Pancreatic islet transplantation: studies on the technique and efficacy of islet isolation and transplantation

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7 Function and survival of intrasplenic islet autografts in dogs*

Introduction

Transplantation of isolated islets of Langerhans has been successfully performed in large mammals, including man [1–10]. Physiological glucose regulation is of utmost importance for prevention or halting the progression of secondary diabetic complications, but as yet metabolic control by islet grafts has not been well established. Reports on normoglycemia and insulin independence after islet transplantation in man are anecdotal, and the metabolic data are difficult to interpret [1–5]. The main focus of most large animal studies has been on the technical aspects of islet transplantation, and functional assessment generally received scant attention [6–10]. We studied metabolic control after fasting, intravenous glucose, mixed meals, and an intravenous arginine bolus during 35 mM glycemia, before operation and up to three years after intrasplenic islet autotransplantation in dogs. The autotransplantation model was applied to circumvent the confounding effects of immunosuppressive drugs and rejection; the spleen was chosen for physiological, portal, drainage of the islet hormones. In addition, the pancreatic polypeptide response to intravenous insulin-induced hypoglycemia was measured — a vagal, cholinergic, mediated stimulus [11] — to monitor possible cholinergic re-innervation of the graft.

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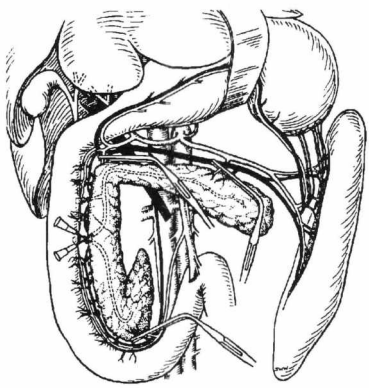
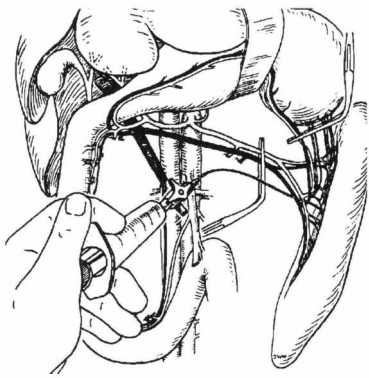
Fig.VII.0 A–B

Schematical representation of pancreatectomy and subsequent intrasplenic islet autotransplantation.



A
The pancreas was mobilised with its major vascular connections preserved; two stub adapters were inserted into the main pancreatic duct, one directed to the left, and the other to the right lobe; the blood vessels were clamped, and the gland was removed.



B
After islet isolation and purification, the suspension was autotransplanted by reflux via two splenic vein tributaries, while clamping the splenic pedicle and short gastric vessels.

Materials and methods

Animals and posttransplant monitoring

Experiments were performed in adult, female, outbred, beagles (Harlan, Zeist, The Netherlands) weighing 11–14 kg. Care and use of animals was in accordance with the national ethical standards for animal experimentation. Dogs were housed in pairs, in kennels of 12 m^2. They were maintained on a regular diet of semi-liquid dog food (50 energy% carbohydrate, 20 energy% fat and 30 energy% protein; Complete Dog Food D-B; Hope Farms, Woerden, The Netherlands) twice daily and had free access to water. In the immediate postoperative period derangements of serum potassium levels were corrected, and hydration was maintained by hypodermoclyses (NaCl 76 mM, glucose 1.39 mM). Dogs were allowed to drink from day two and intake was advanced to the regular diet, supplemented with 2 g/day protease-lipase-amylase pellets (Pancreas Pellets; Organon, Oss, The Netherlands) by day three. Graft function was monitored by determining fasting glucose during the first 14 days

posttransplant, and weekly by assessment of fasting and 2-h postprandial plasma glucose and insulin. Posttransplant time to normoglycemia was defined as time to the first of two consecutive fasting plasma glucose measurements less than 6.4 mM (upper limit of 95% confidence interval in 44 normal dogs). Time to functional graft failure was defined as the time to the first day of persistent fasting ≥ 8 mM glycemia. Animals appeared to be in good condition, and had normal stools and steady body weights (102±3 % of preoperative weight) throughout the study.

