Improving the use of donor organs in pancreas and islet of Langerhans transplantation

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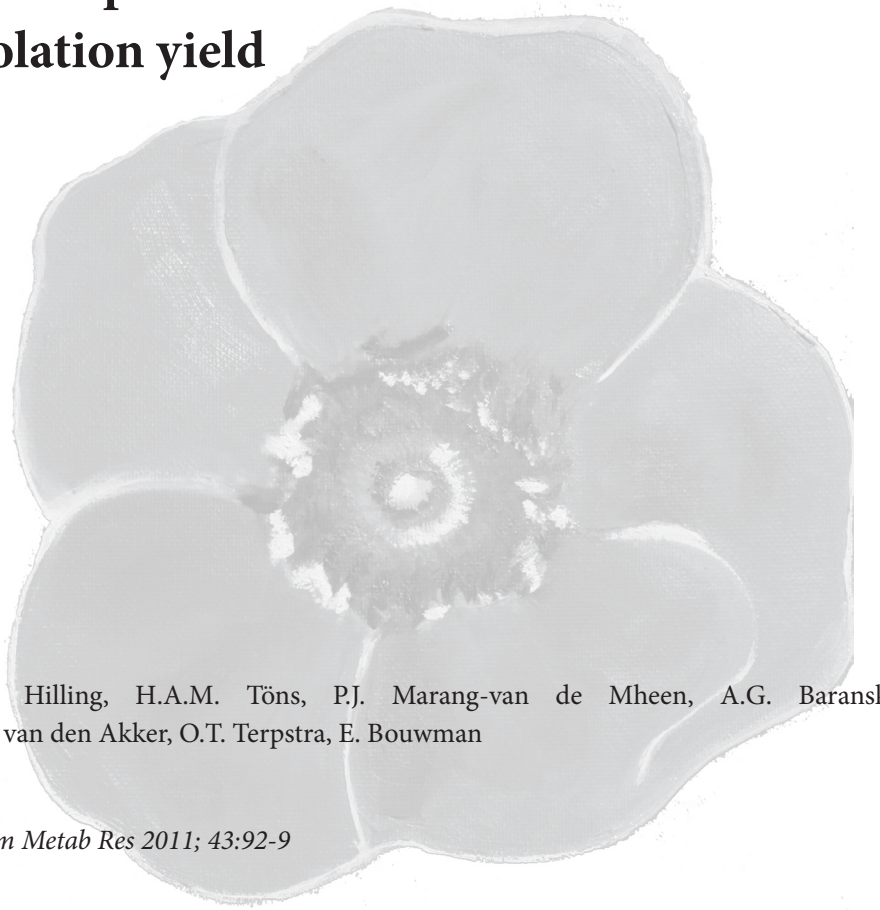
Chapter 5

Presence of hyperemic islets in human donor-pancreata results in reduced islet isolation yield



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ABSTRACT

Background

When studying histological characteristics of human donor-pancreata, a remarkably high number of hyperemic islets (HIs) was encountered. The abnormalities in these HIs ranged from single/multiple dilated vessels to hemorrhages extending into the exocrine tissue. We aimed to determine the relevance of the presence of HIs in human donor-pancreata for isolation outcome and to identify donor and procurement factors associated with the occurrence of HIs.

Methods and Materials

The presence of HIs was scored semi-quantitatively (HI-,HI+) in 102 human donor-pancreata. Islet isolation was performed in 40 cases. Donor and procurement factors were retrospectively analyzed in 94 donors.

Results

HIs were found in 54.6% of all donor-pancreata. However, only 4.5% of all islets in the affected pancreata was hyperemic. The affected pancreata contained slightly more endocrine tissue, but produced significantly lower yields. When corrected for other factors known to influence isolation outcome, the presence of HIs and endocrine content were the only factors significantly influencing isolation outcome. Prolonged ICU stay and pre-procurement hypertension were associated with the presence of HIs.

Conclusions

This study is a first indication that the presence of HIs in human donor-pancreata are associated with reduced isolation outcomes and suggest an impact of the procurement procedure and pre-procurement hemodynamic status of the donor on the islet quality. It is tempting to speculate that this contributes to the generally experienced difficulties in obtaining sufficient amounts of human islets.

INTRODUCTION

Transplantation of islets of Langerhans can improve metabolic control and quality of life in patients with longstanding type 1 diabetes (1-4). Sufficient islet numbers can be obtained from a single donor, but generally more than one islet preparation per recipient is required to obtain insulin independence (1, 3, 5-7). An important factor in determining the islet isolation outcome is the amount of endocrine tissue present in a specific pancreas. However, a high endocrine content does not ensure a high isolation yield. Other factors, such as collagen and other matrix elements are thought to play a role (8-15). When studying histological characteristics of the human pancreas in relation to islet isolation, a remarkably high number of hyperemic islets (HIs) was encountered, a phenomenon that, besides our previous report in pigs (16), has not been described in detail before. The abnormalities observed in these HIs ranged from a single dilated vessel through multiple widely dilated vessels to hemorrhages extending into the surrounding exocrine tissue. In some cases, the endocrine tissue was reduced to a small rim of cells, with only a few scattered cells left, which may consequently affect isolation yield.

The aim of the present study was to assess the frequency and different types of HIs in human donor-pancreata. Furthermore, we studied the occurrence of HIs in relation to the amount of endocrine tissue in situ and the relation to islet isolation outcome. In addition we assessed donor and procurement factors to identify possible factors associated with the presence of hyperemic islets.

