14 (60.8)
DCD	9 (39.2)
Cause of death	
CVA	16 (69.5)
Trauma	6 (26)
Cardiac arrest	1 (4.5)
Medical history	
HBP	8 (34.7)
Diabetes mellitus	0 (0)
Smoking	8 (34.7)
Drug abuse	2 (8.7)
Alcohol abuse	4 (17.4)
Infections	5 (21.7)
Malignancies	3 (13)
Cardiac arrest	9 (39.2)
Vasopressor use	14 (60.8)

CVA, cerebrovascular accident; DBD, donation after brain death; DCD, donation after circulatory death; HBP, high blood pressure.

It is noteworthy that because BMI is a continuous variable, the F_c is therefore associated with a one-unit increase in this variable. Variables related to the AUC included donor gender (male vs. female, $F_c = 0.512$, 95% CI = 0.302–0.864, $p = 0.02$), donor cause of death (cerebrovascular accident vs. cardiac arrest, $F_c = 2.129$, 95% CI = 0.915–4.946, $p = 0.09$; trauma vs. cardiac arrest, $F_c = 2.129$, 95% CI = 1.112–7.106, $p = 0.04$), pancreas weight ($F_c = 1.01$, 95% CI = 1.001–1.019, $p = 0.03$), and IEQ/mg ($F_c = 1.277$, 95% CI = 1.088–1.510, $p < 0.01$) (Figure 5).

Furthermore, no significant interaction effects were observed between the timepoint predictor (Day 7 vs. Day 1) and any of the other predictors for either SI or AUC, suggesting that no donor- or isolation-related variables could reliably predict the temporal evolution of these outcomes between Day 1 and Day 7.

Discussion

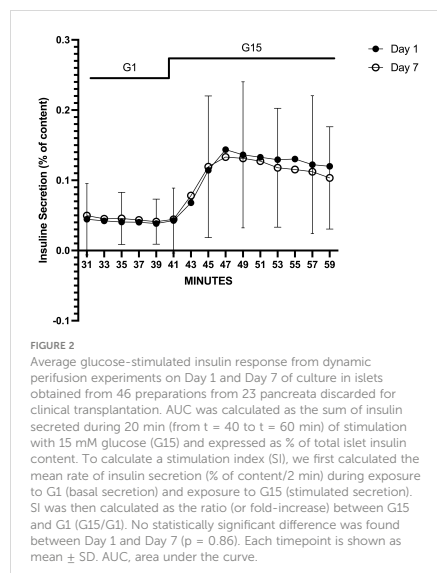
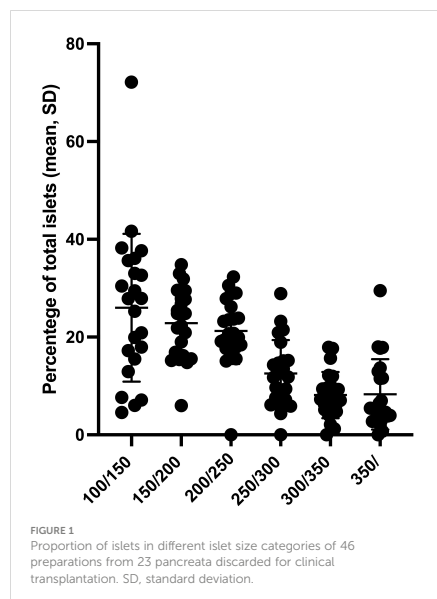
The present study has identified donor- and isolation-related factors that are significantly positively or negatively associated with the islets' secretory function after 1 day and 7 days of culture.

We first demonstrated that islet yield expressed as IEQ/g from islet isolation procedures is positively correlated to the islet secretory function expressed by the AUC of the insulin secretion.

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Although this observation appears intuitive, it can have encouraging implications in the clinical setting.

Islet yield is the most common indicator in predicting both islet isolation and transplantation outcomes in diabetic patients (3–13).

Clinical studies have generated inconsistent findings concerning the correlation between transplanted IEQ and the likelihood of achieving insulin independence (14, 15). The inaccuracies associated with manual quantification of islet mass, characterized by subjectivity and the potential for error, likely contribute to these inconsistencies. Substantial efforts have been dedicated to overcoming the limitations of standard manual islet mass evaluation through the exploration of computer-assisted and digital-assisted approaches (27–30). Nevertheless, as of now, no precise method has been established.

In contrast, *in vitro* islet function tests are an objective method much less prone to subjective variability, possibly becoming a potentially more accurate indicator than islet quantification.

Nevertheless, since no specific pre-transplant test for evaluating islet quality has demonstrated predictive efficacy for the success of islet transplantation (31), our findings need validation in transplanted islets. This validation process may pave the way for the widespread acceptance of this predictive indicator.

Our study also allowed us to determine several donor characteristics affecting *in vitro* islet secretory function, including the donor BMI, gender, HBP cause of death, and pancreas weight.

Numerous studies have highlighted a positive correlation between donor BMI and successful islet yield outcomes (13, 32, 33). Conversely, donors with a low BMI (<21 kg/m²) often yield less satisfactory results in terms of islet production (34). However, as previously discussed, the critical focus lies in evaluating the functionality of islets obtained from obese donors. In a specific study, islets isolated from donors with a BMI exceeding 30 kg/m² displayed nearly identical viability (33). This was determined through *in vitro* assessments, including insulin response to glucose in static incubation assays, as well as *in vivo* evaluations, such as the time required to reverse chemical diabetes in a nude mouse bioassay.

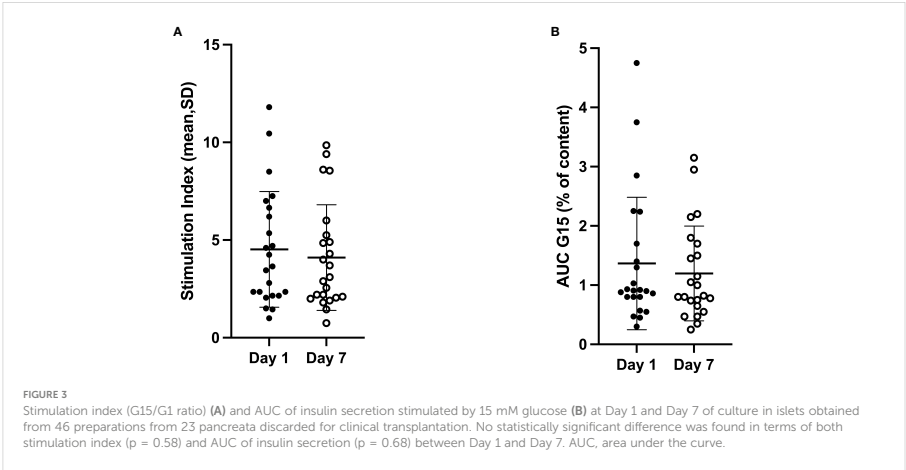
Remarkably, our research indicates that although there is no statistically significant correlation between BMI and the AUC of insulin secretion, SI increases with the gradual reduction of BMI. These findings, prompting further exploration, may be influenced by confounding factors. On the one hand, the origin of donor organs—rejected for clinical use—often entails higher overall risk factors than commonly utilized organs. On the other hand, longer ischemia times, exceeding those typically accepted by the scientific community, could also contribute to these outcomes.

Furthermore, our study provides valuable insights by establishing a positive correlation between organ weight and the functional activity of isolated islets. Numerous studies have consistently shown a positive association between pancreas weight and both pre-purification and post-purification islet yields (13, 35). However, it is crucial to note that this parameter remains indeterminable prior to organ procurement, prompting a focus on identifying metrics reliably correlated with pancreas weight. As a result, common practice has adopted body weight and body surface area as crucial metrics for organ selection and predicting outcomes

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in islet isolation (35). The crucial impact of organ weight on islet function underscores the importance of accurately predicting this parameter.

Recent evidence indicates that recipients of islets from at least one female donor experienced prolonged graft survival in comparison to recipients exclusively receiving male donor islets (36). This observation is believed to be associated with the cell-intrinsic properties of female islets (37). A study reported that the higher β -cell content in transplanted islets from female donors led

to significant improvements in recipient outcomes compared to islets transplanted from male donors (38).

Our data also show that male donor is negatively related to the *in vitro* islet secretory function.

Our study additionally reveals that donor hypertension (HBP) is linked to decreased *in vitro* functional performance of isolated islets in terms of AUC. Drawing insights from kidney transplant research, the authors posit that the adverse impact of HBP on islet function might stem from vascular damage, resulting in a

TABLE 3 Positive and negative correlations between the islet secretory function results on Day 1 and Day 7 and the donor and islet isolation variables.