Graft preparation and transplantation

In six consecutive experiments total pancreatectomy was performed for islet isolation and subsequent intrasplenic islet autotransplantation. After a 24-h fast, the animals were anesthetised as described previously [12]. The pancreas was mobilised with its major vascular connections preserved. Two 20 or 23-gauge stub adapters were inserted into the main pancreatic duct, one directed to the left, and the other to the right lobe (Fig. VII.0 A). The blood vessels were clamped, and the gland was removed, weighed, and perfused at a flow rate of 20 ml/min for 10–15 min with 350 ml of an ice-cold recirculating solution of 1633 U/ml collagenase (type XI; Sigma, St. Louis, Mo., USA) in a modified Hanks' solution [13]. The animals remained anesthetised with the abdomen provisionally closed for approximately 4 h while the pancreas was processed. The gland was incubated in the remaining collagenase solution at 38°C for 20 min in a water bath. The solution was decanted, and the tissue was dispersed in ice-cold University of Wisconsin solution (ViaSpan; Du Pont Pharmaceuticals, Wilmington, Del., USA) by gentle aspiration and flush through a 14-gauge needle, and filtration through a 400–μm steel mesh. Trapped tissue was discarded. The islet suspension was pooled by centrifugation at 200 *g* and 4°C for 2 min. After resuspension, samples were taken for assessment of the islet yield (as described below), and the suspension was aliquoted to eight 50-ml tubes. For density gradient purification, tissue was pelleted and resuspended in the bottom layer of step density gradients (1.095, 1.085, 1.075, and 1.045 g/ml) of dextran (D3759; Sigma) in Hank's solution, as described previously [14]. Gradients were centrifuged 40 *g* for 4 min and 500 *g* for 12 min at 4°C. Purified islets were collected from the two uppermost layers. Because of the expected variable islet yield after density gradient separation [14] the procedure was performed in two steps: first, one of the tubes was used to test the efficacy of the procedure; next, tissue in either part or all of the remaining tubes underwent density separation — to ensure a final dose of the combined

purified and non-purified islets ≥ 3000 islet equivalents/kg body weight. One islet equivalent (IEq) equals the volume of an islet with an 150 μm diameter [1]. The purified and any non-purified islet suspensions were combined, washed thrice in RPMI-1640 (Flow Laboratories, Irvine, Ayrshire, UK), and resuspended in RPMI supplemented with 10% autologous serum. Aliquots of the islet suspensions were stained supravital with dithizone (Sigma) for morphometric assessment of the total volume of isolated islets and purity (relative volume of islet tissue) as described previously [14]. The suspension was autotransplanted after systemic injection of 100 U heparin per kg body weight, by reflux via two splenic vein tributaries, while clamping the splenic pedicle and short gastric vessels (Fig. VII.0 B).

Metabolic studies

Metabolic studies were performed two weeks before operation, and one month and six months (4–7 months) posttransplant in a crossover design in four dogs, under the following conditions: in fasting animals (18 h overnight); after meal stimulation, to study physiological glucose regulation; after — conventional — intravenous bolus glucose infusion (i.v. glucose tolerance test, IVGTT); after intravenous bolus arginine infusion during steady state 35 mM glycemia, to estimate the insulin secretory capacity and functional beta- and PP cell mass of the grafts; and after intravenous bolus insulin-induced hypoglycemia to study the PP response, as a measure of possible re-innervation of the graft.

The tests were carried out at least two days apart in conscious dogs after an overnight fast. With the exclusion of meal tests, dogs were resting in Pavlov slings. Intravenous infusions were delivered using syringe pumps (Terufusion STC-521; Terumo, Tokyo, Japan) through 20-gauge Teflon cannulas inserted in hindleg veins. Blood samples were drawn and boluses administered through 17-gauge Teflon cannulas inserted in foreleg veins. Blood samples were collected on ice in EDTA-containing tubes supplemented with aprotinin (1000 KIE/ml blood; Trasylol, Bayer, Leverkusen, Germany) for glucagon and GIP assay. Plasma was separated at 4°C within 15 min, and samples for RIA were kept below –70°C pending assay.

Meals were given in the kennel after separation of the dogs to ensure ingestion within 15 min of 500 ml of the regular semi-liquid meal. Blood specimens were drawn by puncturing external jugular veins at 0, 15, 30, 60, 90, and 120 min for assessment of the integrated responses above baseline of plasma glucose, insulin, glucagon, PP, and GIP during two postprandial hours. Baseline GIP levels were below the detection limit of the assay (40 pM), and

were assumed to equal the detection limit for analysis.

IVGTTs were performed by bolus infusion over 30 s of 0.5 g/kg glucose as a 40 % solution, and samples for measurement of glucose and insulin were obtained at 0, 1, 3, 5, 10, 15, 30, 45, and 60 min to determine glucose clearance (*Kg*) from 10 min as reported previously [12], and the weighed integrated insulin responses above baseline from 0–3 min (acute response) and from 0–60 min.

