Functional islets and where to find them

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Chapter 4. Clinical Use of Donation After Circulatory Death Pancreas for Islet Transplantation

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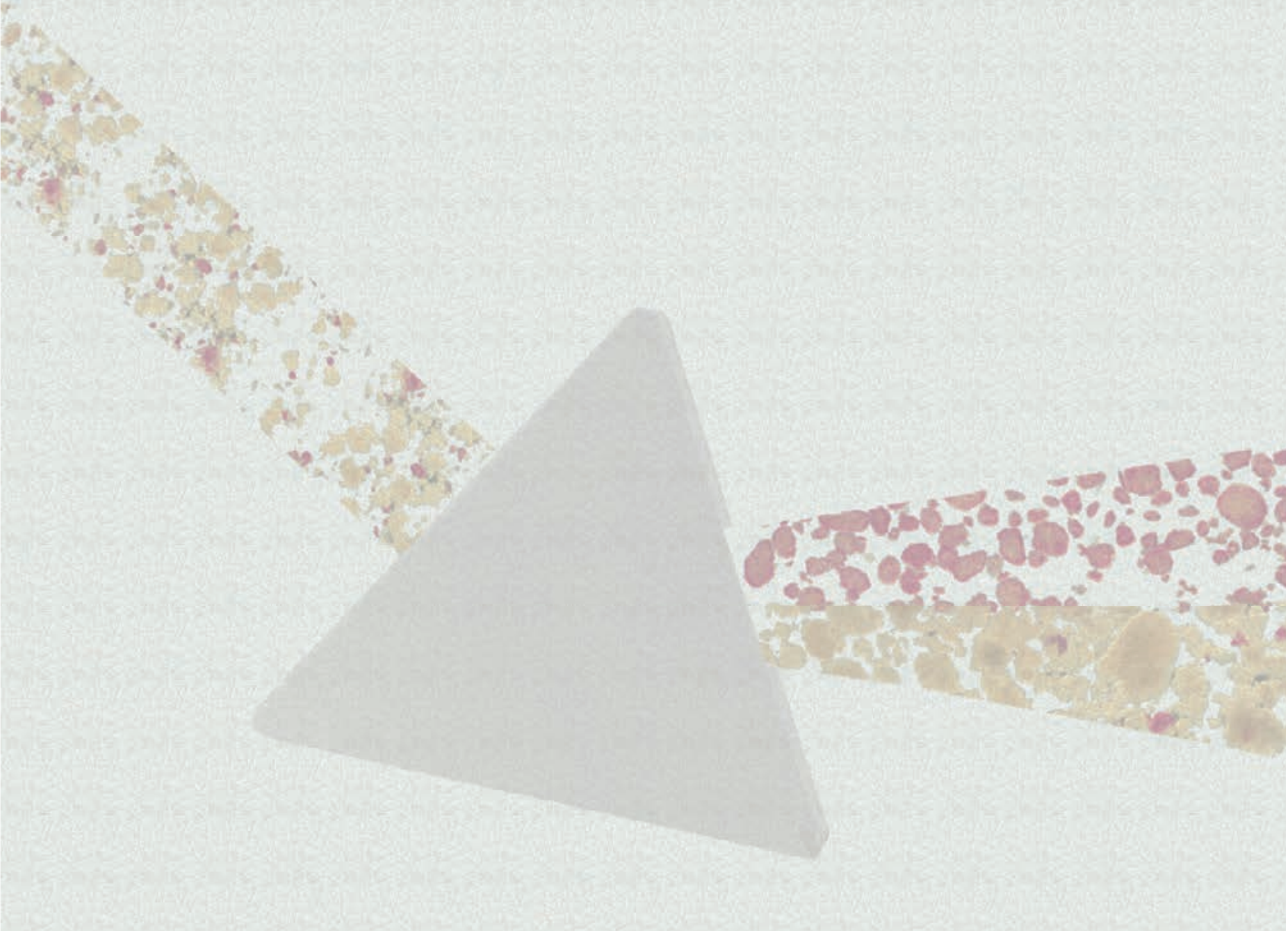

Abstract:

Due to a shortage of donation after brain death (DBD) organs, donation after circulatory death (DCD) is increasingly performed. In the field of islet transplantation, there is uncertainty regarding the suitability of DCD pancreas in terms of islet yield and function after islet isolation. The aim of this study was to investigate the potential use of DCD pancreas for islet transplantation. Islet isolation procedures from 126 category 3 DCD and 258 DBD pancreas were performed in a nine-year period. Islet yield after isolation was significantly lower for DCD compared to DBD pancreas (395,515 islet equivalents (IEQ) and 480,017 IEQ, respectively; p=0.003). The decrease in IEQ during two days of culture was not different between the two groups. Warm ischemia time was not related to DCD islet yield. *In vitro* insulin secretion after a glucose challenge was similar between DCD and DBD islets. After islet transplantation DCD islet graft recipients had similar graft function (AUC C-peptide) during mixed meal tolerance tests and Igls score compared to DBD graft recipients. In conclusion, DCD islets can be considered for clinical islet transplantation.

1. Introduction

Allogeneic transplantation of pancreatic islets is an effective treatment for patients with longstanding type 1 diabetes mellitus[1,2]. However, pancreatic islet isolations do not always yield a sufficient number of islets necessary for transplantation. Consequently, multiple donor pancreas are often needed in order to achieve a good clinical outcome[3]. In most Western countries, the availability of suitable donation after brain death (DBD) organs does not meet the current demand[4]. Due to this shortage, the acceptance criteria for donor pancreas have been extended, such as donation after circulatory death (DCD)[5]. DCD procurement can either be uncontrolled (category 1 or 2) or controlled (category 3, 4 or 5)[6,7]. In the Netherlands, about 50% of all organ procurement procedures are category 3 DCD[8], providing a large source of donor pancreas for potentially transplantable islets.

In DBD procedures, cold preservation fluid can be perfused almost immediately after the aorta is clamped while there is still cardiac activity[9]. In controlled DCD procedures, however, death occurs after cardiac arrest[10]. The time period between the withdrawal of life support and cardiac arrest, known as the agonal phase can vary greatly[11]. This can range from a couple of minutes to two hours in most jurisdictions[12].