	SI			AUC		
	Fold-change*	95% CI	p	Fold-change*	95% CI	p
Timepoint (Day 7 vs. Day 1)	–	–	NS	–	–	NS
Specimen (head vs. tail)	–	–	NS	–	–	NS
Age (years)	–	–	NS	–	–	NS
BMI	0.961	0.927–0.996	0.05	–	–	NS
Gender (F vs. M)	0.702	0.524–0.942	0.04	0.512	0.302–0.864	0.02
Type donor (DCD vs. DBD)	–	–	NS	–	–	–
HBP (yes vs. no)	0.623	0.466–0.832	<0.01	–	–	NS
Vasopressors (yes vs. no)	–	–	NS	–	–	NS
Cause of death (ref = CA)						
CVA	–	–	NS	2.129	0.915–4.946	0.09
Trauma	–	–	NS	2.811	1.112–7.106	0.04
Pancreas weight (g)	–	–	NS	1.01	1.001–1.019	0.03
IEQ/mg	–	–	NS	1.277	1.088–1.510	<0.01

AUC, area under curve of insulin secretion; BMI, body mass index; CA, cardiac arrest; CVA, cerebrovascular accident; DBD, donation after brain death; DCD, donation after circulatory death; HBP, high blood pressure IEQ, islet equivalent; SI, stimulation index (G15/G1 ratio).

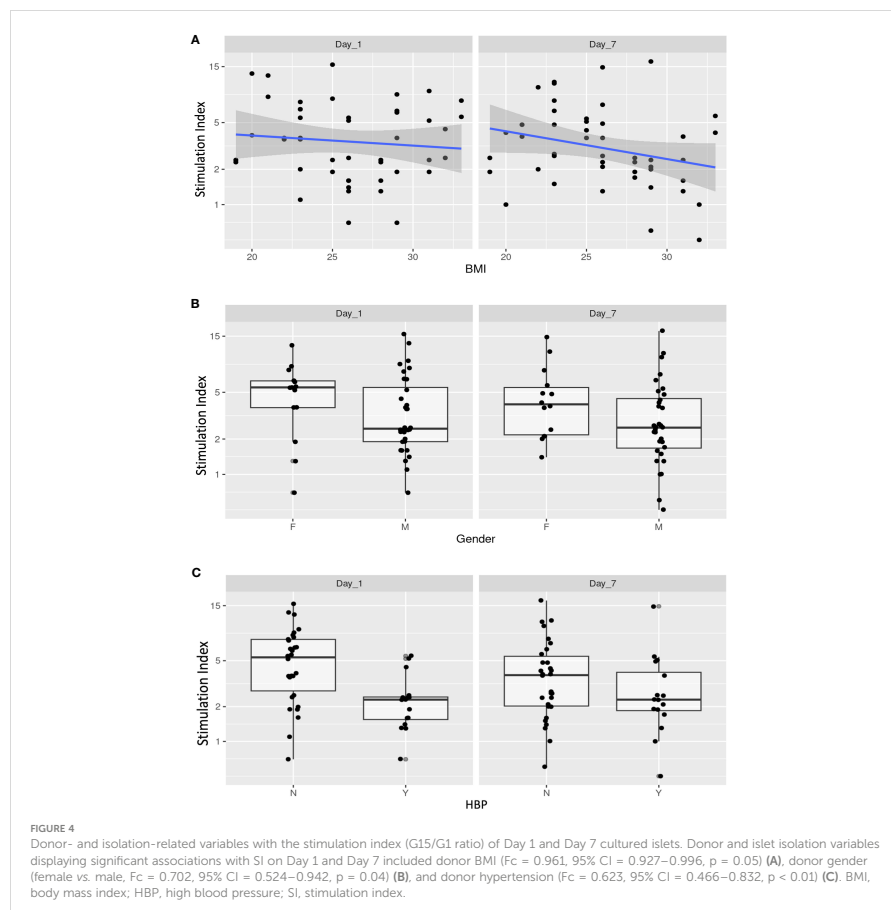
*Coefficient (exponentiated) refers to the impact of donor- and isolation-related variables on the islet secretory function.

NS, Not Significant.

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compromised organ blood supply (39). Furthermore, a recent study reported that donors with a history of HBP exhibited increased pancreatic lipid content and islet lipid content, irrespective of gender. This association was correlated with poorer islet performance after isolation (40).

Finally, our findings substantiate a noteworthy negative correlation between cardiac arrest as a cause of death and islet function when contrasted with alternative causes like trauma or cerebrovascular accidents.

This observed correlation could be closely tied to the varying durations of low or absent blood flow during the phases of donor resuscitation. The prolonged periods of diminished perfusion or complete cessation of blood flow during these critical stages exert a detrimental influence on pancreas perfusion, thereby

contributing to the development of ischemia-reperfusion injuries (41, 42).

This study has certain limitations, primarily stemming from the relatively low number of cases and the presence of numerous potential confounding variables, mainly associated with the nature of the pancreas discarded for clinical purposes and the extended ischemia times. Additionally, *in vivo* function tests were not conducted in this study.

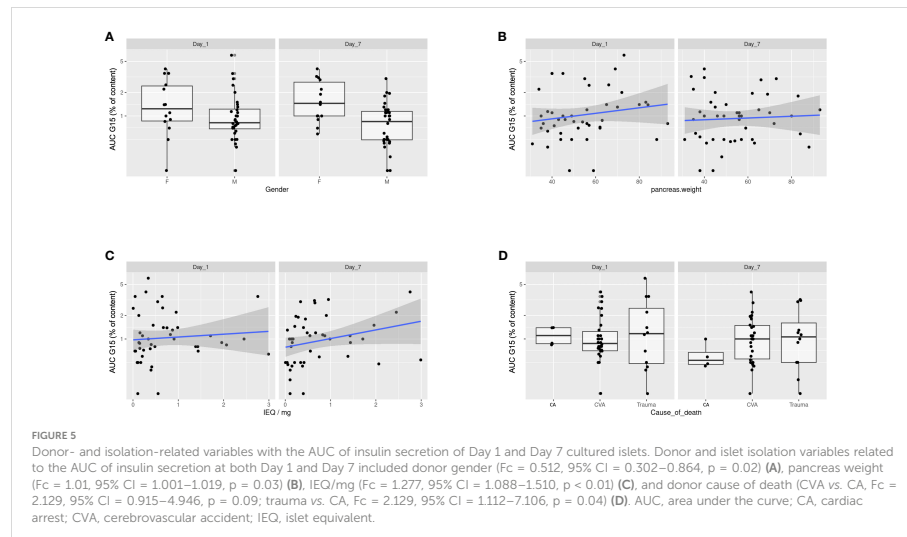
Conclusions

In conclusion, this study identified donor- and isolation-related factors that influence *in vitro* islet secretory function. Further

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studies are essential to evaluate the reliability of these results in clinical practice.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Cliniques Universitaires Saint Luc. The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from primarily isolated as part of your previous study for which ethical approval was obtained. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

AB: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. NM: Investigation, Methodology, Validation, Visualization, Writing – review & editing. JA: Data curation, Methodology, Validation, Writing – review & editing. DH:

Methodology, Writing – review & editing. AD: Validation, Visualization, Writing – review & editing. TD: Visualization, Writing – review & editing. NK: Visualization, Writing – review & editing. PG: Visualization, Writing – review & editing. MM: Validation, Writing – review & editing.

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Conflict of interest

AB received a grant from the “Fondation Saint Luc,” and the “Fondation Recherche Clinique”.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Summary Paper 3

Aims of this study:

Human islet preparations designated for research exhibit diverse insulin-secretory profiles. This study aims to assess the impact of donor- and isolation-related factors on in vitro islet secretory function using organs discarded from clinical pancreas and islet transplantation.

Take Home message

1. The study has identified several donor- and isolation-related factors significantly associated with islet secretory function after one day and seven days of culture. Islet yield, expressed as islet equivalents per gram (IEQ/g), is positively correlated with islet secretory function, indicating its potential as a predictive indicator in clinical settings. In vitro islet function tests may offer a more objective and potentially accurate measure of islet quality compared to manual quantification methods.
2. Factors such as higher BMI, female donors, absence of hypertension, and causes of death other than cardiac arrest are positively associated with better islet function. Conversely, male donors, hypertension, and cardiac arrest as a cause of death are negatively correlated with islet function.
3. There was no predictor significantly associated to the temporal evolution between Day 1 and Day 7 for both SI and AUC outcomes.