For assessment of the insulin secretory capacity, a variable rate infusion of a 40% glucose solution was started from 0 min at 150 ml/h to raise and maintain plasma glucose at approximately 35 mM, guided by on-line plasma glucose analysis every five min to manually control the glucose infusion rate. At 50 min, 2 g arginine hydrochloride was administered as a 10% solution over 30 s, and blood for hormone assay was drawn at 0, 15, 30, 45, 50, 52, 53, 54, 55, 60, 70, and 80 min. The secondary insulin response to 35 mM glucose was expressed in the mean increment from 45 to 50 min above baseline. The acute insulin and PP responses to arginine were expressed in the mean increment from 52 to 55 min over the mean prestimulus level from 45 to 50 min.

The plasma PP response to insulin-induced hypoglycemia was tested by intravenous infusion of an insulin bolus (0.15 U/kg, Actrapid, Novo, Copenhagen, Denmark). Blood samples were taken at 0, 10, 20, 30, 45, and 60 min for assay of plasma PP and glucose. The peak PP response was expressed in the peak increment above baseline.

Histology

In a separate group of normal beagles (n = 14) three wedge-biopsies were taken from the tail, body, and head of the pancreas respectively, for assessment of the native islet mass of the pancreas by the point counting method as described previously [13]. Briefly, after Bouin fixation and paraffin embedding of pancreatic specimens, ten serial 5-µm sections were taken at 150-µm intervals, stained with hematoxylin-eosin, and examined using a 400-point grid at x 200 final magnification. The fractional islet volume of the whole pancreas was expressed in the mean of the three regional fractional islet volumes. The mean value in these animals served as a reference for estimation of the native islet mass in the pancreases of experimental animals by multiplying this reference value by the wet weight of their glands — assuming a density for pancreatic tissue of 1.000 g/ml.

After functional failure of the graft, specimens of the spleen were fixed immediately after surgical removal in formalin, and embedded in paraplast.

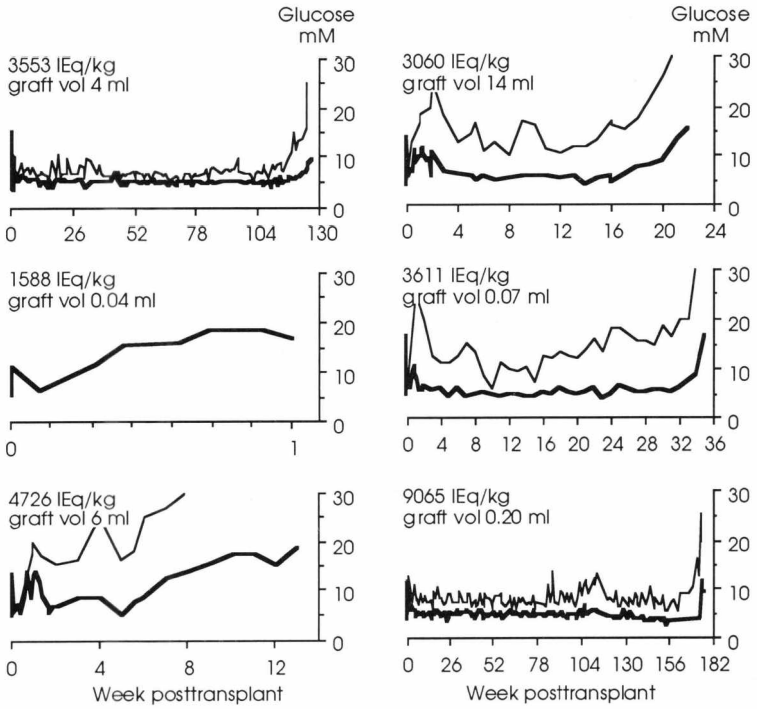


Fig. VII.1

Profiles of weekly assessed fasting (fat line) and 2-h postprandial plasma glucose in each animal, until onset of overt fasting hyperglycemia. The number of transplanted islets per kg body weight is expressed in islet equivalents (IEq); one IEq equals the volume of an islet with a 150 μm diameter. Graft vol indicates packed tissue volume

Sections were stained with hematoxylin-eosin, azan, or immunostained for insulin as described previously [15].

Analytical procedures

Glucose was measured using a glucose oxidase method with a Glucose Analyser 2 (Beckman Instruments, Brea, Ca., USA). The detailed methods used for radioimmunoassay of plasma insulin, PP, pancreatic glucagon [12], and GIP [16] have been reported.