DCD procurement of other abdominal organs have shown that this procedure can provide suitable grafts for patients[13]. Although there is a 50% higher incidence of early graft loss, and an almost 150% higher incidence of delayed graft function in DCD kidney transplantation, 10-year graft survival differs only slightly from DBD kidneys[14,15]. DCD liver transplantations are shown to have a higher risk of complications and higher rates of retransplantation[16]. Due to high mortality rates on the waiting lists DCD livers are also used for liver transplantation although they are associated with higher postoperative complications[17]. A recent retrospective analysis showed that DCD pancreas transplantation did not differ from DBD pancreas transplantation in terms of patient survival, 1-year graft survival, or HbA1c after 1 year[18]. Still, an increased risk of graft thrombosis and bleeding has been reported[19–22].

Due to the inherent presence of a warm ischemia period in category 3 DCD compared to DBD procedures, the potential use of category 3 DCD pancreas for islet isolation and subsequent transplantation is unclear as there is a lack of larger studies[23–26]. Here we report on our extensive experience in isolation of pancreatic islets from DCD pancreas and our initial results on clinical outcome after transplantation of DCD islets.

2. **Materials & Methods**

2.1 Procurement

Donor pancreas were allocated to patients on the islet transplantation waiting list according to Eurotransplant guidelines. Pancreas were declined for clinical use when one or more of the following conditions were met: history of diabetes mellitus or HbA1c ≥ 6.5% (48 mmol/mol Hb) age > 65 years, multiple cardiac arrests (DBD) or a combination of cardiac arrest and DCD, abdominal trauma, signs of current infection, or aberrant laboratory tests indicating pancreatic damage. For DCD procurements, pancreas were declined for allocation when the agonal phase (time from switch-off to cardiac arrest) was longer than 120 minutes. During DCD procurement procedures, systolic pressure, diastolic pressure and oxygen saturation were monitored during the agonal phase. A mandatory 5-minute no touch period after withdrawal of life support was observed for all DCD procurements. Also, the time of cardiac arrest and the start of the perfusion of cold preservation solution were recorded. In addition, for DBD and DCD pancreas, lukewarm ischemia time (LIT) was defined as the time between the start of cold preservation fluid perfusion and pancreatectomy. Cold ischemia time (CIT) was defined as the time between pancreatectomy and infusion of digestive enzymes into the pancreatic duct. These time periods are summarized in figure 1. Organs were transported in either UW (University of Wisconsin) solution, or HTK (Histidine-tryptophan-ketoglutarate) solution on ice to the human islet isolation laboratory of the Leiden University Medical Center in the Netherlands.

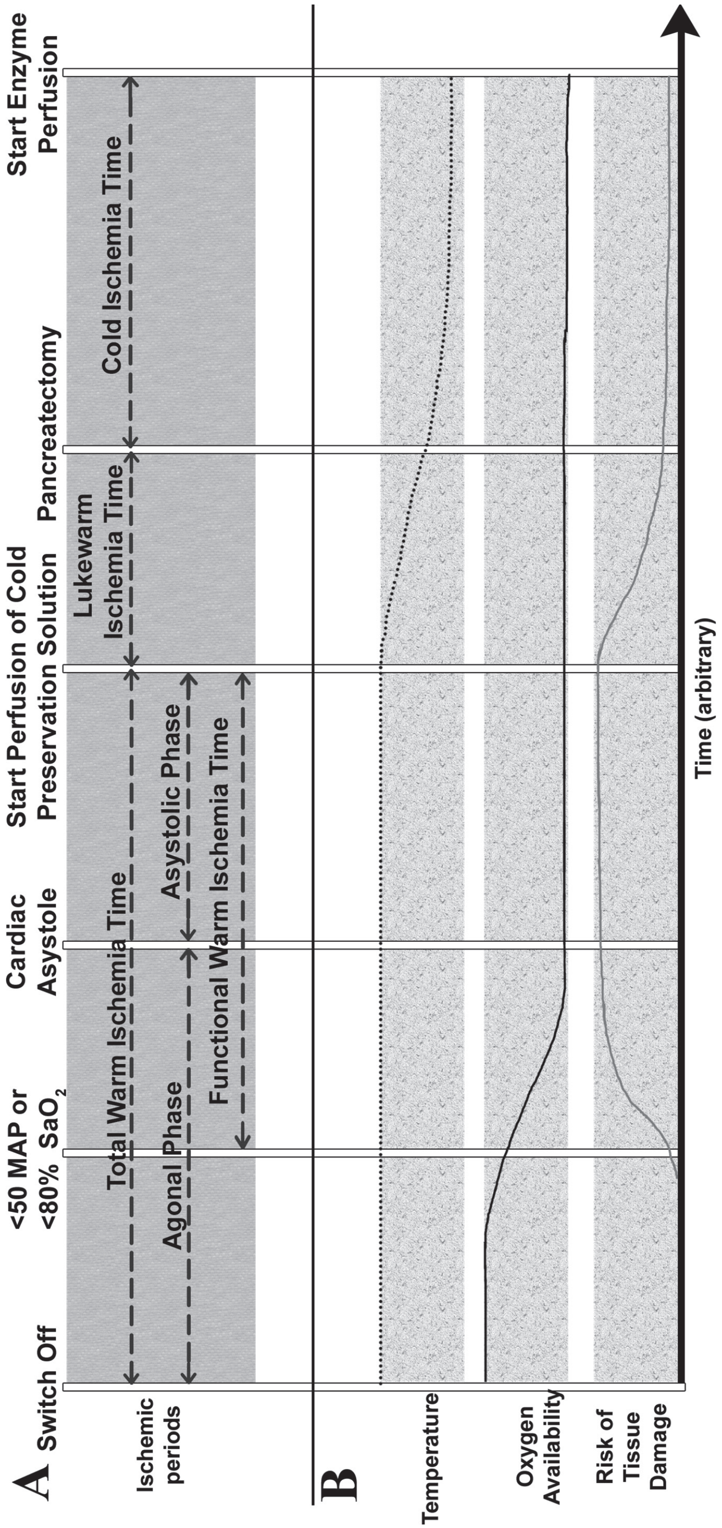
A
Switch Off
<50 MAP or <80% SaO2
Cardiac Asystole
Start Perfusion of Cold Preservation Solution
Pancreatectomy
Start Enzyme Perfusion
Ischemic periods
Total Warm Ischemia Time
Agonal Phase
Asystolic Phase
Functional Warm Ischemia Time
Lukewarm Ischemia Time
Cold Ischemia Time
B
Temperature
Oxygen Availability
Risk of Tissue Damage
Time (arbitrary)