CHAPTER 3 ~ General discussion

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Nearly 40 million individuals are diagnosed with type 1 diabetes mellitus, characterized by the immune-mediated destruction of pancreatic beta cells. While standard treatment involves insulin injections, some patients opt for whole pancreas transplantation or islet transplantation to restore beta-cell function¹⁹⁹. Pancreas transplantation outcomes have notably improved due to advancements in surgical techniques and immunosuppressive strategies²⁰⁰. However, pancreas transplantation remains complex, with significant perioperative risks and stringent recipient screening, unlike islet transplantation, which is less complex but still faces challenges such as considerable islet loss during transplantation²⁰¹.

Despite promising results, pancreas and islet transplantation procedures are less common compared to kidney and liver transplantation due to a shortage of high-quality pancreas grafts²⁰². The pancreas is particularly susceptible to

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injury during brain death and organ retrieval, leading centers to exercise caution in accepting pancreas grafts, especially considering that beta cell replacement is not immediately life-saving¹⁸⁴. To address the shortage of donor organs, many centers now consider organs from extended criteria donors, including those from DBD and DCD donors^{203,204}. However, defining acceptable factors for DCD pancreata remains a challenge due to concerns about warm ischemia and ischemia-reperfusion injury²⁰⁵.

The current challenge is to expand the donor pool without compromising transplantation outcomes. One potential solution is to improve pancreas preservation techniques. While SCS is the current standard, HMP offers a promising alternative by providing continuous circulation of a dedicated preservation solution. HMP has demonstrated superiority over SCS in kidney lung and liver transplantation, attributed to its ability to maintain tissue ATP levels and remove toxic waste products²⁰⁶⁻²¹¹. However, the impact of HMP on enhancing human pancreas quality by reducing reperfusion injury remains fairly unexplored.

Some studies have explored the potential advantages of HMP as a pancreas preservation method¹⁸⁶⁻¹⁸⁸. While initial findings are promising, the inter-subject variability among pancreases studied, coupled with the absence of a reliable control group, limits the ability to draw definitive conclusions regarding the efficacy of HMP compared to traditional preservation methods.

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The overall aim of this thesis was to assess the feasibility and potential advantages of active oxygenated and non-active oxygenated hypothermic machine perfusion compared to static cold storage for preserving donor pancreata using discarded pancreases from deceased donors through a human split pancreas model considering DBD and DCD donors.

However, it's crucial to address certain factors to ensure an accurate interpretation of the results acquired:

-Is a split pancreas a genuinely reliable model for evaluating islet isolation outcomes?

-Is it possible to process pancreas fragments of the same organ in parallel without affecting the islet function?

-What specific intrinsic features of the pancreas must be considered in the development of a model to evaluate novel preservation techniques?

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3.1 Pancreas split model: pro and cons.

The pancreas split model applied in this pilot validation study provided the opportunity to undertake a controlled comparison of islet function from pancreatic fragments of the same donor bypassing inter-subject variabilities. Moreover, the internal control provided by the pancreas split model permitted validation of these findings using a small number of human pancreases. Nevertheless, the heterogeneous architecture of the endocrine component of the pancreas, which includes variations in islet distribution and cellular composition across different regions of the organ, complicates outcome selection significantly²¹²⁻²¹⁴. While the exocrine pancreas exhibits a consistent lobular organization, the endocrine pancreas displays remarkable heterogeneity, evident in both the total number of islets and their sizes. Previous studies have documented a wide variation in the total number of islets, ranging from 3.6 to 14.8 million, with total islet volumes reported between 0.5 and 1.3 cm³²¹⁵⁻²¹⁸. Detailed analyses of human islets indicate a composition of approximately 60% β cells and 30% α cells, with the remaining fraction divided among δ cells (less than 10%, somatostatin-secreting), γ cells (less than 5%, secreting pancreatic polypeptide), and ϵ cells (secreting ghrelin)²¹⁹⁻²²¹. During embryonic development, the pancreas arises from dorsal and ventral pancreatic protrusions from the primitive gut endoderm. Anatomically, the adult pancreas is divided into head, body, and tail regions, with the head region located on the right side of the abdomen attached to the duodenum,

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while the body and tail extend to the left side of the abdomen next to the spleen (Figure 18).

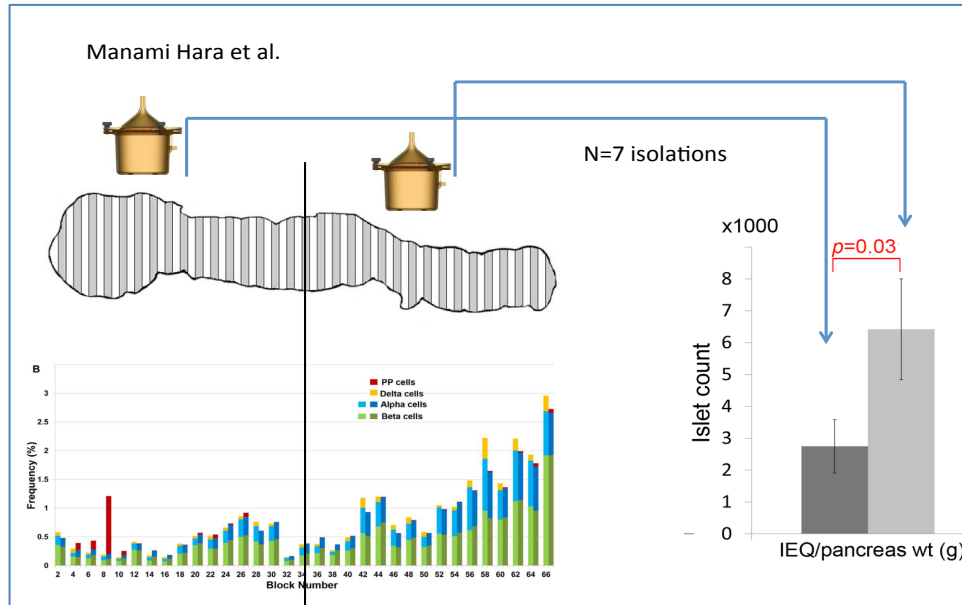


Figure 18. Regional differences in islet distribution and cellular compositions in human pancreas. (Wang, X. et al. Regional differences in islet distribution in the human pancreas—preferential beta-cell loss in the head region in patients with type 2 diabetes. PLoS. One. 8, e67454 (2013).)

Regional variations in islet histology have been extensively studied in human, monkeys and rodents, generally showing lower density of beta-cell mass in the head and body regions compared to the tail region (in average ~1:1:2, respectively)²²²⁻²²⁶. However, a recent sophisticated three-dimensional imaging study of the mouse pancreas by Hörnblad et al. reported conflicting findings regarding regional differences in beta-cell volume, suggesting an order of duodenal > gastric > splenic lobe²²⁷.

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A comprehensive study by Hara et al. conducted in human pancreas revealed significant intra-individual regional variability in the mass of beta-cells/islets, showing notable fluctuations within each region and across the entire pancreas through stereological quantification. This variability was observed regardless of the donor's age, sex, and BMI (Figure 19)²²⁸.

These parameters have been extensively studied in control subjects, who are chosen based on matching criteria such as age, sex, and BMI. Studies have generally suggested a positive correlation between beta-cell mass and BMI, indicating that beta-cell mass tends to increase with higher BMI levels²²⁹. However, it's important to note that researchers have observed a significant amount of variability in beta-cell mass that cannot be solely explained by BMI, as indicated by a correlation coefficient of 0.5. Additionally, Rahier et al²³⁰. found that there is substantial overlap in beta-cell mass between individuals with lean and obese body compositions. They concluded that the impact of obesity on beta-cell mass in humans is relatively modest, with a correlation coefficient of 0.3, especially when compared to findings in rodents. While a study conducted in Korea demonstrated a positive correlation²³¹, indicating that higher BMI is associated with increased beta-cell mass, a study in Japan reported a negative correlation, suggesting the opposite relationship²³². These discrepancies underscore the complexity of the relationship between BMI and beta-cell mass, which may be influenced by various factors