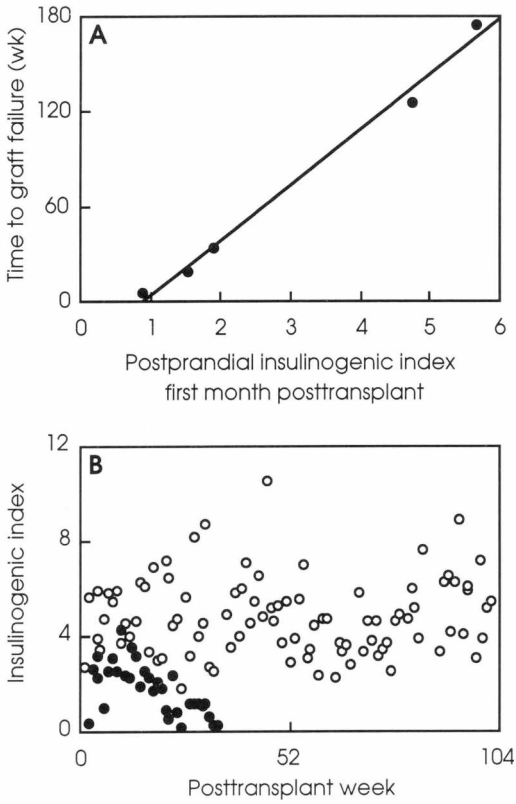


Fig. VII.2 A–B

*Correlation **A** of the postprandial insulinogenic index (ratio of 2-h postprandial plasma insulin to glucose) at one month posttranspant (mean value 2–6 weeks) in each animal vs the time to functional graft failure (r = 0.99);*
*and **B** serial, weekly, postprandial insulinogenic index up to two years posttransplant in two representative dogs, illustrating the steadily declining insulin secretory capacity from one month (r =0.7, $p < .001$) in grafts that failed within one year (closed circles), and no deterioration of graft function in the grafts that functioned over two years (open circles)*

Calculations and statistical analysis

Results are expressed as mean ± SE. Integrated responses were calculated using the trapezoidal rule, and weighed by dividing by the respective periods. Logarithmic transformation of data was used when appropriate to normalise the distribution of the data. Differences between means were evaluated by single factor analysis of variance with repeated measures and multiple comparisons were performed by Scheffé's test and Fisher's protected least significant difference procedure. Differences were considered not significant at $p > .05$.

Table VII.1

Graft function after intrasplenic islet autotransplantation in dogs

Parameter	Preoperation	Postoperation	
		1 month	6 months
Fasting			
Glucose (mM)	5.2±0.1	5.1±0.1	4.9±0.2
Insulin (mU/l)	10±1	9±1	10±0
Glucagon (ng/l)	48±12	56±17	52±14
PP (pM)	35±9	10±1†	11±2†
Intravenous glucose tolerance test			
Glucose clearance, K_g(–%/min)	2.2±0.4	1.1±0.2†	1.0±0.2†
Integrated insulin (mU/l)	25±2	6±2‡	4±1‡
Acute insulin response (mU/l)	54±7	7±1‡	6±3‡
Glucose clamped at 35 mM			
Insulin response (mU/l)	235±45	23±6‡	14±4‡
Arginine i.v. at 35 mM glycemia			
Acute insulin response (mU/l)	116±40	16±6‡	16±6‡
Acute PP response (pM)	40±18	3±1†	4±2†
Postprandial			
Glucose (mM)	0.3±0.2	3.6±1.6†	4.2±1.5†
Insulin (mU/l)	22±4	21±5	26±7
Glucagon (ng/l)	44±13	56±18	91±17*
PP (pM)	275±42	3±3‡	5±3‡
GIP (pM)	198±13	250±33	281±43*
Insulin tolerance test			
Trough glucose (mM)	2.6±0.4	3.4±0.3	2.5±0.2
Incremental peak PP (pM)	201±58	ND	1±1‡

* $p < .05$, † $p < .01$, ‡ $p < .001$ compared with preoperative values. Values are means ± SE of four animals. Individual fasting levels of glucose, insulin, and PP were calculated as means of three values obtained on separate days. PP, pancreatic polypeptide; GIP, glucose-dependent insulinotropic polypeptide; ND, not determined.