Figure 1. Definitions regarding periods of ischemia in organ procurement and transportation until the start of islet isolation for DCD pancreas procedures. A). Critical hemodynamic moments leading to the start of cold preservation solution perfusion are recorded: life support "switch off", the moment when organ perfusion is inadequate (mean arterial pressure (MAP) drops below 50 and/or O_2 saturation drops below 80%), cardiac asystole (and thereafter a "no touch" period), the start of cold preservation fluid perfusion, pancreatectomy, and the start of enzyme perfusion. Several periods can be defined between these time points. The total warm ischemia (tWIT) time starts at the switch off of life support and ends when cold preservation solution is perfused. The agonal phase measures the length of time between ceasing circulatory support and cardiac asystole. Functional warm ischemia time (fWIT) indicates the time that organs experience warm, inadequate oxygenation and perfusion (time between the MAP dropping below 50 mm Hg and/or the O_2 saturation dropping below 80% until the start of cold preservation fluid perfusion). The lukewarm ischemia time (LIT) is defined as the time between the infusion of cold preservation fluid perfusion and pancreatectomy. The cold ischemia time (CIT) is the time from pancreatectomy until the infusion of digestive enzymes in the pancreas in the islet isolation facility. B). During the tWIT, a normothermic temperature persists. The temperature decreases during LIT when ice is packed into the abdominal cavity resulting in a gradual decrease in cell metabolism. In the CIT period, the temperature of the pancreas slowly plateaus. At the start of the agonal phase, the pancreas receives sufficient oxygenation. When perfusion becomes inadequate, at the start of the fWIT, the availability of oxygen to the pancreas decreases. Around the start of the asystolic phase there is anoxemia. The combination of a suboptimal temperature and insufficient oxygenation results in an increasing risk of tissue damage. Actual damage is an outcome of time combined with the risk of tissue damage.

2.2 Islet Isolations

Between 2008-2017 all human pancreatic islet isolations were performed at the Leiden University Medical Center using an adapted version of the semi-automated method[27]. The same islet isolation procedure was performed for both DBD and DCD organs. Briefly, after removal of peripancreatic tissue, the main pancreatic duct was cannulated with either an intravenous catheter at the head of the pancreas for retrograde enzyme infusion, or with 2 intravenous catheters inserted in the main pancreatic duct in the body of the pancreas for antegrade and retrograde enzyme infusion. When using the single catheter technique, a second 3.5 CH catheter was also inserted through the original catheter to reach the end of the tail of the pancreas. Hereafter, the pancreas was perfused by a pump with a blend of Collagenase NB1 (2000 Wünsch units, dissolved in 20 ml) and Neutral Protease NB1 (100-200 dimethyl-casein units, dissolved in 10-20 ml) for 15-45 mins (both enzymes from Serva Electrophoresis GmbH, Kuhlenwall, Germany). After full distention, the pancreas was cut, and the pieces were transferred to a digestion chamber. A 400 µm mesh was placed on top of the chamber to prevent outflow of undigested tissue. A continuous flow of Ringer's acetate (B. Braun, Melsungen, Germany) solution, supplemented with 5% 0.1 M sodium pyruvate (Lonza, Basel, Switzerland), 2.1 mg/ml nicotinamide (LUMC Pharmacy), 4.5 mM glucose (LUMC Pharmacy), 0.17 mM $NaHCO_3$ (Lonza), 25 mM HEPES (Lonza), Pulmozyme (Roche AB, Stockholm, Sweden), adjusted pH to 7.4 with 1M NaOH (LUMC Pharmacy) at 200 ml/min circulated through the system, while maintaining a temperature of 37^0C. The digestion chamber (Ricordi Isolator, Biorep, Miami) was shaken during the digestion phase. Digested pancreatic tissue was collected in 250ml conical tubes with 3 ml freshly thawed human serum, pooled, washed with UW solution (Bridge to Life, London, UK or Viaspan, DuPont, Wilmington, DE, USA), supplemented with 5% 0.1M sodium pyruvate, 1.2 mg/mL nicotinamide (LUMC Pharmacy), pulmozyme (Roche AB, Stockholm, Sweden), and stored at 4^0C. The digest was then purified in a continuous density gradient, made by mixing Biocoll (Biochrom Seromed KG, Germany) with a density of 1.100 g/ml with either UW solution (density 1.045 g/ml) or Biocoll (with a density of 1.077 g/ml) using two computer-controlled peristaltic pumps (Lambda, Switzerland) in an air-cooled COBE 2991 centrifuge (Terumo BCT, USA). A maximum of 30 ml pancreatic digest was loaded into the centrifuge for each purification run. After 5 minutes of spinning at 400g, the digest was harvested in 12 fractions and washed in Ringer Acetate solution (supplemented with 1% freshly thawed human serum (Sanquin, Amsterdam, the Netherlands). Selected fractions, based on purity and amount of embedding, were cultured using CMRL 1066 (Mediatech, Herndon, VA, USA), supplemented with 10% human serum, 10 mM HEPES, 2 mM L-glutamin, 50 µg/mL gentamycin, 0.25 µg/mL Fungizone (GIBCO BRL), 20 µg/mL Ciproxfloxacin (Bayer healthcare AG, Leverkusen, Germany) at 37^{0}C in 5% CO_2. The purity and degree of embedding was assessed visually using dithizone staining and were verified by a second operator. The culture medium was refreshed one day later, and subsequently every day or every other day for up to 5 days.

Islet yield (in islet equivalents, IEQ) was determined[28] after isolation (day 0) and the number of IEQ was also assessed after the first medium change (MC1) one day after isolation, and after the second medium change (MC2), generally 2 or 3 days after isolation.