MATERIALS AND METHODS

Organ Procurement and Pancreatic Sampling

A total of 102 human pancreata were obtained through Eurotransplant. The organs were obtained from 66 multi-organ donations after brain death (DBD) and 36 multi-organ donations after cardiac death (DCD), for which consent for islet isolation and transplantation related research was given by relatives. The majority of the obtained organs was unsuitable for whole organ transplantation. However, all organs were procured and handled in the same way as the organs that were procured for whole organ transplantation. Organs were flushed in situ via the abdominal aorta with either cold University of Wisconsin (UW) or Histidine Tryptophane Ketoglutarate (HTK) organ preservation solution and removed “en bloc” with the spleen and stapled loop of the duodenum.

After dissection, the pancreata were stored in cold (4°C) UW or HTK (with the exception of 2 pancreata which were stored in another preservation solution) and transported according to Eurotransplant regulations, on ice, to the laboratory for

further processing. Mean (± SD) cold ischemia time (CIT, the interval between the aortic cross-clamp and initiation of the digestion procedure in the laboratory) was 8.0 ± 3.2 h. The pancreata were dissected free of spleen, duodenum, surrounding fat and vessels in the laboratory. Before the isolation procedure, biopsies of head, neck and body of the pancreas were taken, immersed in Formalin (Klinipath, Duiven, The Netherlands) fixative for 24-48 h and subsequently cleared and stored in ethanol 70%.

Histology

From each pancreas, paraffin-embedded sections of 5 μm were stained using the Aldehyde Fuchsine-Halmi (AF) technique: Rehydration, 90 s 2.5% potassium permanganate, 2' distilled water, 2' 1% oxalic acid, 10' running water, 1' distilled water, 15' AF, 3' 90% ethanol (2 times), 1' 70% ethanol, 1' 50% ethanol, 5' aquadest, 10-60 s Halmi, 10 seconds 0.2% acetic acid, 2' 100% ethanol (2 times), 5' xylene (2 times). Sections were embedded in malinol (Chroma-Gesellschaft, Köngen, Norway).

Microscopic evaluation

Islet sizes were determined in each pancreas using a calibrated grid and grouped into 8 categories: >50-100, 100-150, 150-200, 200-250, 250-300, 300-350, 350-400 and >400 μm diameter. As is generally practiced in the endocrine quantification in islet isolation procedures, islets smaller than 50 μm were not included because of their neglectable contribution to the total endocrine content. For determination of the endocrine content and the islet numbers, all islets in a tissue area of 1 cm^2 per slide were assessed. The endocrine area density was determined by summation of all calculated (πr^2) islet areas and expressed as percentage of the total area. According to the principle of Delesse (area density = volume density) and the assumption that islets are spherical, the determined endocrine area represents the endocrine content.

Islets described as hyperemic in this study ranged from islets showing only a single slightly dilated vessel to hemorrhages extending into the surrounding exocrine tissue (Fig.1). The presence of HIs was scored semi-quantitatively (HI-, HI+). HI- pancreata contained none and HI+ pancreata at least one hyperemic islet.

To assess the influence of the severity of the hyperemia, the HI+ pancreata were subdivided into HI+ and HI++ pancreata, also on a semi-quantitative basis. HI++ pancreata were more severely affected than the HI+ pancreata. Because of the enormous diversity in the occurrence of hyperemic islets both the amount of affected islets and the severity of hyperemia in the tissue sample were considered. For example, a tissue sample with 2 severely affected islets (with only a rim of islet cells left, as shown in Fig 1F) was scored as ++, while another tissue sample with 3 only slightly affected islets (with 1 dilated vessel, as shown in Fig 1B) was scored as +.

The pancreata were independently allocated to the different categories by 2 observers. The observers were blinded for the isolation outcome when allocating