Results

Animals and graft survival

The islet dose at transplantation ranged from 1588–9065 islet equivalents (IEq) per kg body weight. The fractional islet volume of the pancreas in control dogs was 1.07 ± 0.07 %. Based on this reference value, and the pancreas and body

weights of the experimental dogs, the native islet mass in the pancreas of experimental dogs averaged 14668 IEq/kg before operation, and the mean dose of transplanted islets (4267 IEq/kg) corresponded to 29% of the preoperative islet mass. Purity (relative islet volume) of the fully purified grafts was >95%, and total graft volumes were less than 0.3 ml. Packed tissue volume of the partially purified grafts ranged from 4 to 14 ml. The individual graft data and the fasting and 2-h postprandial glucose values until onset of hyperglycemia in the animals are shown in Figure VII.1. The recipient of only 1588 IEq per kg, became overt hyperglycemic within a few days. The other animals received > 3000 IEq/kg and four dogs became normoglycemic 10–12 days after transplantation of a packed tissue volume ≤ 6 ml. Time to normoglycemia was delayed to five weeks in the recipient of the 14 ml graft. Microscopic examination of the spleens after functional failure of the partially purified grafts, demonstrated prominent scarring, well-formed acini and flattened epithelial cells surrounding cystic spaces, and fibrotic reaction surrounding islets. By contrast, a normal histology of the spleen in recipients of the >95% pure grafts was observed.

The individual postprandial insulinogenic index (ratio of 2-h postprandial insulin to glucose levels) during the first month posttransplant (mean of serial weekly indices from 2–6 weeks) predicted (r = 0.99, p <.001; Fig. VII.2 A) the time to functional graft failure (at 6, 19, 34, 125, and 175 weeks, respectively). Serial weekly assessment of the postprandial insulinogenic indices up to two years posttransplant demonstrated a declining insulin secretory capacity from one month in all three grafts that ultimately failed within one year (r = 0.7–0.8, p < .005), but no deterioration of graft function in the other grafts (Fig. VII.2 B). Correlation analysis of the time to graft failure *vs* other parameters of graft function at one month, demonstrated no correlation of graft life with IVGTT data, and less, though significant (p < .02), correlation *vs* the acute insulin response to arginine at 35 mM glycemia.

Metabolic studies

Results from metabolic studies in four dogs at six months (4–7 months) were similar to the data at one month posttransplant (Table VII.1). We will therefore mainly focus here on the data obtained at six months. Posttransplant, normal fasting glucose and hormones except for reduced PP levels, were observed. The integrated insulin responses to intravenous glucose and arginine were reduced to roughly 15 % of preoperative values. Corresponding response curves are illustrated in Figure VII.3. By contrast, a normal to increased postprandial

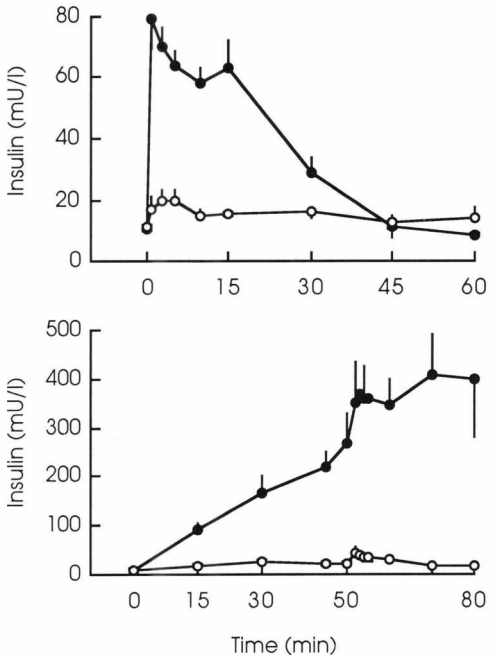


Fig. VII.3

*Plasma insulin response before operation (closed circles) and six months posttransplant (open circles) to i.v. bolus glucose infusion (**top** panel), and i.v. arginine bolus infusion (at 50 min) during a 35 mM i.v. glucose clamp (**bottom** panel)*

insulin response was observed (Table VII.1, and Fig. VII.4), albeit at a mean 10 mM glycemia (Fig. VII.4). Postprandial glucagon and the early GIP response to a meal had increased at six months (Table VII.1, and Fig. VII.4). Comparison of the posttransplant insulinemia after meal stimulation versus intravenous arginine stimulation, demonstrated the meal to be more challenging (Fig. VII.5).