2.3 Glucose stimulated insulin secretion test

Functionality of isolated islets was tested at MC1 using a dynamic glucose stimulated insulin secretion (GSIS) test. Islet samples (±20 islets), were collected and were placed in filter-closed chambers (Suprafusion 1000, Brandel, Gaithersburg, USA) and perifused at 500 µl/min at 37^{0}C. First, islets in each channel were preconditioned by perifusion with a low-glucose solution (1.7 mM glucose) solution (20 mM HEPES, 11.5 mM NaCl, 0.5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 2.4 mM $NaHCO_3$, supplemented with 0.2% human serum albumin in demineralized water) for 90 minutes. Thereafter, the islets were perifused with low-glucose solution for 15 minutes followed by a high-glucose solution (same as low-glucose solution, but with 20 mM glucose) for 60 minutes and finally with a low-glucose solution for 75 minutes. Fractions were collected at 7.5-minute intervals. The fractions were then measured for insulin using an immunoassay specific for human insulin (Mercodia AB, Uppsala, Sweden). The insulin concentration at each time-point was then divided by the average insulin concentration of the last three insulin concentrations during the low-glucose phase. This stimulation index per time point was averaged for DCD (n=27) and DBD (n=102) donors. To calculate the area under the curve of the stimulation indices, the stimulation index curves were integrated over time.

2.4 Islet Transplantations

Islet preparations were used for transplantation if the islet preparation was >5,000 IEQ/Kg recipient, the medium containing the islets was negative for Gram staining, the endotoxin test of the medium was ≤0.1 EU/Kg recipient, proper islet morphology was present, the islet purity was ≥30%, and the stimulation index of static glucose stimulated insulin secretion test was >1.5. If the yield from a single preparation was insufficient for transplantation, islet preparations could be maintained in culture for up to 6 days, to allow the possibility of combining two islet preparations in one infusion procedure.

2.5 Patients

Patients in our study had severe beta cell failure and were referred to the transplantation outpatient clinic of the Leiden University Medical Center (LUMC) for beta cell replacement therapy. They were considered eligible for islet-after-kidney, islet-after-lung or islet alone transplantation. Data regarding inclusion criteria and immunosuppression therapy have been published previously[29]. If after three months the preset treatment goals (i.e. HbA1c < 53 mmol/mol Hb without severe hypoglycemia, simplification or abrogation of the insulin regimen) were not met, additional transplantations could be performed.

2.6 Assessment of islet graft function

Three months after transplantation, mixed meal tests were performed in order to evaluate islet graft function[29]. In short, blood samples were drawn at -10, 0, 15, 30, 60, and 120 minutes. The values obtained for stimulated C-peptide (pmol/L) and glucose (mmol/l) were corrected for the transplanted islet dose (IEQ/kg recipient) for each test. Results were grouped according to type of islet graft (patients receiving only DBD preparations (n=31) or patients receiving only DCD islet preparations (n=9). Three patients received a combined DBD+DCD transplantation and where excluded from analysis.). The area under the C-peptide curve and area under the glucose curve were calculated for both groups. To evaluate clinical outcome the Igls[30] score at 1 year and 2 years after the last transplantation was determined for DBD and DCD islet graft recipients (supplementary table S2). Igls score 1 (Optimal) and score 2 (Good) were considered treatment success, and score 3 (Marginal) and score 4 (Failure) were considered unsuccessful treatments.

2.7 Statistical analysis

UNIANOVA (IBM SPSS Statistics v21, Chicago, IL, USA) was used for multivariate analysis of significant and relevant donor characteristic differences between DCD and DBD groups, namely age, BMI, sex, CIT, last reported glucose concentration, height. Student's t-test was used to calculate p-values for comparisons between DCD and DBD islets, ANOVA when more than two groups were compared, and chi-square test when comparing dichotomous variables, using GraphPad Prism 5.01 (La Jolla, CA, USA). Values are given as mean±standard deviation (SD), unless otherwise specified.

3. Results

3.1 Donor characteristics

In a nine-year period, islets were isolated from 384 donor pancreas. There were 126 category 3 DCD pancreas and 258 DBD pancreas. Donor characteristics are presented in Table 1. DCD donors were younger (46.6±13.1 vs. 51.8±12.0 years, *p<0.001*) and more often male (59.7% vs. 47.9%, *p=0.02*) compared to DBD donors. Furthermore, DCD donors had non-significantly longer hypotensive periods (i.e. systolic blood pressure < 80 mmHg prior to donation) than DBD donors (41.2±66.5 vs. 23.0±26.6 minutes, p=0.08), but were also given vasopressor support less often (56.5% vs. 87.4%, *p<0.001*). The last measured glucose concentration was lower (7.9±2.7 vs. 9.4±3.1 mmol/L, *p<0.001*) in DCD donors. The average functional WIT in the DCD group was 23.2±6.4 min. Other donor characteristics did not differ significantly between the groups.

3.2 DCD and DBD pancreas characteristics before and during islet isolation

The mean trimmed weight of the pancreas was similar in the DCD group compared to the DBD group (112±25.4 vs. 107±26.5 grams, p=0.06), as presented in Table 2. Also, mean cold ischemia time was not significantly different in the DCD group (9.17±3.40 vs. 8.50±3.20 hours, DCD vs DBD respectively, p=0.06). Until 2013, HTK solution was preferred for DCD procedures, and this resulted in a significant difference in the type of perfusate used during procurement (60.1% UW usage for DCD procedures, 87.7% UW usage for DBD procedures, *p<0.001*).

During islet isolation, enzymatic digestion was more complete during DCD pancreas than DBD pancreas isolation (14.1±0.8% vs. 16.5±1.0% undigested tissue after digestion *p=0.03*). However, after multivariate analysis, this difference was no longer significant (difference DCD-DBD: -0.054±0.759, p=0.84). The total volume of tissue to be purified did not significantly differ between DCD and DBD pancreas. (43.8±18.0 vs. 40.9±17.7 ml, p=0.14.

