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Hypothermic Oxygenated Machine Perfusion of the Human Donor Pancreas

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Background. Transplantation of beta cells by pancreas or islet transplantation is the treatment of choice for a selected group of patients suffering from type 1 diabetes mellitus. Pancreata are frequently not accepted for transplantation, because of the relatively high vulnerability of these organs to ischemic injury. In this study, we evaluated the effects of hypothermic machine perfusion (HMP) on the quality of human pancreas grafts. **Methods.** Five pancreata derived from donation after circulatory death (DCD) and 5 from donation after brain death (DBD) donors were preserved by oxygenated HMP. Hypothermic machine perfusion was performed for 6 hours at 25 mm Hg by separate perfusion of the mesenteric superior artery and the splenic artery. Results were compared with those of 10 pancreata preserved by static cold storage. **Results.** During HMP, homogeneous perfusion of the pancreas could be achieved. Adenosine 5′-triphosphate concentration increased 6,8-fold in DCD and 2,6-fold in DBD pancreata. No signs of cellular injury, edema or formation of reactive oxygen species were observed. Islets of Langerhans with good viability and in vitro function could be isolated after HMP. **Conclusions.** Oxygenated HMP is a feasible and safe preservation method for the human pancreas that increases tissue viability.

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W orldwide almost 40 million people currently have been diagnosed with type 1 diabetes mellitus, which is best characterized by immune-mediated destruction of

pancreatic beta cells.[1] Patients with uncomplicated type 1 diabetes mellitus are usually treated with insulin injections. In a selected group of patients, replacement of functioning beta cells by whole pancreas transplantation (PT) or islet transplantation (IT) is the treatment of choice.[1-3] Results of PT have significantly improved over the last decade, mainly due to better surgical techniques and improved immunosuppressive strategies.[4] Pancreas transplantation is, however, a complex procedure with considerable perioperative morbidity and mortality, requiring a strict recipient screening. In contrast, IT is technically less complex and has lower morbidity. However, because of the exposure of islets to multiple series of physiochemical stress factors during isolation and immediate blood-mediated inflammatory reaction during transplantation, there is considerable islet loss requiring often 2 and sometimes 3 donor pancreata per recipient to achieve insulin independence.[5] Islet transplantation has the potential to stabilize blood glucose levels, stop hypoglycemic unawareness and may contribute to the prevention of diabetic sequelae.[2,6]

Despite the encouraging results, pancreas and IT are less often performed compared to kidney and liver transplantation.[3] A key factor is the shortage of good quality pancreas grafts. Compared to other organs, the pancreas is more vulnerable to injury related to brain death and the actual organ retrieval operation.[7] 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.[3] Due to donor organ shortage many centers will now accept organs from older and higher-risk

donors, that is, from extended criteria donation after brain death (DBD) donors and from donation after circulatory death (DCD) donors. Donation after circulatory death donation for islet isolation is still uncommon.[8,9] Andres et al showed that DCD pancreata gave similar islet yield and clinical outcomes as DBD pancreata. However, acceptable factors for acceptation of DCD pancreata remain to be defined.[10] Donation after circulatory death pancreata are thought to be inferior because the warm ischemia period and more pronounced ischemia-reperfusion injury leads to a significant depletion of adenosine 5′-triphosphate (ATP) and accumulation of toxic metabolites.[3,11] The current challenge is to increase the donor pool by utilization of these extended criteria donor pancreata without compromising transplantation outcomes. One method to achieve this is by improvement of pancreas preservation techniques. Currently, static cold storage (SCS) is the standard preservation method for the pancreas. A plausible alternative is hypothermic machine perfusion (HMP), providing a continuous arteriovenous circulation of a dedicated preservation solution. In kidney transplantation, clinical trials and registry data have demonstrated superiority of HMP over SCS, while in liver transplantation there is now evidence that HMP may enhance the quality of higher-risk livers before transplantation.[12-17] It is suggested that the positive effect of HMP is due to the continuous perfusion of the microvascular system, resuscitating ATP levels in the tissue and removing toxic waste products.[9,12,18-20] While effective for kidneys and livers, the impact of HMP on enhancing human pancreas quality, by reducing reperfusion injury has remained unexplored.

In this study, we evaluated the feasibility of oxygenated HMP in discarded human donor pancreata. We developed a customized dual arterial system, allowing separate perfusion of both main arteries supplying the pancreas graft. Viability and injury markers were analyzed and compared with those of SCS preserved pancreata. Both DCD and DBD donor pancreata were included to analyze preservation effects in both groups.