At 20–30 min post intravenous bolus insulin infusion, trough glucose values less than 3.0 mM were obtained both before operation and at six months posttransplant, but not at one month — therefore, PP data at one month were not studied (Table VII.1). The PP responses to a meal, intravenous arginine, and insulin-induced hypoglycemia were severely reduced posttransplant (Table VII.1, Fig. VII.6). In contrast to insulin-induced hypoglycemia, however, both a meal and intravenous arginine still significantly stimulated PP release from the transplanted islets (bottom panel of Fig. VII.6).

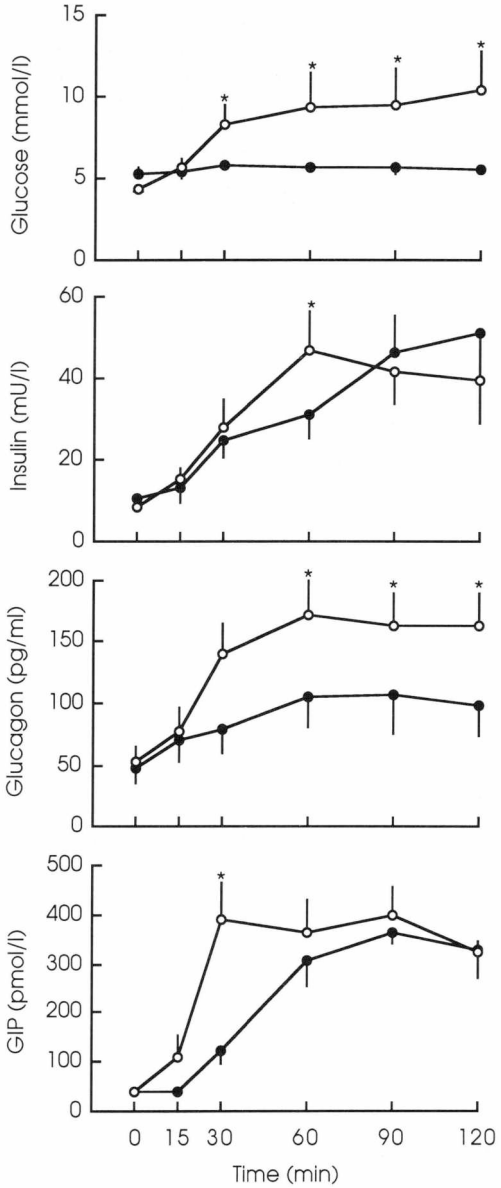


Fig. VII.4

Plasma glucose, insulin, glucagon, and glucose-dependent insulinotropic polypeptide (GIP) responses to a mixed-meal, before operation (closed circles) and six months posttransplant (open circles).
Asterisks indicate significant differences at the indicated postprandial time points before- vs post islet transplantation ($p < .05$)

Discussion

We studied metabolic function and the functional survival of isolated canine islets after intrasplenic autotransplantation. In the autotransplant model

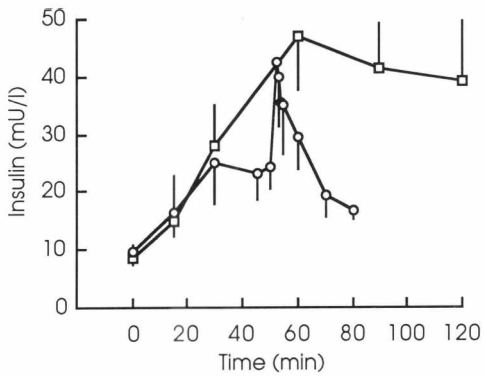


Fig. VII.5

Comparison at six months posttransplant of the postprandial insulinemia (squares) vs the insulinemia during an i.v. 35 mM glucose clamp with arginine bolus stimulation at 50 min (circles), demonstrating similar beta cell stimulation

important factors to be considered for functional graft survival are both the dose, purity, and implantation site of the graft, as well as the metabolic state of the recipient. The prolonged fasting normoglycemia after transplantation of >3000 IEq/kg body weight — corresponding to a volume of 5 µl islet tissue per kg — confirmed reports of the threshold islet dose for successful transplantation in dogs [8, 10]. The functional graft life varied greatly, from one month to three years. In contrast to a previous study by Kaufman et al. [8] graft survival time did not correlate with the islet dose, because in our study purity was varied rather than the islet dose. The importance of purification of the islet graft is controversial, at least in autotransplantation [1, 10, 17]. Successful autotransplantation of non-purified islets in the canine spleen [10, 18] and human liver [17] indicates that these organs can accommodate a large volume of dispersed tissue. However, as yet no direct comparison of the function and survival of pure and nonpurified grafts at these sites has been reported. Both our finding that a small graft volume is associated with longevity of the grafts, and our finding that fibrosis was restricted to the partially purified grafts, support the contention that contaminating acinar tissue may impair islet engraftment [8, 10, 19]. In addition to the aforementioned factors, hyperglycemia may be a factor that precipitates graft failure. Chronic hyperglycemia augments the beta cell mass of the normal pancreas [20], but may reduce the marginally adequate beta cell mass of an islet graft [21, 22]. Hyperglycemia may therefore have contributed to the declining insulinogenic index in the grafts that failed within the first year.