	DCD	DBD	*p* value
Age (yrs)	46.6±13.1	51.2±12.0	*p<0.001*
Sex (% Male)	59.7	47.9	*p=0.02*
Weight (kg)	80.1±16.6	78. 9±16.4	p=0.49
Height (cm)	176.6±9.2	174.3±9.6	*p=0.03*
BMI (kg/m^2)	25.4±4.3	26.0±4.2	p=0.21
Stroke or SAB (%)	45.2	69.6	*p<0.001*
Hypotensive period registered	22%	32%	p=0.05
Hypotensive period duration (min)	41.2±66.5	23.0±26.6	p=0.08
Creatinine (μmol/l)	77.1±34.3	73.7±33.7	p=0.37
Bilirubin (μmol/l)	10.4±6.6	11.7±8.2	p=0.15
GGT (U/L)	63.7±90.8	63.8±135.1	p=0.99
ALT (U/L)	66.6±167.5	51.7±81.4	p=0.26
AST (U/L)	80.1±141.6	63.8±91.4	p=0.19
Lipase (U/L)	42.4±68.3	33.3±41.5	p=0.29
Amylase (U/L)	130.6±227.3	144.2±223.5	p=0.61
HbA1c (mmol/mol)	35.0±4.1	36.0±5.0	p=0.13
HbA1c (%)	5.3±0.37	5.4±0.46	
Vasopressor use (%)	56.5	87.4	*p<0.001*
Blood Glucose (mmol/L)	7.9±2.7	9.4±3.1	*p<0.001*
Donor Points[33]	64.6±12.1	66.6±10.4	p=0.09
NAIDS[71]	35.6±14.8	33.4±16.2	p=0.21

Table 1. Donor characteristics of included donation after brain death (DBD) and donation after circulatory death (DCD) donors. Values are given as mean±SD (n). BMI: Body Mass Index, SAB: Subarachnoidal Bleeding, GGT: Gamma-glutamyl. Transferase, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, HbA1c: Hemoglobin A1c. NAIDS: North American Islet Donor Score. DCD n=126. DBD n=258.

	DCD	DBD	*p* value
UW solution (%)	60.2	87.7	*p<0.001*
Pancreas Weight (g)	112.2±25.4	106.9±26.5	p=0.06
Functional WIT (min)	23.2±6.4	NA	*NA*
CIT (hr)	9.2±3.4	8.5±3.2	p=0.06

Table 2. Pancreas, preservation and procurement parameters of donation after brain death (DBD) and donation after circulatory death (DCD) donors. Values are given as mean±SD (n). CIT: Cold Ischemia Time. UW: University of Wisconsin solution. WIT: warm ischemia time. DCD n=126. DBD n=258.

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3.3 Isolation Outcome

Post-purification, the islet yield from DCD pancreas was 84,502 IEQ lower compared to DBD pancreas (*p=0.003;* Figure 2). When accounting for the mass of a pancreas, DCD pancreas also yielded fewer islets per gram tissue (1432 IEQ/g less, *p<0.001*). There was also a difference in volume between DCD and DBD preparations post-purification. After density separation, the culture tissue volume was 353 µL lower for DCD pancreas, than for DBD pancreas, (*p=0.02;* Table 3*)* Not only did DCD pancreas have a lower islet yield and a lower culture tissue volume, islets from DCD pancreas separated worse during density separation. This was reflected in the maximum purity obtained after purification which was lower in DCD pancreas (84.7±13.9%) than DBD pancreas (88.2±12.9%, *p=0.01*). However, when examining the average purity of islets brought into culture, DCD and DBD preparations did not differ (55.1±14.2 vs. 56.5±16.2%, p=0.43. During culture, the number of IEQ decreased in both groups but the percentage decrease did not differ between DCD and DBD islets.

After multivariate analysis, the differences in IEQ, IEQ/g, maximum purity, and islet culture volume persisted (*p<0.01, p<0.01*, *p=0.01,* and *p=0.01* respectively). No significant correlations were found between different warm ischemia periods and islet yield (Figure 3).

3.4 In Vitro Functionality

Islet preparations considered for transplantation were assessed by GSIS. No significant differences were found between DCD and DBD islets in terms of peak stimulation index or area under the stimulation index curve (Figure 4).

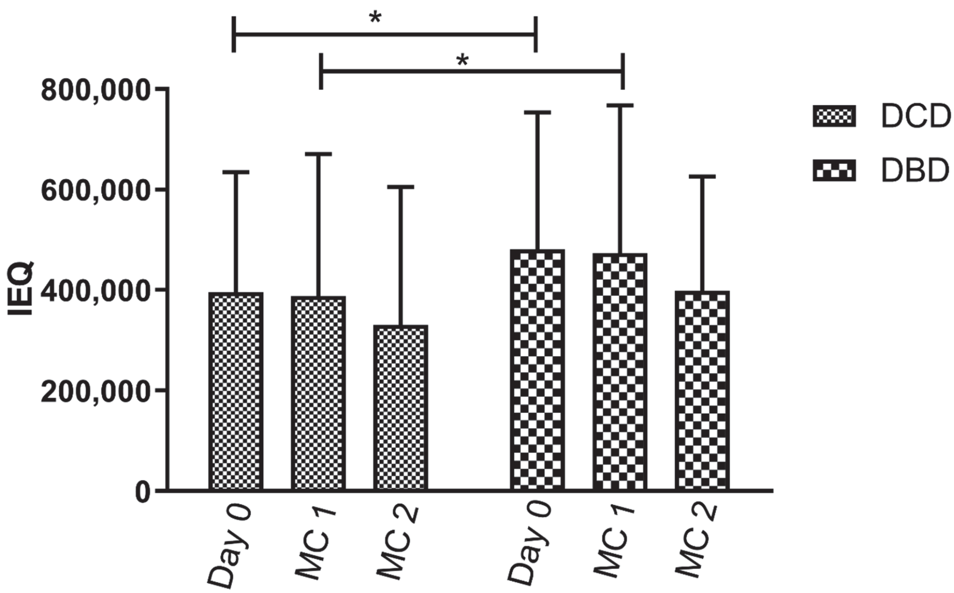

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Figure 2. IEQ of DBD and DCD isolations immediately after isolation (Day 0), at one day (MC1) and at 2-3 days (MC2) after isolation. At day 0 DCD islet yield was 395,515 IEQ (239,287) and DBD islet yield was 480,017 IEQ (273,449; p=0.003). The decrease in IEQ after successive medium changes (MC1 and MC2) was similar in DCD and DBD pancreas. DCD n=126. DBD n=258.