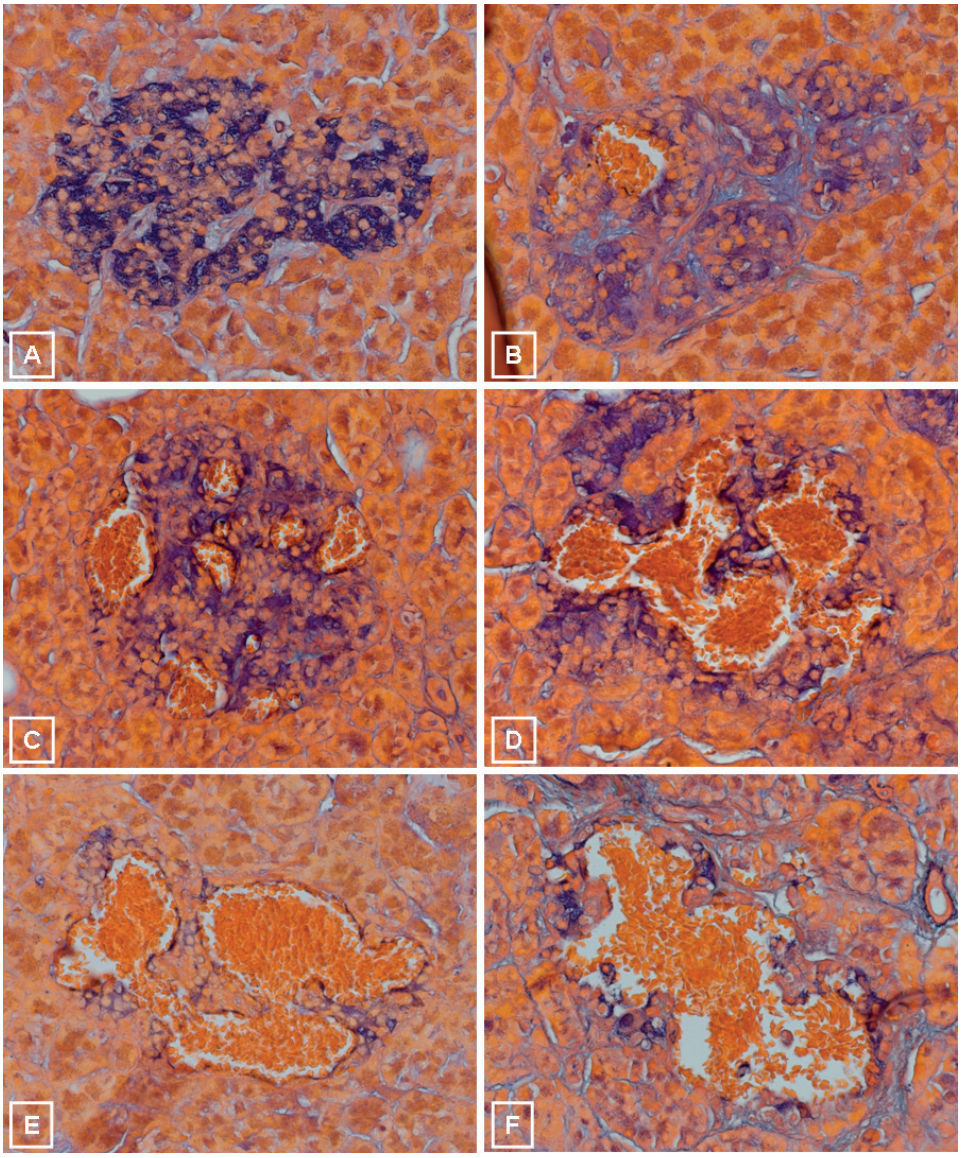

Figure 1: Histology of pancreata (AF staining) showing different stages of hyperemic islets.
(A) Normal islet. (B) Islet with a single dilated vessel. (C) Islet with multiple dilated vessels. (D) Islet with multiple, more severely dilated vessels. (E,F) Swollen islet with vastly dilated vessels and only a rim of islet cells left.

the pancreata to the different categories. Any differences between observers in the allocation of the pancreata, were resolved by discussion.

Donor and Procurement factors

To identify donor and procurement factors associated with the presence of HIs, we compared donor and procurement factors in HI+ and HI- pancreata. From the 102

pancreata, 8 (5 HI+ and 3 HI-) were excluded due to incomplete data, leaving 94 pancreata (52 HI+ and 42 HI-) for analysis.

Variables of interest were identified based on the literature (17-21). Donor variables included: age, sex, height, weight, body mass index (BMI = weight / height^2), ABO blood group, vascular co-morbidity (cardiac, cerebral and/or peripheral vascular event or disease), DBD/DCD donors, presence and duration of cardiac arrest and hypotensive periods, length of stay on the intensive care unit (ICU), cause of death, use of vasopressors and steroids, as well as routine biochemical blood screen (levels before organ removal) and pre-procurement hemodynamic measurements. Procurement variables included: CIT, procurement team and preservation solution.

Average beta cell/endocrine content ratio per islet

To assess possible swelling of the islets, the average beta cell/endocrine content ratio per islet was compared in HI+ and HI- pancreata. This was done in a smaller sample of 40 randomly selected pancreata (20 HI+ and 20 HI-).

In paraffin-embedded sections of 5 μm, beta cells were stained with a polyclonal peroxidase-labeled rabbit anti-human insulin antibody (Santa Cruz Biotechnology, Heidelberg, Germany) at a dilution of 1:100 overnight and visualised with 3,3'-diaminobenzidine (DAB) dissolved in Tris/HCl (0.05 M) with 15 μl H_2O_2. An average of 75 islets per tissue sample was assessed. To quantify the beta cell content (Fig. 2A), the brown stained area was quantified using the Zeiss KS400 image analysis system (Zeiss-Vision, Germany). Only islets larger than 50 μm in diameter were included. To quantify the endocrine content, every islet used in the assessment of the beta cell content was surrounded by a drawn line, manually delineated with a pen tablet using ImageJ (freeware image processing tool) (Fig. 2B). In human islets, beta cells represent 50-60% of the endocrine content. Because alpha, beta and delta cells have been shown to appear scattered throughout the islet (22, 23), the area inside the surrounding line was considered to represent the endocrine content (Fig 2C). This area was also quantified using the Zeiss KS400 image analysis system. To quantify a possible swelling of the islets, the ratio between the beta cell and endocrine content was calculated. The greater the swelling of the islets due to vasodilatation or edema, the lower the beta cell/endocrine content ratio would be.