MATERIALS AND METHODS

Donor Pancreata

Between May 2013 and May 2015, 20 human pancreata retrieved from multiorgan donors in The Netherlands were included in this study. The pancreata were declined for pancreas and islet transplantation and permission for research was given by the relatives of the donor. This study was approved by the medical ethical committee of the University Medical Center Groningen and the Dutch Transplantation Foundation as the competent authority for Organ Donation in the Netherlands. The pancreata were retrieved by regional multiorgan recovery teams according to standard methods. Just before stapling the duodenum, 50 mL of povidone iodine was administered via the nasogastric tube. All pancreata were stored in University of Wisconsin Cold Storage Solution (Bridge to Life, London, United Kingdom).

Experimental Design

After a period of SCS during transportation to the study center, pancreata were assigned to 2 preservation groups. The experimental group underwent 6 hours of HMP (n = 10), the control group underwent an additional 6 hours of SCS (n = 10). In both groups, 5 DCD and 5 DBD pancreata were

included (Figure 1). Characteristics of the donor pancreata and ischemia times are shown in Table 1. No significant differences were observed in donor age, body mass index, donor serum amylase and lactate dehydrogenase (LDH), warm ischemia time (WIT), back table procedure time and total cold ischemia time (CIT).

Back Table Procedure

After the pancreata arrived at the study center, a back table procedure was performed, which included tissue biopsies, splenectomy and ligation of the gastroduodenal artery. The pancreata in the experimental group were prepared to be connected to the HMP device: the superior mesenteric artery (SMA) and splenic artery (SA) were cannulated by using a hose barb with a luer lock end, connecting a cannula. The artery was secured to the hose barb with a cable tie. The portal vein was left open to ensure passive drainage of perfusion fluid. The pancreas was placed in a pancreas chamber of the device, partly submerged in cold University of Wisconsin Machine Perfusion Solution (Bridge to Life, London, United Kingdom). The arteries were flushed with perfusion solution using a syringe to detect leaks of small vessels, which were corrected using vascular clips.

The HMP device consisted of a dual perfusion system using a pressure controlled recirculation circuit, with 2 centrifugal pumps (Deltastream DPII; MEDOS Medizintechnik AG, Stolberg, Germany) providing a pulsatile flow through the mesenteric and splenic arteries, separately. Perfusion pressure was set at 25 mm Hg in both systems. The perfusion solution (1 L) was oxygenated by 2 hollow fiber oxygenators (HILITE 800 LT; MEDOS Medizintechnik AG) with a fixed oxygen flow of 100 mL/min. The temperature of the perfusion solution was kept between 4°C and 7°C by placement of the system in a cool box filled with ice. To measure perfusion efficacy, Acridin Orange (AO, 0.1 mg/L) was added to the perfusion solution in 6 out of 10 HMP preserved pancreata. When duodenal distention occurred, a duodenal drain was needed. Figure 2 shows the setup of the HMP device. Because the machine is running on batteries, HMP could be performed during transport.

Data Collection

Baseline wedge biopsies (0.5 cm³) were taken from all pancreata: 2 from the head, 1 from the body and 1 from
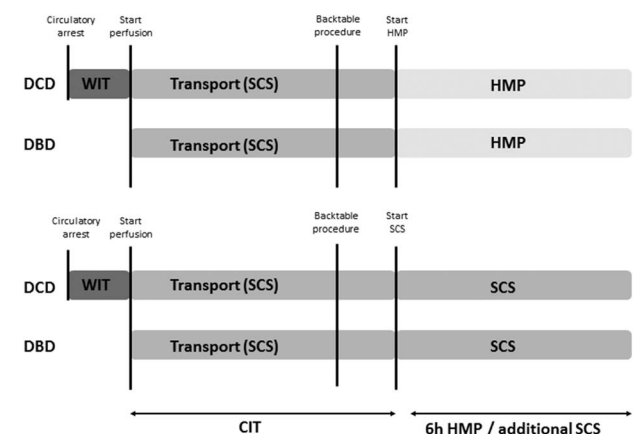

FIGURE 1. Schematic overview of the injurious periods in the pancreata as reflected by color intensity in the 4 different groups.

Donor characteristics

	HMP DCD (n=5)	HMP DBD (n=5)	SCS DCD (n=5)	SCS DBD (n=5)	*P*
Male, %	80	80	100	40	
Age, y	58 (51-64)	71 (53.5-73.5)	50 (20.5-63.5)	60 (51.5-67)	0.271
BMI, kg/m^2	23 (20.5-30)	26 (2.5-29)	27 (25-28.5)	29 (26.5-32)	0.259
Donor serum LDH, U/L	226 (188-182)	346 (180-510)	480 (199-480)	241 (230-440)	0.617
Donor serum amylase, U/L	88 (26-88)	121 (21-300)	47 (22-125)	106 (38-270)	0.673
WIT, min	18 (14-20)	-	21 (15-24.5)	-	0.292
CIT1, min	236 (219-268)	183 (143-299)	227 (212-228)	292 (250-300)	0.297
Back table procedure	60 (60-60)	90 (60-90)	30 (30-60)	30 (30-35)	0.262
CITtot, min	596 (579-628)	543 (503-659)	587 (572-588)	652 (610-660)	0.297