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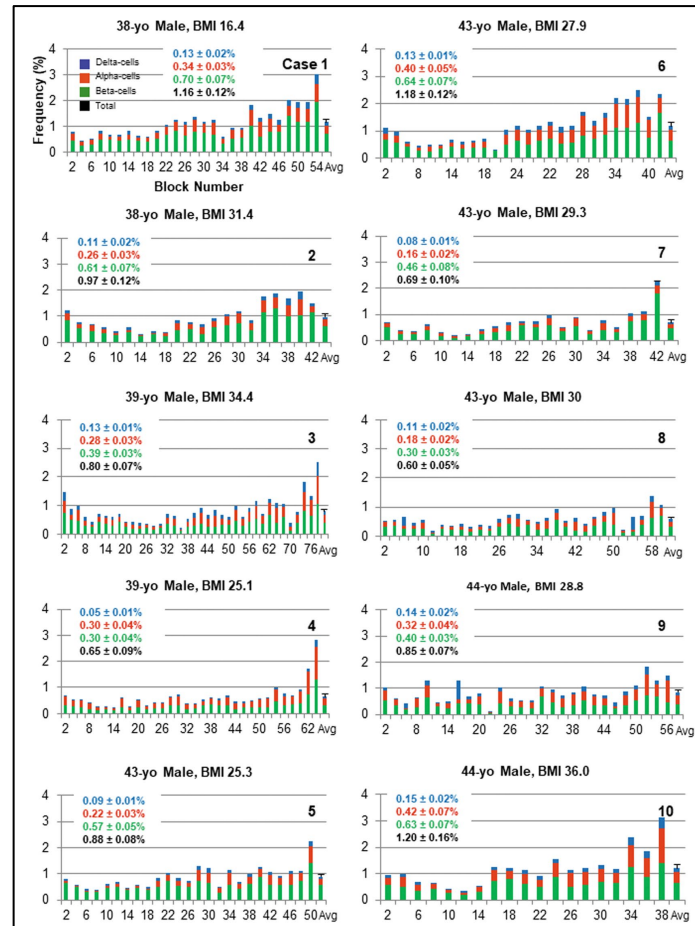


Figure 19. Regional Distribution of Endocrine Cells in the Whole Pancreas: A Comparative Analysis in Non-Diabetic Male Subjects.

X-axis: Block number from head, body to tail region (from left to right). All the graphs are plotted in the same scale for a better comparison. Each pancreas was divided into consecutive tissue blocks, with preparation alternating between fresh-frozen and paraffin embedding. The latter set of tissue blocks was used in the present study. The last bar in each plot depicts average $\pm$ SEM, which value is shown in the inset. (Olechnik SK, Fowler JL, Avramovich G, Hara M. Quantitative analysis of intra- and inter-individual variability of human beta-cell mass. Sci Rep. 2017 Nov 27;7(1):16398. doi: 10.1038/s41598-017-16300-w. PMID: 29180621; PMCID: PMC5703964.)

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including genetic, environmental, and lifestyle differences among populations.

Given the multifaceted nature of pancreas architecture, solely relying on quantitative measures of islet yield obtained from fragments of the same organ for evaluating preservation methods would be methodologically inadequate. Therefore, we have opted to prioritize islet function, particularly focusing on insulin secretion through an islet perfusion analysis, as the primary outcome measure.

A potential approach to reduce the variability in pancreatic architecture among different subjects could involve normalizing isolation outcomes through quantitative analysis of islets from pancreas biopsies.

Take Home message

Pros of the pancreas split model:

1. Controlled comparison: Facilitates a controlled comparison of islet function using pancreatic fragments from the same donor, minimizing inter-subject variations and ensuring a more precise assessment of preservation methods' effects.
2. Internal control: The inherent internal control of the split model allows for validation of findings using a limited number of human pancreases, enhancing the robustness of study results.

Cons of the pancreas split model:

1. Heterogeneous nature of the pancreas: The intricate and heterogeneous nature of the endocrine component of the pancreas presents challenges in selecting appropriate outcome measures, particularly due to variations in islet distribution and cellular composition across different regions.
2. Variability in islet characteristics: Previous studies have documented a wide range of variation in the total number of islets and their sizes, as well as differences in cellular composition among different regions of the pancreas.
3. Conflicting findings: Recent advancements, such as three-dimensional imaging studies, have presented conflicting findings regarding regional differences in islet characteristics, adding complexity to interpretation.

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3.2 The purification step: a European challenge

In order to obtain reliable results, each step of the isolation process was carried out in parallel in each paired pancreas fragments.

Improvements such as consistently assigning the same operator for pancreas preparation, utilizing a single enzyme blend, and implementing miniaturization and duplication of the Automated "Ricordi" isolation system significantly enhanced the dissection and digestion phases of the pancreas. Rather the purification phase proved to be the most challenging aspect of the process, especially considering the discontinuation of COBE 2991 distribution in Europe. This system has been widely utilized for human islet purification in numerous islet processing centers. Now, alternative purification methods are being sought, aiming to maintain consistent isolation results in terms of both islet yield and function.

The first study of Chapter 2 aims to compare the efficacy of the COBE method vs the "bottle" method (Figure 20) for the purification of human islets from deceased human pancreas donors and reveals that a discontinuous purification method, employing a "bottle" technique, demonstrates similar performance to the conventional COBE 2991 isolation procedure in terms of the isolation process and islet secretory function. While these preliminary results suggest the potential efficiency of this procedure as an alternative to the established purification process for human pancreases, we acknowledge the poor outcomes in terms of islet yields in both groups. Critical parameters

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for transplantability, such as islet number, purity, sterility, and potency, were not achieved in our study due to prolonged ischemia times and the nature of donors rejected for clinical purposes. However, despite these challenges, both groups exhibited satisfactory islet function, as indicated by stimulation index and insulin secretion. This suggests that the discontinuous purification method, by preserving islet function, could replace continuous purification, emphasizing the need to adhere to donor selection criteria and minimize organ ischemia time for transplantable results.

Notably, islets purified by the bottle method were significantly larger than those purified by COBE, potentially attributed to reduced shear stress during centrifugation. The impact of islet size on transplantation outcomes is a debated topic, with some evidence suggesting larger islets may have poorer therapeutic values. However, our experience and recent research indicate that larger islets, if cultured appropriately, can be equally suitable.

Additionally, the bottle purification method proved to be relatively simple to implement and associated with lower costs than the COBE 2991 method. In conclusion, our study demonstrates that discontinuous purification using the "bottle" method is a viable and efficient alternative in the absence of COBE 2991, achieving comparable results in terms of insulin secretory function and isolation process performance from discarded organs. Implementing this technique with higher quality organs and shorter ischemia times could potentially replace the established islet purification gold standard of

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the past two decades. Furthermore, the results of this study allowed to integrate this purification method into the split-model pancreas, facilitating the simultaneous and parallel purification of digested pancreas fragments from a single donor. Crucially, this integration does not compromise the outcomes, ensuring comparability with those attained through the conventional continuous purification method using COBE 2991.

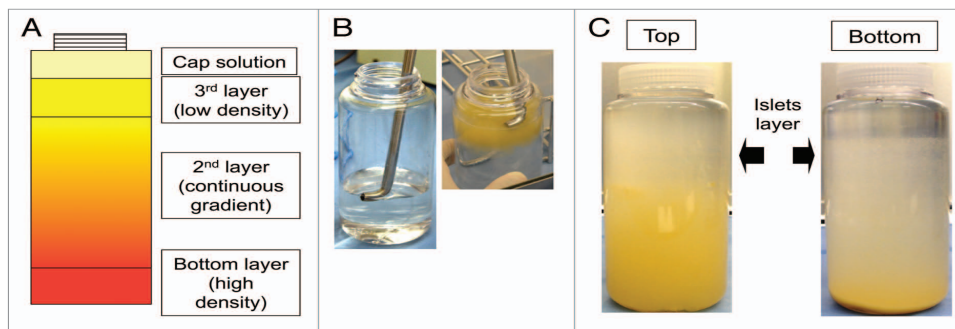


Figure 20. Bottle purification method.

(A) The schema of the continuous gradient solution for the bottle purification method. (B) A “tip-bent” candy cane-shaped stainless pipe used for making the gradient (left panel). The pipe was useful to load the tissue into the top layer in the Top loading method (right panel). (C) Pictures of the bottles after loading and centrifugation. Left: top loading. Right: bottom loading. Islets exist in the upper layer in both methods (arrows). (Shimoda M, Itoh T, Iwahashi S, Takita M, Sugimoto K, Kanak MA, Chujo D, Naziruddin B, Levy MF, Grayburn PA, Matsumoto S. An effective purification method using large bottles for human pancreatic islet isolation. *Islets*. 2012 Nov-Dec;4(6):398-404. doi: 10.4161/isl.23008. Epub 2012 Nov 1. PMID: 23221740; PMCID: PMC3605168.)