Islet isolation

Islet isolation was performed in a smaller sample of 40 pancreata. These were different pancreata than those used in the determination of the beta cell/endocrine content ratio. Islet isolations were performed by a modification of the automated method previously described by Ricordi et al. (24). In short: the body and tail of the pancreas were used in the islet isolation procedure. The main pancreatic duct was identified, cannulated and perfused for 10 min (5 min at 80 mmHg and 5 min at 180 mmHg) with

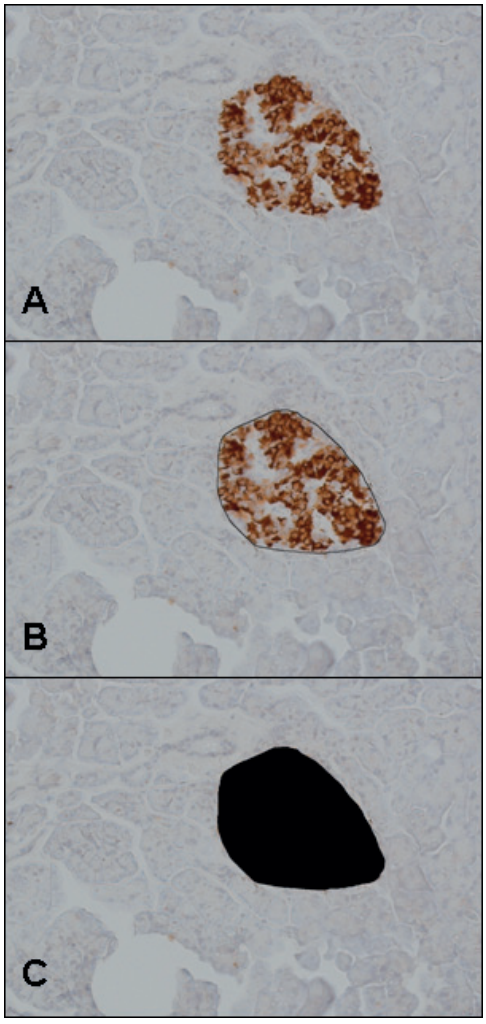

Figure 2: Calculation of the beta cell/endocrine content ratio
The brown stained (beta cell) area was quantified using an image analysis system (A). To quantify the endocrine content, every islet used in the assessment of the beta cell content, was surrounded by a manually drawn line (B). The area inside the surrounding line was considered to represent the endocrine content (C). To quantify the swelling of the islets, the ratio between the beta cell and endocrine content was calculated: area A / area C.

a chilled solution of Collagenase NP (Serva Electrophoresis, Heidelberg, Germany), Neutral Protease NP (25U, Serva Electrophoresis, Heidelberg, Germany) and (Aminoethyl)-benzene sulfonyl fluoride hydrochloride (AEBSF-HCl, 20 mg/ml, Serva Electrophoresis, Heidelberg, Germany) in Hanks Balanced Salt Solution (HBSS). The distended tissue was cut into 5 or 6 pieces and placed in the Ricordi chamber, in a closed recirculating system and heated to 37°C to activate the enzyme blend. During the course of enzymatic and mechanical dissociation of the gland, samples were taken and evaluated in real-time using Dithizone (DTZ, diphenylthiocarbazone, 13 mM DTZ dissolved in 91.2% ethanol supplemented with 1.2% NH_4OH, Sigma, Steinheim, Germany). After the appearance of islets unembedded in acinar tissue, the circulation circuit was cooled and the digest collected. The digested tissue was placed in a beaker on ice and islet samples of 50 µl were stained with an equal volume of DTZ solution,

freshly prepared by the addition of 5% DTZ to HBSS. Islet numbers, expressed as islet equivalents (IE, representing islets of 150 μm diameter), were determined in duplicate.

Data analysis

Statistical analysis was performed using SPSS 16.0 statistical software. HI- and HI+ pancreata were compared on endocrine content, number of islets, isolation outcome and the average beta cell/endocrine content ratio per islet using student *t*-tests. HI-, HI+ and HI++ pancreata were compared to assess the influence of the severity of hyperemia on the above outcomes using one-way analysis of variance. Post hoc Bonferroni's multiple comparisons tests were performed to determine which groups differed from each other.

To assess whether the relation between the presence of HIs and isolation outcome was confounded by other factors known to influence isolation outcome (17-21, 25), multivariate analyses was performed using linear regression with isolation outcome as dependent variable. Independent variables included: the presence of HIs, endocrine content, donor age, sex, BMI, cause of death, DBD/DCD donors, ICU stay, cardiac arrest, use of vasopressors, hypotensive periods (last measured blood pressure before procurement, SBP < 90 mm Hg and/or DPB < 60 mm Hg), pre-procurement hypertension, local/distant procurement, CIT and preservation solution.

To identify possible relevant variables in the occurrence of HIs, HI+ and HI- pancreata were compared on differences in donor and procurement factors using student *t*-tests for continuous variables and χ^2 analyses for categorical variables. In case of expected count less than 5, the Fisher exact test was used. Multivariate analysis was consequently performed using binary logistic regression with presence of HIs as the dependent variable. As independent variables were included: age, sex, BMI, cause of death, vascular co-morbidity, ICU stay, use of vasopressors, use of steroids, hypotensive periods, blood creatinine, amylase, glucose and sodium levels, pre-procurement hypertension, duration of cardiac arrest, DBD/DCD donors, local/distant procurement team, CIT and preservation solution.

All p-values < 0.05 were considered to be statistically significant.

RESULTS

Presence of HIs

Histological analysis showed that 57 of the 102 pancreata (54.6%) contained at least one hyperemic islet and were scored as HI+. However, only an average of 4.5% of all assessed islets in the HI+ pancreata was hyperemic. Hence, 45 pancreata (45.4%) contained no HIs and were scored as HI-.

We assessed the presence of HIs in different parts of the pancreata. There were no differences in the occurrence of HIs in the head, neck or body of the pancreata (results not shown).

Donor and Procurement factors in relation to HIs

Differences in donor and procurement factors between the HI- and HI + groups are listed in Table 1, 2. ICU stay was significantly longer in the HI+ group compared to the HI- group. Furthermore, pre-procurement hypertension (last measured blood pressure before procurement, Systolic Blood Pressure (SBP) > 140 mmHg and/or Diastolic Blood Pressure (DBP) > 90 mm Hg) was more frequently found in the HI+ than in the HI- group. We also found a trend ($p = 0.07$) towards longer cold ischemia times in the HI+ group.