	DCD	DBD	*p*
IEQ day 0	395,515±239,287	480,017±273,449	*p=0.003*
IEQ MC1	387,341±283,763	472,831±294,768	*p=0.014*
IEQ MC2	330,112±275,280	398,399±227,854	p=0.132
Change in IEQ day 0→MC1 (%)	-6.0±38.2	-6.88±32.3	p=0.83
IEQ drop >30%, >40%, >50% (%)	26.7, 16.8, 8.9	23.4, 14.0, 5.4	
Change in IEQ day 0→MC2 (%)	-30.1±32.2	-25.0±27.62	p=0.34
Undigested tissue (%)	14.1±0.81	16.5±1.02	*p=0.03*
Prepurification pellet volume (ml)	43.8±18.0	40.86±17.71	p=0.14
Culture tissue volume (µl)	1724±1093	2077±1554	*p=0.02*
IEQ/g digested tissue	4254±2577	5686±3220	*p<0.001*
Average Purity (%)	55.1±14.2	56.5±16.16	p=0.43
Maximum Purity (%)	84.7±13.9	88.2±12.9	*p=0.01*

Table 3. Islet isolation outcome parameters. Values are given as mean±SD. IEQ=islet equivalents. MC1=First medium change on day 1. MC2=second medium change usually on day 2-3. In multivariate analysis IEQ day 0 (p<0.01), IEQ MC1 (p=0.03), Culture tissue volume (p=0.01), IEQ/g digested tissue (p=0.01) and maximum purity (p=0.01) remained significantly different. DCD n=126. DBD n=258

3.5 Transplantations with DCD and/or DBD islets

Included patients were administered a single DCD or a single DBD islet preparation or a combined preparation (two DCD or two DBD islet preparations). Three months after the islet transplantation, insulin secretary capacity of the grafts was measured after a mixed meal challenge. The area under the C-peptide curve was similar between DCD and DBD graft recipients (figure 5b, p=0.41). In addition, the area under the glucose curve was not different between the 2 groups (figure 5d, p=0.94)

To determine clinical graft function, Igls scores 1 year and 2 years after the last transplantation were calculated (figure 6). After one year, treatment success was attained in 89% DCD recipients (n=9) and in 74% of DBD recipients (n=31, p=0.65). After two years, this diminished to 75% (n=8) and 74% respectively (n=30, p>0.99).

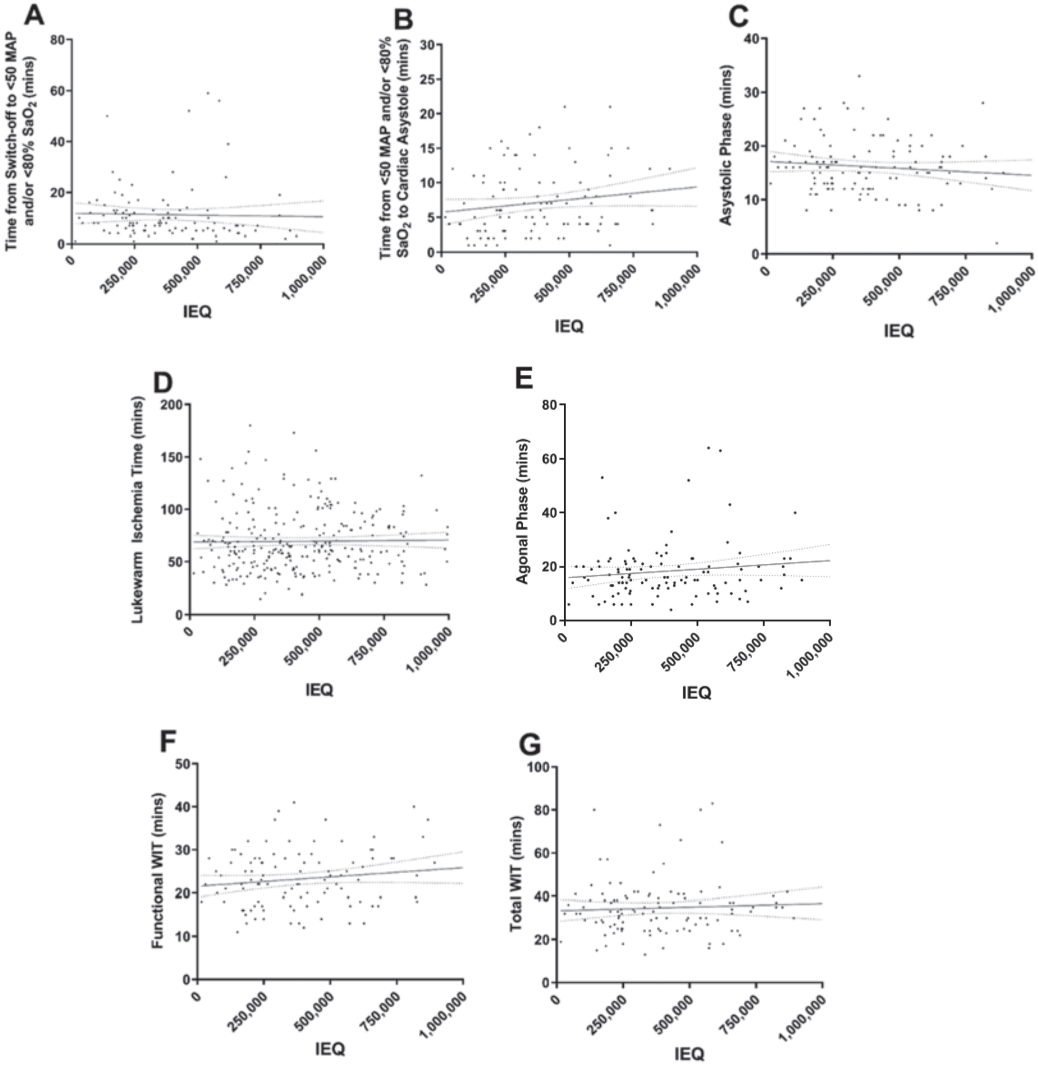

Figure 3. Association of ischemia periods with islet yield. A-D show the relation between ischemic time periods and islet yield. E-G show the relation between islet yield and agonal phase (E), functional WIT (F), and total WIT (G). No associations are significant.