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Take Home message

1. The discontinuation of the COBE 2991 system in Europe has posed challenges for islet purification. Alternative purification methods, such as the "bottle" method, are being explored to maintain consistent isolation results in terms of both islet yield and function.
2. Preliminary results comparing the COBE method with the "bottle" method for islet purification indicate similar performance in terms of the isolation process and islet secretory function. Despite poor outcomes in terms of islet yields, both methods exhibited satisfactory islet function.
3. Critical parameters for transplantability were not achieved due to prolonged ischemia times and the nature of donors rejected for clinical purposes. However, both purification methods preserved islet function, emphasizing the importance of adhering to donor selection criteria and minimizing organ ischemia time for transplantable results.
4. Islets purified by the bottle method were significantly larger than those purified by COBE, potentially attributed to reduced shear stress during centrifugation. Despite debates on the impact of islet size on transplantation outcomes, larger islets, if cultured appropriately, can be equally suitable.
5. The bottle purification method is relatively simple to implement and associated with lower costs than the COBE 2991 method. It is considered a viable and efficient alternative, achieving comparable results in terms of insulin secretory function and isolation process performance from discarded organs.
6. Integrating the bottle purification method into the split-model pancreas facilitates simultaneous and parallel purification of digested pancreas fragments from a single donor without compromising outcomes. This integration ensures comparability with results attained through the conventional continuous purification method using COBE 2991.

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3.3 HMP and pancreas preservation: more or no more

The scarcity of pancreases from traditional donors has sparked interest in DCD and ECD pancreas transplantation. While reports on DCD pancreas transplantation are limited, they have shown promising outcomes. Comparisons of DCD pancreas transplants with DBD transplants have demonstrated comparable results. However, it's crucial to note that the selection criteria for DCD donors are typically stricter. Consequently, DCD donors tend to be younger with a lower BMI, and recipients also have lower immunologic risk, which may favor DCD transplants.

The mechanisms underlying tissue injury from pancreatic ischemia and subsequent reperfusion have not been extensively studied compared to other organs like the kidney or liver. Nevertheless, the pathophysiological events are complex and multifaceted. They involve up-regulation of proinflammatory cytokines and endothelial adhesion molecules, resulting in recruitment and activation of leukocytes and platelets, leading to microcirculatory impairment and reduced tissue perfusion. Microcirculatory impairment correlates with acinar cell apoptosis, and the extent of impairment after clinical pancreas transplantation relates to the degree of early graft damage. Reduced tissue perfusion is compounded by ATP depletion due to absent oxidative phosphorylation, which is crucial for organ damage. Release of oxygen free radicals and proteolytic enzymes from activated neutrophils further contributes to tissue damage.

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Static cold storage remains the standard method for pancreas preservation, typically sufficient for DBD pancreas transplants. However, preservation techniques play a vital role in limiting ischemic insult. Techniques such as hypothermic machine perfusion with or without oxygenation have shown potential to mitigate the deleterious effects of increased ischemia, particularly for the kidney and liver.

The second study described in Chapter 2 compares three preservation methods of discarded human pancreases intended for islet isolation using the split model approach. The methods examined included HMP with or without active oxygenation, and SCS.

While no differences were found in the secretory function between head and tail pancreas portions within each group, the split model enabled a comparison of islet function from fragments sourced from the same donor. However, variability in outcomes between head and tail isolation was noted, emphasizing the need for careful consideration of the pancreas's architectural arrangement.

The study found that neither HMP nor HMP O₂ enhanced the histological attributes of tissues pre- or post-preservation, nor did they impact the expression of markers associated with oxidative stress and apoptosis. These outcomes were attributed, in part, to the distinct characteristics of the studied organs, including their initial rejection for clinical use and prolonged ischemia times.

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While oxygenated HMP has shown benefits in kidney transplant models, its effects on pancreatic islets remain uncertain. The study suggests that high oxygen levels may be toxic to islets, leading to oxidative stress and apoptosis. Conversely, non-oxygenated preservation methods may also contribute to oxidative stress through hypoxia.

In conclusion, the study highlights the need for further research to enhance the applicability of preservation methods for transplantation. This includes testing under conditions involving shorter ischemia times, exploring new donor oxygenation protocols, and normalizing isolation outcomes through quantitative analysis from pancreas biopsies to mitigate inherent variability among procured organs.

Several considerations can be made regarding the ineffectiveness of these preservation methods compared to the traditional SCS method in enhancing the function of isolated islets and future direction of research in this field:

3.3.1 *Avoid barotrauma*

The pancreas, characterized by low-flow vascularization without end arteries like in liver and kidney, is vulnerable to barotrauma during perfusion. High perfusion pressure, such as that used in the liver and kidney HMP, can cause acinar necrosis, edema formation and endothelial injury, which are recognized risk factors for early graft pancreatitis and thrombosis²³³. On the contrary, if the pressure is too low, perfusate solution flow will not be

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appropriate for an effective perfusion of the microvasculature and an adequate oxygenation and nutrient supply. Various studies have investigated the optimal perfusion pressures for different animal models and pancreas sizes. Perfusion of canine pancreas segments at a pressure of 30 mmHg resulted in edema, indicating that this pressure was too high for pancreases from dogs weighing 14–30 kg²³⁴. Lower pressures, such as 10 mmHg, were found to be more suitable, as demonstrated in studies perfusing pancreases from pigs weighing 25–32 kg²³⁵. These transplants exhibited very moderate edema with preserved islet function after 24 hours of perfusion. Similar findings were reported in studies using porcine pancreases, where perfusion pressures of 10 mmHg resulted in no harmful effects on islet function. However, pressures of 40 and 70 mmHg led to pancreatic edema, suggesting that a pressure of 20 to 25 mmHg is more effective without causing harm during hypothermic perfusion²³⁶. In other studies involving pigs weighing 60–90 kg, perfusion at a pressure of 23 mmHg resulted in moderate edema²³⁷. Furthermore, comparisons of low perfusion pressures, such as 5, 10, and 25 mmHg, revealed that higher pressures did not necessarily induce more severe edema, indicating that optimal perfusion pressure may vary depending on the experimental conditions^{238,239}. Overall, these studies highlight the importance of carefully selecting perfusion pressures to achieve effective organ perfusion while minimizing the risk of inducing harmful effects. Based on the findings of these studies, we established our protocol using a pressure of 25 mmHg during the preservation period. Despite observing no instances of

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pancreatic edema, our vision for further experiments is to customize the perfusion pressure based on specific organ (i.e. fibrosis, oedema, pancreatitis) and donor characteristics (i.e age, donor, need of vasopressor use), duration of perfusion and histologic parameters. This “personalized” approach aims to mitigate the effects of barotrauma during the preservation period.

Take Home message

- 1.Pancreatic vulnerability:** The pancreas lacks end arteries like the liver and kidney, making it susceptible to barotrauma during perfusion.
- 2.Effects of high perfusion pressure:** High perfusion pressure can lead to acinar necrosis, edema, and endothelial injury, increasing the risk of early graft pancreatitis and thrombosis.
- 3.Impact of low perfusion pressure:** Conversely, low perfusion pressure may compromise microvascular perfusion, resulting in inadequate oxygenation and nutrient supply.
- 4.Optimal perfusion pressures:** Studies have investigated optimal perfusion pressures across various animal models and pancreas sizes. Lower pressures, around 10 mmHg, are suitable for preserving islet function in pigs, while higher pressures, like 40-70 mmHg, induce edema.
- 5.Importance of pressure selection:** Careful selection of perfusion pressures is crucial to achieve effective organ perfusion while minimizing adverse effects.

3.3.2 Reduce ischemia time, *how long is too long?*

The integration of HMP in the clinical practice for kidney and liver grafts has significantly improved outcomes in ECD and DCD donor transplantations^{206,240-242}. This has led to an expansion of the donor pool and allowed for longer ischemic times. HMP has been associated with a decreased incidence of DGF and improved graft survival compared to SCS in kidney transplantation²⁴³. Notably, these benefits appear consistent regardless of the duration

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of CIT, with positive outcomes even observed in cases with CIT exceeding 24 hours in kidneys, attributed to the potential reconditioning effect of HMP²⁴⁴. HMP is believed to restore graft hemodynamic function, maintain metabolic activity, and stabilize ischemic damage accumulated during initial static cold storage²⁴⁵. However, there is still some debate regarding the extent of cold ischemic injury during HMP compared to SCS. While some reports emphasize the importance of minimizing CIT as much as possible, the growing prevalence of both kidney and liver candidates with multiple comorbidities or advanced age has made pretransplant preparation more complex and time-consuming. In certain scenarios, such as combined liver-kidney or heart-kidney transplantation, there has been a recent trend towards advocating for delayed organ procedures²⁴⁶. This approach aims to stabilize the recipient after the first organ implant and protect the graft from hemodynamic instability. Despite these promising results little information is available in the literature in the context of pancreas transplantation. The pancreas is significantly more vulnerable to injury than other abdominal organs. Despite minimal CIT, stable perfusion and maintenance of acid-base homeostasis, exocrine pancreatic tissue displays acinar damage, inflammation, and thrombosis²⁴⁷. Data from several studies show CIT to be an independent variable that decreases islet yield and/or function (as a continuous variable, or in arbitrary cutoff values of 7, 8, 12, or 16 h CIT)^{93,248,249}. Few studies have analyzed the effect of ischemia time in organs preserved with HMP. Recently in a HMP of porcine pancreases model, Branchereau et al.¹⁸⁸ showed