BMI, body mass index; CIT1, CIT (start cold perfusion at donor hospital - start back table procedure in study center); CITtot, total CIT (start perfusion at donor hospital - end of experiment). Results are shown as median and IQR.

the tail. These biopsies were snap frozen in liquid nitrogen and stored at –80°C for fluorescence microscopy, wet/dry ratio and measurement of ATP concentration. Another 2 biopsies were taken (head and tail) and fixed in 4% formaldehyde for histological analysis.

In the experimental group, perfusion flow was measured after 5, 10, 15, 20, and 30 minutes of HMP, followed by measurements every 30 minutes. Perfusion fluid temperature was recorded every hour. Samples (1 mL) of the perfusion solution were collected at 60-minute intervals during machine perfusion and before and after SCS in the control group. These samples were stored at –20°C for determination of enzyme levels, using standard biochemical assays. Another sample (200 µL) was taken every hour, snap frozen in liquid nitrogen and stored at –80°C for analysis of thiobarbituric acid reactive substances (TBARS) concentration, reflecting reactive oxygen species activity.

Markers of Viability and Injury

ATP concentration was analyzed by using the ATP Bioluminescence assay kit CLS II (Roche Diagnostics GmbH, Boehringer Mannheim, Germany). ATP and TBARS measurements were performed as described earlier by our laboratory.[21]

Wet-to-dry weight ratio of the tissue was measured as an indicator of edema. The weight of the biopsy was measured before (wet weight) and after (dry weight) the biopsy was dried in a heat block (VWR international, Leuven, Belgium) at 95° for 24 hours.

Histological Analysis

Cryosections (4 µm) of pancreas tissue were examined with a fluorescence microscope (Leica, Rijswijk, The Netherlands)
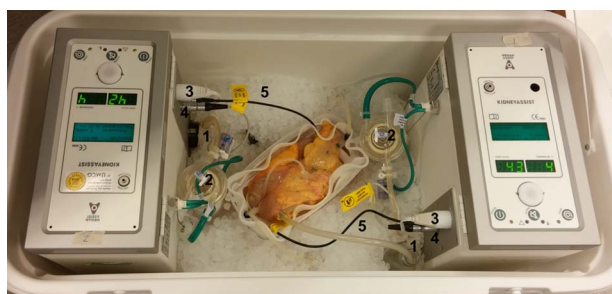

FIGURE 2. Dual arterial perfusion setup. 1, rotary pump; 2, oxygenator; 3, pressure sensor; 4, flow sensor; 5, temperature sensor.

with 495/519 nm filter for direct observation of the AO staining. Biopsies were fixed in 4% formaldehyde, subsequently embedded in paraffin and cut into sections of 4 µm. Light microscopy of hematoxylin and eosin–stained sections was performed to evaluate changes in morphology. No validated scoring system for histological analysis of donor pancreata exists, so a scoring system was developed, partly based on previous work by Karcz et al.[7] Morphometry was performed on the sections, assessing changes in 3 parameters: edema, acinar cell integrity loss and vacuolization. Each parameter was scored using the following semiquantitative scale: grade 0 (not affected), grade 1 (moderately affected) and grade 2 (severely affected). Caspase-3 staining (Cell Signaling, Asp175, Rabbit, 1:100) was performed to evaluate the extent of apoptosis. All slides were scored in a semiquantitative scale: the percentage islets scoring grade 0 (no caspase-3 staining), grade 1 (<50% of the islet stained positive for caspase-3) and grade 2 (≥50% of the islet stained positive for caspase-3). Examples of histological analysis are shown in **Figures S1 and S2**, http://links.lww.com/TXD/A138. The results were analyzed using the H-score of these grades. All sections were analyzed in a blinded fashion by 2 investigators (ML and GL) and confirmed by a blinded pathologist (NtH).

