tion of high islet yields. We conclude that pancreas procurement has an important influence on human islet isolation.

A8

Quantification of islet isolation efficiency in dogs

M. P. M. van der Burg, O. R. Guicherit, M. Frölich, J. P. Scherft, J. A. Bruijn, F. A. Prins, H. G. Gooszen
Leiden, The Netherlands

Overall, islet isolation efficiency in large mammals has improved ~ five-fold over the past decade. No consistent islet yields are warranted however, due to the intertwined effects of many variables like donor characteristics, pancreas preservation conditions and crude collagenases. Sizing of isolated islets has greatly contributed in comparing islet yield from different laboratories, and largely superseded insulin extraction. As yet however no attempts have been made to extend the use of this new parameter by comparing yield with the islet volume of the individual pancreas. We studied the effects of interindividual differences in pancreatic islet and insulin mass, and other donor characteristics on isolation outcome. Islet isolation from the pancreatic tail segment (beagle, n = 31; age 9–67 mo; BW 9–18 kg) was performed by intraductal collagenase digestion, dispersion in cold UW solution ('digest') and density gradient purification. For islet sizing (by stereology of pancreas sections and dithizone staining of isolated islets) and insulin or amylase extraction samples were taken from the pancreatic tail, the digest, and the purified ('pure') and 'rest' fractions of the gradients. The islet volume fraction of the pancreas averaged 1.6 % (16 µl/g) and varied 3.4 fold. Digest islet volume averaged 8 µl/g pancreas and varied 9-fold. Animals too varied in age 8-fold and BW 2-fold. Differences in BW and age together explained 60 % of variance in the islet volume fraction of the pancreas and 50 % of the variance in digest islet yield ($p < .001$; mult. regression and correlation). Both insulin (89 %) and amylase (82 %) recovery reflected the loss of tissue (16 %) in the digest ($p < .001$). In contrast digest islet recovery averaged only 48 % ($p < .001$). From the gradients (pure and rest) ~ 80 % of digest tissue ($p < .05$), 89 % of digest insulin ($p < .05$), 67 % of digest amylase and 64 % of digest islet volume were recovered ($p < .001$). Only 33 % of digest insulin, 53 % of digest islet volume and virtually no amylase were recovered in the purified fraction ($p < .001$). Pancreas islet and insulin content correlated with islet and insulin mass (and vice versa) in digest ($r = .6–.8$; $p < .001$) and purified suspensions ($r = .5–.6$; $p < .001$). Digest islet volume too correlated with islet and insulin values after purification ($r = .7$; $p < .001$). No correlation could be demonstrated for digest-insulin vs. islet or insulin recovery in the purified fraction, though digest insulin did correlate with total insulin recovered from both the pure and rest fractions of gradients ($r = .6$, $p < .001$). Islet isolation neither affected islet size, nor islet-insulin or acinus-amylase content. In conclusion: variability in isolation outcome; 2) islet isolation is best quantified by morphometry and qualified by additional biochemical parameters like insulin and amylase: we underestimated islets entrapped in acinar tissue; 3) by using UW for isolation pure islets were obtained.

A9

Simultaneous pancreatic and kidney transplantation before end-stage chronic renal failure

D. Cantarovich, M. Hourmant, J. Dantal, R. Baatard, M. Giral, J. Paineau, G. Karam, A. Murat, J. P. Soulillou
Nantes, France