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moderate and severe edema after 12 and 24 h of HMP, respectively. Histological examination showed similar results up to 12 h of preservation when compared to SCS. In a study regarding the effect of 24 h of HMP on isolated porcine islets, it was speculated that edema induced by HMP aided the enzymatic digestion during isolation. Leemkuil et al.¹⁸⁶ compared HMP and SCS of human pancreases from DBD and DCD donors (preserved with HMP for a period of 6 h). No significant differences in histology (oedema formation, acinar cell integrity loss) between HMP and SCS were found despite an extension in the oxygenated HMP-preserved pancreases group of 6 hours (on average, CIT was 13 hours in the HMP group and 9 hours in the SCS group). Although amylase, lipase and lactate dehydrogenase levels increased in grafts preserved with both HMP and SCS, ATP concentration only increased in the HMP group. In our study, we observed significantly longer ischemic times, averaging about 19 hours in both groups. We consider this prolonged ischemia as a potential confounding factor in our findings. Moving forward, one of our future research priorities will involve conducting procedures with shorter ischemic times. Additionally, we plan to utilize HMP throughout the entire preservation period rather than for a limited duration, as practiced in previous studies. Currently, the question of "how long is too long" remains unanswered, posing a challenge for the scientific community in this field. Finding optimal ischemic times cut off for the pancreas preservation will be crucial for improving transplant outcomes.

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Take Home message

1.Integration of HMP: HMP has significantly improved outcomes in kidney, liver, and lung graft transplantations from ECD and DCD donors, expanding the donor pool and allowing for longer ischemic times.

2.Benefits in kidney transplantation: HMP has been associated with decreased DGF incidence and improved graft survival compared to SCS, even with CIT exceeding 24 hours, possibly due to a reconditioning effect.

3.Mechanisms of action: HMP restores graft hemodynamic function, maintains metabolic activity, and stabilizes ischemic damage accumulated during initial SCS.

4.Challenges in Pancreas Transplantation: Unlike other abdominal organs, the pancreas is more vulnerable to injury despite minimal CIT, stable perfusion, and maintenance of acid-base homeostasis. Ischemic time has been shown to decrease islet yield and function, and while few studies have analyzed the effect of ischemia time in organs preserved with HMP, finding optimal ischemic time cutoffs for pancreas preservation remains crucial for improving transplant outcomes.

5.Future Research Priorities: Addressing the challenges of prolonged ischemic times and optimizing preservation techniques, such as utilizing HMP throughout the entire preservation period, are key priorities for future research in pancreas transplantation. Determining the optimal ischemic time cutoffs will be essential for enhancing transplant outcomes and advancing the field.

3.3.3Adapt the oxygenation time and pressure:

One key consideration in pancreas preservation through HMP is the approach and timing of oxygenation. While this aspect of preservation technique is extensively studied in the context of kidney transplantation²⁵⁰, there is comparatively less information available in the literature regarding pancreas preservation. From a physiological perspective, the pancreas exhibits unique characteristics that provide insights for tailoring oxygenation techniques during the preservation period.

Several studies have shown that pancreatic islets, despite constituting only 1% of the total pancreas mass, receive 10% to 15% of arterial blood, indicating

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a relatively high oxygen supply compared to other pancreatic tissues^{251,252}. However, the availability of oxygen to a specific tissue does not solely depend on blood volume but also on the tissue's microvascular density²⁵³. For instance, the pancreas, supplied mainly by arteries, tends to have higher oxygen partial pressure (pO₂) compared to the liver, which primarily receives blood from the portal vein. However, poorly vascularized tissues can experience oxygen imbalance, leading to gradients in oxygen concentration, like what happens in brain areas exposed to systemic hypotension²⁵⁴. This phenomenon can occur in isolated islets in culture or after transplantation due to the loss of vascular supply, resulting in localized oxygen depletion²⁵⁵.

Due to the dense vascular network in the pancreatic tissue, both excessively low and high pO₂ levels can have adverse effects on delicate structures like pancreatic islets.

Research has investigated the impact of hypoxia on the pancreas endocrine component, revealing that both acute and chronic anemia in fetal sheep lead to elevated blood glucose levels and impaired β -cell function²⁵⁶. Intriguingly, despite no functional disparities observed in vitro between islets isolated from anemic sheep and those from normal sheep, indicating potential reversibility of islet dysfunction, anemic sheep maintained similar arterial oxygen levels to control sheep, implying that oxygen diffusion supported the function of native islets. Conversely, exposure of neonatal rats to intermittent hypoxia resulted in irreversible β -cell dysfunction²⁵⁷. Intermittent hypoxia-

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treated rats exhibited persistently elevated blood glucose and decreased C-peptide levels even weeks after exposure, indicating the importance of oxygen supply via hemoglobin for in vivo islet function²⁵⁸.

Hypoxic conditions can also be induced in vitro by seeding islets at high density²⁵⁹. Studies have demonstrated a decrease in oxygen tension in culture media with increasing islet seeding density, leading to reduced islet viability and impaired β -cell function²⁶⁰. Inflammatory responses were also observed under hypoxic conditions, with upregulated gene expressions and increased release of inflammatory markers²⁶¹. Mechanisms underlying impaired β -cell function by hypoxia involve metabolic transition to anaerobic glycolysis, changes in gene expressions related to β -cell transcription and insulin secretion pathways, and dysregulation of adaptive unfolded protein response genes critical for endoplasmic reticulum homeostasis²⁶²⁻²⁶⁸. Additionally, calcium influx into islet cells induced cell damage, highlighting the potential therapeutic target of preventing calcium influx to mitigate hypoxia-induced islet dysfunction²⁶⁹.

In contrast to the extensive research on hypoxia, the effects of hyperoxia (>160 mmHg) on islets have been largely overlooked. Understanding how islets respond to hyperoxia is crucial as additional oxygen could potentially enhance islet survival. Only a few studies have investigated whether hyperoxia improves islet function and have aimed to determine the optimal oxygen partial pressure (pO₂) for islet survival²⁷⁰. Islets function optimally

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within the pancreas where sufficient oxygen is available to meet their physiological needs. However, after isolation, islets are typically cultured under air plus 5% CO₂, providing 21% oxygen or 160 mmHg pO₂ in culture media, a level much higher than that found in the native pancreas. Nevertheless, this culture condition still induces "central necrosis" in the core of larger islets. Recent studies have shown that hyperoxic cultures (35% and 50% oxygen) reduce the occurrence and size of islet central necrosis by maintaining higher pO₂ in the islet core²⁵⁹. However, exposure to hyperoxia levels above toxic thresholds can cause significant damage, especially to cells on the islet surface, highlighting the importance of considering oxygen toxicity when treating islets with hyperoxic conditions²⁷¹⁻²⁷⁴.

Recent attempts to assess the high toxicity threshold of oxygen using isolated human islets showed that viability significantly decreased when exposed to 75% and 95% oxygen, while exposure to 21% and 50% oxygen resulted in the highest viability²⁷⁵. These findings suggest that exposure to 21% to 50% oxygen in culture maintains islet cell viability and function optimally, whereas exposure to levels above 75% oxygen is toxic. Additionally, moderate ranges of hyperoxia (35% to 50% oxygen) have been shown to benefit islet culture, maintaining volume, function, and viability, especially in larger islets (>250 μ m in diameter), thus preventing hypoxia in the core of isolated islets²⁷⁶.

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The findings from these studies, primarily conducted on isolated islets, underscore the importance of exercising caution when applying oxygenation during the preservation phase.

As described in the Chapter 1, numerous investigations focused on oxygen delivery and maintaining the quality of donor pancreases during cold preservation to enhance the yield and quality of islets, including immersing the pancreas in oxygenated solutions. The two-layer method, employing PFC, has been commonly used, where the pancreas is placed between oxygenated PFC and cold preservation solution, allowing gradual oxygen release into the tissue^{171,277}. While promising results were initially seen in canine pancreata, subsequent attempts with human pancreases revealed limitations due to the thick tissue hindering oxygen diffusion²⁷⁸⁻²⁸⁰.