Islet Isolation Procedure

Two pancreata preserved by HMP were used for an islet isolation procedure at the human islet isolation laboratory (GMP facility) of the Leiden University Medical Center as described previously.[22-24] During transportation to Leiden, the pancreata continued to be perfused in the HMP device. Islet isolation started directly after arrival at the isolation laboratory. Perfusion time and total CIT did not differ from the other pancreata. After termination of HMP, the organ was surgically prepared; the pancreatic duct was cannulated with a cannula, after which the organ was perfused at 4°C to 10°C with collagenase NB1 and neutral protease (Serva Electropheresis, Germany), aiming for mild pancreas distension with minimal enzyme leakage. After distension, the pancreas was digested in a closed loop at 37°C, during which the released pancreatic tissue was collected and washed. Subsequently the islets were purified using density gradient separation in a COBE 2991 Cell separator (Terumo BCT, Lakewood, CO). Finally, the islets were cultured in CMRL-1066 solution (Corning-Mediatech, Manassas, VA) supplemented with 10% ABO-compatible human serum (Sanquin Bloodbank, The Netherlands) until they were assessed for quality. Islet

equivalent (IEQ) was calculated after assessment of purity and pellet volume of the islet product.[23] The number of isolated islets (expressed in IEQ and IEQ/g) as well as islet viability was tested.[25] In addition, islet preparations were subjected a static glucose-stimulated insulin secretion test performed at a low glucose concentration of 1.67 mmol/L and a high glucose concentration of 16.7 mmol/L. Briefly, pancreatic islets (n = 20) were subsequently incubated in Krebs buffers containing 1% human serum albumin and with a low or a high (45 minutes each) glucose concentration. From these incubations samples were taken and the concentration of secreted human insulin was determined by ELISA (Mercodia, Uppsala, Sweden). The stimulation index was calculated as (insulin concentration at 16.7 mM glucose) / (insulin concentration at 1,67 mM glucose).

Statistical Analysis

Continuous data were presented as median and interquartile range (IQR). For statistical analysis of independent samples, Mann Whitney U tests were performed. For paired samples, Wilcoxon signed-rank tests were performed. Kruskal-Wallis tests were performed to analyze data of >2 groups. Categorical variables were compared using the Fischer exact test. Results of the HMP and SCS preserved pancreata were compared in DCD and DBD pancreata separately. The level of significance was set at P less than 0.05. IBM SPSS Statistics version 23 for Windows was used.

RESULTS

HMP of the Human Pancreas Is Technically Feasible

In the DCD group, perfusion flow in the SMA was higher than in the SA during the first minutes of HMP ($P < 0.05$). Flow in the SA increased during HMP and became similar to flow in the SMA. After 360 minutes, median flow in both arteries was 36 mL/min. In the DBD group, flow increased nonsignificantly in both arteries and stabilized after 4 hours of HMP to 52 mL/min and 38.5 mL/min in the SMA and SA, respectively (Figure 3). There was no significant difference in flow between the DCD and DBD group. In the 6 pancreata where AO was added to the perfusion solution, AO staining was visible in all biopsies throughout the pancreas, demonstrating uniform perfusion (Figure 4). Median temperature of the perfusion fluid during HMP varied between 5.5°C and 7.0°C.

In 7 of 10 pancreata a duodenal drain was placed. In DCD pancreata 80 mL (IQR, 0-98 mL) and in DBD pancreata 70 mL (IQR, 40-120 mL) of fluid passively drained, which was a combination of duodenal content, povidone iodide and perfusion fluid.

Maintenance of Tissue Integrity

No significant differences were seen in edema formation, acinar cell integrity loss or vacuolization between HMP and SCS in both DBD and DCD pancreata. In both DCD and DBD pancreata, wet/dry ratio did not change after preservation and was equal in both the HMP and SCS group (Figure 5).

HMP Provides Increased Viability of the Pancreatic Tissue Without Signs of Injury

ATP concentration in the tissue is shown in Figure 6. At arrival in our hospital, ATP concentration in DCD pancreata was 8.4 (IQR, 3.0-15.6) μmol/g protein. ATP concentration

in DBD pancreata at this moment was significantly higher: 48.2 (IQR, 29.5-67.3) μmol/g protein ($P = 0.003$). After prolonged SCS, ATP concentration decreased further to 2.9 (IQR, 2.8-5.7) μmol/g protein in DCD pancreata and 22.8 (IQR, 20.6-46) μmol/g protein in DBD pancreata ($P < 0.05$). In contrast, after HMP ATP concentration in both DCD and DBD pancreata increased significantly ($P < 0.05$). In the DCD group a 6.8-fold increase and in the DBD group a 2.6-fold increase was observed. Interestingly, ATP concentration in DCD pancreata reached the same level of DBD pancreata: 100.5 (IQR, 49.4-169.8) and 109.3 (IQR, 65.2-301.5) μmol/g protein in DCD and DBD pancreata, respectively. In our laboratory using the same ATP assay, ATP level in normothermic sham livers was found to be around 60 μmol/g protein.[26]

Thiobarbituric acid reactive substance concentration in the preservation fluid was determined to analyze the formation reactive oxygen species. In all samples TBARS concentration remained low and almost nondetectable. No significant differences were seen between HMP and SCS preserved pancreata (data not shown). To evaluate the extent of apoptosis in the tissue, caspase-3 staining was performed. Only islets of Langerhans showed a positive caspase-3 staining, with no staining in the exocrine tissue of the same pancreas. No significant differences in caspase-3 staining were observed before and after HMP/prolonged SCS or between DBD and DCD pancreata.