Introduction: Pancreatic transplantation is commonly performed simultaneously with the kidney in type-1 diabetic patients with end-stage chronic renal failure.
Aim of the study: To compare the results of simultaneous pancreatic and kidney (SPK) transplantation according to the recipient pre-transplant chronic renal failure stage.
Methods: Since the beginning of our pancreatic transplant program in November 1987, a total of 64 patients underwent SPK transplants. Twenty-two patients (35 %) were not on dialysis at the time of transplantation (the non-dialyzed group). Their serum creatinine level ranged from 165 to 833 µmol/l (mean 373 ± 137) and their 24-hour creatinine clearance from 10 to 30 ml/min (mean 21 ± 7). They were aged from 22 to 55 years (mean age 40 ± 8), and were insulin-dependent from 15 to 35 years (mean 23.6 ± 7). The remaining 42 patients (65 %) were on dialysis before transplantation from 1 to 108 months (mean 23 ± 21; the dialyzed group). Their mean age was 38 ± 9.6 years (20–61) and duration of diabetes ranged from 5 to 37 years (mean 23.6 ± 6.7). All 64 patients underwent cadaveric SPK transplants (segmental duct-occluded pancreatic transplants) and were similarly immunosuppressed with antithymocyte globulin or the anti-IL2-R LoAb 33B3.1 for 10 days, prednisolone for 30 to 45 days, cyclosporine and azathioprine.
Results: The 3-year patient, pancreas and kidney actuarial survival rates were respectively 95, 82 and 90.5 % in the non-dialyzed group and 87, 60 and 79 % in the dialyzed one (p = ns). In the non-dialyzed group, a total of 4 pancreata and 2 kidneys were lost because of 1 death (cerebral bleeding 7 months after transplantation), 3 pancreatic venous thrombosis during the first post-transplant week and 1 kidney rejection at month 5. In the dialyzed group, a total of 16 pancreata and 8 kidneys were lost because of 3 deaths (probable pulmonary embolism at day 27, infectious pneumonia at month 9 and multiple myeloma at month 18), 9 pancreatic technical failures (5 venous thrombosis during the first post-transplant week, 3 transplant bleedings during the first post-transplant month and 1 arterial thrombosis at month 3), 3 pancreatic rejections at month 4, 7 and 36, respectively, 3 kidney rejections at day 11, month 18 and month 40, respectively, 1 immediate renal artery thrombosis and 1 death due to sepsis following a leg amputation at month 33. During the first 3 months, 9 patients in the non-dialyzed group (41 %; onset 39 ± 13 days) and 12 in the dialyzed one (29 %; onset 35 ± 20 days) experienced 1 rejection episode respectively (ns; all but one reversible and all biologically restricted to the kidney). The incidence of CMV was 96 % in both groups. When analyzed at 1 year, no statistical differences were noted in fasting blood glucose (5.4 ± 0.9 and 5.2 ± 0.8 mmol/l), 2-hour OGTT glycemia) ± 3 and 8.5 ± 2 mmol/l), fasting serum C-peptide (2.5 ± 1.3 and 2.3 ± 1.1 ng/ml), glycosylated A1C haemoglobin (5.8 ± 0.8 and 5.7 ± 0.7 %), serum creatinine (140 ± 34 and 150 ± 51 µmol/l), 24-hour creatinine clearance (62 ± 19 and 65 ± 31 ml/min), and cyclosporine dosage (9.3 ± 4.7 and 7 ± 2 mg/kg/day) in the non-dialyzed and dialyzed groups, respectively.
Conclusion: Similar patient and transplant survival rates, rejection episode incidence, and functional results were observed following SPK transplantation in non-dialyzed diabetic uraemic patients when compared to patients on dialysis at the time of transplantation. Whether correction of uraemia and normalisation of glucose homeostasis before end-stage chronic renal failure may have the advantage of improving diabetic degenerative complications, theoretically less advanced at this stage, still requires long-term follow-up to be acertained.

A10

Effect of culture and cryopreservation on insulin release from isolated, perifused human islets

A. di Carlo, D. W. Scharp, R. L. Gingerich, M. Ansara, B. Olack, R. Navalesi, C. Swanson
Pisa, Italy; St. Louis, USA

Pancreatic islet transplantation therapy is currently being evaluated in several clinical trials. Islets are transplanted into patients either shortly after the isolation, or following a period of culture (usually 5–7 days), or after cryopreservation. So far, no detailed study is available that compares the function of the islets according to the storage technique. In this paper, we perifused human islets, isolated from 7 cadaver organ donors, after overnight culture (ONCIs), 6 day culture (6DCIs), and 6 day culture plus cryopreservation (CPIs). The islets were sequentially perifused with 3.3 mmol/l glucose (3.3G), 16.7 mmol/l glucose (16.7G), 16.7 mmol/l glucose plus 10 mmol/l theophylline (GT), and 3.3G again. Basal and stimulated insulin release results are detailed in the table, showing that secretion was similarly reduced both basally (p