Alternative solutions like perfluorohexyloctan have shown improved intrapancreatic oxygen levels and enhanced islet viability and function in porcine models^{281,282}. However, simply immersing the organ still restricts oxygen availability to the core. Unlike passive diffusion, perfusion through blood vessels may offer more uniform oxygen distribution. Although liver perfusion has been extensively studied, pancreas perfusion faces challenges due to its complex vascular anatomy and involvement of exocrine tissue²⁸³⁻²⁸⁵. Direct injection of gaseous oxygen into vessels (persufflation) has also been explored, showing promising results in reducing cell death and improving islet

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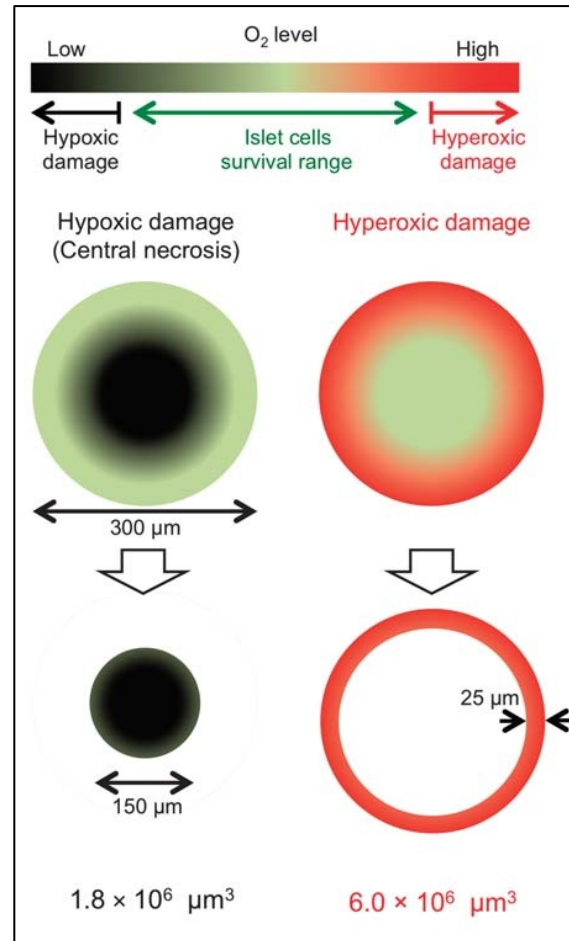


Figure 21. Comparative Analysis of Hypoxic and Hyperoxic Damage to Isolated Islets

The comparison between hypoxic and hyperoxic damage to isolated islets reveals that islet cells have a specific range of oxygen levels within which they can survive. When exposed to oxygen environments outside this range, cells incur damage. Hypoxia leads to cell damage by depleting oxygen in the islet core, whereas hyperoxia causes damage primarily on the islet surface or mantle. Notably, hyperoxic damage tends to affect larger volumes compared to hypoxia. For instance, the volume of hyperoxic damage for a 25 μm-thick islet surface is more than three times larger than the volume of hypoxic damage for a 150 μm-diameter islet core. (Komatsu H, Kandee F, Mullen Y. Impact of Oxygen on Pancreatic Islet Survival. *Pancreas*. 2018 May/Jun;47(5):533-543. doi: 10.1097/MPA.0000000000001050. PMID: 29621044; PMCID: PMC5943071.)

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yield, but further research is needed to determine the optimal method for whole pancreas oxygenation²⁷⁸.

While there isn't a definitive consensus on the optimal oxygen administration method during HMP, an increasing number of researchers are advocating for its use, particularly under hypothermic conditions²⁰⁸⁻²¹¹. Hypothermia reduces metabolism, thereby decreasing the tissue's oxygen requirements. For example, at 4°C, metabolism is approximately 10% lower than normal. Oxygenated HMP is believed to sustain residual metabolism and prevent further declines in cellular energy levels²⁸⁶.

As metabolism shifts from aerobic to anaerobic, lactate and hydrogen ions accumulate in the tissue, leading to acidosis²⁸⁷. This acidosis can trigger lysosomal instability, enzyme activation, disruption of ion binding to transporter proteins, and reduced mitochondrial function. A continuous oxygen supply, combined with adenosine as an ATP precursor, may increase ATP concentration during storage²¹¹.

Higher ATP levels in pancreas transplants have been linked to improved transplantation outcomes²⁸⁷. This underscores the potential importance of optimizing oxygenation strategies during HMP to enhance the viability and function of preserved pancreas grafts, ultimately improving transplantation success rates.

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In a recent study, Doppenberg et al.¹⁸⁷ demonstrated a significant increase in ATP levels by administering 100% oxygen during a 6-hour period. Specifically, they observed a 6.8-fold increase in ATP levels in DCD pancreases and a 2.6-fold increase in DBD pancreases after 6 hours of oxygenated HMP. However, their study found no difference in isolation performance or function between DCD pancreases preserved with O₂HMP and DBD pancreases preserved with SCS.

Our study, which involved DBD and DCD organs preserved with both HMP and O₂HMP with full oxygenation for longer periods (13 and 11 hours, respectively), yielded similar results. We found no significant differences in isolation performance, islet function and both apoptosis and oxidative stress markers compared to organs preserved with SCS.

Our perspective is that future endeavors should focus on exploring lower oxygen administrations and shorter durations to optimize the benefits of this procedure, as observed in other organs.

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Take Home message

1.Unique Considerations in Pancreas Preservation: Despite extensive research on oxygenation techniques in kidney transplantation, less literature exists on pancreas preservation, which exhibits unique physiological characteristics influencing oxygenation techniques.

2.Physiological Insights: The pancreas receives a relatively high oxygen supply due to its vascularization, but tissue oxygenation also depends on microvascular density. Poorly vascularized tissues can experience oxygen imbalance, leading to localized oxygen depletion.

3.Effects of Hypoxia and Hyperoxia: Both excessively low and high oxygen levels can harm pancreatic islets. Hypoxia induces cell damage in the islet core, while hyperoxia damages the islet surface. Moderate hyperoxia (35%-50% oxygen) benefits islet culture, but exposure to oxygen levels above 75% is toxic.

4.Importance of Oxygenation During Preservation: Caution is necessary when applying oxygenation during the preservation phase. Various methods, including perfusion and direct oxygen injection, have been explored, but further research is needed to determine the optimal method for whole pancreas oxygenation.

5.Optimizing Oxygen Administration: While no definitive consensus exists on optimal oxygen administration during HMP, increasing evidence supports its use, particularly under hypothermic conditions. Oxygenated HMP sustains residual metabolism, prevents energy decline, and increases ATP levels, potentially improving transplantation outcomes.

6.Research Insights: Studies have demonstrated significant ATP increases with 100% oxygen administration during HMP, with no difference in performance between DCD pancreases preserved with O2HMP and DBD pancreases preserved with SCS.

3.3.4 Use organ acceptable for clinics: *Can a rejected organ be a reliable model for clinics?*

Considering the disappointing findings concerning the impact of HMP preservation on islet function and the notable variability in results, we explored other parameters related to the donor or the isolation process itself that could potentially contribute to this variability.

Specifically, we aimed to identify which of these factors could reliably predict either poor or favorable islet function as the ability to reliably identify and reject poor-quality donor pancreases not only enhances overall isolation success but also mitigates the costs associated with unsuccessful isolations.

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Several donor variables, such as age, BMI, BSA, and hemodynamic stability without the need for vasopressors during donor management, have been linked to higher islet yields. This has led to the development of various scoring systems aimed at predicting the suitability of potential pancreatic donors before organ processing.

However, achieving effective isolation, as indicated by high islet yields, does not necessarily guarantee optimal islet graft function post-transplantation. Recent studies have highlighted the importance of non-quantitative indicators in predicting the ultimate goal of any islet transplantation procedure: normalizing blood sugar levels and alleviating diabetic symptoms in transplanted recipients.

The third study described in Chapter 2 explores donor- and isolation-related factors associated with the secretory function of islets after one and seven days of culture. It demonstrates a positive correlation between islet yield, expressed as IEQ/g, and islet secretory function, as measured by the area under the curve of insulin secretion. While the correlation seems intuitive, it holds significant implications for clinical practice, where islet yield is a common predictor of isolation and transplantation outcomes in diabetic patients.

In contrast to manual quantification methods, which have limitations due to subjectivity and potential errors, *in vitro* islet function tests emerge as objective indicators, less prone to variability. However, no specific pre-transplant test for evaluating islet quality has shown predictive efficacy for

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transplantation success, highlighting the need for further validation in transplanted islets.