Pancreatic Enzyme Levels

Amylase and lipase concentration in the perfusion solution increased significantly after 6 hours of HMP, as well as after 6 hours of SCS ($P < 0.05$). Amylase increased significantly during the entire period of HMP. Lipase increased predominantly during the first 3 hours of HMP after which a plateau was reached. The absolute increase in amylase and lipase was significantly higher after HMP compared to SCS in DCD pancreata (Figure 7A-D). No significant difference in absolute amylase and lipase increase between the 2 preservation methods was observed in DBD pancreata. Lactate dehydrogenase concentration in the perfusate was measured as a marker for cellular damage. Lactate dehydrogenase increased significantly after HMP as well as SCS ($P < 0.05$). The absolute increase in LDH after 6 hours of preservation did not differ between the groups (Figure 7E, F).

Islet Isolation (n = 2)

Islets were isolated from 2 HMP preserved DCD pancreata, retrieved from a 50- and a 52-year old donor (both males). Islet yield was 378.261 (3999 IEQ/g tissue) and 400.000 (6452 IEQ/g tissue) respectively. Viability was 94 and 98% after isolation and 90 and 92% after 3 days in culture. The insulin stimulation index was 2.2 and 1.9, respectively.

DISCUSSION

This is the first report describing a dual arterial hypothermic perfusion system for preservation of the human donor pancreas. With our system, uniform perfusion of the pancreas was achieved, without edema formation or tissue injury in hypothermic conditions. A substantial increase in ATP concentration was seen, indicating an improved viability after HMP. Viable and functioning islets could be isolated.
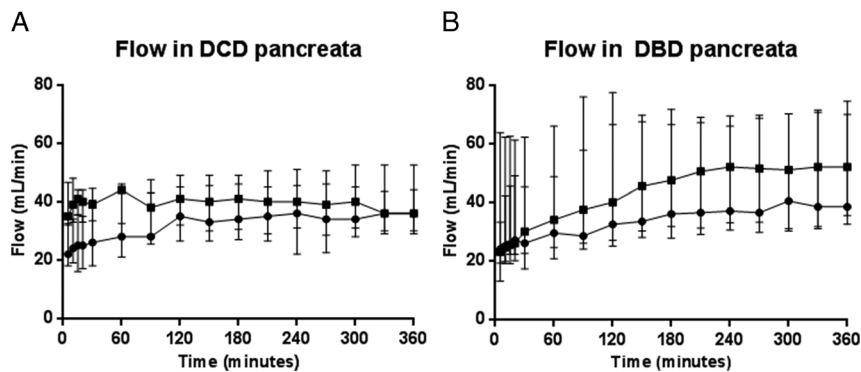
FIGURE 3. Perfusion flow during HMP. A, In DCD pancreata, perfusion flow in the SMA was significantly higher than in the SA in the first 5 minutes of HMP ($P < 0.05$). After 6 hours, median flow in both arteries was equal. B, In DBD pancreata, flow in both arteries showed a nonsignificant increase during HMP. Flow in both arteries did not differ during 6 hours of HMP. SA, represented by circles, SMA, represented by squares. Results are shown as median and IQR.

In this study, 20 human pancreata were preserved for 6 hours either by HMP or prolonged SCS after an initial period of SCS. The period of 6 hours was chosen to ensure a total cold preservation time of 9 to 11 hours, comparable to the average CIT in clinical pancreas and IT.[4] HMP was performed at 25 mm Hg using a pressure controlled perfusion system. In hypothermic conditions the pressure should be low to prevent the organ from barotrauma. 't Hart et al showed that 25% of the normal physiological pressure seems optimal in hypothermic liver preservation.[27,28] In pancreas perfusion studies, various perfusion pressures were used, ranging from 10 to 30 mm Hg in canine, porcine and human pancreata.[7,29-32] In a pilot study on porcine pancreata, different perfusion pressures were analyzed (10, 25, and 40 mm Hg). Twenty-five mm Hg seemed optimal for complete perfusion of the pancreas. In this study, perfusion flow stabilized after an initial increase in the first hours of HMP, which was also described earlier.[7,33] The quality of perfusion was assessed by fluorescent staining with AO in 6/10 pancreata. Staining with AO was visible in all these pancreata, indicating a complete and uniform perfusion.