Multivariate analyses confirmed that length of ICU stay (Odds ratio (OR) 1.32, 95% Confidence Interval (CI) [1.02 – 1.71]) and the presence of pre-procurement hypertension (OR 3.43 [1.07 – 11.03]) significantly increased the probability of HIs. Last sodium level before organ procurement was found to significantly reduce the probability of HIs (OR 0.85 [0.76 – 0.95]).

Endocrine content in relation to HIs

The mean endocrine content in the HI+ pancreata was 0.66%. This was significantly higher than in the HI- group where this was 0.55% ($p = 0.04$) (Fig. 3A).

To assess the influence of the severity of the hyperemia on the endocrine content, the HI+ pancreata ($n = 57$) were subdivided into 31 HI+ and 26 HI++ pancreata (Fig. 3B). The mean endocrine content was 0.55% in the HI-, 0.58% in the HI+ and 0.75% in the HI++ group. One way analysis of variance showed that the mean endocrine content differed between the groups ($p = 0.004$). Bonferroni's test revealed that endocrine content was significantly higher in the HI++ group compared to the HI- ($p = 0.004$) and the HI+ group ($p = 0.03$).

The mean number of islets (in 1 cm^2 tissue) was 72 in the HI- and 78 in the HI+ group. We found no significant differences in numbers of islets between these groups ($p = 0.19$).

These results suggest that the higher endocrine content in the affected pancreata is a result of swelling of the islets, due to vasodilatation, edema or both.

In a smaller sample of 20 HI+ and 20 HI- randomly selected pancreata, we observed that the mean beta cell/endocrine content ratio in the HI- group was 0.67%, which is significantly higher ($p = 0.02$) than the 0.55% shown for the HI+ pancreata. This supports our hypothesis that the higher endocrine content in the most affected pancreata is the result of islet expansion.

Table 1. Differences in donor characteristics between HI+ and HI- pancreata

Donor characteristic	HI + (n = 52)	HI - (n = 42)	Test of difference
Age (y) (± SD)	47.4 ± 13.0	46.9 ± 14.3	t = -0.181, p = 0.86
Sex			X^2 = 0.416, p = 0.52
Male	27 (51.9%)	19 (45.2%)	
Female	25 (48.1%)	23 (54.8%)	
ABO Blood Group			X^2 = 0.614, p = 0.94
O	18 (34.6%)	14 (33.3%)	
A	25 (48.1%)	19 (45.2%)	
B	6 (11.5%)	5 (11.9%)	
AB	3 (5.8%)	4 (9.5%)	
Body mass index (kg/m^2)	25.2 ± 5.3	24.4 ± 4.3	t = -0.823, p = 0.41
Cause of Death			X^2 = 0.314, p = 0.86
Cerebrovascular accident	30 (57.7%)	26 (61.9%)	
Trauma	11 (21.2%)	9 (21.4%)	
Other	11 (21.2%)	7 (16.7%)	
DBD/DCD donors			X^2 = 0.105, p = 0.75
DBD	33 (63.5%)	28 (66.7%)	
DCD	19 (36.5%)	14 (33.3%)	
ICU stay (days)	3.4 ± 3.5	2.2 ± 2.1	t = -2.033, p = 0.05
Cardiac arrest			X^2 = 0.024, p = 0.88
Yes / No	19 (36.5%) / 33 (63.5%)	16 (38.1%) / 26 (61.9%)	
Cardiac arrest duration (min)	5.5 ± 10.7	8.1 ± 13.8	t = 1.004, p = 0.32
Hypotension [a]			X^2 = 0.001, p = 0.97
Yes / No	20 (38.5%) / 32 (61.5%)	16 (38.1%) / 26 (61.9%)	
Hypotension duration (min)	6.5 ± 13.2	9.0 ± 20.4	t = 0.738, p = 0.46
Hypertension [b]			X^2 = 3.753, p = 0.05
Yes / No	29 (55.8%) / 23 (44.2%)	15 (35.7%) / 27 (64.3%)	
Vascular comorbidity [c]			X^2 = 0.171, p = 0.68
Yes / No	22 (42.3%) / 30 (57.7%)	16 (38.1%) / 26 (61.9%)	
Sodium (mmol/l) [d]	145 ± 7	147 ± 7	t = 1.470, p = 0.15
Creatinine (mg/dl) [d]	77.9 ± 37.5	72.5 ± 27.0	t = -0.786, p = 0.43
Amylase (U/l) [d]	240 ± 349	206 ± 161	t = -0.595, p = 0.55
Glucose (mmol/l) [d]	8.1 ± 2.8	8.5 ± 3.7	t = 0.623, p = 0.54
Hyperglycemia [e]			X^2 = 0.211, p = 0.65
Yes / No	26 (50.0%) / 26 (50.0%)	23 (54.8%) / 19 (45.2%)	
Vasopressors			X^2 = 0.004, p = 0.95
Yes / No	35 (67.3%) / 17 (32.7%)	28 (66.7%) / 14 (33.3%)	
Vasopressor (µg/kg/min)			
Noradrenaline [1]	0.32 ± 0.74	0.27 ± 0.40	t = -0.301, p = 0.77
Dobutamine [2]	5.60 ± 3.15	5.37 ± 4.92	t = -0.083, p = 0.94
Dopamine [3]	7.10 ± 5.42	5.87 ± 2.95	t = -0.612, p = 0.55
Steroids			X^2 = 0.369, p = 0.54
Yes / No	12 (23.1%) / 40 (76.9%)	12 (28.6%) / 30 (71.4%)	