Tentatively, because metabolic control — as outlined above — may affect graft survival, and because graft function may reflect the interplay of other factors — such as the islet dose and purity — that affect graft survival,

parameters of early graft function may best predict graft life. Our study corroborated previous reports of a poor correlation of graft life with IVGTT data early posttransplant [6, 8, 9, 18]. The acute insulin response to arginine at roughly 30 mM glycemia is a sensitive indicator of the reduction in beta cell mass in partial pancreatectomised and diabetic subjects [23, 24]. Therefore correlation of this parameter with the engrafted beta cell mass in our study probably underlay the correlation with graft life. Whereas this test is considered to normally elicit the maximum possible (or near-maximum) insulin response [23, 25], posttransplant in our study a meal — resulting in lasting insulinemia similar to the peak insulinemia after intravenous arginine — was clearly more challenging. This may account for the close correlation of the postprandial insulinogenic index and time to graft failure.

The reasons for spontaneous failure of islet grafts within a few years in the present and other studies [6, 8, 10, 17, 18] are not clear, but larger doses of purified islets are generally expected to extend graft life. We estimated the infused islet dose to correspond to approximately 30% of the native islet mass of the pancreas. With the assumption that the reduction in beta cell mass by islet transplantation is reflected by the arginine-stimulated insulin response — the reduction of the insulin response to 15% of the preoperative value would indicate that overall only half of the infused dose eventually became engrafted. However, the reduction of the arginine-stimulated insulin response may, actually, have overestimated the reduction in beta cell mass [24].

The postprandial normo- to hyperinsulinemia as opposed to the roughly 85 % reduction of the intravenous glucose- and arginine-stimulated insulin response in the established autografts may be explained both from the different stimuli with these tests, as well as from the postprandial contribution of the enteroinsular axis. GIP and other insulinotropic gut hormones such as glucagon-like peptide 1 (GLP-1) are known to have a progressively greater stimulatory effect with higher glucose levels on insulin release from the pancreas [16, 26–28]. We recently demonstrated that physiological, circulating, levels of these peptides potentiate glucose-stimulated insulin from isolated perifused canine islets as well [29]. Thus, since the postprandial GIP response had rather increased after islet transplantation, the normo- to hyperinsulinemia was probably attained as a consequence of a postprandial hyperglycemia enhanced activation of the enteroinsular axis.

Although postprandial hyperglycemia as outlined above may account for postprandial normo-insulinemia, these glucose excursions remain to be explained. Apart from the reduced beta cell mass, both a deranged insulin secretion and PP deficiency may have been conducive to glucose intolerance.

Since postprandial insulin was not quantitatively affected, but insufficient with respect to the prevailing glycemia, fine regulation of insulin release seems deranged. Firstly, the largely or entirely (vagal) cholinergic mediated preabsorptive insulin response is important for postprandial glucose tolerance [30] — and either is absent or drastically curtailed due to denervation of the graft [30, 31]. Histological evidence for parasympathetic and sympathetic re-innervation from two months after intrahepatic and subcapsular renal implantation in rodents has been reported [32], and re-innervation of exclusively sympathetic origin has been observed in intrasplenic canine grafts [33]. Physiological confirmation of these findings has not been presented. In our study, the PP response to a meal and arginine demonstrated the posttransplant presence of functionally active PP cells. Since the PP response to hypoglycemia is exclusively mediated by vagal cholinergic nerve fibers [11], the abolished response to hypoglycemia after transplantation corroborated the latter histological report of persistent cholinergic denervation of the intrasplenic graft in dogs. Second, a growing body of evidence suggests that an intrinsic autonomously functioning intrapancreatic neuronal network co-ordinates the secretory activity of islets, to produce pulsatile peptide secretion [34]. Pulsatile compared to nonpulsatile insulin delivery requires far less insulin to obtain normoglycemia [35]. Pulsatile insulin secretion from isolated islets has been demonstrated [36–38], but pulse intervals were abnormal, and small changes in the periodicity are known to affect insulin sensitivity, and glucose handling [39]. Preliminary data from hyperinsulinemic euglycemic clamps at 6–9 months in three of the grafts (data not shown) indeed indicated insulin resistance, albeit non-significant, compared to values in a large (n = 26) control group. By contrast, a normal insulin sensitivity has been reported in a previous study of canine islet autografts [40]. This issue therefore requires further investigation. Studies of other conditions characterised by a reduced insulin secretory capacity and insulin resistance — such as pancreatectomy and pancreatitis — indicate that these defects may also account for the hyperglucagonemia and the normal to augmented postprandial GIP levels in our model [41–43]. Finally, apart from a deranged insulin secretion, PP deficiency may be related to impaired glucose handling after islet transplantation. Exogenous PP administration has been shown to improve insulin sensitivity in congenitally PP deficient obese rodents [44]. Duct-ligation induced chronic pancreatitis and glucose intolerance in the dog is also associated with PP deficiency and a diminished inhibiting effect of exogenous insulin on hepatic glucose production — and, exogenous PP has been shown to partially reverse these aberrations [45]. Thus, the postprandial PP response may be important to