One of the concerns related to pancreas preservation is the development of edema, which is generally regarded as undesirable as it has been shown to correlate with a lower IEQ after isolation.[11,34] In our study, no signs of edema formation were observed after HMP. The impact of edema formation due to HMP is however questionable since Taylor showed in a porcine study that despite edema formation after 24 hours of HMP, a more uniform digestion of the pancreas and thereby higher IEQ was realized.[31]

Histological analysis revealed neither loss of acinar cell integrity nor vacuolization which can be seen in apoptosis. This corresponds with the results from the caspase-3 staining in which both the DBD and DCD pancreata showed equal levels of apoptosis before and after HMP or prolonged SCS. It should however be acknowledged that these results might be an underestimation since no normothermic reperfusion was performed, which will lead to full expression of tissue injury.

In this study, the preservation fluid was oxygenated with 100% oxygen during HMP. There is no clear consensus about the need for oxygen supply during HMP but many researchers advocate the use of oxygen even in hypothermic conditions.[14-17] Hypothermia induces lowered metabolism reducing the oxygen requirement of the tissue. Hence, at 4° of Celsius the metabolism is 10% of normal.[35] Oxygenated

HMP is considered to support remaining metabolism and prevent a further decline of cell energy levels. When metabolism switches from an aerobic to anaerobic state, lactate and hydrogen ions accumulate in the tissue, leading to acidosis. This causes induction of lysosomal instability, activated enzymes, disrupted binding of transition ions to carrier proteins and decreased mitochondrial function.[2,35] A constant supply of oxygen along with adenosine as a precursor for ATP formation might result in increased ATP concentration during preservation.[14,36] Increased ATP concentration in pancreas grafts is associated with better outcome after transplantation.[2,37,38] Our results show that DCD pancreata are almost ATP deplete in contrast to DBD pancreata. This is thought to be the result of the period of warm ischemia, as has also been shown for kidneys and livers.[16,35,39] After HMP, a significant increase in ATP was seen in both DCD and DBD pancreata.

After HMP, no difference in ATP concentration between DCD and DBD pancreata is present anymore. This indicates that especially the more injured DCD organs benefit more of oxygenated HMP, which is in line with studies in kidneys and livers.[14,16,35] We hypothesize that HMP has the potential to improve viability of DCD pancreata toward DBD pancreata. In our experiments, formation of TBARS could not be observed, indicating that oxygenated HMP is a safe preservation method regarding to the formation of free oxygen radicals as is also shown by other groups.[36]
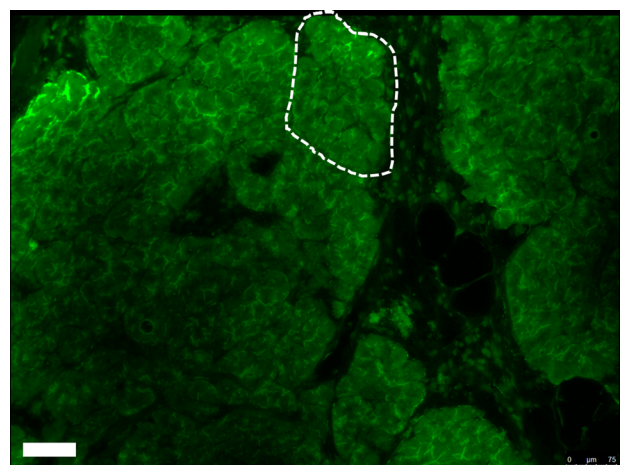

FIGURE 4. AO fluorescent staining after 6 hours of HMP. Scale bar indicates 75 μm. Dotted line indicates one of the pancreas lobuli.
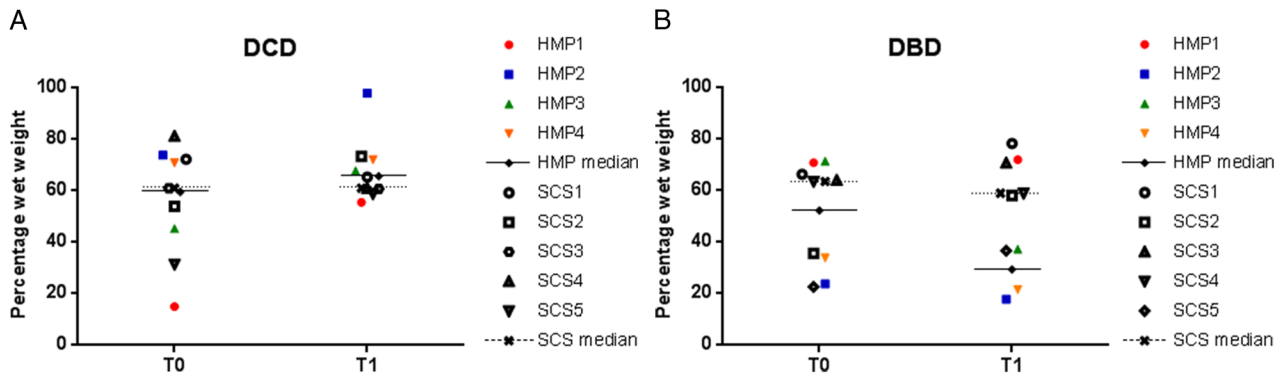
FIGURE 5. Percentage of wet tissue per pancreas. A, In DCD pancreata, wet tissue content did not change at T1, no significant differences between groups were seen. B, In DBD pancreata, wet tissue content decreased after HMP, however no significant differences were seen between groups. Closed colored figures represent HMP pancreata, open figures represent SCS pancreata. T0, before preservation; T1, after preservation.