DBD: Donation after brain death, DCD: Donation after cardiac death, ICU: Intensive care unit

[a] Hypotension: last measured blood pressure before procurement, SBP < 90 mm Hg and/or DPB < 60 mm Hg
[b] Hypertension: last measured blood pressure before procurement, SBP > 140 mm Hg and/or DBP > 90 mm Hg
[c] Vascular co-morbidity: cerebral, cardiac and/or peripheral vascular event or disease
[d] Levels before organ procurement
[e] Hyperglycemia: last serum glucose level before organ procurement > 10.0 mmol/l
[1] Means were calculated for 51 donors who received Noradrenalin before procurement
[2] Means were calculated for 8 donors who received Dobutamine before procurement
[3] Means were calculated for 20 donors who received Dopamine before procurement

Table 2. Differences in pancreas characteristics between HI+ and HI- pancreata

	HI + (n = 52)	HI - (n = 42)	Test of difference
Pancreas characteristic			
Cold ischemia time (h) (± SD)	8.5 ± 3.4	7.3 ± 2.5	t = -1.871, p = 0.07
Procurement team			X^2 = 1.098, p = 0.30
Local	18 (34.6%)	19 (45.2%)	
Distant	34 (65.4%)	23 (54.8%)	
Preservation solution [1]			X^2 = 0.128, p = 0.72
UW	34 (66.7%)	26 (70.3%)	
HTK	17 (33.3%)	11 (29.7%)	

UW: University of Wisconsin Solution, HTK: Histidine Tryptophane Ketoglutarate
[1] Data missing for 1 HI+ and 5 HI- donors. Percentages calculated for 51 and 37 donors, respectively.

Islet isolation yield in relation to HIs

In a different series of 40 pancreata (20 HI- and 20 HI+), we assessed whether the presence of HIs might influence isolation outcome. In spite of their histologically assessed higher endocrine content, the mean isolation yield was significantly lower in the HI+ pancreata compared to the HI- group (2634 IE/g and 4069 IE/g, respectively p = 0.05).

However, the HI+ pancreata contained significantly higher endocrine content. When this higher endocrine content was taken into account and the isolation results were expressed as the ratio of yield and content, the results were even more pronounced and illustrated a severely compromised endocrine quality (p = 0.003) (Fig.4A).

We also assessed the influence of the severity of hyperemia on isolation outcome and subdivided the HI+ group (n = 20) into 13 HI+ and 7 HI++ pancreata (Fig. 4B). One way analysis of variance showed that the mean yield/endocrine content ratio differed between the groups (p = 0.008). Bonferroni's test revealed that the yield/endocrine content ratio was significantly lower in the HI++ group compared to the HI- group (p = 0.02). Furthermore, a trend towards a lower yield/endocrine content ratio was found in the HI++ group compared to the HI+ group (p = 0.06).

Furthermore, we assessed whether the reduced isolation outcome in the HI+ groups was confounded by other factors know to influence isolation outcome. We corrected for endocrine content, donor age, sex, BMI, cause of death, DBD/DCD donors, ICU stay, cardiac arrest, use of vasopressors, use of steroids, hypotensive periods, local/distant procurement, CIT, preservation solution. The presence of HIs (B -1648.9, p = 0.01) and the endocrine content (B 6809.4, p < 0.001) were the only factors significantly influencing isolation outcome.

Etiology of HIs

To gain more insight in the etiology of HIs and validate whether other differences existed between HI groups, an experienced pathologist assessed a random selection of 10 HI+ and HI- pancreata. The exocrine tissue and the nonhyperemic islets appeared to be visually normal in both groups. The pathologist found no macrophages or iron