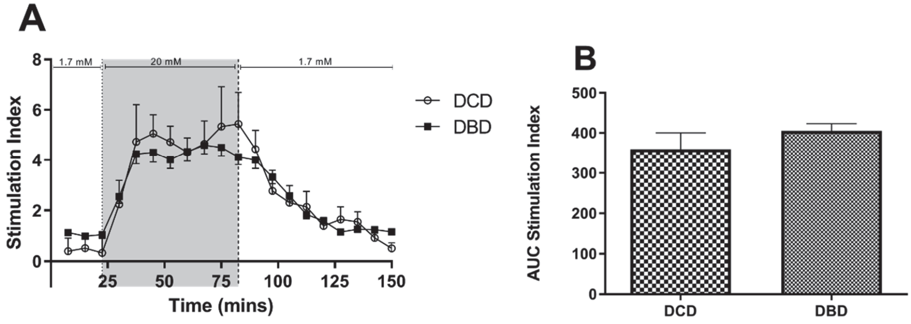

Figure 4. Dynamic glucose stimulated insulin secretion test. A.) After 1 day of culture, islets were perifused with a low glucose (1.7mM) solution, a high glucose (20mM) solution and finally the low glucose solution. The average of the last three low glucose values was defined as the baseline value. The insulin concentration at each time point was then divided by the baseline to give the stimulation index at each time point. A similar response is present in both DCD and DBD islets. Peak stimulation index values of DCD islets (5.4±2.7, n=27) and DBD islets (4.6±2.9, n=102) are not significantly different (p=0.30). B) The stimulation index curves were integrated over time to calculate the area under the curve of the stimulation index for DCD and DBD islets. No significant difference between DCD islets (295.0 ± 49.7, n=27) and DBD islets (270.7± 19.0, n=102) islets was observed, p=0.64.

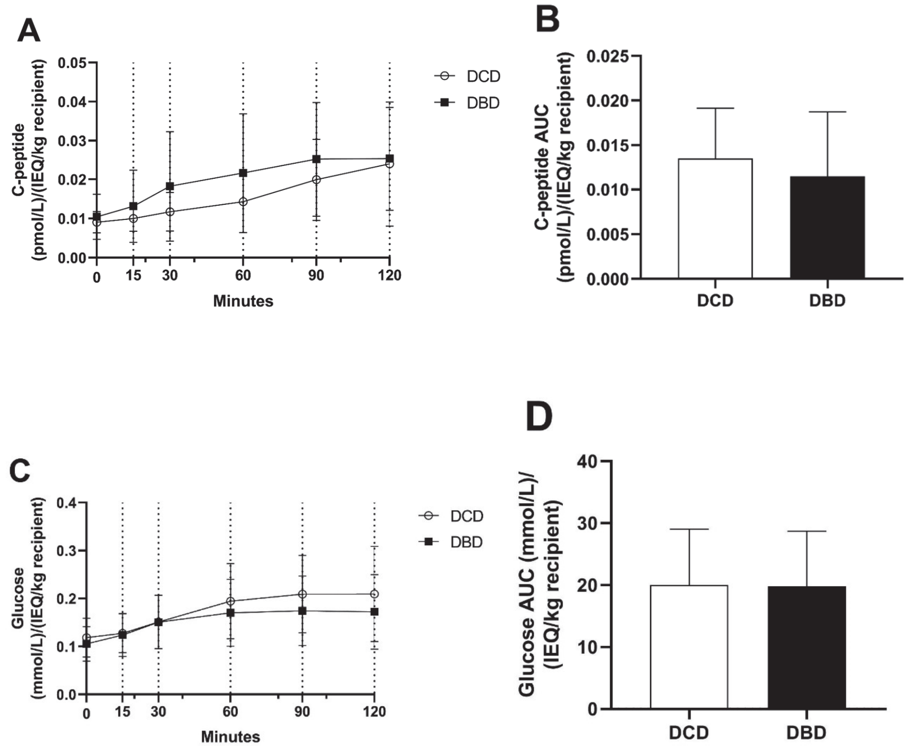

Figure 5. Graft function three months after islet transplantation. Mixed meal tests were performed in single or double DCD graft recipients (DCD, n=9)) and in single or double DBD graft recipients (DBD, n=31). C-peptide (pmol/l) and glucose (mmol/l) were corrected for the number of islets per kg recipient.. A) C-peptide concentrations during the mixed meal test. The increase in C-peptide was similar in DBD and DCD graft recipients. B) The area under the C-peptide curve was not different between DCD and DBD graft recipients (DCD 0.013±0.0057, DBD 0.011±0.0072, p=0.41). C) Glucose concentrations during the mixed meal test. The increase in glucose was similar in DBD and DCD graft recipients. D) The area under the glucose curve was not different between DCD and DBD graft recipients (DCD 20.0±9.0, DBD 19.8±8.9, p=0.94).