Different amounts of duodenal drainage fluid were present in the pancreata. Unfortunately, because of fluid consistency and color, enzyme levels could not be measured reliably. Amylase and lipase concentration in the preservation solution increased significantly in both HMP and SCS preserved pancreata. As no differences in acinar cell integrity throughout the tissue was seen by histological analysis, we assume that this is partly due to the injury induced by taking multiple biopsies before preservation. This might lead to leakage of enzymes in the preservation solution. However, the absolute increase of amylase and lipase was significantly higher after HMP compared to SCS in the DCD group, and nonsignificantly higher in the DBD group. We hypothesize that this can be ascribed to the constant flush during HMP. The fact that LDH levels were the same in SCS and HMP preserved pancreata strengthens our hypothesis that the increased amylase and lipase levels are not due to acinar cellular injury during HMP.

Islet isolation was performed in 2 pancreata, to determine the potential to use perfused pancreas for islet isolation. An adequate amount of good quality islets could be isolated from both pancreata. According to the IEQ, both isolation procedures could be called an isolation success.[40,41] Islets were viable directly after isolation and after 3 days in culture

with a normal in vitro function. This results are in line with 2 other studies in which islet isolation after a period of HMP showed improved IEQ and viability compared to prolonged SCS.[32,42]

Limitations

The main limitation of this study is the lack of post-transplant evaluation. To evaluate the effect of preservation on the tissue in a more physiological environment, a normothermic (37°C) reperfusion model could be used to mimic transplantation. In this study we did not perform this kind of reperfusion, so we have to be careful with our conclusions. However, we were able to show that viable and functional islets could be obtained from 2 pancreata after HMP. Although numbers are small, we believe that more research into the use of HMP in pancreata from extended donor criteria donors for islet isolation is warranted.

CONCLUSIONS

In conclusion, this study demonstrates that oxygenated HMP of the human pancreas is technically feasible. Uniform perfusion of the pancreas was achieved and no signs of injury were seen under hypothermic conditions. Viability improved after HMP and functional isolated islets could be obtained.
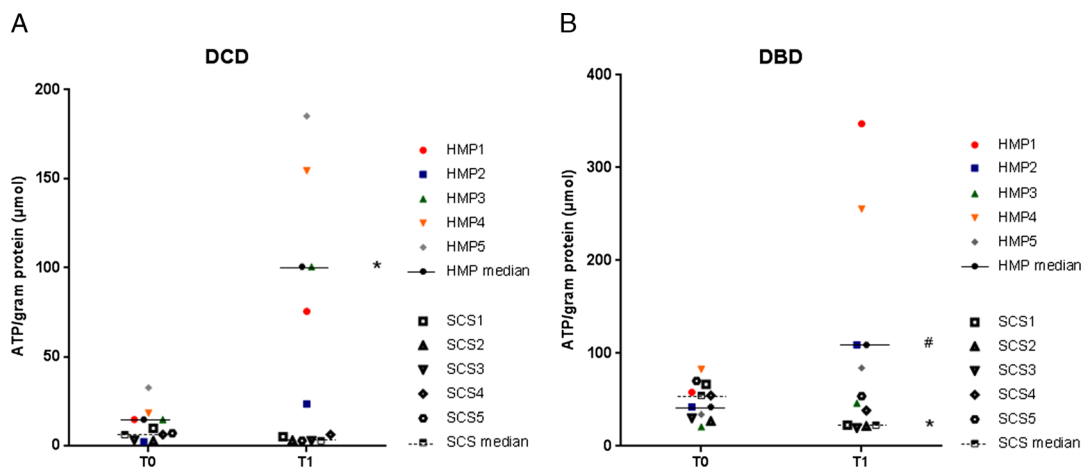