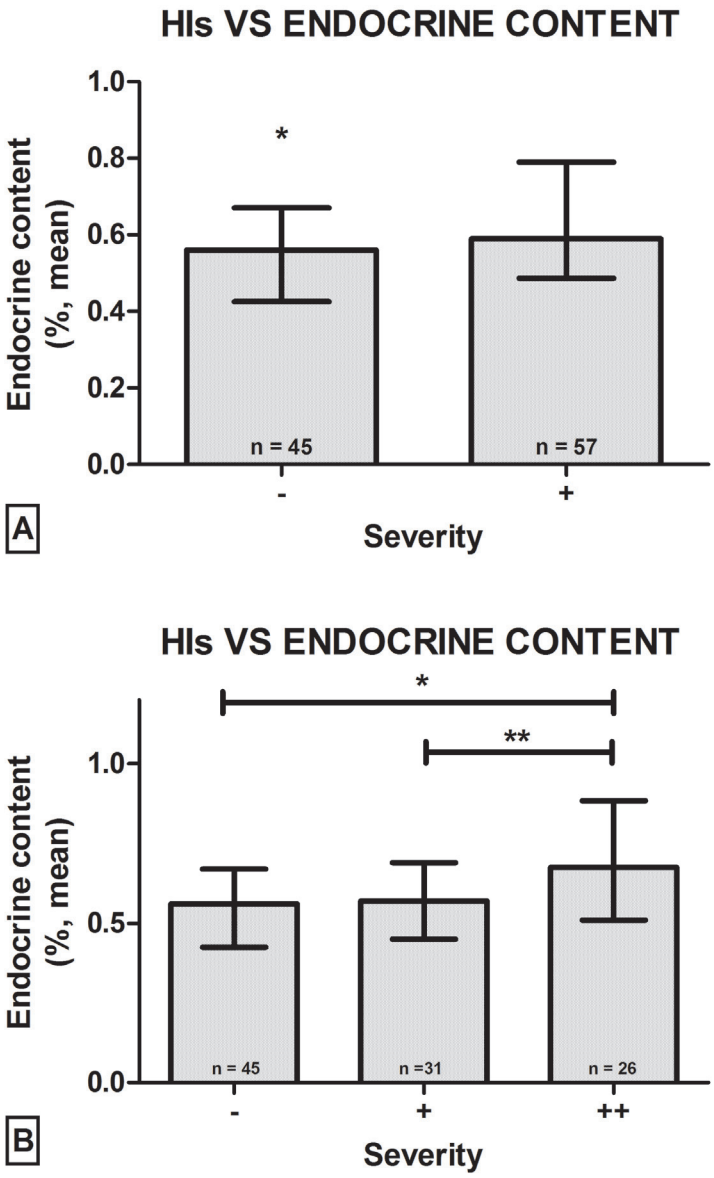

Figure 3: The endocrine content in relation to hyperemic islets.
The bars represent the mean endocrine content per hyperemic islet category. The error bars represent the standard deviation.
(A) The mean endocrine content in 45 HI- and 57 HI+ pancreata was 0.55, and 0.66%, respectively. Significantly higher endocrine content was found in the HI+ group, * p = 0.04
(B) The influence of the severity of the hyperemia, by subdividing 57 HI+ pancreata into 31 HI+ and 26 HI++ pancreata, in relation to the endocrine content. Significant higher endocrine content was found in the HI++ group compared to the HI- and HI+ group, * p = 0.004, ** p = 0.03.

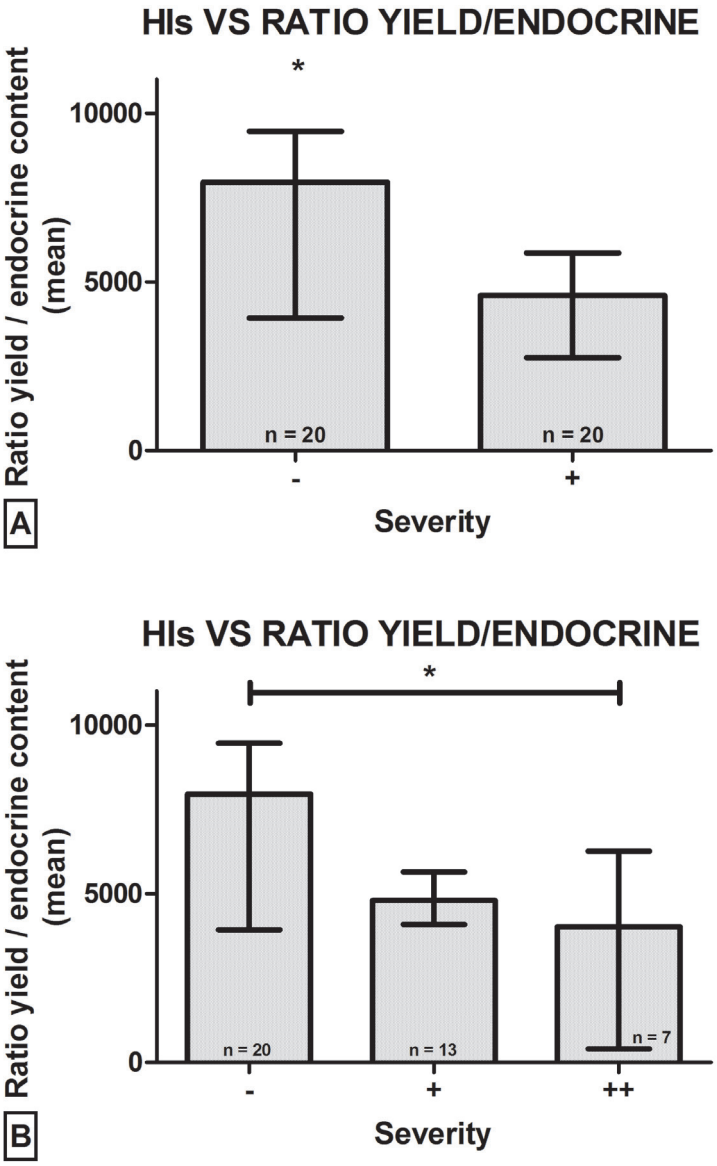

Figure 4: Yield/endocrine content ratio in relation to hyperemic islets.
The bars represent the mean yield/endocrine content ratio per hyperemic islet category. The error bars represent the standard deviation.
(A) The mean yield/endocrine content ratio in 20 HI- and 20 HI+ pancreata. A significantly lower ratio was found in the HI+ group, * p = 0.003.
(B) The influence of the severity of the hyperemia, by subdividing 20 HI+ pancreata into 13 HI+ and 7 HI++ pancreata, in relation to the yield/endocrine content ratio. A significantly lower yield / endocrine content ratio was found in the HI++ group compared to the HI- group, * p = 0.02.

deposits and no evidence for an ongoing chronic process in the affected pancreata. Beside the presence of HIs, no differences were seen between the HI- and HI+ pancreata.