The study also identifies donor characteristics impacting in vitro islet secretory function, including BMI, gender, cause of death, and pancreas weight. Notably, while higher donor BMI correlates with successful islet yield outcomes, there's no statistically significant correlation between BMI and the area under curve of insulin secretion. Recent evidence suggests that recipients of islets from female donors may experience prolonged graft survival compared to those receiving exclusively male donor islets. This observation is attributed to the intrinsic properties of female islets, particularly their higher β -cell content, which leads to improved recipient outcomes. Our own data corroborate these findings, indicating a negative association between male donor status and in vitro islet secretory function.

Furthermore, our study reveals a correlation between HBP and decreased in vitro functional performance of isolated islets, as measured by area under the curve. Building on insights from kidney transplant research, we propose that the adverse effects of HBP on islet function may arise from vascular damage, compromising organ blood supply.

Moreover, recent research indicates that donors with a history of HBP exhibit increased pancreatic and islet lipid content, regardless of gender. This association is linked to poorer islet performance post-isolation.

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Finally, there's a negative correlation between islet function and donor cause of death, particularly cardiac arrest, possibly due to prolonged periods of diminished perfusion during donor resuscitation, leading to ischemia-reperfusion injuries.

In conclusion, the study highlights important donor- and isolation-related factors influencing in vitro islet secretory function.

Hence, the utilization of research organs introduces variability that can obscure results and impede the ability to reach consistent conclusions. To tackle this challenge, forthcoming studies should prioritize the utilization of clinically acceptable organs. This approach will bolster the reliability and relevance of findings, thereby advancing our comprehension of preservation methods and their implications for transplant outcomes.

In the future, increased efforts will aim to develop a scoring system based on function rather than the quantity of isolated islets. This advancement will enable operators to select criteria more accurately, leading to improved outcomes and decision-making processes.

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Take Home message

- 1. Predicting Islet Function in Pancreas Transplantation:** Despite disappointing findings regarding the impact of HMP preservation on islet function, identifying factors related to donor or isolation process variability can enhance success rates and reduce costs. Donor variables such as age, BMI, BSA, and hemodynamic stability without vasopressors are linked to higher islet yields, prompting the development of scoring systems to predict donor pancreas suitability.
- 2. Quality vs. Quantity:** While high islet yields are desirable, they don't guarantee optimal graft function post-transplantation. Recent studies emphasize the importance of non-quantitative indicators in predicting successful outcomes, such as normalizing blood sugar levels in recipients.
- 3. Correlation Between Islet Yield and Function:** Positive correlations between islet yield and secretory function underscore the importance of islet yield as a predictor of isolation and transplantation outcomes. In vitro function tests offer objective indicators less prone to variability.
- 4. Donor Characteristics Impacting Islet Function:** Donor BMI, gender, cause of death, and pancreas weight influence in vitro islet secretory function. Female donor islets may lead to prolonged graft survival due to their higher β -cell content, while high blood pressure and a history of cardiac arrest negatively impact islet function.
- 5. Implications for Transplant Outcomes:** Understanding donor and isolation-related factors influencing in vitro islet secretory function is crucial for improving transplant outcomes. Utilizing clinically acceptable organs can reduce variability and enhance the reliability of research findings, advancing our understanding of preservation methods and their impact on transplantation outcomes.

Conclusion and perspectives

Conclusion and future perspectives

The aim of my thesis was to evaluate the impact of two innovative preservation techniques (HMP and O₂HMP) on the efficiency of islet isolation, utilizing the pancreas split model. This model facilitated a comparative analysis that surpassed the limitations of inter-subject variability. Our findings revealed the following key insights:

Compared to traditional purification methods, implementing a discontinuous purification approach with a "bottle" method yields comparable results in terms of isolation yield and islet secretory function. Notably, islet size was significantly larger in the discontinuous purification group, likely due to reduced shear stress during centrifugation. This technique, especially when applied to high-quality organs and shorter ischemia times, has the potential to establish a durable alternative to the conventional gold standard in islet purification.

For the first time, a split model was conducted in human discarded pancreases for clinical purposes, demonstrating consistent outcomes across three different organ preservation methods. Islets from DCD organs exhibited greater purity in the HMP study group compared to SCS and O₂HMP groups. However, neither HMP nor O₂HMP showed improvements in islet secretory function or histological changes. Further studies are needed under conditions involving shorter ischemia times, along with exploring new donor oxygenation protocols, to enhance transplantation outcomes.

Conclusion and perspectives

Various donor- and isolation-related factors significantly influence islet secretory function after one day and seven days of culture. Islet yield, expressed as IEQ/g, correlates positively with islet secretory function, suggesting its potential as a predictive indicator in clinical settings. In vitro islet function tests may offer a more objective measure of islet quality compared to quantification methods.

Factors such as higher BMI, female donors, absence of hypertension, and non-cardiac arrest causes of death are associated with better islet function, while male donors, hypertension, and cardiac arrest as a cause of death are negatively correlated. No predictors were significantly associated with the temporal evolution between Day 1 and Day 7 for both stimulation index and AUC outcomes.

In conclusion, these findings highlight the importance of innovative preservation techniques and comprehensive donor and isolation assessments in enhancing the outcomes of islet transplantation as predicted by the improvement of in vitro secretory function.

Conclusion and perspectives

...Indeed, every arrival marks not an end, but a fresh beginning...

Perspectives

1.Enhancing Pancreatic Architecture Consistency: A promising strategy to minimize variability in pancreatic architecture across different subjects involves normalizing isolation outcomes through quantitative analysis of islets from pancreas biopsies. By employing rigorous quantitative techniques, researchers can obtain objective measurements of islet quantity, size, and distribution. This standardized approach facilitates accurate comparisons of pancreatic architecture among subjects, leading to improved understanding and more reliable research outcomes.

2.Preventing Pancreas Barotrauma during HMP Preservation: Our vision for future experiments entails customizing perfusion pressure during hypothermic machine perfusion (HMP) based on specific organ characteristics (e.g., fibrosis, edema, pancreatitis) and donor parameters (e.g., age, need for vasopressors). By adopting a personalized approach, we aim to mitigate the potential effects of barotrauma during the preservation period, ensuring optimal organ viability and function.

3.Reducing Ischemia Time: Moving forward, our research priorities include conducting procedures with shorter ischemic times and utilizing HMP throughout the entire preservation period rather than for a limited duration. Addressing the critical question of optimal ischemic times for pancreas preservation is paramount for improving transplant outcomes and minimizing ischemia-related complications.

4.Adapting Oxygenation Strategies: We advocate for exploring lower oxygen administrations and shorter durations to optimize the benefits of oxygenation during preservation, drawing insights from successful approaches used in other organs. By refining oxygenation protocols, we aim to enhance graft viability and function, ultimately improving transplant success rates.

5.Utilizing Clinically Acceptable Organs: The utilization of research organs introduces variability that can obscure results and hinder consistent conclusions. To overcome this challenge, future studies should prioritize the use of clinically acceptable organs. This approach enhances the reliability and relevance of research findings, advancing our understanding of preservation methods and their impact on transplant outcomes.

6.Developing Function-Based Scoring Systems: In the future, efforts will focus on developing scoring systems based on functional parameters rather than isolated islet quantity. By prioritizing functional assessments, operators can make more informed decisions, leading to improved transplantation outcomes and enhanced patient care.

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La transplantation d'îlots de Langerhans est une thérapie prometteuse pour les patients souffrant de diabète de type 1. Cependant, la pénurie d'organes capables de fournir des résultats efficaces chez les receveurs diabétiques rend ce procédé destiné à un petit nombre de patients. Les machines de perfusion hypothermique semblent être un moyen efficace d'élargir les critères de sélection des organes en raison de leur capacité à réduire les dommages d'ischémie-reperfusion pendant la période de préservation. Cette thèse vise à apporter de nouvelles connaissances dans la préservation dynamique du pancréas destiné à l'isolement des îlots de Langerhans et à identifier leur impact sur la fonction des îlots. Afin d'obtenir des résultats fiables contournant la variabilité interindividuelle, nous avons créé un modèle de pancréas fractionné à partir d'organes humains destinés à la recherche, permettant d'obtenir des îlots du même organe préservés selon trois différents types de processus de préservation.

Islet transplantation holds promise as a therapy for patients with type 1 diabetes. However, the shortage of organs capable of yielding effective outcomes for diabetic recipients restricts this procedure to a limited number of patients. Hypothermic perfusion machines emerge as an effective tool for broadening organ selection criteria, thanks to their ability to mitigate ischemia-reperfusion damage during the preservation phase. The objective of this thesis is to contribute fresh insights into the dynamic preservation of the pancreas for Langerhans islet isolation and to evaluate their impact on islet functionality. To achieve consistent results while circumventing inter-individual variability, we have developed a split pancreas model using human organs earmarked for research. This model facilitates the isolation of islets from the same organ, preserved using three distinct preservation technique