FIGURE 6. ATP concentration in the tissue before and after preservation in DCD and DBD pancreata. A, ATP concentration in DCD pancreata increased significantly after 6 hours of HMP (*$P < 0,05$). B, ATP in DBD pancreata decreased significantly after 6 hours of SCS (*$P < 0.05$) and increased significantly after 6 hours of HMP (#$P < 0,05$). Closed colored figures represent HMP pancreata, open figures represent SCS pancreata.
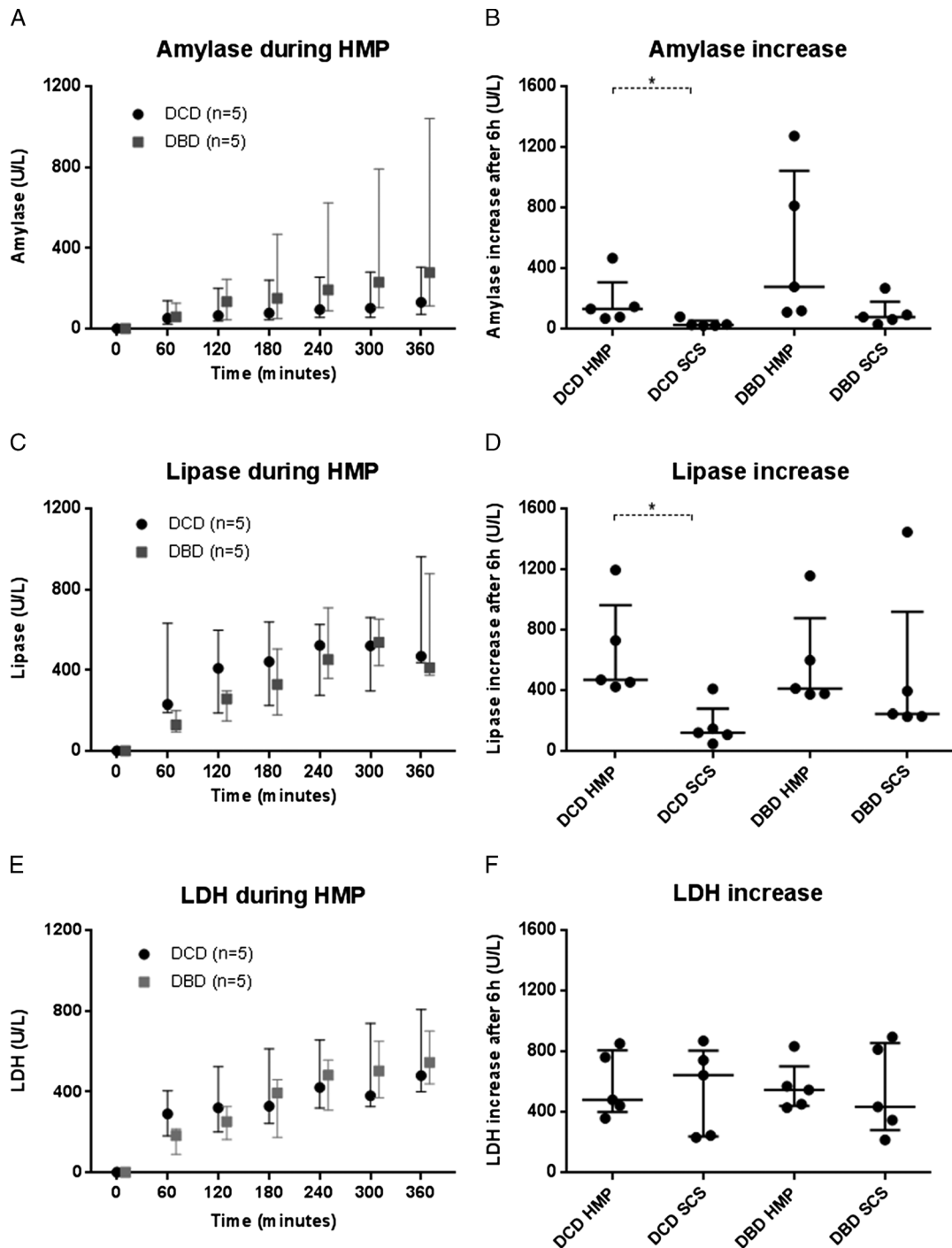
FIGURE 7. Enzyme concentration in perfusion fluid. A, B, Absolute amylase content in the perfusion fluid increased significantly after 6 hours of HMP in DCD and DBD pancreata ($P < 0.05$). The absolute increase in amylase content after HMP was higher than after SCS in the DCD group ($P < 0.05$). C, D, Lipase content increased significantly after 6 hours of HMP in DCD and DBD pancreata ($P < 0.05$). In the DCD group, the absolute increase in lipase was higher after HMP compared to SCS ($P < 0.01$). E, F, LDH increased significantly after 6 hours of HMP in both groups ($P < 0.05$). The absolute increase in LDH after preservation did not differ between the 4 groups. Results are shown as median and IQR.

We suggest therefore that HMP might help to increase the donor pool by improving the quality of pancreas grafts.

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