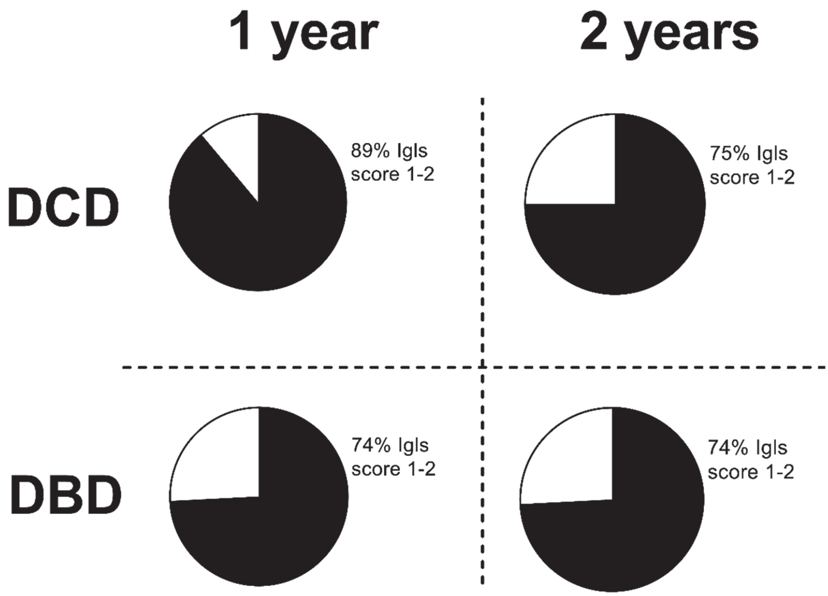

Figure 6. Igls scores were assessed at 1 year (A and C) and 2 years (B and D) after the islet transplantation. A) After one year, 89% of DCD islet graft recipients and 74% of DBD islet graft recipients has Igls score 1 or 2 indicating treatment success (p=0.65). At two years after the last transplantation this was 75% and 74% in DCD and DBD islet graft recipients, respectively (p>0.99). DCD n=9, DBD n=31 at 1 year. DCD n=8, DBD n=30 at 2 years.

Discussion

The main findings of our study on islet isolation from 126 category 3 DCD pancreas indicate that DCD pancreas can be used for islet isolation and transplantation. Islet secretory function and clinical outcome were similar up to 2 years after islet transplantation in recipients receiving only DCD islet grafts compared to recipients receiving only DBD grafts.

Donor characteristics from the DCD and DBD groups differed in several aspects. The DCD donors were younger, were more often male, had a longer hypotensive period, required less vasopressor support and the last glucose concentration was lower. Of these factors, age and sex of the donor have been shown to have an influence on the isolation results in several previous publications[31,32,41–47,33–40]. These data indicate that in our center donor characteristics are more favorable for DCD donors compared to DBD donors. Also in other transplantation fields, DCD organ acceptance is characterized by more favorable other donor characteristics[15,48].

In a porcine study, a warm ischemia time of more than 30 minutes impaired *in vitro* islet function[49]. In our cohort of category 3 DCD and DBD pancreas, islet functionality *in vitro* is not affected by organ procurement type, as evidenced by dynamic glucose-stimulated insulin secretion. It should be noted that the average functional warm ischemia time was 23.2 minutes with a maximum of 41 minutes. Our findings of the responsiveness of DCD islets to glucose are in line with previous studies which used static glucose stimulated insulin secretion tests[23,25,26].

Using identical isolation protocols, our results showed an approximately 85,000 lower post-purification IEQ after islet isolation from DCD pancreas compared to DBD pancreas. Our study on 126 DCD pancreas is in line with one other study that observed 100,000 IEQ less from 10 DCD pancreas[24]. Two previous studies with relatively small numbers of category 3 DCD pancreas (≤ 15 per study) reported similar islet yields obtained from category 3 DCD and DBD donors[23,25] and one small study reported 100,000 more IEQ from 10 DCD pancreas[26],

Several studies have been reported on controlled DCD procedures from Japan in which a rapid *in situ* regional organ cooling technique was used[34,50–52]. This allows for much shorter warm ischemia times (WIT of 4.2±0.7 mins[51]), compared to what is possible using category 3 DCD procedures. The initial results from 10 category 4 DCD pancreas yielded a mean IEQ >400,000, but were not compared to DBD pancreas[52].

Islet isolation from DCD pancreas resulted in a lower islet yield, but the warm ischemia, that is present during DCD and that can potentially adversely affect islet viability, does not appear to lead to a decline in islet number during a culture period of several days. Studies in rats have shown improved viability in DCD rat islets, compared to DBD rat islets after isolation[53,54]. Small human studies (<15 DCD pancreas for islet isolation) reported no difference in viability between DCD and DBD islets after isolation[25,26]. In islets from DCD pancreas, a correlation between longer WIT and lower ATP and GTP contents was found, suggesting that DCD islets may contain less energy reserves than DBD islets. Importantly, DCD islets were able to maintain blood glucose levels as well as DBD islets in mice seven days post-transplantation[26].

Three months after islet transplantation, no difference was observed in islet functionality after a mixed meal challenge in our cohort. Reports on transplantations with DCD islets are scarce. A single islet transplantation isolated from a DCD pancreas was reported in 2003, with a reduction in daily insulin requirement, improved glycemic control and absence of hypoglycemic events[24]. Another report using nine category 3 DCD and 196 DBD islets also reported no difference in insulin independence and decrease in insulin requirement between procurement types[25]. The Japanese Islet Transplant Registry published results from 18 recipients receiving category 4 DCD islet preparations[55]. After three years, 33.6% maintained a C-peptide level more than or equal to 0.3 ng/mL. All recipients remained free of severe hypoglycemic events and three achieved insulin independence for 14, 79, and 215 days in that study. We observed that DCD islet preparations did not negatively affect patient's clinical treatment outcome using the Igls score at one and two years after transplantation.

Different recommendations for a maximal total WIT for islet isolations have been made in the past (30, 45, and 60 minutes). These were based on either data from kidney transplantations, porcine studies or were inferred from small studies in which a statistically significant cut-off could not be found[24,25,56,57]). In our study, the duration the total warm ischemia time or other periods within this time frame did not affect the yield. Thus, other factors during the DCD procedure are likely to play a more important role. Exactly which physiological differences occur during DCD procurement that lead to a lower IEQ yield remains a question for further research.

Previous research has found a multitude of donor characteristics which can have an effect on islet isolation outcome: a well-trained local procurement team[40,41,45,58], donor age[31,32,41–46,33–40], batch and type of collagenase[38,40,43,59–62], sex of the donor [40–42], CIT[35,43,46,63–66], amylase[67], preservation solution[68,69], and BMI[40,42,45,46,70,71]. The discrepancy in islet isolation yield between DCD and DBD pancreas may diminish in the future due to developing technologies such as normothermic regional perfusion prior to procurement and machine perfusion (after procurement)[68,72].

When a cautious approach is used related to donor characteristics, islet isolations from category 3 DCD pancreas result in a lower yield than isolations from DBD pancreas, but DCD islets are as functional in vitro and after clinical transplantation as DBD islets.

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