To assess whether HIs also occurred in normal pancreatic tissue, we examined a series of 30 "normal" pancreatic tissue samples excised at elective surgical procedures (e.g. after pancreatoduodenectomy for pancreatic carcinoma) at the Academic Medical Centre, Amsterdam, The Netherlands. Areas with pancreatic pathology were identified by an experienced pathologist and were left out of the assessment. Only areas with "normal" pancreatic tissue were used in our assessment. At least 1 HI was found in 43.3% of these pancreata. Apparently, the presence of HIs is not limited to donor organs, but they can also be found in pancreatic tissue excised in elective surgery, suggesting that besides the pre-procurement hemodynamic status, the handling of the pancreas during surgery could also be a contributing factor in the development of HIs.

DISCUSSION

Human islet isolations produce unpredictable, highly variable islet yields. We previously reported HIs as a possible explanatory factor in the varying porcine islet yields (16). When we studied histological characteristics of the human pancreas in relation to islet isolation, we found a similar remarkably high number of HIs. Similar islets have only been reported anecdotally in the literature but no mechanisms were described regarding their origin and no relevance has been determined from the perspective of the isolation of islets for transplantation (26-30). Slight hyperemia in islets has been reported in normal metabolic situations when higher insulin release is demanded (30, 31). However, like in our pig study, the phenomena encountered in the present study are far more dramatic and are most likely not related to the normal glucose metabolism.

In the present study, HIs were found in more than half of the pancreata. However, the HI+ category consisted of pancreata with at least 1 HI. This could potentially lead to an overestimation of the HI- group, when a hyperemic islet was missed by an observer or when an islet appeared just outside the sectioning plane. However, because 2 observers independently assessed the tissue samples and since we have found no differences in the presence of HIs between different parts of the pancreata, it is unlikely that our results were influenced by this. Because no macrophages or lytic erythrocytes were seen and no evidence for an ongoing chronic process was found, it is most likely that the HIs arose shortly before or during the procurement of the pancreas. Since length of ICU stay and pre-procurement hypertension were shown to increase the probability of HIs, it can be speculated that a rise of blood pressure just before procurement and hemodynamic instability associated with prolonged ICU stay, are responsible for the formation of HIs. This in line with our porcine study where we

reported a rise in blood pressure, induced by the slaughtering process, as a possible causative moment for the presence of hyperemic islets in pigs. It is not clear how our findings with respect to the last sodium level fit in this explanation. Hence, since the mean sodium level of both the HI positive and HI negative groups are around the upper limit of normal values and the mean values only differed by 2 points, this finding might not be of clinical relevance. However, HIs were also found in 43.3% of "normal" pancreatic tissue samples obtained from elective surgical procedures. Since we have very little donor specifications of these tissue samples, we cannot make any statement about whether these influenced the presence of HIs, but apparently the presence of HIs is not limited to donor organs. However, these "elective patients" were most likely not admitted to the ICU before undergoing surgery and were probably hemodynamically more stable than the pancreas donors, especially when compared to DBD donors. Therefore, it seems likely that, besides the pre-procurement hemodynamic status, the handling of the pancreas during surgery could also be a contributing factor in the development of HIs.

We found no microscopic hemorrhages in the exocrine pancreas, which makes up 98-99% of the organ, indicating that the islet vasculature is probably very sensitive for blood pressure rises and hemodynamic instability. This vulnerability may be reflected in its unique structure: blood vessels from the surrounding exocrine tissue abruptly increase in diameter upon entering the islet, have a thinner wall and are extensively fenestrated inside the islet, as reported in a rat study (32).

We have also investigated the possible relevance of the presence of HIs from the perspective of islet isolation. A higher presence and severity of HIs was accompanied by a small increase in the endocrine content. This increase, however, is not paralleled by a similar increase in islet numbers, and could therefore be caused by expansion of the individual islets. Additionally, we found a lower beta cell/endocrine content ratio in the most affected pancreata, indicating that islets in these pancreata are more swollen, probably through vasodilatation, edema or both. In spite of the slightly higher endocrine content of the HI+ pancreata, substantial lower yields were found. When we corrected for the amount of endocrine tissue, even more pronounced lower yields were found in the affected groups. Furthermore, when corrected for other factors that are known to influence isolation outcome, the presence of HIs and endocrine content were the only factors significantly influencing isolation outcome. This is remarkable since even in the affected pancreata only a few percent of all islets were histologically abnormal. Most likely, the substantial increase in islet volume, or the reduced isolation outcome is not caused by this small fraction itself. A possible explanation could be that the entire islet population is compromised and that the abnormal islets are just an indicator of the actual damage of the islets. It can be speculated that besides the reduced isolation outcome, this phenomenon could also provide a possible explanation for the variable, unexplained loss of islets during culture and after transplantation. When

islets are obtained from donor-pancreata containing HIs, these islets would appear to be "normal" when in fact, the entire islet population of these pancreata is affected. Therefore, these islets are more likely to fail in culture or have impaired function when transplanted. In whole organ transplantation this would probably be less of a problem because of the intact integrity of the exocrine pancreas, providing external support for the islets and a possibility for recovery and angiogenesis. However, further research would be necessary to support this hypothesis, Furthermore, in this study, we have assessed the relation between the presence of HIs and pre-purification yield. To establish the importance of HIs for islet transplantation, further research, involving post-purification yield, purity, viability etc. would be necessary.

In conclusion, these data are a first indication that the presence of hyperemic islets negatively influence isolation outcome and suggest an impact of the procurement procedure and pre-procurement hemodynamic status of the donor on the islet quality. It is tempting to speculate that this contributes to the generally experienced difficulties in obtaining sufficient amounts of human islets.

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