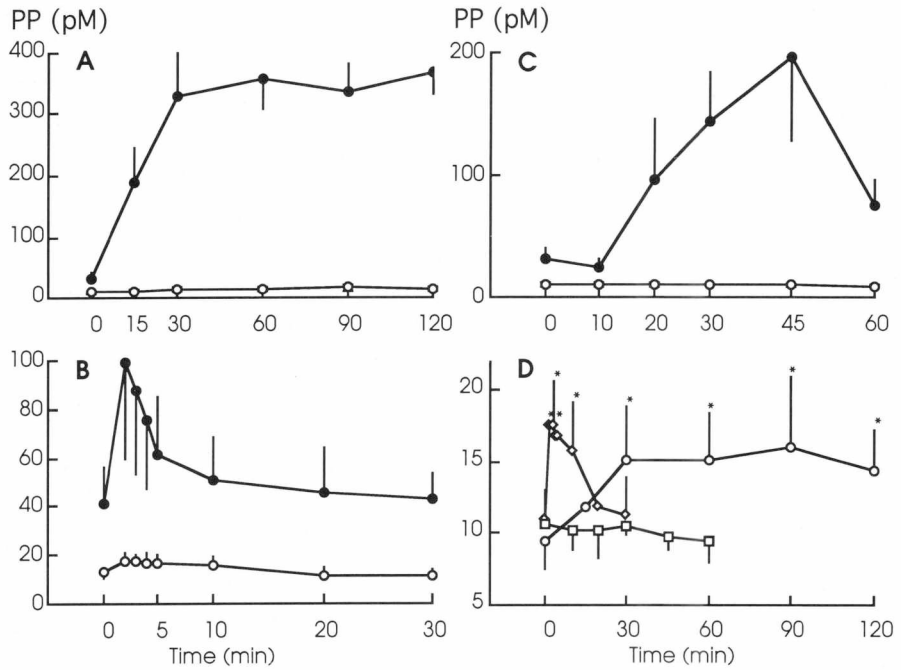


Fig. VII.6 A–D

Plasma pancreatic polypeptide (PP) responses, before (closed circles) and six months after islet transplantation, to ***A*** *mixed-meal stimulation,* ***B*** *an i.v. arginine bolus, and* ***C*** *i.v. bolus insulin-induced hypoglycemia.* ***D*** *Overlay plot of the posttransplant PP responses to a meal (circles), arginine (diamonds) and i.v. insulin-induced hypoglycemia (squares). Asterisks indicate significant differences with baseline PP levels. Insulin-induced hypoglycemia did not stimulate PP secretion, indicating no cholinergic re-innervation of transplanted islets*

mediate the inhibiting effect of insulin on hepatic glucose production.

In conclusion, both the islet dose, purity of the graft, and resultant glycemia may determine graft life. The acute insulin response to arginine at >30 mM glycemia probably best reflected the engrafted beta cell mass, but both the insulin secretory capacity of the grafts, graft life expectancy, and glucose regulation were best documented by a meal challenge. Tentatively, the postprandial normoinsulinemia as opposed to a severely reduced insulin response to intravenous glucose and arginine can be explained by the postprandial hyperglycemia-enhanced activation of the enteroinsular axis; and the moderate postprandial hyperglycemia may probably be attributed to insulin resistance due to a deranged pulsatile delivery of insulin, and PP

deficiency.

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